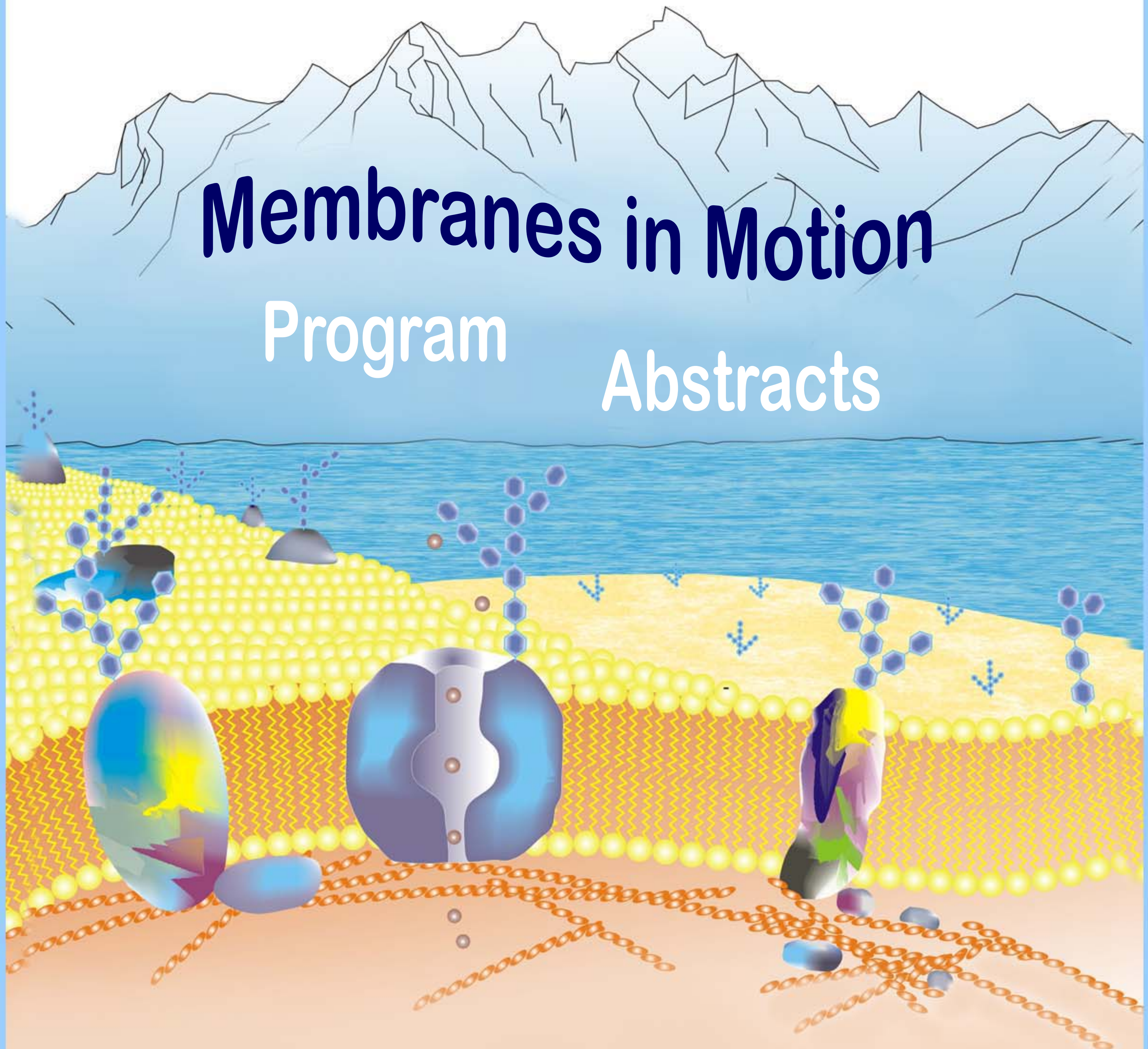
USSBE USGEB

40 Years USGEB

UNIVERSITÄT BERN

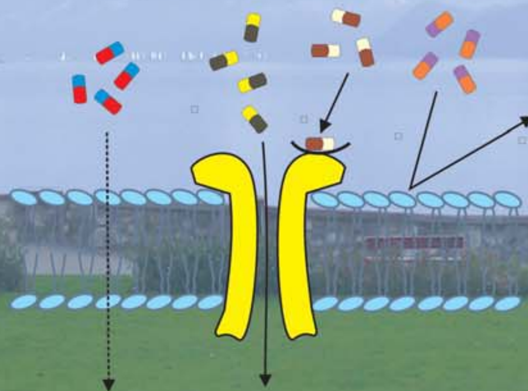
USGEB 2009

January 29/30 Casino Kursaal Interlaken



http://www.usgeb2009.ch

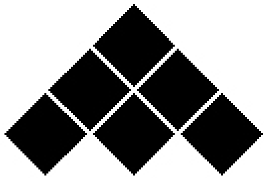
Membrane transporters and their impact on drug discovery

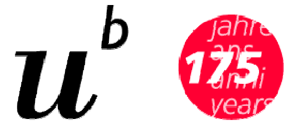


Seepark Congress Center, Thun, Switzerland
August 9-13, 2009

Organizers:
Matthias Hediger
(University of Bern, Switzerland)
Robert Burrier
(EMD Chemicals, Inc.USA)
Ken-ichi Inui
(Kyoto University, Japan)
Yoshikatsu Kanai
(Osaka University, Japan)
Bruno Stieger
(University of Zürich, Switzerland)

http://www.bioparadigms.org/





b
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41ST ANNUAL MEETING OF THE SWISS SOCIETIES FOR EXPERIMENTAL BIOLOGY (USGEB/USSBE)

29 – 30 January 2009
Casino Kursaal Interlaken

Organizing Committee:

Matthias Hediger, Chairman	Institute of Biochemistry and Molecular Medicine
Rupert Bruckmaier	Veterinary Physiology
Peter Bütikofer	Institute of Biochemistry and Molecular Medicine
Stephan Christen	Institute for Infectious Diseases
Annette Draeger	Institute of Anatomy
Britta Engelhardt	Theodor Kocher Institute
Bernhard Erni	Department of Chemistry and Biochemistry
Pascal Maeser	Institute of Cell Biology
Ernst Niggli	Department of Physiology
Peter Ott	Institute of Biochemistry and Molecular Medicine
Doris Rentsch	Institute of Plant Science
Uwe Simon	Institute of Pharmacology

Congress Website http://www.usgeb2009.ch:

Informatikdienste der Universität Bern

Conference Coordinator:

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WELCOME TO USGEB 2009

Message from the USGEB President

Welcome to Interlaken and to this year's USGEB/USSBE congress. This year we celebrate the 40th birthday of USGEB/USSBE and are proud to offer you again a great program of scientific activities to look forward to over the next two days. Thus, although USGEB/USSBE gets older, we are a young and vibrant society. Matthias Hediger and his team from the University of Berne have closely worked with the different member and associated societies of USGEB/USSBE to develop a program that covers the full range of life sciences in Switzerland. Besides the lectures, we have a large poster exhibition and we are making a special effort to give time and space for discussion around the posters. I hope those of you who are presenting posters get plenty of comments and feedback at your poster. For those who are not already members of one of the USGEB/USSBE societies, I encourage you to join us and participate in our various activities. If you are already a member, please encourage your friends and colleagues to join one of our societies. Everyone interested in life sciences is welcome! Have a great time in Interlaken and as always, if you have any ideas for further improvements of our meetings, do let us know!

Hans-Uwe Simon, University of Berne
President, USGEB/USSBE

Message from the USGEB 2009 Chair

Welcome to USGEB 2009 entitled "Membranes in Motion"! This meeting is a great opportunity to review and discuss the latest breakthroughs in membrane biology and other fields of biomedical research, while celebrating the 40th anniversary of USGEB. In recent years, spectacular new insights into the cell biology, biophysics and structural biology of membrane proteins and the associated membrane lipids have been obtained. Let's "set in motion" all "membranes" and "membrane proteins" through stimulating interactions and discussions among young scientists, world-renowned leaders in the membrane biology field and the delegates from industry. The meeting will be attended by scientists from both academia and industry all over Switzerland, as well as from surrounding countries.

USGEB 2009 features 6 plenary lectures. We have selected a diverse group of speakers - all prominent scientists and leaders in the field of membrane biology: David E. Clapham (Harvard University, USA), Sirpa Jalkanen (University of Turku, Finland), Kaspar P. Locher (ETH Zürich, Switzerland), Nathan Nelson (Tel Aviv University, Israel), Krzysztof Palczewski (Case Western Reserve University, USA), and Erich Gulbins (University of Duisburg-Essen, Germany). In addition, all USGEB member societies are organizing dedicated symposia. The scientific diversity of USGEB 2009 will greatly enhance networking between scientists from different disciplines.

The USGEB society marks its 40th anniversary. The Union of Swiss Societies for Experimental Biology USGEB has been established in 1969 in response to rapid progress in biological research. USGEB was founded to generate a platform to stimulate interdisciplinary scientific interactions. The Swiss societies for physiology, pharmacology, biochemistry, cell biology and molecular biology, as well as additional specialty societies later on, joined forces. The "roots" of USGEB are solidly implanted in the scientific community and its "shoots" are lively and growing. Thus, to celebrate this special anniversary, Ewald R. Weibel (USGEB founding member) will give a special lecture on the history and development of the society.

The Friedrich Miescher Prize is awarded each year by a jury of scientists from Swiss Universities for outstanding achievements in the field of biochemistry. This year we are happy to announce that the Friedrich Miescher Prize will be given to Theresa Fitzpatrick, Department of Botany and Plant Biology, University of Geneva. Congratulations!

We hope that you will enjoy USGEB 2009 and you will find the lectures, sessions, poster presentations, scientific interactions, discussions and the cocktail dinner pleasant, beneficial and stimulating.

Please also visit the booths of the exhibitors to get the latest information on technical advances and novel reagents.

Matthias A. Hediger, University of Berne
Chair USGEB 2009

USGEB SOCIETIES ORGANIZING A SYMPOSIUM

Swiss Society for Anatomy, Histology and Embryology (sgAHE)
http://www.unifr.ch/sgahe

Swiss Society for Biochemistry (BIO)
http://www.swissbiochem.unibe.ch

Swiss Society for Pharmacology and Toxicology (PHA)
http://www.swisspharmtox.ch

Swiss Physiological Society (PHY)
http://www.swissphysio.org

Swiss Society for Oncology (SSO-SGO)
http://www.sso-sgo.ch

Società Ticinese della Scienze Biomediche e Chimiche (STSBC)
http://www.stsbc.ch

Swiss Society for Cell Biology, Molecular Biology and Genetics (ZMG)
http://zmg.scnatweb.ch

USGEB AFFILIATED SOCIETIES ORGANIZING A SYMPOSIUM

Swiss Laboratory Animal Science Association (SGV)
http://www.sgv.org

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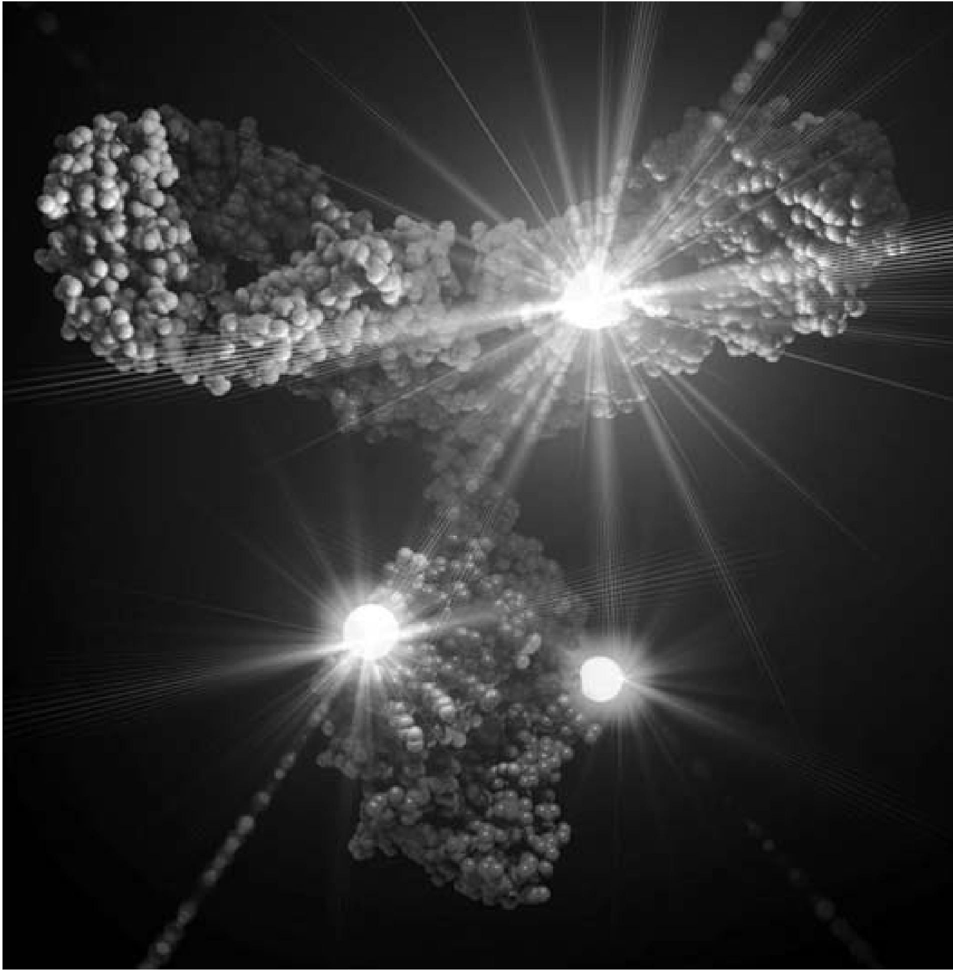

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PROGRAM OVERVIEW

Thursday, January 29, 2009

08:00 - 09:30	Registration / Mounting of Posters
09:30 - 09:50	Opening & Welcome
09:50 - 10:30	Plenary Lecture 1
10:30 - 11:00	Coffe and Tea / Exhibition / Posters
11:00 - 13:00	3 Concurrent Symposia – PHA / STSBC / ZMG
13:00 - 14:30	Lunch / Exhibition / Posters
14:30 - 16:30	3 Concurrent Symposia – BIO / Transporters / SGV
16:30 - 17:00	Coffe and Tea / Exhibition / Posters
17:00 - 17:40	Plenary Lecture 2
17:40 - 18:20	Plenary Lecture 3
18:20 - 18:50	40 Years USGEB
18:50 - 21:00	Cocktail Dinner

Friday, January 30, 2009

09:10 - 09:50	Plenary Lecture 4
09:50 - 10:30	Plenary Lecture 5
10:30 - 11:00	Coffe and Tea / Exhibition / Posters
11:00 - 13:00	3 Concurrent Symposia – PHY / AHE / SGO-SSO
13:00 - 14:30	Business Meetings of the USGEB Societies
13:00 - 14:30	Lunch / Exhibition / Posters
14:30 –15:10	Plenary Lecture 6
15:15 –15:55	Friedrich Miescher Award
16:00 - 16:15	sgAHE Morphologiepreis
16:20 - 16:40	Presentation of Poster Awards
	Closing Remarks

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GENERAL INFORMATION

Registration and Information

Desk:

The desk is located on "Eingang Ost" (Foyer Ost)
It will be open from 08:30 to 17:00 on Thursday and from 08:30 to 14.30 on Friday
Backoffice phone: +41 76 302 52 30
Meeting Address: Casino Kursaal Interlaken, Strandbadstrasse 44, CH-3800 Interlaken

Badges:

Must be obtained from the registration desk. They give access to the conference rooms, the coffee stands and the Thursday evening Dinner. **Please wear them at all times.**

Message Board:

A message board will be located near the registration desk. Personal messages can also be left there.

Posters:

Presenters of posters are kindly asked to be available during the coffee and lunch breaks.

Accreditation:

The SGV Symposium at USGEB 2009 has been accredited as a half day of continued training by the State Veterinary Office. The accreditation will be forwarded to you electronically providing that you register yourself at the Symposium.

Coffee Breaks and Lunches:

All participants and exhibitors are invited to the coffee/tea breaks. They are served in the exhibition/poster rooms. You can buy your lunch in the exhibition/poster rooms. We kindly ask you to stay at the Conference Center for lunch so you have enough time to visit the exhibition and posters.

Dinner:

All participants and exhibitors are invited to attend the cocktail dinner on Thursday, January 29.

Internet Access:

Internet Access is possible via a Swisscom Hotspot

Travel Information:

To Basel									
Interlaken West dep	17:06	17:31	18:06	18:36	19:06	19:31	20:06	21:06	22:06
Spiez arr		17:50				19:50			
Spiez dep		17:54				19:54			
Basel SBB arr	18:55	19:32	19:55	20:32	20:55	21:32	21:55	22:55	23:55

Travel Information continued:

To Bern, Zürich

Interlaken West dep	17:06	17:31	18:06	18:36	19:06	19:31	20:06	21:06	22:06
Spiez arr		17:50				19:50			
Spiez dep		17:54				19:54			
Bern arr	17:52	18:23	18:52	19:23	19:52	20:23	20:52	21:52	22:52
Bern dep	18:02	18:32	19:02	19:32	20:02	20:32	21:02	22:02	23:02
Zürich HB arr	18:58	19:28	19:58	20:28	20:58	21:28	21:58	23:01	23:58

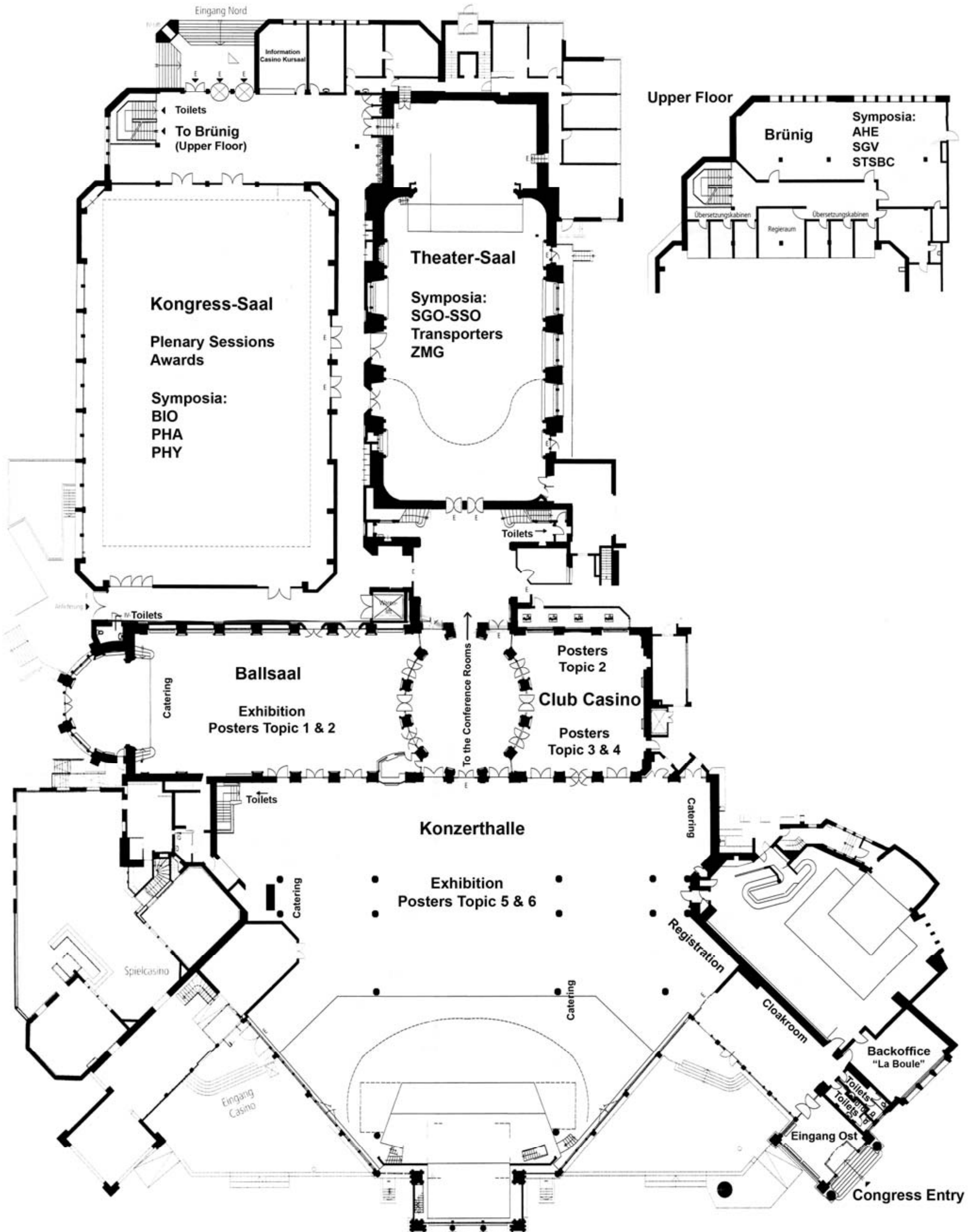
To Bern, Fribourg, Lausanne, Geneva

Interlaken West dep	17:06	17:31	18:06	18:36	19:06	19:31	20:06	21:06	22:06
Spiez arr		17:50				19:50			
Spiez dep		17:54				19:54			
Bern arr	17:52	18:23	18:52	19:23	19:52	20:23	20:52	21:52	22:52
Bern dep	18:04	18:34	19:04	19:34	20:04	20:34	21:04	22:04	23:08
Fribourg arr	18:25	18:54	19:25	19:54	20:25	20:54	21:25	22:25	23:29
Lausanne arr	19:15	19:40	20:15	20:40	21:15	21:40	22:15	23:15	00:19
Geneva arr	19:50	20:15	20:50	21:15	22:04	22:15	23:04	23:50	01:05

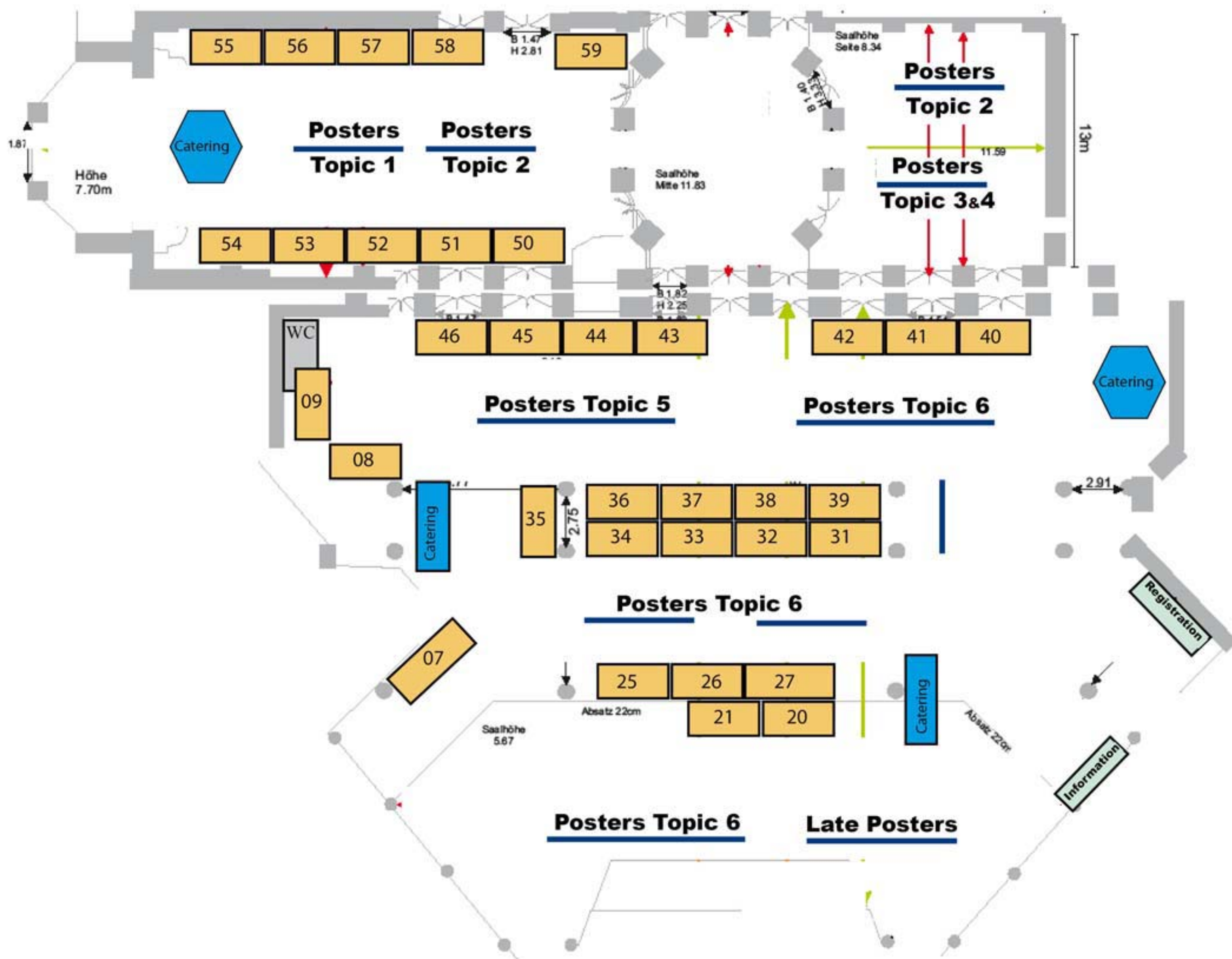
More travel information at: http://www.sbb.ch

SITE MAP

All rooms are located on the Ground Floor except “Brünig”



EXHIBITION AND POSTERS



LIST OF EXHIBITORS

Company Name	Place	Booth No.
ADInstruments GmbH	D-74937 Spechbach	53
AMS Biotechnology (Europe) Ltd.	CH-6934 Bioggio Lugano	58
Applied Biosystems	CH-6343 Rotkreuz	51
Axon Lab	CH-5405 Baden-Dättwil	38
Berthold Technologies (Schweiz) GmbH	CH-8105 Regensdorf	44
BioConcept	CH-4123 Allschwil	27
Biolabo Scientific Instruments SA	CH-1618 Châtel St.Denis	25
Bio-Rad Laboratories AG	CH-4153 Reinach	09
Bucher Biotec AG	CH-4051 Basel	42-43
Carl Zeiss AG	CH-8714 Feldbach	31
Chemie Brunschwig AG	CH-4009 Basel	08
Huber & Co. AG	CH-4153 Reinach	39
IG Instrumenten-Gesellschaft AG	CH-8045 Zürich	45
Jackson ImmunoResearch Europe Ltd.	UK-Newmarket	55
Labforce AG	CH-4208 Nunningen	07
Leica Microsystems Schweiz AG	CH-9435 Heerbrugg	34
LuBioScience GmbH	CH-6000 Luzern 6	50
Macherey-Nagel AG	CH-4702 Oensingen	46
Microsynth AG	CH-9436 Balgach	57
Nikon AG	CH-8132 Egg	41
Olympus Schweiz AG	CH-8604 Volketswil	54
Omnilab AG	CH-8932 Mettmenstetten	36
Roth AG	CH-4153 Reinach	20
Science Products AG	CH-4055 Basel	26
Sigma-Aldrich Chemie GmbH	CH-9471 Buchs	35
Skan AG	CH-4123 Allschwil	32
Socorex ISBA S.A.	CH-1024 Ecublens	21
Stiftung biobank-suisse	CH-3001 Bern	52
Thermo Fisher Scientific (Zürich) AG	CH-8045 Zürich	59
Thermo Scientific	CH-1002 Lausanne	56
Vaudaux-Eppendorf AG	CH-4124 Schönenbuch	37
Vitaris AG	CH-6340 Baar	33
Witec AG	CH-6014 Littau	40

LIST OF SPONSORS

The organizers would like to acknowledge the sponsors of USGEB 2009 for their generous financial support.

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ADMINISTRATIVE MEETINGS OF THE MEMBER SOCIETIES

The administrative meetings of the member societies will take place on

Friday, January 30, 2009 from 13:30 to 14:30

in the following rooms:

Society	Room
BIO	**Theater-Saal**
SGO-SSO	**Kongress-Saal**
ZMG	**Brünig**

AWARDS

Friday, January 30, 2009 from 15:15 to 16:40

Friedrich Miescher Award	**Kongress-Saal**
sgAHE Morphologiepreis	**Kongress-Saal**
Presentation of Poster Awards	**Kongress-Saal**

PLENARY LECTURES THURSDAY

Room: Kongress-Saal

Thursday, January 29, 09:30 – 09:50

Uwe Simon – USGEB President

Matthias Hediger – Chair USGEB 2009

Opening and Welcome

Thursday, January 29, 09:50 – 10:30

David E. Clapham
Department of Neurobiology, Harvard University, Boston, MA, USA

TRP Ion Channels
Abstract P1-1

Chair: Matthias Hediger, Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland

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Thursday, January 29, 17:00 – 17:40

Nathan Nelson
Department of Biochemistry, Tel Aviv University, Tel Aviv, Israel

Molecular evolution of perfection and imperfection
Abstract P2-1

Chair: Pascal Mäser, Institute of Cell Biology, University of Bern, Switzerland

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Thursday, January 29, 17:40 – 18:20

Erich Gulbins
Department of Molecular Biology, University of Duisburg-Essen, Germany

Lipid rafts and redox signaling
Abstract P2-2

Chair: Annette Draeger, Institute of Anatomy, University of Bern, Switzerland

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Thursday, January 29, 18:20 – 18:50

Ewald Weibel
USGEB Founding Member and Former Chairman, Professor Emeritus of Anatomy, Institute of Anatomy, University of Bern, Switzerland

40 Years USGEB – Of Roots and Shoots
Abstract P2-3

Chair: Annette Draeger, Institute of Anatomy, University of Bern, Switzerland

PLENARY LECTURES FRIDAY

Room: Kongress-Saal

Friday, January 30, 09:10 – 09:50

Krzysztof Palczewski
Department of Pharmacology, Case Western Reserve University, Cleveland, Ohio, USA

Chemistry of Vision: Inherited Retinal Diseases and Rhodopsin
Abstract P3-1

Chair: Dimitrios Fotiadis, Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland

Friday, January 30, 09:50 – 10:30

Kaspar Locher
Department of Biology, Institute of Molecular Biology and Biophysics, ETH Zürich, Switzerland

Structure and mechanism of ABC transporters
Abstract P3-2

Chair: Bernhard Erni, Department of Chemistry and Biochemistry, University of Bern, Switzerland

Friday, January 30, 14:30 – 15:10

Sirpa Jalkanen
MediCity Research Laboratory, University of Turku, Finland

Ectoenzymes in control of cell trafficking
Abstract P4-1

Chair: Britta Engelhardt, Institute Theodor Kocher, University of Bern, Switzerland

Friday, January 30, 15:15 – 15:55

Teresa Fitzpatrick
Department of Botany and Plant Biology, University of Geneva, Switzerland

Vitamin B6 biosynthesis: Steps towards unraveling a complex puzzle
Abstract P4-2

Winner of the 2009 Friedrich Miescher Award

CONCURRENT SYMPOSIA PROGRAM THURSDAY

Society	Title	Speakers	Day	Time	Room
PHA	**Ion channels as drug targets: from function to therapeutic application** Symposium 1	D. Beech, A. Brüggemann, A. Arcangeli V. Bize, G. Shapovalov, K. Bolanz	**Thursday**	11:00 - 13:00	Kongress-Saal
STSBC	**Viruses and Membranes** Symposium 2	A. Helenius, A. Trkola, A. Macagno R. Bernasconi, D. Bausch, P. Masson	**Thursday**	11:00 - 13:00	Brünig
ZMG	**Genes and Development** Symposium 3	M. Noll, R. Zeller, H. Riezmann Ch. Gallo, N. Aeple, St. Meier	**Thursday**	11:00 - 13:00	Theater-Saal
BIO	**Functionalizing membranes by proteins and lipids** Symposium 4	R. Jahn, G. van der Goot, A. Engel J.-M. Segura, P. Philippsen, H. Ding	**Thursday**	14:30 - 16:30	Kongress-Saal
	Transporters: Determinants for Nutrient and Drug Absorption and Waste Disposal Symposium 5	H. Lennernäs, B. Stieger, Y. Hagos, H. Koepsell, F. Winkler	**Thursday**	14:30 - 16:30	Theater-Saal
SGV	**Biosafety** Symposium 6	P. Baehler, A. Foletti, S. Baechler, K. Summermatter	**Thursday**	14:30 - 16:30	Brünig

CONCURRENT SYMPOSIA PROGRAM FRIDAY

Society	Title	Speakers	Day	Time	Room
PHY	**Channelopathies** Symposium 7	F. Lehmann-Horn, I. Decosterd, E. Niggli Y. Suzuki, L. Sintra, M.Grunnet	**Friday**	11:00 - 13:00	Kongress-Saal
AHE	**Advanced Imaging of Membrane Protein Function** Symposium 8	J. Peti-Peterdi, A. Stemmer, A. Engel E. Babiychuk, S. Tabone-Eglinger, R. Hilf	**Friday**	11:00 - 13:00	Brünig
SGO-SSO	**Membranes and Cancer** Symposium 9	B. Borisch, N. Hynes, Y. Shai P. Forny, D. Ilgen, D. de Beer	**Friday**	11:00 - 13:00	Theater-Saal

SYMPOSIA DETAILS

PHA

Ion channels as drug targets: from function to therapeutic application

Recent advances in the pharmacological investigation of ion channels

Symposium S1

Thursday, January 29, 11:00 - 13:00

Room: Kongress-Saal

Chair: **Olivier Staub**, Dept. of Pharmacology and Toxicology, University of Lausanne

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11:00 – 11:25
David Beech
Institute of Membrane & Systems Biology, University of Leeds, UK

TRP channels in human rheumatoid arthritis
Abstract S1-1

11:25 – 11:50
Andrea Brüggemann
Research & Development
Nanion Technologies, Munich, Germany

Large scale screening of ion channels by automated patch clamping
Abstract S1-2

11:50 – 12:15
Annarosa Arcangeli
Experimental Pathology and Oncology
University of Florence, Firenze, Italy

Targeting ion channels in cancer: a novel frontier in antineoplastic therapy
Abstract S1-3

Short Oral Presentations

12:15 – 12:30
Vincent Bize
Department of Pharmacology and Toxicology, University of Lausanne, Switzerland

Sodium self-inhibition of the epithelial sodium channel: Identification of residues involved in sodium sensing
Abstract S1-4

12:30 – 12:45
George Shapovalov
Pharmacology, University of Geneva, Switzerland

Role of TRPC1 and Orai1 channels in the regulation of store-operated currents in muscular dystrophy
Abstract S1-5

12:45 – 13:00
Katrin A. Bolanz
Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland

Tamoxifen inhibits TRPV6 activity via estrogen receptor independent pathways in TRPV6 transfected MCF-7 cells
Abstract S1-6

Viruses and Membranes

Symposium S2

Thursday, January 29, 11:00 - 13:00

Room: Brünig

Chair: **Antonio Lanzavecchia**, Laboratory of Immune Regulation, Institute for Research in Biomedicine, Bellinzona Switzerland

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11:00 – 11:25
Ari Helenius
Institute of Biochemistry, ETH Zürich, Switzerland

The role of membrane lipids in virus entry
Abstract S2-1

11:25 – 11:50
Alexandra Trkola
Division of Infectious Diseases, University Hospital Zürich, Switzerland

HIV entry inhibition
Abstract S2-2

11:50 – 12:15
Annalisa Macagno
Laboratory of Cellular Immunology, Institute for Research in Biomedicine, Bellinzona, Switzerland

Selection of novel HCMV antigens for vaccination
Abstract S2-3

Short Oral Presentations

12:15 – 12:30
Riccardo Bernasconi
Molinari Lab, Institute for Reserach in Biomedicine, Bellinzona, Switzerland

Role of ERAD substrate topology in selection of E3 ubiquitin ligases
Abstract S2-4

12:30 – 12:45
Damaris Bausch
Institute for Molecular Systems Biology, ETH Zürich, Switzerland

The Cell Surface Protein Atlas
Abstract S2-5

12:45 – 13:00
Patrick Masson
Virus Program, Swiss Institute of Bioinformatics, Geneva, Switzerland

Virus-cell membrane interactions in ViralZone
Abstract S2-6

Genes and Development

Symposium S3

Thursday, January 29, 11:00 - 13:00

Room: Theater-Saal

Chairs: **François Karch**, Zoology and Animal Biology, University of Geneva, Switzerland

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11:00 – 11:25
Markus Noll
Institute of Molecular Biology, University of Zürich, Switzerland

Formation of bicoid morphogen gradient: an mRNA gradient dictates the protein gradient
Abstract S3-1

11:25 – 11:50
Rolf Zeller
Center for Biomedicine, University of Basel, Switzerland

A Self-Regulatory Feedback Signaling System controls Vertebrate Limb Development
Abstract S3-2

11:50 – 12:15
Howard Riezman
Biochemistry, Sciences II, University of Geneva, Switzerland

Sterols and Sphingolipids in Biological Membranes: an Intimate Relationship
Abstract S3-3

Short Oral Presentations

12:15 – 12:30
Chiara Gallo
Section des Sciences pharmaceutiques, Laboratoire de Pharmacologie, University of Geneva, Switzerland

Role of the cationic channel TRPC1 in Duchenne muscular dystrophy: Analysis of double knock-out TRPC1 -/- DYS -/- skeletal muscles
Abstract S3-4

12:30 – 12:45
Natalia Aeple
Institute of Molecular Biology, University of Zürich, Switzerland

The Role of Ku80 in DSB Repair in Drosophila melanogaster
Abstract S3-5

12:45 – 13:00
Stefan Meier
Department for Plant Physiology, University of Bern, Switzerland

AtGAT1 and AtGAT2 the GABA transporters of Arabidopsis thaliana
Abstract S3-6

Functionalizing membranes by proteins and lipids

Symposium S4

Thursday, January 29, 14:30 – 16:30

Room: Kongress-Saal

Chairs: **Marcus Thelen**, Institute for Research in Biomedicine, Bellinzona, Switzerland

Margot Thome, Department of Biochemistry, University of Lausanne, Switzerland

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14:30 – 14:55
Reinhard Jahn
Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany

Membrane fusion in the secretory pathway of eukaryotic cells
Abstract S4-1

14:55 – 15:20
Gisou van der Goot
Global Health Institute, EPFL, Lausanne, Switzerland

Ubiquitin dependent quality control of a membrane protein in the ER
Abstract S4-2

15:20 – 15:45
Andreas Engel
CINA, Maurice E. Müller Insitut, Biozentrum, Basel, Switzerland

To visualize membrane proteins in their native environment
Abstract S4-3

Short Oral Presentations

15:45 – 16:00
Jean-Manuel Segura
Life Technologies, HES-SO Valais, Sion, Switzerland

Visualizing cytotoxic T cell activation at the single-molecule level
Abstract S4-4

16:00 – 16:15
Peter Phlippsen
Applied Microbiology, Biozentrum, University Basel, Switzerland

Exocytic membrane fusions and endocytic membrane internalisation: Two interdependent processes in fast growing fungal cells
Abstract S4-5

16:15 – 16:30
Heidrun Ding
Pathology and Immunology, University of Geneva, Switzerland

Raft-based signalosomes in human lymphoma cell membranes
Abstract S4-6

Transporters: Determinants for Nutrient and Drug Absorption and Waste Disposal

Symposium S5

Thursday, January 29, 14:30 – 16:30

Room: Theater-Saal

Chairs: **Bruno Stieger**, Division of Clinical Pharmacology and Toxicology, University of Zürich, Switzerland

Matthias Hediger, Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland

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14:30 – 14:55
Hans Lennernäs
Department of Pharmacy, Uppsala University, Sweden

Gastrointestinal permeability and drug absorption
Abstract S5-1

14:55 – 15:20
Bruno Stieger
Division of Clinical Pharmacology and Toxicology, University Hospital, Zürich, Switzerland

Hepatic Bile Salt and Drug Transport
Abstract S5-2

15:20 – 15:45
Yohannes Hagos
Georg-August-University Göttingen, Department Vegetative Physiology und Pathophysiology, Germany

Transport Systems in Renal Drug Elimination
Abstract S5-3

15:45 – 16:10
Hermann Koepsell
Institute of Anatomy and Cell Biology, University Würzburg, Germany

Investigation of structure and function of organic cation transporters using homology modeling and mutagenesis
Abstract S5-4

16:10 – 16:30
Fritz K. Winkler
Paul Scherrer Institut, Villigen, Switzerland

Bacterial Transport of Ammonia
Abstract S5-5

Biosafety

Symposium S6

Thursday, January 29, 14:30 – 16:30

Room: Brünig

Chairs: **Beat Riederer**, Department of Cell Biology and Morphology, University of Lausanne, Switzerland

Ingrid Kohler, Institute for Immunology and Immunoprophylaxis, Mittelhäusern, Switzerland

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14:30 – 15:00
Pascal Baehler
Security Officer, University of Lausanne, Switzerland

Safety around the animal house
Abstract S6-1

15:00 – 15:30
Alesandro Foletti
Biosecurity Officer CHUV, Lausanne, Switzerland

Biosafety in the animal house
Abstract S6-2

15:30 – 16:00
Sebastien Baechler
University Institute for Radiation Physics, University Hospital & University of Lausanne (CHUV-UNIL), Switzerland

Radioprotection in the animal house
Abstract S6-3

16:00 – 16:30
Kathrin Summermatter
Institute of Virology and Immunoprophylaxis (IVI), Federal Veterinray Office FVO, Mittelhäusern, Switzerland

Biosafety of highly contagious animal pathogens
Abstract S6-4

Channelopathies

New insights in physio(patho)logical mechanisms provided by investigation of dysfunctional ion channels

Symposium S7

Friday, January 30, 11:00 – 13:00

Room: Kongress-Saal

Chairs: **Hugues Abriel**, Pharmacology and Toxicology, University of Lausanne, Switzerland

Eric Raddatz, Department of Physiology, University of Lausanne, Switzerland

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11:00 – 11:25
Frank Lehmann-Horn
Institute of Applied Physiology, Ulm University, Germany

Pathogenetic patterns in channelopathies
Abstract S7-1

11:25 – 11:50
Isabelle Decosterd
University Hospital Center (CHUV) and Lausanne University, Switzerland

Dysregulation of voltage-gated sodium channels in the context of chronic pain
Abstract S7-2

11:50 – 12:15
Ernst Niggli
Department of Physiology, University of Bern, Switzerland

Cardiac ryanodine receptor Ca^{2+} release channels: channelopathies and hypersensitivity
Abstract S7-3

Short Oral Presentations

12:15 – 12:30
Yoshiro Suzuki
Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland

A haplotype of the epithelial Ca^{2+} channel TRPV6 is a risk factor for renal Ca^{2+} stone formation: Possible selection pressure during the migration from Africa to Europe
Abstract S7-4

12:30 – 12:45
Liliana Sintra Grilo
Pharmacology and Toxicology, University of Lausanne, Switzerland

Characterization of a novel 7-amino-acid duplication located in the PAS domain of hERG found in a patient with congenital long QT syndrome
Abstract S7-5

12:45 – 13:00
Morten Grunnet
Biomedical Sciences, University of Copenhagen, Denmark

Increased repolarization reserve as a new anti-arrhythmic principle
Abstract S7-6

Advanced Imaging of Membrane Protein Function

Symposium S8

Friday, January 30, 11:00 – 13:00

Room: Brünig

Chairs: **Johannes Loffing**, Institute of Anatomy, University of Zürich, Switzerland

Andres Käch, Center for Microscopy and Image Analysis, University of Zürich, Switzerland

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11:00 – 11:25
Janos Peti-Peterdi
Physiology & Biophysics, Medicine, University of Southern California, USA

In vivo multiphoton imaging of ion transporting renal epithelia
Abstract S8-1

11:25 – 11:50
Andreas Stemmer
Nanotechnology Group, ETH Zürich, Switzerland

Sub-100-nm resolution in TIRF microscopy
Abstract S8-2

11:50 – 12:15
Andreas Engel
CINA, Maurice E. Müller Insitut, Biozentrum, Basel, Switzerland

Imaging membrane proteins by atomic force microscopy in their native environment
Abstract S8-3

Short Oral Presentations

12:15 – 12:30
Eduard Babiychuk
Cell Biology, Institute of Anatomy, University of Bern, Switzerland

Fluorescent Annexin A1 Reveals Dynamics of Ceramide Platforms in Living Cells
Abstract S8-4

12:30 – 12:45
Séverine Tabone-Eglinger
Cellular Physiology and Metabolism, Centre Médical Universitaire, Geneva, Switzerland

Dimerization and ER-export of Kit-ligand is induced by a conserved glycine repeat motif in its transmembrane domain
Abstract S8-5

12:45 – 13:00
Ricarda Hilf
Department of Biochemistry, University of Zürich, Switzerland

Open and Closed Structures of two Pentameric Ligand Gated Ion Channels
Abstract S8-6

Membranes and Cancer

Symposium S9

Friday, January 30, 11:00 – 13:00

Room: Theater-Saal

Chair: **Bruno Fuchs**, Department of Orthopedics, University of Zürich, Balgrist, Switzerland

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11:00 – 11:25
Bettina Borisch
Santé et médecine communautaire, University of Geneva, Switzerland

Cell Membranes in Human Tumours as Therapeutic Targets
Abstract S9-1

11:25 – 11:50
Nancy Hynes
Growth Control, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Targeting receptor tyrosines in breast cancer
Abstract S9-2

11:50 – 12:15
Yechiel Shai
Dept. of Biological Chemistry, The Weizmann Institute of Science Rehovot, Israel

A lesson from nature: Membrane disrupting peptides and ultrashort lipopeptides as novel weapons against bacteria fungi and cancer
Abstract S9-3

Short Oral Presentations

12:15 – 12:30
Patrick Forny
Institute of Anatomy, University of Zürich, Switzerland

Photodynamic effects of hypericin and Foslipos in head and neck squamous cell carcinoma in-vitro
Abstract S9-4

12:30 – 12:45
Denise Ines Ilgen
Pharmaceutical Sciences, ETH Zürich, Switzerland

Investigation on lipid bilayer permeation of P-glycoprotein substrates
Abstract S9-5

12:45 – 13:00
Dirk de Beer
Haematology, University of Bern, Switzerland

Deficient nuclear expression of the Ku86 autoantigen in lymphocytes of patients with chronic lymphocytic leukaemia (CLL)
Abstract S9-6

ABSTRACTS – PLENARY LECTURES (P)

P1-1
TRP Ion Channels

David E. Clapham[1]
[1]Harvard University, Boston, USA

No abstract available.

P2-1
Molecular evolution of perfection and imperfection

Nathan Nelson[1]
[1]The Daniella Rich Institute for Structural Biology, Tel Aviv University, Tel Aviv 69978, Israel

Despite its enormous complexity, the plant PSI is arguably the most efficient nano-photochemical machine in Nature. It emerged as a homodimeric structure containing several chlorophyll molecules over 3.5 billion years ago, and has perfected its photoelectric properties ever since. The recently determined structure of plant PSI, which is at the top of the evolutionary tree of this kind of complexes, provided the first relatively high-resolution structural model of the supercomplex containing a reaction center (RC) and a peripheral antenna (LHCI) complexes. The RC is highly homologous to that of the cyanobacterial PSI and maintains the position of most transmembrane helices and chlorophylls during the last 1.5 years of separate evolution. The LHCI is composed of four nuclear gene products (Lhca1-Lhca4) that are unique among the chlorophyll a/b binding proteins in their pronounced long-wavelength absorbance and their assembly into dimers. Recently we solved the structure of PSI supercomplex at 3.4 Å resolution. The structure provides a first glimpse at the fine architecture of nature's efficient nano-photochemical machine that was perfected during long evolutionary time. In contrast, metal-ion transporter DCT1 operates with a variable stoichiometry of proton to metal ion and under optimal transport conditions more then ten protons are co-transported with a single metal ion. This imperfection is maintained during evolution suggesting that the proton slippage provides a physiological advantage and the proton slip was positively selected during the evolution of DCT1.

P2-2
Lipid rafts and redox signaling

Erich Gulbins[1]
[1]Dept. of Molecular Biology, University of Duisburg-Essen, Hufelandstrasse 55, 45122 Essen, Germany

Hydrolysis of sphingomyelin by activity of the acid sphingomyelinase triggers the formation of ceramide that spontaneously forms small ceramide-enriched domains. The fusion of these domains results in the formation of large ceramide-enriched membrane platforms. Ceramide-enriched membrane domains are critical in many aspects of cellular stress, for instance the mediation of cellular effects of irradiation, UV-light, death receptors and several pathogenic bacteria. For instance, ceramide-enriched membrane domains mediate internalisation of *Pseudomonas aeruginosa* into mammalian cells as well as the induction of cell death upon infection with this pathogen. The latter is mediated by a signaling cascade involving activation of the acid sphingomyelinase, release of ceramide, clustering of CD95 in ceramide-enriched membrane domains, stimulation of caspases and mitochondrial changes finally leading to death. Much less is known about the molecular mechanisms that mediate bacterial internalisation via activation of acid sphingomyelinase and release of ceramide. Several data suggest that ceramide-enriched membrane domains serve clustering of *P. aeruginosa* receptor molecules, for instance Cftr, that may mediate internalisation. However, the details require definition. Furthermore, ceramide-enriched membrane domains serve the clustering and activation of NADPH-oxidases upon infection of macrophages with *P. aeruginosa*. The pathogens trigger a rapid activation of NADPH-oxidases and a release of reactive oxygen species, which is abolished in macrophages lacking the acid sphingomyelinase. Reactive oxygen species further activated the acid sphingomyelinase in a positive feedback loop and promoted the formation of ceramide-enriched membrane domains that finally resulted in JNK activation and cell death. The significance of ceramide released by the acid sphingomyelinase for the host response to *P. aeruginosa* infections is indicated by experiments that investigated pulmonary infection of acid sphingomyelinase-deficient mice. These mice were unable to adequately respond to a pulmonary *P. aeruginosa* infection with an increase of ceramide in the lung, internalisation of the pathogens by bronchial epithelial cells was abrogated, bronchial epithelial cells failed to undergo apoptosis upon infection, the mice responded with an overshooting release of cytokines and, finally, died. This indicates that an adequate host response upon pulmonary infection with *P. aeruginosa* requires the formation of ceramide and ceramide-enriched membrane domains in bronchial epithelial cells and pulmonary macrophages.

P2-3
40 Years USGEB — of Roots and Shoots

Ewald R. Weibel[1]
[1]Institute of Anatomy, University of Bern, Switzerland

The Foundation of the Union of Swiss Societies for Experimental Biology USGEB in 1969 must be considered part of the broad academic reform movements of the late 1960s, of which "1968" was a prominent element. The explosive development of scientific research since the 1950s (SNF founded 1952), with cell and molecular biology becoming established new disciplines in the biomedical field, resulted in marked specialization. New institutes were formed and each discipline needed its own society to promote its own progress. This called for counteractions. The reform of the medical curriculum (Rossi plan 1967-72) was based, in the preclinical years, on integrated teaching of anatomy, physiology and biochemistry with a focus on cell and molecular biology. In the same spirit USGEB was founded in March 1969 as a platform for interdisciplinary discourse and collaboration in research. The Swiss societies for physiology, pharmacology, biochemistry and cell and molecular biology joined forces, later joined by other specialty societies. While about 80 scientists took part in the first and founding scientific meeting in May 1969 — that occurred in the presence of Bundesrat H.P. Tschudi, Federal Minister of the Interior, to mark the importance of the initiative — the attendance at recent annual USGEB meetings has been approaching 1000. The roots of USGEB are thus solidly implanted in the scientific community and its shoots are lively and growing.

P3-1

Chemistry of Vision: Inherited Retinal Diseases and Rhodopsin

Krzysztof Palczewski[1]
[1]Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106-4965, USA

Rhodopsin, which absorbs a photon to initiate visual phototransduction, belongs to the superfamily of G protein (guanine nucleotide-binding protein)-coupled receptors (GPCRs), encoded by ~950 genes of the human genome. Mutations in the rhodopsin gene may cause human diseases like retinitis pigmentosa (RP) that usually result in late-onset blindness. Like all GPCRs, rhodopsin has seven transmembrane helices and consists of an apoprotein, the opsin, together with a covalently bound chromophore, 11-cis-retinal.
Mutations in the genes encoding many proteins involved in production of this chromophore have been implicated in causing recessive blinding diseases of humans such as Leber's congenital amaurosis (LCA), Stargardt macular degeneration, congenital cone-rod dystrophy, and retinitis pigmentosa (RP). Promises of therapy for incurable blinding hereditary retinal diseases are more frequently discussed owing to major scientific advances that have increased our understanding of basic disease mechanisms. For instance, mutation in the RPE genes encoding RPE65 is one of several known molecular causes of these blinding diseases, and several therapeutic approaches to treat LCA have been proposed: RPE transplantation, gene replacement therapy, and pharmacological intervention. For example, some of these diseases can be treated by dietary intake of active chromophores or their 9-cis-precursors. We wish to capitalize on recent progress and continue working to understand how vision is triggered by light, is maintained in healthy individuals, and how it can be rescued or regenerated in patients who experience environmental insults on their vision or are prone to vision loss due to their genetic background.

P3-2

Structure and mechanism of ABC transporters

Kaspar Locher[1]
[1]Institute of Molecular Biology and Biophysics, ETH Zurich, Switzerland

ABC transporters are ubiquitous membrane proteins that couple the hydrolysis of ATP to the translocation of diverse substrates across cellular membranes. Whereas ABC importers (only present in prokaryotes) catalyze the uptake of essential nutrients from the environment, ABC exporters facilitate the extrusion of various compounds, including drugs and antibiotics, from the cytoplasm. ABC transporters share a conserved architecture: two nucleotide-binding domains (NBDs) hydrolyze ATP, while two transmembrane domains (TMDs) provide a pathway for the substrate.
Recent crystal structures of full ABC transporters suggest that a key step of the molecular mechanism is conserved in importers and exporters. Binding of ATP appears to promote an outward-facing conformation, whereas the release of the hydrolysis products ADP and phosphate promotes an inward-facing conformation. This basic two-state scheme can in principle explain ATP-driven drug export and binding protein-dependent nutrient uptake.

P4-1

Ectoenzymes in control of cell trafficking

Sirpa Jalkanen[1]
[1]University of Turku and National Public Health Institute, Turku, Finland

Leukocyte traffic from the blood into tissues is a fundamental element in normal immune surveillance and in inflammation. According to the existing dogma, leukocyte extravasation from the blood stream is mediated by selectins, sialomucins, chemokines and their receptors, integrins and members of the immunoglobulin super-family, which function in a well-coordinated manner constructing a multi-step adhesion cascade between leukocytes and endothelial cells. Recently, emerging evidence indicates that enzymes expressed on the surface of leukocytes and vascular endothelial cells significantly regulate the leukocyte extravasation cascade. Vascular adhesion protein-1 (VAP-1) and CD73 are examples of two ecto-enzymes controlling leukocyte migration from the blood into the tissues. Specific enzyme inhibitors, gene-manipulated mice and recombinant enzymes have recently demonstrated that the catalytic activities of these enzymes regulate leukocyte migration. The whole concept of enzymatic regulation of leukocyte migration provides new insight into the adhesion cascade and permeability of vascular endothelium. In summary, the involvement of ecto-enzymes opens new possibilities to control harmful inflammations with small molecule enzyme inhibitors.

P4-2

Vitamin B6 biosynthesis: Steps towards unraveling a complex puzzle

Teresa B. Fitzpatrick[1]
[1]University of Geneva, Department of Botany and Plant Biology, 1211 Geneva, Switzerland

Vitamin B6 is well known in its biochemically active form as pyridoxal 5´-phosphate (PLP), an essential cofactor of numerous metabolic enzymes. The vitamin is also implicated in numerous human body functions ranging from modulation of hormone function to its recent discovery as a potent antioxidant. Its *de novo* biosynthesis occurs only in bacteria, fungi and plants, making it an essential nutrient in the human diet. Despite its paramount importance, its biosynthesis was predominantly investigated in *Escherichia coli*, where it is synthesized from the condensation of deoxyxylulose-5-phosphate and 4-phosphohydroxy-L-threonine catalysed by the concerted action of PdxA and PdxJ. However, it has now become clear that the majority of organisms capable of producing this vitamin do so via a different route, utilizing ribose 5-phosphate, glyceraldehyde 3-phosphate and glutamine. This alternative pathway is characterized by the presence of two genes, Pdx1 and Pdx2. Their discovery has sparked renewed interest in vitamin B6 and numerous studies have been conducted over the last few years to characterise the new biosynthesis pathway. Indeed, enormous progress has been made in defining the nature of the enzymes involved and important insights have been provided into their mechanisms of action, where *Pdx1* and *Pdx2* function together as a glutamine amidotransferase now known as PLP synthase. The structure of the PLP synthase complex has been solved and displays an ornate architecture made up of 24 subunits, two hexameric rings of 12 Pdx1 subunits to which 12 Pdx2 subunits attach, with the glutaminase and synthase active sites remote from each other. The multiple catalytic ability of Pdx1, the remote glutaminase and synthase active sites, as well as the elaborate structure provide a fertile and challenging area of research on several levels. A summary of the current advances in our knowledge of the biosynthesis of this versatile molecule and the most recent results from the laboratory will be provided.

ABSTRACTS – SYMPOSIA (S)

S1-1
TRP channels in human rheumatoid arthritis

David Beech[1]
[1]Institute of Membrane & Systems Biology, Faculty of Biological Sciences, University of Leeds, Leeds, LS2 9JT, UK.

Rheumatoid arthritis is a debilitating inflammatory disease of joints, affecting millions of people world-wide. Treatments are available and new important biological therapies are emerging, but it remains a problematic disease in which the underlying mechanisms are poorly understood. TRP channels are a relatively recently discovered family of non-selective cationic channels, numbering at least 25. Many are polymodal sensors that show promise for helping us understand chemical sensing mechanisms in the human body and as potential novel therapeutic targets. Although often associated with sensory nervous systems, TRP channels are widely expressed across mammalian systems. An aspect we have taken interest in is the relevance to human arthritic joints, using biopsies taken during arthroscopy as a source of tissue and cells. We have found TRP expression in the fibroblast-like synovial cells, which line the synovium and are important for secretion of a range of factors as well as remodeling of the joint during disease. Subtypes of TRP expressed in joints are the widely-expressed TRPC1 and TRPC5 proteins, which heteromultimerise to form channels. We have shown that TRPC5 contains a repressive disulphide bridge in its extracellular turret region, making it susceptible to activation by reducing agents. A feature of rheumatoid arthritis is high levels of secreted thioredoxin, which we show is capable of breaking extracellular disulphide bridges. Importantly, endogenous TRPC5-containing channels of the synovial cells are activated by concentrations of thioredoxin that occur in the inflamed joint (Xu et al 2008, Nature 451, 69-72). Furthermore, the TRP channels have significant negative impact on secretion of the tissue remodeling factors, matrix metalloproteinases. Constitutive activity of the channels is important, as is enhanced activity in response to thioredoxin. Therefore, we suggest TRPC channels have protective function in the context of the rheumatoid arthritic joint. Our work is continuing in this area, expanding to the TRPM subtype of TRP channels and revealing potential for therapeutic approaches. The research is supported by the Wellcome Trust.

S1-2
Automated patch clamp for large scale screening of ion channels

Andrea Brüggemann[1]
[1]Nanion Technologies, 80335 München, Germany

In recent years, ion channels have received increasing interest as drug targets, because their malfunction underlies many chronic and acute disease states. The importance of ion channels for drug safety issues (e.g. hERG), is also becoming widely recognized in industry. This has motivated the pharmaceutical industry to search for techniques that can combine accurate determination of ion channel function with an acceptable data throughput and reasonable cost per data point.
The highest throughput combined with the lowest cost per data point in ion channel screening is still based on fluorescent techniques. These screening techniques are not ideal because of the time consuming assay development required, coupled with high false positive rates and, most strikingly, large false negative rates.
Patch clamp electrophysiology remains the gold standard for obtaining highly relevant information about ion channels and their effectors. However, this technique is limited because it is laborious, requires highly skilled personnel, and is notoriously low throughput. This has spurred the development of automated patch clamp platforms that are capable of high quality recordings at a much higher throughput. Although several automated patch clamp platforms are commercially available which allow for high quality recordings, so far none of these meet the throughput requirements of high throughput screening in drug discovery.
Over the last several years, the advances in automated electrophysiology have become immense. They range from devices that allow for single channel recordings of primary cells, up to pharmacological recordings from 384 cells simultaneously. Some devices are optimized for higher throughput on highly expressing cell lines, whilst others also allow bilayer recordings of reconstituted ion channels.
This talk will give an overview of the various advances that have been made in automated electrophysiology. Examples of recordings of voltage-gated and ligand-gated ion channels will be shown.

S1-3
Targeting ion channels in cancer: a novel frontier in antineoplastic therapy

A. Arcangeli[1], O. Crociani[1], E. Lastraioli[1], A. Masi[1], S. Pillozzi[1], A. Becchetti[2]

[1]Department of Experimental Pathology and Oncology, University of Firenze, Italy, [2]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy

Abstract will followTargeted therapy is considerably changing the treatment and prognosis of cancer. Progressive understanding of the molecular mechanisms that regulate the establishment and progression of different tumors is leading to ever more specific and efficacious pharmacological approaches.

In this picture, ion channels represent an unexpected, but very promising, player. The expression and activity of different channel types mark and regulate specific stages of cancer progression. Their contribution to the neoplastic phenotype ranges from control of cell proliferation and apoptosis, to regulation of invasiveness and metastatic spread.

Evidence is particularly extensive for K^+ channels. Their expression is altered in many primary human cancers, especially in early stages, and they frequently exert pleiotropic effects on the neoplastic cell physiology. For instance, by regulating membrane potential they can control Ca^{2+} fluxes and thus the cell cycle machinery. Their effects on mitosis can also depend on regulation of cell volume, usually in cooperation with chloride channels. However, ion channels are also implicated in late neoplastic stages, by stimulating angiogenesis, mediating the cell-matrix interaction and regulating cell motility. Not surprisingly, the mechanisms of these effects are manifold. For example, intracellular signaling cascades can be triggered when ion channels form protein complexes with other membrane proteins such as integrins or growth factor receptors.

This has been extensively proven by our group for ion channels encoded by the *human ether-a-gò-gò-related gene 1 (herg1)*, hERG1 channels. hERG1 channels are often aberrantly expressed in many primary human cancers and exert pleiotropic effects in cancer cells. Some of them are strictly related to the modulation of adhesive interactions with the extracellular matrix. This role often depends on the formation, on the plasma membrane of tumor cells, of macromolecular complexes with adhesion receptors of the integrin family. The link between hERG1 and integrins is twofold: integrins, mainly the β1 integrin subunit, can activate hERG1. Conversely, the channels, once activated by integrins, can modulate signaling pathways downstream to integrin receptors. Based on current evidence, we hypothesize that the activity of

hERG1 channels inside the complex modulates the function of the partner protein(s) mainly through conformational coupling, instead of alterations of ion flow.

Altered channel expression can be exploited for diagnostic purposes or for addressing traceable or cytotoxic compounds to specific neoplastic tissue. What is more, recent evidence indicates that blocking channel activity impairs the growth of some tumors, both *in vitro* and *in vivo*. This opens a new field for medicinal chemistry studies, which can avail of the many available tools, such as blocking antibodies, antisense oligonucleotides, small interfering RNAs, peptide toxins and a large variety of small organic compounds. The major drawback of this approach is that some ion channel blockers produce serious side effects, such as cardiac arrhythmias. Therefore, drug developing efforts aimed at producing less harmful compounds are needed and we discuss possible approaches toward this goal. Finally, we propose that a novel therapeutic tactic could be developed by unlocking ion channels from multiprotein membrane signaling complexes.

S1-4

Sodium self-inhibition of the epithelial sodium channel: Identification of residues involved in sodium sensing

Vincent Bize[1], Jean-Daniel Horisberger[1]

[1]Département de Pharmacologie et de Toxicologie de l'Université de Lausanne

The Epithelial Na+ Channel (ENaC) is located in the apical membrane of „tight“ epithelia in the distal nephron, distal colon and airways. Its activity controls the rate of transepithelial sodium transport. Among several regulatory factors, ENaC activity is modulated by the extracellular N^{a+} concentration, a phenomenon named self-inhibition: high extracellular Na+ induces a decrease of the open probability of the channel and thus affects the rate of transepithelial N^{a+} transport with a fast time course (the time constant is about 3 s). The molecular mechanism by which extracellular Na+ concentration is detected is not known. It has yet been proposed that this regulation involves a site (distinct from the conduction site) able to sense the extracellular Na+ concentration. We previously confirmed the existence of this extracellular sensing site and characterized its physiological properties in terms of ionic selectivity and affinity. We indeed studied the effects of extracellular cations on steady state amiloride-sensitive outward currents in Na+ loaded Xenopus laevis oocytes expressing human ENaC and compared them with self-inhibition of inward current after fast solution changes. Self-inhibition by extracellular Li+ was similar to that of Na+ except for slightly slower kinetics. Ionic selectivity of the inhibition for steady state outward current was Na+ ≥ Li+ > K+. We estimated an apparent inhibitory constant (KI) of about 40 mM for extracellular N^{a+} and Li^+, and found no evidence for a voltage dependence of the KI.
Concerning the localization of this sensing site, experimental evidence shows that the extracellular loops of the channel subunits are involved in self-inhibition and plausibly contain residues responsible for the extracellular Na+ detection. Recent results highlight the central role of the α and γ subunits (and not β). Moreover studying α and “α-like” (δ, ε) subunits in different species (rat, Xenopus, human), we observe that δβγ ENaC displays an inverted ionic selectivity of the self-inhibition for extracellular Na+ and Li^+. This suggests that both subunits α and δ contain sensing site (or part of it), but also that the residues involved in extracellular Na+ detection are probably not conserved between α and δ. Sequence alignment of the α and δ subunits shows that about 30 amino acids are not conserved in the extracellular domain. We substituted some of these residues in α human ENaC by those of δ, using site-directed mutagenesis. The ionic selectivity of the self-inhibition of two of the mutated channels (αG366Sβγ and αG389Sβγ ENaC) is inverted, as in the case of δβγ channels. These results suggest that these two positions are close to the extracellular cation site.
Poster Topic 2: BIOLOGY OF ION CHANNELS AND TRANSPORTERS

S1-5

Role of TRPC1 and Orai1 channels in the regulation of store-operated currents in muscular dystrophy

George Shapovalov[1], Chiara Gallo[1], Emmanuelle Roulet[1], Nadège Zanou[2], Philippe Gailly[2], Urs T. Ruegg[1]

[1]Laboratory of Pharmacology, University of Geneva, CH-1211 Geneva, Switzerland, [2]Laboratory of Cell Physiology, University of Louvain, B-1200 Brussels, Belgium

Store operated ion channels play an important role in regulation of Ca^{2+} homeostasis in skeletal muscle at rest as well as during contraction. Thus, a dysregulation of SOC activity is considered an important factor contributing to cell degradation and death in many musculopathies, such as Duchenne or Becker muscular dystrophies. Transient receptor potential (TRP) proteins are a novel class of cation channels exhibiting variety of regulatory functions. In particular, TRPC channels exhibit a mild selectivity for Ca2+ and are considered likely candidates to convey SOC activity. Using single-channel patch clamping of TRPC1 positive and negative isolated muscle fibers we have identified a mode of activity associated with the TRPC1 protein. Currents with a characteristic conductance of 15 pS, reported in systems overexpressing TRPC1 could be observed in $TRPC1^{+/+}$ but not $TRPC1^{-/-}$ cells and could be also be stimulated by application of stretch, thus confirming mechanosensitive properties of TRPC1 channels. This TRPC1 associated Ca^{2+} current however, was minor compared to the more abundant and higher conductance Orai1 associated Ca^{2+} current, which additionally exhibited SOC properties (P. Avdonin, et al., in preparation). While this Orai1 associated current could be observed in both $TRPC1^{+/+}$ and $TRPC1^{-/-}$ cells, absence of TRPC1 protein influenced the kinetic properties of this current, suggesting that principal endogenous ion channel responsible for Ca^{2+} entry in skeletal muscle cells has a heteromeric structure involving both Orai1 and TRPC1 subunits.
Poster Topic 2: BIOLOGY OF ION CHANNELS AND TRANSPORTERS

S1-6

Tamoxifen inhibits TRPV6 activity via estrogen receptor independent pathways in TRPV6 transfected MCF-7 cells

Katrin A. Bolanz[1], Gergely G Kovacs[1], Christopher P. Landowski[1], Matthias A. Hediger[1]

[1]*Institute of Biochemistry and Molecular Medicine, University of Berne, 3012 Berne, Switzerland*

TRPV6 expression has been shown to be up regulated in breast cancer tissue compared to normal mammary gland tissue. In several cell lines, it was shown that knockdown of TRPV6 inhibits cell growth. Tamoxifen is a selective estrogen receptor modulator widely used in breast cancer therapy. Previously, we showed that tamoxifen down regulates TRPV6 mRNA expression and that it also inhibits calcium uptake in TRPV6-transfected oocytes. In the present study we examined the effect of tamoxifen on TRPV6 function in MCF-7 breast cancer cells transiently transfected with TRPV6, tagged with EYFP. TRPV6 function was measured with fluorescence microscopy using Fura-2. The influx of calcium, barium and manganese was significantly larger in cells transfected with this construct, compared to non-transfected cells. In transfected cells, 10 µM tamoxifen induced a decrease in intracellular calcium concentration in transfected cells down to the basal calcium level of non-transfected cells, following a large, transient increase. In nominally calcium free buffer, tamoxifen induced a smaller transient increase. The transport rate of calcium and barium but not manganese was decreased 50-70% by tamoxifen. This inhibitory effect was not mediated by estrogen receptors as the antiestrogen ICI 182,720 had no inhibitory effect. Inhibition of PI3K with 25 µM LY294002 had no effect on TRPV6 activity. These findings give novel insights into how tamoxifen might be effective in estrogen receptor negative breast cancer patients. Our data suggest that TRPV6 is involved in the therapeutic mechanism of tamoxifen during breast cancer treatment. This study was supported by the Novartis Stiftung and the Bernische Krebsliga (PI's Hediger and Landowski).

Poster Topic 2: BIOLOGY OF ION CHANNELS AND TRANSPORTERS

S2-1

The role of membrane lipids in virus entry

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The small DNA tumor-virus Simian virus 40 (SV40) is unusual among animal viruses in that it uses a lipid, the GM1 ganglioside, as a cell entry receptor. After binding to GM1 in the outer leaflet of the plasma membrane, the virus induces transmembrane signaling leading to internalization by a clathrin-independent pathway and infection. Here we show that the structure of the hydrocarbon-chains of GM1 controls SV40 binding-induced membrane-invagination, endocytosis and infection in cells. Unlike the natural native glycosylceramides molecules, GM1 analogues with shorter hydrocarbon chains supported binding but failed to induce invagination of the plasma membrane, endocytosis and infection. In a reconstituted minimal membrane system we found that binding to GM1 with long hydrocarbon-chains alone is not sufficient for membrane deformation, but the organization of the GM1 binding sites in the SV40 capsid contributes to efficient catalysis of membrane invagination. Our data suggest that several pathogens (SV40 and polyoma viruses) or pathogenic products (Shiga and cholera toxins) exploit binding to specific glycolipids to induce a common membrane mechanics process to drive their cellular uptake via induced plasma membrane invagination.

Vaccinia virus, a enveloped virus of the poxvirus family, is also critically dependent on a lipid for entry but in a different fashion. In this case, it is an abundant lipid component, phosphatidylserine (PS), of the envelope of the mature virus (MV) particle. This phospholipid is exposed and serves as a signal to trigger a complex signaling pathway in host cells that results in membrane blebbing and subsequent macropinocytic endocytosis of the virus particle.

S2-2

HIV entry inhibition

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Inhibition of HIV entry is key for both therapeutic and preventive interventions. So are neutralizing antibodies considered a central component of protective vaccines against HIV-1. Neutralizing antibodies have been shown to act through limiting viral entry by blocking HIV attachment to its receptors or by inhibiting membrane fusion. The enormous effort put into investigating the neutralizing antibody response to HIV-1 over the past two decades has generated detailed information on epitope specificity, potency, breadth and in vivo activity of neutralizing antibodies. Less clear is still the role of effector functions mediated by these antibodies. The increasing need for a vaccine to control the HIV pandemic is undoubted, but recent failures of vaccine programs have made clear that it will be years to decades before a successful vaccination program can be installed. In the meantime, drug based intervention strategies must be found to fill the gap and put the continuous spread of HIV at halt. HIV infection is predominantly acquired via heterosexual transmission across mucosal surfaces. Strategies that prevent mucosal transmission are therefore thought to significantly impact on diminishing viral spread. Microbicides, agents that by topical application on mucosal surfaces protect from HIV infection, are regarded as one of the most promising preventive intervention strategies in the absence of effective vaccination programs. Prime targets for microbicide attack are viral and cellular proteins involved in the early events in infection: the entry receptors CD4, CCR5 and CXCR4, as well as the viral envelope proteins gp41 and gp120. In this presentation principles and obstacles of HIV entry inhibition by neutralizing antibodies and engineered compounds will be discussed.

S2-3

Selection of novel human cytomegalovirus antigens for vaccination

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Neutralizing antibodies can be used not only as therapeutics in a passive vaccination setting, but also as tools to identify critical molecular patterns of a pathogen that are required for infectivity. Once identified and produced in an appropriate form, these structures should be able to elicit a neutralizing antibody response. This approach can be effectively implemented using the memory B cell immortalization method recently developed in our laboratory. We explored the potential of this antibody-driven target antigen discovery procedure ("analytic vaccinology") by screening for neutralization of human cytomegalovirus infection (HCMV) on different target cells (either fibroblasts or endothelial/epithelial cells), with the aim of identifying glycoproteins that are essential for HCMV infectivity of endothelial/epithelial cells versus fibroblasts. Using cells transfected with HCMV genes in various combinations, we provided evidence that human monoclonal antibodies allow the identification of novel HCMV structures that elicit a protective response. Namely, we found that the majority of potent HCMV neutralizing antibodies bind to epitopes defined by the expression of one or more HCMV proteins UL128, UL130, and UL131A, and few of them bind to glycoproteins gH or gB. This knowledge should help the design of efficacious vaccines that eventually lead to the control of infection.

S2-4

Role of ERAD substrate topology in selection of E3 ubiquitin ligases

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More than one third of mammalian gene products attain their native structure in the lumen of the endoplasmic reticulum (ER). Folding-defective polypeptides are ultimately extracted from the ER folding machinery and are degraded by cytosolic proteasomes in a series of tightly regulated events named ER-associated degradation (ERAD). Many aspects regulating protein disposal are still uncharacterized, especially how misfolded proteins are recognized, targeted to the retrotranslocon and translocated across the ER membrane.

In Saccharomyces cerevisiae, two different pathways are involved in protein disposal from the ER: the DOA10 pathway regulates disposal of proteins with cytosolic defects (ERAD-C), while the HRD1 pathway serves aberrant proteins with transmembrane or luminal lesions (ERAD-M and ERAD-L).

In mammalian cells, two Hrd1p orthologs, namely Synoviolin/HRD1 and gp78, play crucial roles in ERAD. Here, I will show how intrinsic ERAD substrate features determine selection of the Synoviolin/HRD1 or of the gp78 pathway for dislocation into the cytosol and proteasomal degradation.

Poster Topic 6: OTHERS

S2-5

The Cell Surface Protein Atlas

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Introduction

Cell surface-exposed proteins embedded in the plasma membrane are crucial for cell-cell communication, interaction with pathogens and responding to environmental perturbations and are indicators of the differentiation state. The knowledge about the expression of specific receptors, ion channels, transporters and adhesion molecules is crucial for the elucidation of cellular functions.

Due to a lack of enabling technology cell surface protein analysis has been limited to the measurement of a few 12 CD molecules in parallel via available antibodies and flow cytometry. Therefore, in spite of its successes, the current gold standard approach for cell classification is limited to the availability of antibodies and therefore relies on hypotheses and existent knowledge.

Methods

We developed a methodology for the multiplexed, quantitative, mass-spectrometric identification of cell surface glycoproteins, and their N-glycosites, which can be used to phenotype cells without antibodies, in an unbiased fashion, without a priori knowledge or to identify novel targets for antibody generation. This highly specific cell surface capturing (CSC) technology allows for the isolation, identification and quantification of N-glycosites from the extracellular domains of cell surface proteins in a discovery-driven mode. In contrast to other technologies the CSC identifies nearly exclusively proteins from the plasma membrane without contamination from intracellular membrane proteins and is therefore able to identify the key players of a given cellular state on the cell surface.

Results

We used the CSC technology to investigate and compare the cell surface proteome of nearly 40 different cell types. Among the investigated cell types are immune cells, neuronal cells, epithelial cell and stem cells of mouse and human origin. The identified cell surface proteins were integrated into a new systems biology resource - the Cell Surface Protein Atlas. To date, the Atlas contains 2200 human and 1500 mouse mass-spectrometric identified cell surface glycoproteins and their glycosites, including 232 CD antigens. The identified cell surface glycoproteins contained a range from one to twenty-eight transmembrane domains. The set of identified cell surface proteins on a single cell is relatively unique which is shown by the fact that 70% of all identified proteins are only expressed on one single celltype. In contrast, less than 1‰ of all proteins in the atlas are found on more than 80% of cells. The fact, that unsupervised hierarchical clustering of identified cell surface proteins from different cell types allowed for the grouping of closely related cell types contradicts the argument of undersampling. This indicates, that even non-quantitative cell surface protein signatures could be used to classify unknown cells into functional categories. The Cell Surface Atlas is a prerequisite to identify cell type specific marker proteins and to develop quantitative cell surface protein barcodes relevant in predictive and preventive medicine.

Poster Topic 6: OTHERS

S2-6

Virus-cell membrane interactions in ViralZone

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Viruses are ubiquitous companions of cellular life forms, infecting every known cellular organism. All of them have to cross the host membrane upon primo-infection. Typically the first step of infection occurs through binding to one or several cell receptor(s) present on the cell membrane. Enveloped viruses then fuse their virion envelope with the cellular membrane through fusion proteins. Although all viruses have to cross host cell membrane, the strategy adopted by different virus families is rather diverse in term of entry, replication or budding.

This extreme diversity is complicating the understanding of viruses' molecular biology. Indeed each virus family has a different virion organization and replication cycles: virion can be enveloped or not, entry can occur through endosomes or direct fusion at the plasma membrane, genome replication happens in cytoplasm, nucleus or in association with ERG, budding starts through nuclear envelope, ERG or plasma membrane…

ViralZone is a web site that summarizes molecular biology, and epidemiology knowledge for all known viruses. They are first classified in groups according to the nature of their genome: DNA, RNA, single or double stranded. For each group, a listing describes all known interactions between viral proteins and cell surface membrane proteins. Each group page also leads to specific description pages for the 78 families and the 293 genus identified in virology. These description pages contain concise information about a given virus, as well as links to all related protein. Original pictures or virion shapes describe in a user friendly way whether a given virus is enveloped or not, and if enveloped, by how many membranes. A summary of the replication cycle provides details on the virus molecular biology.

A listing of UniProtKB/Swiss-Prot viral entries follows the general description of the virus. It allows users to see at once all given proteins entries of a specific virus. Viral proteins that have been crystallized are clearly indicated on the list, with a direct link to the 3D structure database (PDB). Several tools allow the alignment of selected protein sequences, retrieval of their nucleotide sequence, or display of only the strains/isolates for which the complete genome is available. Each protein name links to fully manually annotated UniprotKB/Swiss-Prot entries, consisting in a small review of all the information gathered on the protein: correct protein and gene names, function description, interactions, keywords, known domains, active sites, references, and the amino acid sequence.

Poster Topic 3: CELL-CELL INTERACTIONS

S3-1

Formation of *bicoid* morphogen gradient: an mRNA gradient dictates the protein gradient

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The Bicoid (Bcd) protein gradient is generally believed to be established in the pre-blastoderm *Drosophila* embryo by the diffusion of Bcd after translation of its maternal mRNA, which serves as a strictly localized source of Bcd at the anterior pole. However, already when proposed, this model ignored our conflicting results, which demonstrated that the Bcd protein gradient is preceded by a *bcd* mRNA gradient. Here, we extend our old results by showing that the *bcd* mRNA and protein gradient profiles are virtually identical at all times, which proves that the Bcd gradient is produced by its mRNA rather than by diffusion. Based on our observation that *bcd* mRNA colocalizes with Staufen (Stau), we conclude that the *bcd* mRNA gradient is formed by a novel mechanism of a quasi-random active transport of a Stau-*bcd* mRNA complex through a non-polar microtubular network, which confines the *bcd* mRNA to the cortex of the embryo.

S3-2

A Self-Regulatory Feedback Signaling System controls Vertebrate Limb Development

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I will present the results of our genetic analysis of mouse limb bud development which reveals how interlinked dual-time signaling feedback loops involving the SHH, BMP and FGF pathways impact on transcriptional regulation of the BMP antagonist Gremlin1 to generate a robust and largely self-regulatory limb patterning system. We have also used mathematical simulations of these processes and I will discuss the predictive power this analysis with respect to the changes in signaling hierarchies and activities over time. The limb patterning system is able to compensate heterozygosity in one pathway by compensatory regulation involving the other signaling pathways in the feedback loop, revealing the molecular nature of distributed robustness These studies establish developing vertebrate limb is a good model for systems biology type approaches to studying cell-cell interactions during vertebrate organogenesis.
Our studies were carried out in collaboration with M. Bischofberger and F. Naef (EPFL Lausanne) and J. Martin (Texas A&M Health Center, Houston/USA)

S3-3

Sterols and Sphingolipids in Biological Membranes: an Intimate Relationship

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Sterols and sphingolipids are largely eukaryotic lipids that have been proposed to self associate in vitro to form membrane microdomains called rafts. In order to examine sterols and sphingolipid functions we have engineered new yeast strains with altered sterols and/or sphingolipids. Using these strains we could show that cholesterol could substitute for some ergosterol functions. We have also shown that yeast with altered sterol compositions adjust their glycerolphospholipid and sphingolipid compositions in precise and specific ways. In particular, sphingolipid amounts are much more strongly affected in single erg mutants than other lipids. This led to the hypothesis that sphingolipids and sterols may be acting in common pathways to complete cellular functions. In order to test this genetically we created a series of double mutant between enzymes in sphingolipids and sterols. These results reveal a surprisingly complex functional interaction between sphingolipids and sterols in yeast cells and provide genetic proof that these two classes of lipids function together in a wide variety of cellular pathways. For example, a particular combination of sterol and sphingolipid composition renders cells hyperensitive to caffeine and rapamycin. The direct molecular target of caffeine and rapamycin is the TOR complex 1. However, in this double mutant the TOR complex 2 is less active. Sphingolipid and sterol composition also affects function of multidrug resistance-like plasma membrane transporters. Measurements of protein trafficking, resistance to various inhibitors and membrane fluidity as a measure of membrane order, suggest that the functions of sterols and sphingolipids are probably dependent more upon their structure than upon the physical properties they confer to the membrane. This suggests that most of the phenotypes we observe are probably the result of fairly direct protein-lipid interactions, rather than the protein functions being controlled by entering into rafts.

S3-4

Role of the cationic channel TRPC1 in Duchenne muscular dystrophy: Analysis of double knock-out *TRPC1 -/- DYS -/-* skeletal muscles

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Duchenne Muscular Dystrophy (DMD) is a muscular disease leading to progressive muscle degeneration due to the lack of dystrophin, a cytoskeletal protein. Muscle degeneration occurring in this disease is thought to be partly caused by increased Ca2+ entry through store operated channels (SOC) and stretch activated channels (SAC). Since TRPC1 seem to be one of the main candidates to SOC and SAC structure and function, we have generated a new double knock out mouse *TRPC1* $^{-/-}$ *dys* $^{-/-}$ (mdx) to further analyze its involvement especially in dystrophic skeletal muscles. To investigate a possible over-expression of other TRPC family members or other cytoskeletal proteins in the double k.o. mice *TRPC1* $^{-/-}$ *dys* $^{-/-}$, qtPCR was performed on different muscles and showed no significant differences. Interestingly, electrophysiological analysis showed a difference between TRPC1 $^{-/-}$ and TRPC1 $^{+/+}$ original mice. Indeed we have isolated a current of 15 pS, which seems to correspond to TRPC1 channel. FURA-2 experiments were performed on FDB muscle fibres to measure calcium influx and we showed that TRPC1 is not essential for basal and Thapsigargin induced calcium influx. Works are in progress to determine the function of this cationic channel.
Poster Topic 2: BIOLOGY OF ION CHANNELS AND TRANSPORTERS

S3-5

The Role of Ku80 in DSB Repair in *Drosophila melanogaster*

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Nonhomologous end joining (NHEJ) is the major double strand break (DSB) repair mechanism in mammalian cells. In this repair pathway, DSB's are directly joined end-to-end. During NHEJ, end processing (resection and/or insertion of nucleotides) before the final ligation step is often observed. Work in mammals and yeast has shown that for accurate NHEJ the ku70/80 heterodimer is required. In an initial step ku80 binds the DNA ends, protects them from degradation and prepares for ligation. To elucidate the role of Ku antigen in Drosophila, we generated Ku80 knock-out flies by the method of targeted gene disruption (Rong and Golic 2002). Under normal laboratory conditions, these flies are viable and fertile. We found that the frequency of single strand annealing (SSA) events was elevated in Drosophila ku80 mutants. Preliminary data suggest that NHEJ events are at least as frequent in these ku80 mutants as in controls. In ionizing irradiation experiments the ku80 mutant embryos showed an enhanced sensitivity relative to controls. This demonstrates an important role of Ku80 in coping with genotoxic damage and maintaining genome stability.

Poster Topic 6: OTHERS

S3-6

AtGAT1 and AtGAT2 the GABA transporters of *Arabidopsis thaliana*

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4-Aminobutyric acid (GABA) is a well characterized inhibitory neurotransmitter in the central nervous system of animals. In contrast, little is known about the function of GABA in plants. Accumulation of GABA via increased synthesis is observed in response to a variety of stress conditions. However, the contribution of intracellular and intercellular transport to changes in GABA concentrations remains unclear.

We characterized AtGAT1 (At1g08230) as the first high affinity GABA transporter in plants, by measuring uptake of radiolabled GABA in *Saccharomyces cerevisiae* expressing AtGAT1 and by using two electrode voltage clamp experiments with *Xenopus laevis* oocytes (Meyer *et al.*, 2006). The transient expression of AtGAT1/GFP fusion proteins in protoplasts revealed a localization at the plasma membrane. In *A. thaliana*, expression of *AtGAT1* is highest in flowers and under conditions of elevated GABA concentrations.

The highly homologous gene *AtGAT2* (At5g41800) encodes for a transporter with a much lower affinity for GABA. AtGAT2/GFP fusion proteins are also targeted to the plasma membrane. Expression of the *uidA* gene under the control of the *AtGAT2* promotor shows expression of *AtGAT2* in the anthers of young flowers and in the vascular tissue. Further characterization of AtGAT1 and AtGAT2 will help to reveal the role of GABA transport in plants.

Poster Topic 2: BIOLOGY OF ION CHANNELS AND TRANSPORTERS

S4-1

Membrane fusion in the secretory pathway of eukaryotic cells

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Membrane fusion in the secretory pathway is mediated by SNARE proteins. According to current ideas, assembly of SNAREs residing in the membranes destined to fuse operate as nanomachines that pull membranes tightly together and thus cause fusion. Both structural studies on SNARE complexes, and reconstitution of SNAREs in artificial membranes have lend support to this model, strengthening the idea that SNAREs are both necessary and sufficient to mediate fusion.

In our work, we have characterized the structural changes associated with the SNARE assembly-disassembly cycle, and we study mechanism and regulation of SNARE-mediated fusion of vesicles in vitro using either artificial or native vesicles. Main emphasis is on the exocytotic fusion of synaptic vesicles and on the fusion of endosomes that are used as model organelles. Furthermore, we have carried out a quantitative analysis of the synaptic vesicle protein and lipid composition as model trafficking organelle, allowing us for the first time to obtain accurate data on the stoichiometry of proteins essential for fusion. Our data provide new insights into the kinetic control and the underlying molecular mechanisms of SNARE-mediated membrane fusion.

S4-2

Ubiquitin dependent quality control of a membrane protein in the ER

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Canonical Wnt signaling is initiated by binding of Wnt proteins to members of the Frizzled family and subsequent complex formation with LRP5/6. Here we show that LRP6 is palmitoylated on a juxtamembranous cysteine and that palmitoylation is required for exit from the endoplasmic reticulum. We propose that palmitoylation serves to tilt the long, 23 residues, transmembrane domain of LRP6 with respect to the plane of membrane to prevent a hydrophobic mismatch and subsequent recognition by the ER quality control. We found that palmitoylation deficient LRP6 was retained in the ER by a completely novel mono-ubiquitination dependent ER retention mechanism. Mutation of a specific lysine indeed abolished ubiquitination of palmitoylation deficient LRP6 and led to a rescue form ER retention.

On the basis of these findings, we propose a novel mechanism of ER quality control, that would be somewhat the cytosolic counter part of the calnexin/calreticulin cycle and which would be based on ubiquitin addition and removal, instead of glucose, as in the calnexin/calreticulin cycle.

S4-3

Structure and Function of Aquaporins

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Progress in the structure determination of AQPs has led to a deep understanding of water and solute permeation by these small integral membrane proteins. The atomic structures now available allowed the water permeation and exclusion of protons to be monitored by molecular dynamics simulations, and have provided a framework for assessing the water and solute permeation in great detail by site-directed mutations. In spite of this, further structural and molecular dynamics analyses are required to elucidate the basis for regulation as well as for gas permeation, processes that are still to be deciphered. Structure and function of AQP1 will be discussed in detail and the assessment of the water transport capacity of SoPIP2;1, a plant aquaporin with putative gating capability will be presented.

S4-4

Visualizing cytotoxic T cell activation at the single-molecule level

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CD8+ cytotoxic T lymphocytes (CTL) play a key role in the immune system, for example for the elimination of viral infections. T cell receptors (TCR) recognize specific peptides bound to major histocompatibility complex I molecules (MHC) on antigen-presenting cells (APC). T cells are able to kill infected cells even in the presence of very low amounts of cognate MHC-peptide complexes (pMHC). This high sensitivity requires an efficient scanning of a vast number of highly diverse MHC I-peptide complexes by the TCR in the contact site of transient conjugates formed mainly by non-specific interactions of ICAM-1 and LFA-1.

We have used single-molecule imaging (SMI) to unravel the complex processes occurring during CTL antigen recognition. SMI has the advantage of allowing the investigation of T cell recognition at physiological pMHC surface concentrations. Tracking of single MHC molecules loaded with fluorescent peptides on target cells and nascent conjugates with CTL showed dynamic transitions between states of free diffusion and immobility. The immobilizations were explained by association of MHC I-peptide complexes with ICAM-1. The reduced mobility in presence of ICAM-1 strongly increased the local concentration of MHC molecules in cell adhesion sites and hence optimised their scanning by TCR.

SMI revealed the existence of a sorting mechanism in nascent immunological synapses: cognate complexes became immobile, whereas non-cognate ones diffused out again. This mechanism has a strong physiological role as interfering with this mobility-modulation-based concentration and sorting of MHC I-peptide complexes strongly impaired the sensitivity of antigen recognition by CTL, which demonstrates that it constitutes a new basic aspect of antigen presentation by MHC I molecules.

Segura J.-M., Guillaume P., Mark S., Dojcinovic D., Johannsen A., Bosshard G., Angelov G., Legler D. F., Vogel H., and Luescher I. F., “Increased Mobility of Major Histocompatibility Complex I-Peptide Complexes Decreases the Sensitivity of Antigen Recognition”, J. Biol. Chem. 283 (2008) 24254

Poster Topic 5: MEMBRANE TRAFFICKING AND DYNAMICS

S4-5

Exocytic membrane fusions and endocytic membrane internalisation: Two interdependent processes in fast growing fungal cells

Michael Köhli[1], Hanspeter Helfer[1], Philippe Laissue[1], Mark Finlayson[1], Philipp Knechtle[1], Riccarda Rischatsch[1], Virginie Galati[1], Kamila Boudier[1], Robert W. Roberson[2], **Peter Philippsen[1]**

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Surface growth of filamentous fungi is restricted to hyphal tips and exceeds by far surface growth of yeast cells. We study this process in the model fungus *A. gossypii* which, on the genome level, is closely related to the yeast *S. cerevisiae* but exclusively grows as multinucleated continously elongating cells (hyphae). We monitored by *in vivo* time lapse microscopy GFP-fusions of 15 *A. gossypii* proteins potentially involved in vesicle transport, exocytosis, endocytosis and polarisome assembly. In slow growing hyphae, the investigated fusions mostly localized to the apical cortex. Surprisingly, in fast growing hyphae, at least four functional zones defined by distinct sets of polarity factors were observed. These data together with phenotypes of gene deletions indicate a coordination between efficient exocytosis at the hyphal tip centers and endocytosis focussed at the adjacent tip regions.

Poster Topic 5: MEMBRANE TRAFFICKING AND DYNAMICS

S4-6

Raft-based signalosomes in human lymphoma cell membranes

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Accumulated sphingolipids or rafts constitute privileged sites for the assembly of functional signaling complexes. Neoplastic cells such as lymphomas use these signaling complexes to activate antiapoptotic signaling pathways, and secure their continous proliferation and survival.
The raft-seeking transmembrane protein Cbp/PAG, a multiply tyrosine-phosphorylated protein adaptor with a short transmembrane portion and a long cytoplasmic extension, was investigated for its capacity to anchor signaling proteins and generate a constitutive signaling module in lymphoma cell membranes. The main partner of Cbp/PAG is the Src-family kinase Lyn, which phosphorylates Cbp/PAG and provides docking sites for the signaling proteins STAT3 and phosphatidyl 3'-kinase. These proteins represent upstream proteins in survival pathways.
Pharmacologic inhibition and decreased expression of Lyn compromise survival by blocking proliferation and initiating apoptosis.
The assembly of a Cbp/PAG-Lyn complex makes the Lyn kinase independent of its negative regulator Csk, a cytoplasmic kinase that favors the auto-inhibited configuration of Lyn.
Consequently rafts provide a favourable membrane docking site for Cbp/PAG and Lyn, which then allow the building up of a signalosome containing other signaling proteins that support proliferative and anti-apoptotic pathways. Therefore rafts could also be considered as membrane contexts that enable the oncogenic assembly of otherwise regulatable signaling proteins.

Poster Topic 1: STRUCTURE OF MEMBRANE PROTEINS

S5-1

Gastrointestinal permeability and drug absorption

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Improved mechanistic understanding of the intestinal and hepatobiliary disposition of drugs and metabolites in vivo will facilitate the prediction of, drug absorption, bioavailability, clearance, drug-drug interactions and other pharmacological and toxicological issues. The number of drugs classified as substrates or inhibitors of different transporters is continually progressing as is the understanding of the molecular properties of these transporters. Extrapolating results from non-clinical studies to the clinical situation is however challenging. Aiming at providing in vivo data for a better understanding of the in vivo role of intestinal and hepatobiliary transporters we developed different advanced models: a model for direct estimation of the effective intestinal permeability, apparent biliary clearance and identification of bile-specific metabolites in humans, and a pig model that enables simultaneous intestinal perfusion and collection of bile. In healthy volunteers, the intestinal permeability and biliary excretion are investigated by means of an orally introduced perfusion tube creating a closed or a semi-open segment positioned close to papilla of Vater. In pigs, bile is collected directly from the bile duct and intestinal excretion is estimated from a perfused intestinal segment. The majority (~85%) of the 50 most-sold pharmaceutical products are given orally. This route of administration presently dominates drug therapy and is likely to continue to do so in the foreseeable future because in addition to causing minimal discomfort to the patient, it is safer, more efficient and more easily accessible than alternative routes such as intramuscular, subcutaneous, rectal and pulmonary delivery. Human jejunal permeability (Peff) is determined in the intestinal region with the highest expression of carrier proteins. Intestinal Peff are often based on multiple parallel transport processes. Site-specific jejunal Peff cannot reflect the permeability along intestinal tract, but they are useful for approximations of the fraction oral dose absorbed. It seems like drugs with a jejunal Peff > $1.5 \cdot 10^{-4}$ cm/s will be completely absorbed no matter which transport mechanism(s) that are utilized. Many drugs that are significantly effluxed in vitro have a rapid and complete intestinal absorption (i.e.>85%) mediated by passive transcellular diffusion. The determined jejunal Peff for drugs transported mainly by absorptive carriers (such as peptide and amino acid transporters) will accurately predict the fraction of the dose absorbed as a consequence of the regional expression. The data also show that (1) the human intestinal epithelium has a large resistance towards large and hydrophilic compounds; (2) the paracellular route has a low contribution for compounds larger than MW 300.

S5-2

Hepatic Bile Salt and Drug Transport

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Two key functions of the liver are bile formation and detoxification. Both processes are functionally linked as the bile fluid provides a vehicle for the excretion of poorly water soluble compounds. Detoxification includes metabolic conversion of poorly water soluble compounds into better soluble compounds within hepatocytes, followed by the export of the metabolites for biliary or renal excretion. Bile formation is a vectorial process involving uptake of cholephilic compounds, e.g. bile salts, from the sinusoidal blood across the basolateral membrane into hepatocytes followed by their export into bile via the canalicular membrane. These multiple transport steps require a large array of transporters in the hepatocellular plasma membrane. Uptake of bile salt into hepatocytes is largely sodium dependent and to a minor extent sodium independent. The latter part is mediated by organic anion transporting polypeptides (OATPs), which are among other transporters the entry site for drugs, such as for example statins. The transport mechanism of OATPs is not yet fully understood. Recent evidence supports the concept that many OATPs act as anion exchangers, mediating bicarbonate efflux in exchange for the uptake of organic anions. Bile acids are exported by the bile salt export pump BSEP, a member of the ABC-transporter superfamily, from hepatocytes into the canaliculi against a steep concentration gradient. Drug metabolites are often exported by members of the mulitdrug resistance ABC transport family or MRPs. Drugs or their metabolites can interfere with the function of export systems, e.g. by inhibition or by stimulation. Inhibition of transporters (e.g. BSEP) will lead to intracellular accumulation of cytotoxic compounds, while stimulation of transporters (e.g. MRP2) will alter canalicular bile flow. Both processes can ultimately lead to acquired liver disease. Since the canalicular bile salt concentration is higher than their critical micellar concentration, the canalicular membrane needs to be resistant to the detergent action of bile salts. One potential mechanism could be the presence of microdomains (also called lipid rafts) in the canalicular membrane. We have recently obtained evidence for the presence of two different types of microdomains, which are either resistant to extraction by Triton X-100 or to extraction by Lubrol WX. These two distinct types of canalicular microdomains differ considerably in their protein and lipid composition. In conclusion, hepatocytes express a large array of transporters, which are either directly or indirectly energized by metabolic energy. These transporters are essential for drug detoxification and bile formation and hence constitute an important defense line of hepatocytes against toxic compounds. In addition, the canalicular membrane has a unique composition and contains microdomains, protecting this membrane against the detergent action of canalicular bile salts.

S5-3

Transport systems in renal drug elimination

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Many water-soluble drugs and drug metabolites can be efficiently excreted by the kidneys. Proximal tubular secretion is a powerful way to extract drugs from the blood and deliver them to the urine. Secretion is a two-step process involving drug uptake at the basolateral membrane and release at the apical membrane of proximal tubule epithelial cells. For cationic drugs, uptake across the basolateral membrane is facilitated by the inside negative membrane potential and involves the electrogenic organic cation transporters 1 (OCT1; in rodents) and 2 (OCT2; rodents, man). Efflux across the apical membrane involves multidrug and toxin extrusion 1 (MATE1; rodents, man) and 2K (man). MATE transporters probably perform electroneutral organic cation/H+ exchange. Organic cations may be also released by the ATP-driven P-glycoprotein (MDR1). For organic anions, uptake across the basolateral membrane occurs against the membrane potential and requires the input of energy stored in the in-to-out gradient of alpha-ketoglutarate. Organic anion transporters 1 and 3 (OAT1, OAT3; all species) operate as organic anion/alpha-ketoglutarate antiporters. Interestingly, OCTs and OATs belong to the same solute carrier family (SLC22) and exhibit a broad specificity for exogenous compounds of various chemical structures. The release of anionic drugs across the apical membrane is species-dependent. OAT4 is expressed only in man and works as an asymmetric antiporter, releasing drugs in exchange for chloride ions, and absorbing urate and estrogen sulfate from the lumen in exchange for intracellular dicarboxylates. In addition, an electrogenic transporter, OATv1 (alias: sodium phosphate transporter 1; NPT1), releases organic anions, driven by the inside negative membrane potential. In rodents, OAT2 may be the functional correlate to OAT4. Furthermore, the multidrug resistance related transporters 2 and 4 (MRP2, MRP4), are able to expel anionic drugs at the expense of ATP hydrolysis.

For several reasons, it is important to consider renal drug transporters in pharmacotherapy. First, the rate of transport is an important determinant of pharmacokinetics. Loss-of-function or gain-of-function mutations increase or decrease, respectively, the exposure of the patient to drugs. Second, gender differences have been found for OCT2, OAT1, OAT3, MDR1, and MRP4, all being expressed to a higher extent in male rats. In contrast, OAT2 and

OAT5 showed a female-dominant expression in rats. Third, given the fact that widely prescribed ß-lactam antibiotics, antivirals, diuretics, and analgesics interact with OAT1 and OAT3, drug-drug interactions can occur at the level of the transporter. Fourth, OAT1 and OAT3 provide an entry for nephrotoxic substances such as antivirals, uremic toxins, and organomercurials. Likewise, cancerogenic sulfooxymethylpyrene compounds enter proximal tubule cells via OAT1 and OAT3 which, therefore, may be involved in renal cancerogenesis. On the other hand, if expressed in renal cancer cells, OCT3 may be utilized to direct certain cytostatics into the cells, rendering them more chemosensitive than cells not expressing OCT3. Transporter expression profiling may help in the future to individually tailor chemotherapy.

S5-4

Investigation of structure and function of organic cation transporters using homogy modeling and mutagenesis

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The transporter family SLC22 contains three subtypes of polyspecific transporters for organic cations (OCTs) that have different but overlapping substrate specificities and tissue distributions. The OCTs are involved in small intestinal absorption of cationic drugs and in their excretion in liver and kidney. Functional characterization revealed that OCT1, OCT2 and OCT3 translocate organic cations in both directions across the plasma membrane and transfer positive electric charge. The direction of translocation is determined by the electrochemical potential. Extensive mutagensis studies and detailed functional characterization of the mutants were performed. The functional studies included (i) voltage-clamp-fluorometry after modifying of amino acids with a fluorescent dye, (ii) parallel measurements of translocation of organic cation substrate and electrical charge, and (iii) inhibition experiments using nontransported inhibitors that were applied either from the extracellular or from the intracellular side of the plasma membrane. The obtained data were interpreted with help of tertiary structural models drived from the crystal structure of lactose permease that belongs to the same superfamily as the OCTs. The results indicate that OCT1 contains a large binding cleft that switches during transport between an outward-facing and an inward-facing conformation and contains high-affinity and low-affinity binding sites for organic cations. The cleft contains an innermost cavity that is accessible from both sites of the plasma membrane and is involved in low affinity binding of organic cations and in their translocation. Several amino acids were identified that contribute to the surface of the outward-facing innermost cavity as well as to the surface of the inward-facing innermost cavity. If the overall negative surface charge of the outward-facing innermost cavity was reduced by an exchange of one negative amino acid with an electroneutral one, the surplus of positve charge translocation during organic cation uptake was abolished. The data provide evidence for an alternating access transport mechanism. They suggest that small cations can be translocated together with organic cations substrates by a "pick-a-back-mechanism". In addition the data suggest that high affinity binding of organic cations to peripher sites within the binding cleft may block translocation of organic cation substrates.

S5-5

Bacterial Transport of Ammonia

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Ammonium transport is facilitated by the Amt proteins, found in all domains of life from bacteria to man. In animals they are represented by the homologous Rhesus (Rh) proteins. The nature of the substrate is still controversial for both the Amt and Rh proteins. For Amt proteins, ammonium is the substrate but it is still open whether transport is electrogenic or not. For net electrogenic transport of ammonium, a separate pathway for ammonia and proton is indicated. For Rh proteins it is well established that substrate transport is non-electrogenic but it is not clear whether the physiological substrate is NH_3, CO_2 or both. We have studied the structure and function of Escherichia coli AmtB extensively by analyzing a large number of mutants of highly conserved residues lining the conduction pore [1-3]. We have also determined the structure of a complex of AmtB with the regulatory PII protein GlnK [4]. More recently we have solved the first structure of a Rhesus protein, the Rh50 protein from the bacterium Nitrosomonas europaea[5]. We will present our conclusions on similarities and differences of the substrate transport mechanism in these two families.

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S6-0

SGV symposium on „Biosafety"

Beat M. Riederer[1], Ingrid Kohler[1]

[1]*SGV committee members*

The symposium will address various questions of biosecurity and biosafety around and in the animal house, and topics such as radioprotection and work with highly contagious animal pathogens will be presented.

Participation at the symposium, in combination with the attendance at the USGEB meeting will give half a day credit of continued education in animal welfare.

S6-1

Safety around the animal house

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No abstract available

S6-2

Biosafety and biosecurity in animal facilities

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Bio-hazards can be defined as the potential source of harm from biological agents (pathogenic and/or genetically modified (micro-)organisms) and their products. Formally we can also differentiate between biosafety which involves harm to people and other organisms and biosecurity which encompasses both the accidental and deliberate release of biological material.

From these definitions we can easily understand that many different activities present bio-risks: the agronomy, the terrorism (bio), the biological research, the medical diagnostic laboratories, the animal facilities, etc..

Even if the activities are heterogeneous (different applications, biological material, techniques, working environment), concerning the biosafy/biosecurity domain, they all have a common starting

point: the bio-risk assessment. Where the combination of the likelihood of the occurrence of an adverse event involving biological agents and toxins and the consequences (in terms of accidental infection, toxicity or allergy or unauthorized access, loss, theft, misuse, diversion or release of biological agents) of such an exposure are evaluated.

The risk assessment is then followed by the implementation of countermeasures to reduce the bio-risk to an acceptable level. Based on the principles of precaution and confinement, the measures are generally grouped in four types: a) strategic (biosafety concept, program, BSO, BSC, etc.), b) technical (construction, infrastructure, equipment), c) organization (SOP), d) personal protection (PPE (Personal Protective Equipment), medical prevention).

The variable combination of biohazards combined to the specificity of the activities lead to a great diversity of potential risks and so also to a great diversity of countermeasures. Which, even if specific to an activity, aims to protect the environment, the population and the employes by reducing or blocking the environmental dissemination (by air, water, wastes, transport, contaminated persons) and preventing the transmission (by accidental inoculation, contact, inhalation or ingestion).

S6-3

Radiation protection in the animal house

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Ionizing radiations produce extensive benefits in medicine, scientific research and industry. Conversely, exposure to ionizing radiation may result in adverse health effects. Those effects are commonly divided into deterministic effects (e.g. erythema), having a threshold dose below which the biological response is not observed, and stochastic effects (e.g. induction of cancer), for which the existence of a threshold dose is not demonstrated. The goals of radiation protection are then to prevent the occurrence of deterministic effects by keeping the individual doses below the threshold dose of a given effect and to limit the probability of stochastic effects to an acceptable level.

In most countries, the use of ionizing radiation is regulated by comprehensive legislation. In Switzerland, the Federal Office of Public Health issues the licenses for the use of ionizing radiation and monitors the compliances of facilities as well as the safety of occupational workers. The ultimate responsibility for radiation protection is held by the employer, while the radiation safety officer (RSO) is responsible to establish local rules for ensuring safe working practices.

Ionizing radiation hazards may occurred either from external exposure from a source emitting penetrating radiation (X-ray tube, gamma irradiator) or from internal exposure after intake of radionuclides into the body. External exposure can be minimized by increasing the distance from the source, limiting the time and using shielding protection, whereas internal exposure is reduced by observing rules of safe practice (analogous to chemicals or biohazards).

The use of ionizing radiation in an animal house must be organized in accordance with the legislation. The room design may have to include specific features, such as restriction of access, as well as shielding requirements if x-rays or gamma sources are employed. A separate work area should be devoted to animals injected with radioactivity in order to confine the potential spread of radioactive contamination, e.g. through animal excreta. The staff that handles animals or works with unsealed radioactive sources must be aware of their occupational risk. They must be trained to apply protective measures in order minimize external and internal exposure. Any material, whose radioactivity level lies above the clearance limit and which will not be used anymore, are classified as radioactive waste. Specific limits are set-up on the amount of radioactivity that can leave the site in solid, liquid or gaseous form.

This talk will discuss several practical issues related to the use of ionizing radiation in the animal house and appropriate measures to ensure compliance with radiation protection will be presented.

S6-4

Biosafety of highly contagious animal pathogens

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The Institute of Virology and Immunoprophylaxis (IVI), which is part of the Swiss Federal Veterinary Office, is the ISO 17025 accredited institute for the diagnosis, surveillance and control of highly contagious epizootics such as avian influenza, foot-and-mouth disease, bluetongue and classical swine fever.
The top priority of the institute is therefore work on these diseases which cannot be done in normal laboratories due to the danger of introducing these microorganisms into the surroundings. As some of these pathogenic agents may also cause disease in man, the protection of IVI employees is paramount. Experiments with highly contagious epizootics require stringent safety measures and a coherent biorisk management policy. The technical, structural and organisational safety measures are so well coordinated that an optimum protection of people and the environment is guaranteed. For example, all technical installations are regularly checked for efficiency. The building was cast on the spot from a special concrete which must be as non-porous as possible and possess an extremely low degree of shrinkage in order to be gas-tight and without cracks. All material leaving the high containment unit needs to be decontaminated, either by heat or chemical treatment. The exhaust air from both animal and laboratory sections has to pass through two HEPA filters. The ventilation plant maintains all rooms within the containment boundary at a determined negative pressure.
A thorough showering after a defined procedure is required before leaving the high containment unit. All IVI employees and all visitors must undergo a three day quarantine after being in the high containment unit in order to prevent the likelihood of transmission of animal diseases by people.
The presentation will focus on the practical and technical biosafety measures in place for activities involving highly contagious animal diseases.

S7-1

Channelopathies – Overview and insights from ion channel mutations in skeletal muscle

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Hereditary diseases of ion channels cover the diverse fields of medicine myology, neurology, cardiology, and nephrology. As ion channels do not come alone, but rather in whole families of related proteins conducting each ion type with slightly modified function and varying tissue expression patterns, the underlying mutations are restricted to single genes expressed in a specific tissue such as brain, skeletal and cardiac muscle, and sensory and secretory tissues. Examples are myotonia, periodic paralyses, cardiac arrhythmia, long QT syndrome, migraine, episodic ataxia, epilepsy, and nephrocalcinosis. The localization of the disease-causing mutations in the various channel proteins and their functional consequences can be similar in these disorders. Mutations in the genes encoding voltage-gated chloride and sodium channels expressed in skeletal muscle can cause myotonias, i.e. muscle stiffness characterized by membrane hyperexcitability: i) loss-of-function mutations in the major chloride channel of skeletal muscle, CLC-1, reduce the chloride current which normally counterbalances the membrane depolarization caused by T-tubular K^+ accumulation during electrical activity. The resulting after-depolarization causes bursts of action potentials and thus myotonia and lead to dominant Thomsen myotonia or recessive Becker myotonia; ii) gain-of-function mutations in the muscle sodium channel Nav1.4 cause a pathologically increased inward sodium leak current through the main channel pore and generate

repetitive action potentials. This repetitive activity is pronounced at pre-existing physiological membrane depolarization, e.g. due to elevated serum potassium (potassium-aggravated myotonia) or cold environment (paramyotonia). Next to clinical features such as warm-up or paradoxical myotonia, also electromyography and provocative testing with oral potassium or local cooling are helpful for diagnosing. Familial periodic paralyses (PP) also are typical channelopathies, i.e. caused by functional disturbances of ion channel proteins. The episodes of flaccid muscle weakness observed in these disorders are due to reduced excitability of sarcolemma. Decisive for the phenotype is the type of functional defect brought about by the mutations rather than the channel affected, because contrary phenotypes (hyperkalemic and hypokalemic periodic paralysis) are caused by Nav1.4 point mutations. Mutations which lead to a sodium leak through the central channel pore at membrane depolarization, i.e. elevated serum potassium, cause hyperkalemic PP. In contrast, voltage sensor mutations in Nav1.4 and Cav1.1, the skeletal muscle L-type Ca^{2+} channel, cause a leak through an aberrant pore at resting or more negative potentials, i.e. in response to lowered serum potassium (hypokalemic PP). Loss-of-function mutations in KCNJ2 encoding a potassium channel that is essential for the resting membrane potential cause a third type of PP called Andersen syndrome. The possible pathogenetic mechanisms deduced from both in-vitro and in-vivo studies for the fore-mentioned diseases will be discussed, particularly for hypokalemic PP. Muscle membrane inexcitability is due to a long-lasting depolarization that inactivates sodium channels. This is a common final pathway of the muscle weakness.

S7-2

Dysregulation of voltage-gated sodium channels in the context of chronic pain

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Tissue injury and nerve lead to regulation of expression of different voltage-gated sodium channel subunits as well as alteration of their biophysical properties. contributing to abnormal activity in primary sensory neurons and altering central processing of somatosensory information. However, the exact role of voltage-gated sodium channels in the generation and the sustaining of ectopic firings as well as their contributions to pain remained unclear and matter of study since long years. In the last decade several works partially elucidate the crucial functions of voltage-gated sodium channels in pain. This review intends to summarize recent findings on voltage-gated sodium channels in different animal models of pain with particular emphasis on neuropathic pain.

S7-3

Cardiac ryanodine receptor Ca^{2+} release channels: channelopathies and hypersensitivity

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Channelopathies can not only arise from congenital or acquired malfunctioning of ion channels of the cell membrane, intracellular ion channels located on organelles can also be affected. In cardiac muscle cells, each contraction is governed by Ca^{2+} release from intracellular stores, the sarcoplasmic reticulum (SR). Ca^{2+} release occurs via Ca^{2+} release channels, a.k.a. ryanodine receptors (RyRs), that are located in the SR membrane and activated by the Ca^{2+}-induced Ca^{2+} release mechanism. Mutations in the skeletal muscle isoform of the channel (RyR1) have long been known to cause malignant hyperthermia, a rare but potentially lethal complication of general anesthesia. Analogous mutations in the cardiac channel isoform (RyR2) have only been discovered a few years ago. Most RyR2 mutations are associated with cardiac arrhythmias, often manifest as catecholaminergic polymorphic ventricular tachycardias (CPVTs) induced by emotional stress or physical activity. As a serious complication, these CPVTs can lead to sudden cardiac death (SCD). In a recent molecular autopsy study 1 out of 7 SCD victims was found to have mutations in the RyR2. When heterologously expressed, these mutated RyR2 channels exhibited changes of channel gating resulting in accidental and spontaneous SR Ca^{2+} release and an abnormally sensitive Ca^{2+} dependent activation, both being potentially arrhythmogenic by inducing Ca^{2+} activated depolarizing membrane currents. Like inherited mutations of the channel, acquired modifications of the RyR2 can also lead to similar functional changes on the molecular level, that assemble into an comparable cellular phenotype. For example, hyperphosphorylation of the RyR2 has recently been proposed to be present during heart failure, leading to anomalous RyR2 openings causing an SR Ca^{2+} leak which ultimately contributes to the weak heartbeat. Similarly, cardiomyopathies involving excess production of reactive oxygen species (ROS), such as dystrophic cardiomyopathy, were found to cause hypersensitive Ca^{2+} signaling arising from redox modifications of the RyRs, sometimes also culminating in CPVTs. Taken together these and related observations indicate that malfunctions of intracellular Ca^{2+} release channels are one of the cornerstones of cardiac Ca^{2+} signaling abnormalities and underlie some types of arrhythmias, therefore rendering them attractive as future drug targets. The combined presence or activation of several of these channel sensitizing mechanisms, such as a RyR2 mutation combined with β-adrenergic stimulation with subsequent phosphorylation of the RyR2, may be particularly detrimental. *Supported by SNF, SCRTN and FSRMM.*

S7-4

A haplotype of the epithelial Ca^{2+} channel TRPV6 is a risk factor for renal Ca^{2+} stone formation: Possible selection pressure during the migration from Africa to Europe

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Calcium is essential for many physiological processes in our body. For that reason, Ca^{2+} homeostasis is thoroughly regulated by the coordination of intestinal absorption, renal reabsorption and bone resorption. The late limiting step of dietary Ca^{2+} absorption in the intestine requires the epithelial Ca^{2+} entry channel TRPV6. In this study, the TRPV6 gene was sequenced in renal Ca^{2+} stone patients. The frequency of “ancestral” TRPV6 haplotype (157R+378V+681T) which was reported to represent a positive selection in human evolution, was significantly higher ($p = 0.039$) in Ca^{2+} stone formers (8.4 %, derived = 502, ancestral = 46) compared to non-stone forming individuals (5.4 %, derived = 645, ancestral = 37). Mineral metabolism was investigated on different Ca^{2+} regiments. However, no difference of plasma 1,25-vitamin D, PTH, Ca^{2+} and urinary Ca^{2+} were found when patients homozygous for the derived haplotype were compared with heterozygous patients. In one stone-forming patient, the ancestral haplotype was found to be homozygous, and the patient had absorptive hypercalciuria. We therefore expressed the ancestral protein (157R+378V+681T) in *Xenopus* oocytes and found a significantly enhanced Ca^{2+} permeability when tested by $^{45}Ca^{2+}$ uptake assay. These results suggest that the ancestral gain of function haplotype in TRPV6 is a risk factor for the renal Ca^{2+} stone formation in certain forms of absorptive hypercalciuria. Moreover, excess Ca^{2+} due to the expression of ancestral TRPV6 protein could be a selection pressure during the migration from Africa to Europe in human evolution.

Poster Topic 2: BIOLOGY OF ION CHANNELS AND TRANSPORTERS

S7-5

Characterization of a novel 7-amino-acid duplication located in the PAS domain of hERG found in a patient with congenital long QT syndrome

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Background: Cardiac repolarization is mediated by K^+ currents, mainly I_{Kr} (hERG) and I_{Ks} (KCNQ1), which are both essential for determining the duration of the cardiac action potential. Reduction of these currents may prolong the duration of the QT interval of the ECG, which is a hallmark of the congenital long QT syndrome (LQTS). In this study, a novel heterozygous mutation in the gene KCNH2 (343 363dup) was discovered in a 37-year-old woman with history of syncope and convulsions due to torsades de pointes arrhythmias. Her heart rate corrected QT interval was found to be prolonged up to 620 ms. The mutation resulted in a 7-amino-acid duplication in the PAS domain at the N-terminus of hERG.

Objective: Characterize a novel hERG 343-363dup mutation found in a LQTS patient.

Methods and Results: Wild-type (WT) and mutant (Dupl) hERG channels were transiently re-expressed in mammalian cell lines, and their biophysical properties were assessed using the patch-clamp technique in whole-cell configuration. Since the 7-amino-acid duplication is in the PAS domain, which is involved in the deactivation transition of hERG, we first studied this process. The main deactivation time constant was reduced by 1.4 to 1.8-fold for hERG Dupl compared to WT in tail currents recorded at -150 and -90mV, respectively. Tail current density at 120mV, after a depolarizing pulse at +40mV, was decreased by 66±8% for Dupl alone, and by 83±4% for the heterozygous condition WT:Dupl (1:1), thus suggesting a dominant negative effect. Steady state inactivation showed no significant difference, but activation curve displayed a hyperpolarizing shift of ~-5mV (V50= 29.5 ±0.2mV, V50= 34.8±0.2mV). Cell surface biotinylation experiments with the mutant channel showed a ~50% decrease of the mature, fully glycosylated, form of hERG both in the membrane and intracellular fractions, whereas the immature form was not affected.

Conclusions: This novel hERG 343-363dup mutation causes a reduction of the peak current density and hastening of deactivation process. Both alterations lead to a loss-of-function of the channel. Furthermore, amplitude of current measured in the heterozygous model, i.e. co-expression of hERG WT and Dupl, suggests dominant negative effect of the mutation. The shift towards hyperpolarizing voltages in the activation curve may reflect a gain-of-function; however, we speculate that its contribution is negligible. Biotinylation experiments showed that only the mature form of hERG Dupl is affected by the mutation, unveiling a likely defective maturation/trafficking process. Despite the fact that this novel mutation is not located in the pore, a region where mutations are considered to lead to a higher arrhythmic risk, our findings illustrate that that N-terminally located mutations can also lead to severe ventricular arrhythmias.

Poster Topic 2: BIOLOGY OF ION CHANNELS AND TRANSPORTERS

S7-6

Increased repolarization reserve as a new anti-arrhythmic principle

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Background: A healthy human heart will beat approximately 3.000.000.000 times during a normal lifespan without any disturbances. Any electrical deviation from this regular pathern is termed an arrhythmia. Arrhythmias can result in anything from minor palpations to sudden cardiac death. A number of arrhythmias are due to malfunction of a cardiac ion channel named HERG1. This channel is essential for appropriate repolarisation of the cardiac action potential. It is well known that unintended inhibition of the HERG channel is pro-arrhythmic. We have therefore developed a new concept of HERG channel activation an investigated the anti-arrhythmic properties of such activators.

Methods: The experimental approach is translational. Patch-clamp experiments have been conducted applying native cardiomyocytes or by using heterologous expression systems in oocytes and mammalian cells of the HERG channel. In addition, ex vivo Langendorff experiments and in vivo studies in both conscious and anaestezied animals have been conducted.

Results: A number of anti-arrhythmic properties was demonstrated for the HERG channel activators. In native cardiomyocytes action potential was abbreviated and post-repolarisation refractory period was increased significantly. Further HERG channel activation rendered the cardiomyocytes more resitance towards early-afterdepolarisations (EAD's). In intact hearts investigated in Langendorff set-up extrasystolis could be prevented by HERG channel activation and a tendency towards less dispersion of the length of action potentials was observed. In in vivo studies HERG channel activation could prevent drug induced prolongation of the QT interval and significantly reduce the incidence of extrasystolis and ventricular fibrillations.

Conclusions: In conclusion we believe it is demonstrated that under certain circumstances, activation of the cardiac HERG channel can be a new antyi.-arrhythmic principle

Poster Topic 2: BIOLOGY OF ION CHANNELS AND TRANSPORTERS

S8-1

In vivo multiphoton imaging of ion transporting renal epithelia

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The old concept “seeing is believing” has driven many optical imaging applications in various fields of biomedical research including renal physiology. An advanced confocal laser scanning fluorescence imaging technique, multiphoton microscopy has been especially useful for deep optical sectioning of living tissues. Multiphoton imaging can provide ultra-sensitive detection of fluorescent signals with sub-micron resolution, meaning that even intracellular organelles can be visualized in intact organs. We applied multiphoton imaging to directly and quantitatively visualize the intact kidney and the complex functions of the salt reabsorbing proximal and distal nephron segments. Visualization of cellular variables like cytosolic calcium ($[Ca^{2+}]_i$), pH, cell-to-cell communication and signal propagation, interstitial and tubular fluid flow, real-time imaging of tubuloglomerular feedback (TGF) and renin release mechanisms will be shown. Advantages of several fluorophores, e.g. rhod-2, fluo4-fura red for $[Ca^{2+}]_i$, SNARF for pH, NAD/NADH autofluorescence for reactive oxygen species measurements will be demonstrated and discussed. Complex, in vivo oscillations (a faster 100-200 mHz, and a slower 20-40 mHz component) in glomerular filtration, proximal and distal tubular flow rate, renal epithelial $[Ca^{2+}]_i$, and in the generation of reactive oxygen species mainly at the subapical plasma membrane are due

to the afferent arteriolar myogenic and TGF mechanisms. Tubular flow dependent proximal and distal tubular $[Ca^{2+}]_i$ oscillations may be due to mechanically-induced luminal ATP release via the recently identified connexin 30 hemichannels at the apical cell membrane. Visualization of the concentrating-diluting function of the nephron, the effects of various ion transport blocking diuretics will be demonstrated. Alexa Fluor 546-labeled siRNA uptake in rat proximal and distal nephron segments can be visualized. Within two minutes of injection into the carotid artery, significant levels of AF546-siRNA were detected in the proximal tubule, mainly at the apical brush border membrane indicating uptake from the tubular lumen. Although lower amounts than that in the PT, siRNA uptake was detected in cells of the distal tubule 15-20 minutes after injection. The use of a FRET-based fluorogenic renin enzyme substrate will be demonstrated as a great tool to visualize in real-time and in the living kidney tissue the activity of the prorenin receptor, i.e. basolateral cell membrane-bound angiotensin generation in distal nephron segments. New visual data challenge a number of existing paradigms in renal (patho)physiology. Quantitative in vivo imaging of kidney function with multi-photon microscopy has tremendous potential for the basic scientist as well as to eventually provide novel non-invasive diagnostic and therapeutic tools for future applications in clinical nephrology.

S8-2

Sub-100-nm resolution in TIRF microscopy

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Fluorescence microscopy and highly specific labelling of molecules and molecular assemblies have become indispensable tools in cell biology. Total internal reflection fluorescence (TIRF) microscopy is the method of choice for observing structures and processes close to cell surfaces and adjacent to the cover slip or glass slide. However, for proper analysis many structures and processes require lateral resolution of 100 nm or better. To this end, the resolution of optical microscopes needs to be extended beyond the classical diffraction limit.

Several concepts known to exceed the diffraction limit rely on nonlinear fluorescence transitions, nonlinearities in the saturation regime, or sequential localization of photoswitchable fluorophores. The latter technique is restricted to a small class of fluorophores while nonlinearities generally increase photobleaching and phototoxicity, limiting their application to biological specimens. Hence a technique relying on linear fluorophore response is preferred. In this regime, extending optical resolution is feasible when illuminating the specimen with structured light instead of uniform light. In standard widefield fluorescence microscopy, illuminating the specimen with standing waves increases lateral resolution by up to a factor of 2. In TIRF microscopy, owing to the higher spatial frequency of the evanescent standing wave emerging from the glass substrate, lateral resolution can be improved by a factor of 2.5. To recover high spatial frequencies, three images at different phase shifts are acquired for each direction of the standing wave pattern.

Recent progress in instrumentation, image reconstruction, and biological applications including multi-wavelength imaging will be presented.

S8-3

Imaging membrane proteins by atomic force microscopy in their native environment

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Atomic force microscopy (AFM) is an ideal method to study the surface topography of biological membranes. It allows membranes that are adsorbed to flat solid supports to be raster-scanned in physiological solutions with an atomically sharp tip. Therefore, AFM is capable of observing biological molecular machines at work. In addition, the tip can be tethered to the end of a single membrane protein, and forces acting on the tip upon its retraction indicate barriers that occur during the process of protein unfolding. Recent results achieved on reconstituted and native biological membranes will be presented and discussed.

S8-4

Fluorescent Annexin A1 Reveals Dynamics of Ceramide Platforms in Living Cells.

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Upon its genesis during apoptosis ceramide promotes gross reorganization of the plasma membrane structure involving clustering of signalling molecules and an amplification of vesicle formation, fusion and trafficking. The annexins are a family of proteins, which, in the presence of Ca2+, bind to membranes containing negatively-charged phospholipids. Here, we show that ceramide increases affinity of annexin A1-membrane interaction. In the physiologically-relevant range of Ca2+-concentrations, this leads to an increase in the Ca2+-sensitivity of annexin A1-membrane interaction. In fixed cells, using a ceramide-specific antibody, we establish a direct interaction of annexin A1 with areas of the plasma membrane enriched in ceramide (ceramide platforms). In living cells, the intracellular dynamics of annexin A1 match those of plasmalemmal ceramide. Among proteins of the annexin family, the interaction with ceramide platforms is restricted to annexin A1 and is conveyed by its unique N-terminal domain. We demonstrate that intracellular Ca2+-overload occurring at the conditions of cellular stress induces ceramide production. Using fluorescently-tagged annexin A1 as a reporter for ceramide platforms and annexin A6 as a non-selective membrane marker we visualize ceramide platforms for the first time in living cells and provide evidence for a ceramide-driven segregation and internalization of membrane-associated proteins.

Poster Topic 5: MEMBRANE TRAFFICKING AND DYNAMICS

S8-5

Dimerization and ER-export of Kit-ligand is induced by a conserved glycine repeat motif in its transmembrane domain

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Kit-ligand (Kitl), also known as Stem Cell factor, is a non-covalently bound dimer signaling via the c-kit receptor tyrosine kinase, which is required for migration, survival and proliferation of melanocytes, mastocytes, germ and hematopoetic cells. Despite the fundamental role of Kitl in morphogenesis, the biochemical regulation of the protein is not fully understood. Signals for basolateral sorting and ER-export have recently been described, but the dimerization of the extracellular domains of Kitl is indispensable to activate its receptor c-kit.

By employing a cell permeable cross-linker and a bi-molecular fluorescence complementation of wildtype and mutant forms of Kitl, we determined that Kitl dimerization is initiated by its transmembrane domain in the ER and directly coupled to efficient ER-export. Further biochemical and mutational analysis of the transmembrane dimerization domain revealed a critical and highly conserved glycine repeat motif. This motif appeared in vertebrate evolution during the teleost to tetrapod transition, concomitantly with the role of Kitl in maintaining stem cell populations. This transmembrane motif mediates specific interactions between Kitl, but not with other transmembrane proteins, and is thus the driving force for Kitl dimerization in the ER and efficient cell surface transport and signaling. This provides evidence that fine-tuning of intracellular transport mechanisms can improve morphogenetic activities of growth factors, thus selecting and enhancing paracrine signaling during evolution.

Poster Topic 1: STRUCTURE OF MEMBRANE PROTEINS

S8-6

Open and Closed Structures of two Pentameric Ligand Gated Ion Channels

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The pentameric ligand gated ion channels (pLGIC) constitute a family of selective ion channels that are key players in the control of electric signaling at chemical synapses. The family codes for a structurally conserved scaffold of channel proteins that open in response to the binding of neurotransmitter molecules. We have determined the X-ray structures of two prokaryotic family members from the bacterium *Erwinia chrysanthemi* (ELIC) at 3.3 Å; resolution [1] and from the bacterium *Gloeobacter violaceus* (GLIC) at 3.1 Å; resolution [2]. Both proteins form cation selective channels and bear most of the structural hallmarks of the family including the N-terminal extracellular ligand binding domain and the four helices of the pore domain. Despite the overall similarity, both structures adopt distinct conformations of the ion conduction pathway:
The structure of ELIC shows a nonconductive state with rings of hydrophobic residues at the extracellular side of the pore preventing ion permeation. This hydrophobic barrier has opened in the structure of GLIC to a funnel shaped pore, where a ring of glutamate residues at the intracellular constriction of the pore creates an ion coordination site. GLIC is thus believed to represent a conducting conformation of the channel.
In combination, both structures suggest a novel gating mechanism for pentameric ligand-gated ion channels where channel opening proceeds by a change in the tilt of the pore-forming helices. Our study thus provides a first structural view at high resolution into how a pLGIC may open and selectively conduct ions.

[1] Hilf, RJC., Dutzler, R., *Nature* **452**, 375-379 (2008).
[2] Hilf, RJC., Dutzler, R., *Nature* doi:10.1038/nature07461; Published online 5 November 2008

Poster Topic 1: STRUCTURE OF MEMBRANE PROTEINS

S9-1

Cell Membranes in Human Tumors as Therapeutic Targets

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No abstract available.

S9-2

Targeting receptor tyrosines in breast cancer

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No abstract available.

S9-3

A lesson from nature: Membrane disruption peptides and ultrashort lipopetides as novel weapons against bacteria, fungi and cancer

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All types of living organisms including human and plants use host-defense peptides, known also as antimicrobial peptides (AMPs), to protect themselves from invading pathogens. These peptides are characterized by a net positive charge and threshold hydrophobicity, enabling them to bind and lyse preferentially negatively charged membranes, which are enriched in the outer leaflet of bacteria, fungi, and slightly in cancer cells. Biochemical, biophysical and biological studies suggest that membrane lysis occurs after a threshold concentration has been reached. We named this process a “carpet” mechanism. Based on the “carpet” mechanism, we designed novel families of diastereomeric (containing D- and L-amino acids) peptides and ultra-short lipopeptides with cell specific killing activity against a variety of target cells. They have promising properties which make them attractive templates for the development of future antibiotics and anticancer compounds with new modes of action, to which it will be difficult for pathogens and cancer cells to develop resistance.

S9-4

Photodynamic effects of hypericin and Foslipos in head and neck squamous cell carcinoma in-vitro

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Introduction
Head and Neck Squamous Cell Carcinoma (HNSCC) is the eighth most common malignancy worldwide. However, treatment options are still limited. With the aim to improve the survival rate, tissue preservation and quality-of-life issues photodynamic therapy (PDT) was recently introduced. This treatment regime is based on the uptake of a fluorescent photosensitizer into cancer cells. Following light activation of the photosensitizer, highly toxic metabolites are generated that may cause cell death. In the present study we explored the effects of two photosensitzers alone and in combination in HNSCC in-vitro.

Materials and Methods
HNSCC cells (UMB-SCC 745) were cultivated under standard conditions and incubated with hypericin (Invitrogen, Basel, Switzerland) or the meso-tetrahydroxyphenylchlorin derivative Foslipos (Biolitec, Jena, Germany) in concentrations ranging from 10-0.6 µg/ml for 0-24 h. In addition, a combination of hypericin and Foslipos was used (final concentration 20-0.6 µg/ml). Thereafter cells were washed, illuminated with 5 J/cm^2 for 15 min and further cultivated for 0-24 h.
For each photosensitizer a dark toxicity assay was performed using a BrdU proliferation assay (Roche, Basel, Switzerland). Uptake kinetics of the photosensitizers were investigated by ELISA fluorescence measurements at 540±40nm and by confocal microscopy. Furthermore, PDT effects were monitored by live cell imaging and MTT viability tests.

Results
Dark toxicity assays showed that photosensitizer concentrations of 10 µg/ml are cytotoxic, while concentrations of 2.5 µg/ml were well tolerated. Photosensitizers accumulated over time in the cytoplasm of HNSCC cells, with a maximum after 5 h. Live cell imaging studies showed that single applications of either hypericin or Foslipos (each 2.5 µg/ml) resulted in death of close to 100% of HNSCC cells within 12 h. After treatment with a mixture of both photosensitizers (total concentration 2.5 µg/ml) all HNSCC cells were already killed after 3 h.

Conclusions
Our data indicate that the photosensitizers hypericin and Foslipos may have synergistic phototoxic effects on HNSCC cells in-vitro. We hypothesize that this effect may be the result of the hypericin emission spectrum exciting Foslipos. The application of mixtures with overall lower concentrations of each photosensitizer may be advantageous compared to established conditions.
Poster Topic 6: OTHERS

S9-5

Investigation on lipid bilayer permeation of P-glycoprotein substrates

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Pressure keeps mounting for development and improvement of methods to predict ADME properties of drug candidates early in discovery process. Lipid bilayers and efflux transporters such as P-glycoprotein (P-gp, MDR1) represent the major *in vivo* barriers for drugs. They are involved in all pharmacokinetic processes. The existing models to predict barrier passage of a compound and especially its recognition by efflux transporters are not satisfactory.
To study lipid bilayer permeation and P-gp transport, we purify P-gp and incorporated it into liposomes. Fully functional His-tagged P-gp is overexpressed in HEK293 cells and purified via Cobalt(II)-based immobilized metal ion affinity chromatography. Additional methods are currently being tested to further improve the purity. The goal is to combine these proteoliposomes with a lanthanide-based liposomal permeation assay, developed in our group [1]. It is based on the interaction of lanthanides with aromatic ligands, resulting in a characteristic luminescence signal.
Permeation profiles of the tetracycline group have been measured; tetracycline represents a well characterized P-gp substrate. Europium(III)-containing liposomes were incubated with the drug, excited at the appropriate wavelength and the luminescence at 615 nm was recorded. The resulting luminescence time-curves were fitted with a monoexponential or biexponential function and the rate constant of the faster exponential was used to calculate the apparent permeation coefficient ($Perm_{app}$). The more lipophilic doxycycline (logP -0.22 [2]) shows a faster permeation with a $Perm_{app}$ of 37.8 s^{-1} compared to tetracycline (logP -1.44 [2]) with a $Perm_{app}$ of 10.0 s^{-1}. The estimated $Perm_{app}$ values of chlortetracycline and oxytetracycline do not correlate with their logP values. We are currently determining the membrane affinities of theses compounds to find out wether they correlate better with $Perm_{app}$. In a next step, the lanthanide permeation assay will be adapted to its use with proteoliposomes to measure lipid bilayer permeation and P-gp transport in parallel.

Acknowlegment:
We are grateful for the financial support from OPO Foundation, Switzerland.

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2. Florence, A. T. and Attwood, D., Physicochemical Principles of Pharmacy, 2006

Poster Topic 6: OTHERS

S9-6

Deficient nuclear expression of the Ku86 autoantigen in lymphocytes of patients with chronic lymphocytic leukaemia (CLL)

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Background: Chronic lymphocytic leukemia (CLL) is characterized by an increase in monoclonal, phenotypically mature B-lymphocytes in the blood and lymphatic organs, by short telomeres and cytogenetic aberrations (A. Röth, D. de Beer et al., Brit. J. of Haematol., 2008; H. Döhner et al. NEJM, 2000). One of the major proteins for the regulation of telomere maintenance and the repair of DNA double-strand breaks is the Ku autoantigen (composed of a 70kDa subunit (Ku70) and a 80kDa subunit (Ku86)). Knockdown experiments of the Ku autoantigens in human somatic cell lines results in rapid shortening of telomere ends and genomic instability (Ruis et al., Molecular and Cellular Biology, 2008).
Our aim was to assess the localisation and expression of the Ku proteins in lymphocytes from healthy individuals and from patients with CLL in order to find out whether changes exist. Such changes could point to an involvement of the Ku-autoantigen in the pathophysiology of the disease.
Patients and Methods: We examined the localisation and expression of the Ku-proteins in leukemic cells of 20 patients with CLL, in the cell lines DOHH-2 and Karpas 422 (human follicular B cell lymphomas) and in peripheral blood lymphocytes of 4 healthy individuals by immune staining and Western blot. Additionally, telomere length measurements were performed by automated multicolour Flow-FISH.
Results: Lymphocytes of healthy individuals showed telomere lengths in the normal range (mean: 7.05kb ± 0.95kb) and the Ku autoantigens were exclusively expressed in the nucleus. In the cell lines DOHH-2 and Karpas 422 the majority of the Ku-proteins was expressed in the nucleus. The expression of the Ku proteins was clearly elevated compared to lymphocytes from healthy individuals. Most interestingly, in leukemic cells obtained from patients with CLL no nuclear but only cytoplasmatic Ku86 expression was detected. In addition, Ku70 was detected in the nucleus as well as in the cytoplasm. The telomere length in leukemic cells of all patients with CLL was short (mean: 4.99 ± 1.99 kb) and on average significantly shorter compared to telomere lengths from healthy individuals ($p<0.001$).
Conclusion: Our findings demonstrate for the first time clear differences in the expression and localisation of the Ku-proteins in leukemic cells of patients with CLL and in lymphoid cell lines compared to lymphocytes of healthy individuals. All leukemic cells of patients with CLL with short telomeres demonstrate no Ku86 expression in the nucleus. Deficient nuclear expression of Ku86 and/or Ku70 in CLL cells might be involved in shortening of telomeres and genomic instability as has been seen in heterozygous Ku86 deficient human cell lines. The localisation of Ku86 and Ku70 in the cytoplasm could furthermore point to defects in the Ku proteins and/or in the transport system of the Ku proteins between the nucleus and the cytoplasm. Further molecular and functional studies of the Ku proteins to address these questions are needed and ongoing.

Poster Topic 6: OTHERS

ABSTRACTS – POSTERS (T)

Poster Topic 1 – Structure of Membrane Proteins

T1-01

Localization and quantification of the flavin cofactors of the Na^+-translocating NADH:quinone oxidoreductase from *Vibrio cholerae*

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The Na^+-translocating NADH:quinone oxidoreductase (Na^+-NQR) from *Vibrio cholerae* is a respiratory membrane protein complex that couples the exergonic oxidation of NADH with quinone to the transport of Na^+ across the membrane. Na^+-NQR is composed of six subunits (NqrA to NqrF) and contains one Fe-S centre, ubiquinone-8, one FAD, and two covalently bound FMNs as cofactors.
Preparations of purified Na^+-NQR additionally contained riboflavin. The question whether riboflavin is an additional flavin cofactor or whether it is an artefact originating from the hydrolysis of FAD or FMNs is discussed controversially [1,2].
We present a new method for the quantification of the covalently linked FMNs allowing the determination of the total flavin content. Riboflavin is confirmed as being an intrinsic cofactor of the Na^+-NQR [1], and is demonstrated to be localized on the membrane-bound NqrB subunit.

Refs:
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[2] A. V. Bogachev, Y. V. Bertsova, D. A. Bloch, M. I. Verkhovsky, Thermodynamic properties of the redox centres of Na^+-translocating NADH:quinone oxidoreductase, Biochemistry 45 (2006) 3421-3428.

T1-02

Cardioprotective action of human recombinant erythropoietin: targets and mechanisms

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Evidences accumulating during the past decade reveal impressive cardioprotective potential of human recombinant erythropoietin (hrEpo). Data obtained using isolated cardiomyocytes and in vivo animal models of myocardial infarction indicate that acute administration of hrEpo before or shortly after the coronary occlusion results in reduction of the infarct size and inflammatory response. Since treatment of cardiomyocytes with hrEpo protected the cells for apoptosis, anti-apoptotic effect of the cytokine was suggested as a mechanism of its cardioprotective action.
We have used in vivo rat heterotopic heart transplantation model of ischemia-reperfusion and isolated primary cultures of rat cardiomyocytes and human aortic endothelial cells (HAECs) to assess hrEpo targets in the heart and mechanisms of its cardioprotective action in the settings closely resembling those during on-pump cardiac surgery.
Administration of a single dose of hrEpo (5000 U/kg) intravenously 20 min prior to the onset of reperfusion of the heart transplanted into the abdomen of the recipient animal after 45 min of cold global ischemia significantly reduced myocardial stress and reperfusion injury already 5 min after the restoration of blood flow as follows from the plasma atrial and brain natriuretic peptides concentration and troponin T levels. Apoptosis markers (caspases 9 and 3, cleaved PARP and Tunel-positive staining) were below the detection level in both control and hrEpo-treated animal groups suggesting that cardioprotective effect of hrEpo was not linked to the suppression of apoptosis. Significant reduction of oxidative stress (GSH:GSSG content) in the transplanted myocardial tissue of the hrEpo-treated recipients was at least in part mediated by an increase in NO production detected as NO2- levels in plasma. Immunohistochemical examination indicated that hrEpo was not entering the myocardial tissue but interacted exclusively with the endothelial cells of the coronary vessels when applied intravenously. Therefore, its cardioprotective effect was mediated by the factors secreted by the endothelium where PI3K-Akt pathway was activated almost instantaneously in response to hrEpo treatment. Activation of the endothelial NOS followed Akt phosphorylation. We have furthermore evaluated 125I-hrEpo interaction with isolated rat cardiomyocytes. Binding of Epo occurred within minutes and was followed by internalization (with 30 min) and degradation (within 3 h) of Epo-Epo receptor complexes. Similar to that in endothelial cells, interaction of Epo with the sarcolemmal membrane resulted in activation of the PI3K-Akt pathway and phosphopylation of eNOS at Ser 1177.
We therefore conclude that despite availability of the hrEpo binding sites at the sarcolemmal membrane of cardiomyocytes, cardioprotective effect of intravenously applied hrEpo is mediated by endothelial-derived factors including NO. Characterization of these hrEpo-sensitive cardioprotective factors is currently ongoing.

T1-03

Stability and selectivity assessment of a putative low-K+ inactivated state of the KcsA channel

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Potassium channels constitute a large family of proteins, notably involved in the regulation of the activity of excitable cells. The channels partly exert that function by varying their conductance through a mechanism known as C-type inactivation: Shortly after the activation of K^+ channels, their selectivity filter stop conducting ions at a rate that depends on various stimuli. This inactivation process plays a critical role in controlling the length and frequency of cardiac action potentials, as well as the firing patterns in neurons. The molecular process underlying the C-type inactivation mechanism remains unexplained despite the accumulation of experimental evidences showing the key role played by the channels' selectivity filter and some neighboring residues.
It's been recently shown that the prokaryotic KcsA channel undergoes C-type inactivation like its eukaryotic counterparts (Gao et al., PNAS, 102:17630 (2005)), establishing KcsA as a perfect prototypic model to study the structural basis of the inactivation mechanism. An X-ray structure of the KcsA channel obtained in presence of low K^+ concentration (Zhou et al., Nature 414:43 (2001), pdb code 1K4D) has since then been postulated to correspond to the C-type inactivated state of the channel. While the structural analysis of this static conformation suggests that pore lining amide hydrogens would prevent the permeation of ions, uncertainties remain about its stability under physiological conditions and its ion occupancy state. These questions are of primary importance to better understand the relevance of this structure to the physiological regulation of ion permeation in K+ channels. Using molecular dynamics simulations and free energy calculations, we investigated on the stability, selectivity, and conductance of the selectivity filter of KcsA in this putative inactivated state.

T1-04

Ammonium Transport Mechanisms in the Amt/Rh Protein Family

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Transport of ammonium across cellular membranes is a fundamental physiological process. Although ammonium is highly toxic to animals, it is the preferred source of nitrogen for most microorganisms. Ammonium transport is mediated by a family of ubiquitous membrane proteins (Amt), found in all domains of life and homologous to animals Rhesus (Rh) proteins. Based on the X-ray structure of the E.coli AmtB transporter, it was concluded that the conduction mechanism involves the single file diffusion of electro-neutral ammonia (NH_3) molecules, excluding the presence of water molecules in the narrowest portion of the pore (Khademi et al., Science 2004). This lead the Amt/Rh proteins to be generally considered as ammonia conducting channels. However, this model neglects the fact that X-ray diffraction experiments have shown electronic density in the pore lumen for crystals grown in both presence and absence of ammonium salt (Zheng et al., PNAS 2004). Furthermore, free energy calculations suggest that the hydrophobic pore of AmtB is able to stabilize a file of water molecules at positions in excellent agreement with the experimental electronic density (Lamoureux et al., Biophys. J. 2007).
The possible presence of water molecules in the pore lumen of AmtB calls for the reassessment of the so far accepted permeation model. Functional experiments on plant ammonium transporters and rhesus proteins suggest a variety of permeation mechanisms including the passive diffusion of NH_3, the antiport of NH_4^+/H^+, the transport of NH_4^+, or the cotransport of NH_3/H^+ (Javelle et al., J. Struct. Biol. 2007). In the light of some recent functional and simulation studies on the AmtB transporter, we discuss these mechanisms and illustrate how they can be reconciled with the available high resolution X-ray data.

T1-05

Molecular architecture and conformational rearrangements of the membrane-bound metalloprotease FTSH (HFLB)

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The ATP-dependent membrane-bound metalloprotease FtsH is universally conserved in eubacteria, chloroplasts and mitochondria [1]. Of the 5 energy-dependent AAA+ proteases in E.coli, FtsH is the only essential one and malfunctioning of the close human homolog paraplegin causes one form of hereditary spastic paraplegia. FtsH is involved in quality control and signalling. It degrades non-functional or damaged membrane proteins by pulling them out of the membrane, followed by unfolding and translocation into the proteolytic chamber. In this process, the energy of ATP is converted into a mechanical force by large conformational rearrangements. We have solved the crystal structure of FtsH from Th. maritima in 2 different crystal forms in the ADP-bound and nucleotide-free state ([2] and Bieniossek, unpublished). The tetragonal crystal form has ADP bound and reveals a hexamer composed of two rings, the protease and AAA ring. The protease ring shows virtually perfect C6 symmetry and is responsible for hexamerisation. The AAA ring shows C2 symmetry, where pairs of AAA domains located at opposite sides of the ring have an interdomain angle with respect to the protease which differs from those of the other two pairs. In the hexagonal crystal form the AAA ring has dramatically changed its conformation showing exact C6 symmetry. Analysis of the position of the conserved pore-phenylalanine residues suggests a mechanism for protein unfolding and translocation. The protease domain reveals a novel, all-alpha-helical fold with only a short beta ribbon. In the inhibitor-bound hexagonal crystal form an additional beta strand is formed which serves as an edge for substrate binding. Contrary to earlier reports, the third zinc ligand is an aspartic acid.

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T1-06

Structure of the NanC porin from *Escherichia coli*

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Sialic acids, present mostly on vertebrate cell surfaces, can be metabolized by bacteria and also signal inflammation when found in solution. N-acetylneuraminic acid, the most abundant sialic acid, can enter into *Escherichia coli* through NanC, a monomeric substrate-inducible outer-membrane channel. NanC has a mass of 25kDa and belongs to the KdgM family of small monomeric porins. KdgM homologues are found in gammaproteobacteria, including major plant and human pathogens, and define a large family of putative acidic sugar/oligosaccharide-specific outer-membrane channels. Here, we present the first high-resolution structure (1.8Å) of a KdgM family member. NanC was crystallized in two forms, one obtained from membrane-extracted material, the other from inclusion bodies refolded protein. NanC folds into a 28 Å-long 12-stranded beta-barrel. The open tubular pore is slightly constricted at its centre with an average radius of 3.3 Å. The channel is lined by two conspicuous tracks of basic residues facing each other across the pore. They are likely to guide monomers and oligomers of N-acetylneuraminic acid through the pore.

T1-07

Heterologous expression of the bacterial oligosaccharyltransferase PglB from *Campylobacter lari*

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Glycosylation is the predominant way of protein modification used in nature to enlarge and diversify functionality among a given set of proteins. The recent identification of bacteria with the ability for N-linked protein glycosylation disproves current text book knowledge that this feature is restricted to eukaryotes and archaea.
The central enzyme in this process of post-translational protein modification is the oligosaccharyl transferase (OTase). It catalyzes the *en bloc* transfer of an oligosaccharide from a lipid linked donor (LLO) to the asparagine side chain of a protein. Whereas in eukaryotes the OTase usually consists of a hetero-oligomeric complex with up to nine subunits, the single integral membrane protein PglB is responsible for the transfer of glycans in prokaryotes. Nevertheless PglB shows homology to the eukaryotic integral membrane protein STT3, which is supposed to be the catalytic subunit of the OTase complex. Studies on the substrate specificity could show that both OTases are restricted to the transfer of glycans that contain an acetamido group at the C-2 of the sugar at the reducing end. In addition, PglB requires a negatively charged amino acid in the -2 position of the targeted asparagine (D/E-X-N-Z-S/T), which makes bacterial glycosylation more specific. Besides these requirements only speculations about the catalytic mechanism of N-linked glycosylation were made so far. In order to really understand how the reactivity of the relatively poor nucleophile, the amido group of the asparagine side chain, might be enhanced, structural information seems to be crucial.
For this purpose we established the expression and purification of PglB from *Campylobacter lari* from an *E. coli* expression system. Purified PglB shows activity in an *in vitro* glycosylation assay and is currently tested in crystallization trials.

T1-08

Deubiquitylation Regulates Activation and Proteolytic cleavage of ENaC

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Aldosterone is a mineralocorticoid hormone that plays a key role in Na+ reabsorption in the aldosterone-sensitive distal nephron mediated by the epithelial Na+ channel (ENaC). ENaC, composed of three subunits (α β γ), is known to be regulated by two topologically separated processes, namely the down-regulation via ubiquitylation by the cytosolic ubiquitin-protein ligase Nedd4-2, which interacts via WW domain / PY-motif interactions with ENaC, and by luminal proteases, which cleave ENaC on the extracellular loops of the alpha and gamma subunits. Recently, it has been shown that ubiquitylation of ENaC is reversible involving deubiquitylation by the aldosterone-induced ubiquitin-specific protease Usp2-45. Thereby Usp2-45 interacts with ENaC via its C-terminal part and deubiquitylates all three subunits of ENaC leading to increased amiloride-sensitive currents and cell surface expression of ENaC. This is accompanied by proteolytical cleavage of alpha and gamma ENaC, on predicted furin sites of their respective extracellular loops. This suggests that intracellular ubiquitylation of ENaC controls extracellular cleavage, possibly via conformational changes. To test this hypothesis we expressed ENaC mutant channels in which all cytoplasmic lysines (i.e. putative ubiquitylation sites) were mutated to arginine in Hek293 cells and carried out cell surface biotinylation experiments. We found that such mutated channels displayed strong cleavage of alpha and gamma ENaC at the cell surface, independently of co-expression of Usp2-45, supporting the idea that direct ubiquitylation/deubiquitylation of ENaC influences ENaC cleavage. To test if conformational changes may play a role we carried out limited proteolysis experiments. We expressed ENaC channels in which the putative furin sites on alpha and gamma ENaC were inactivated, together with Usp2-45, or the ubiquitin-ligase Nedd4-2. Dominant-negative dynamin was co-expressed to prevent ENaC endocytosis. Cells were then treated or not with limited amounts of trypsin. We observed proteolysis of alpha and gamma ENaC at the cell surface in the presence of Usp2, but not of Nedd4-2, corroborating the idea that deubiquitylation of ENaC is required to render accessible the extracellular proteolytic sites, most likely by conformational changes.

T1-09

***Moraxella catarrhalis* outer membrane protein m35 – a relevant virulence factor?**

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Aim: To characterize the role of the outer membrane porin M35 of *M. catarrhalis* in host-pathogen interactions.
Methods: M35 knockout mutants of the type 1 strains O35E, 300 and 415 were tested together with their respective wild-type strains for their antimicrobial resistance by E-test. The occurrence of human salivary IgA antibodies and serum IgG antibodies against M35 were investigated by Western Blot. To investigate the degree of conservation of M35 in both phylogenetic subpopulations of *M. catarrhalis* (type 1 and type 2), the M35 sequence of strain 287 was amplified by PCR and sequenced with an AB 3130 Genetic Analyzer (AB, Foster City, USA) and analysed by Lasergene programmes.
Results: From a total number of 14 different antibiotics we observed differences in the MIC (Minimum Inhibitory Concentration) between wild-type and mutant for eight antibiotics. For ampicillin and amoxicillin-clavulanate we observed a two to four fold higher MIC in the M35 mutants than in the wild-type strains. Western Blot analysis demonstrated that both human saliva and serum contain anti-M35 IgA and IgG, respectively. Sequencing analysis of M35 of type 2 strain 287 showed a 94.2% identity on the DNA level and 92.8% identity on the amino acid

level in comparison with type 1 strains. The structural analysis of the protein sequence suggested that this diversity is unlikely to result in functional differences.
Conclusion: The increase of the ampicillin and amoxicillin-clavulanate MIC in the M35 knock-out mutants suggests that this porin affects the outer membrane permeability for these molecules. The presence of IgA and IgG antibodies in healthy human donors indicates that M35 is not only a possible virulence factor but also a relevant antigen and - in connection with this and its high conservation within the different strains - a possible vaccine candidate.

Poster Topic 2 – Biology of Ion Channels and Transporters

T2-01

Acute and chronic adaptation of renal phosphate transporters to dietary phosphate intake in mice

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Inorganic phosphate (Pi) reabsorption in the renal proximal tubule is required to maintain Pi homeostasis. It mainly occurs via NaPi cotransporters in the brush border membrane of proximal tubule cells (BBM). So far, two isoforms have been identified, NaPi-IIa and NaPi-IIc. Recently, mutations in SLC34A3, the gene coding NaPiIIc, have been reported in patients with hereditary hypophosphatemic rickets with hypercalciuria. In mice, dietary Pi levels regulate NaPi-IIa protein but modulation of NaPi-IIc protein level and activity by dietary Pi intake is still only partially understood. Here we investigated the role of NaPi-IIa and NaPi-IIc in chronic and acute adaptation to dietary Pi by using WT and NaPi-IIa KO mice.
In each strain, mice were first fed 5 days with a 1.1% Pi diet (HPD) or 0.1% Pi diet (LPD) in metabolic cages. On day 5, half of each group was switched for 4 hours to LPD or HPD. Serum Pi levels were similar under chronic Pi diets in WT and KO, but KO mice drastically reduced their serum Pi level in the acute HPD-to-LPD. Urinary Pi excretion was similar between WT and KO mice under a chronic HPD, but, under a chronic LPD KO mice exhibited a constant Pi loss in urine, compensated by a higher intestinal Pi absorption. Finally, in acute HPD-to-LPD, KO mice exhibited a delayed decrease in urinary Pi excretion compared to WT mice. In isolated BBM vesicles, sodium-dependent Pi transport activity showed clear adaptation with diets in WT mice. Immunoblots from the same BBM preparation revealed adaptation of NaPi-IIa plasma membrane level both in acute and chronic diets whereas NaPi-IIc plasma membrane abundance was only increased in a chronic LPD. In KO BBM vesicles, transport activity was lower under each conditions and did not adapt. Immunohistochemistry on kidneys from WT mice revealed that NaPi-IIa is expressed at the apical plasma membrane but also in intracellular compartments both in chronic and acute diets. NaPi-IIc, was only detected at the plasma membrane whatever the diet.
NaPi-IIa seems to be the major Pi transporter in mouse kidney adapting to changes in dietary Pi intake, both acutely and chronically. Its lack in NaPi-IIa KO mice is critical under a LPD and seems to be partly compensated by the intestine under this diet. NaPi-IIc activity did not adapt to Pi intake and plasma membrane protein level and is increased only under a LPD.

T2-02

Investigating Phospholipase A_2 Activity in Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is an X-linked muscular disease caused by the absence of the protein dystrophin. Approximately 1 out of 3500 male births is affected by the disorder, which manifests itself by progressive muscle wasting, ultimately leading to wheelchair confinement and shortened life expectancy[1]. On a molecular level, lack of dystrophin results in muscle membrane fragility and it is further accompanied by abnormally elevated levels of intracellular calcium, contributing to cell degradation and apoptosis[2].

Increased activity of store-operated channels (SOC) has been proposed as the cause and entry point for the enhanced calcium influx observed in dystrophic muscles[3]. Additionally, it has been shown that the enzyme calcium-independent phospholipase A_2

($iPLA_2$) is involved in the regulation of SOC. By inhibiting $iPLA_2$ calcium entry in dystrophic fibers is greatly reduced. However, inhibition of $iPLA_2$ will not eliminate the influx completely, as *e.g.* SOC blockers, suggesting that additional mechanisms are sustaining the calcium current[4].

The hypothesis of this work is that the, to $iPLA_2$ closely related enzyme, cytosolic phospholipase A_2 ($cPLA_2$) is accountable for the residual calcium entry. In order to illuminate the role of $cPLA_2$ in DMD and further shed light on the influence of $iPLA_2$, enzyme expression levels and phospholipase A_2 activity have been investigated in dystrophic as well as in healthy human and mouse muscle cells. Both pharmacological tools and gene silencing have been employed to enable the differentiation of $cPLA_2$ and $iPLA_2$ specific activity.

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T2-03
Differential distribution of the Na, K ATPase in the left and right ventricle: causes and consequences

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Na, K ATPase is a major ion gradient keeping enzyme in the myocardial tissue and one of the principle energy consumers. The energy of electrochemical gradient of Na and K is used to support and control contractile function of the heart and even modest suppression of the Na, K ATPase function by pharmacological doses of cardiac glycosides results in an impressive increase in contractile force. It is known that maximal ventricular pressure differs by a factor of 5 between the left (LV) and the right (RV) ventricle.
We have hypothesised that it is at least in part due to the asymmetry in the abundance and activity of the Na, K ATPase in the LV and RV. Wistar rat hearts were perfused with autologous blood equilibrated with 20% O_2 in gas phase for 80 minutes. We have monitored the N^a, K ATPase hydrolytic activity, expression and the resulting tissue Na^+ and K+ levels in rat ventricular tissue. We have furthermore used autoradiography to monitor spatial changes in glucose utilization in the heart and also compared ATP content in the LV and RV.
Steady state sodium content in the LV tissue was exceeding that in the RV. This observation could be at least in part explained by the higher hydrolytic activity of the Na,K ATPase in the RV compared to the LV. Our pilot data indicate that most of the Na,K ATPase in the LV is retained in the inactive state as it is sequestered from the sarcolemma and retained in the vesicular fraction.
Maintenance of the high activity of the Na, K ATPase requires sufficient energy supply but even more energy is spent by myosin ATPases for the force generation. Glucose utilisation showed spatial pattern, with higher glucose utilisation detected in the LV, the side of extended force generation. ATP detection revealed a trend of higher ATP levels in the LV where more glucose is utilised. A difference between the LV and RV ATP levels was however not statistically significant suggesting that ATP turnover in the LV exceeds that in the RV and the energy production is thus matching the demand.
The obtained data supports our hypotheses that both the Na,K ATPase and glycolytic metabolism are involved in control the ventricular pressure development.

T2-04
Mouse lacking aromatic amino acid transporter TAT1

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Basolateral efflux is a necessary step in transepithelial (re)absorption of amino acids from small intestine and kidney proximal tubule. The abundant basolateral amino acid transporters LAT2-4F2hc and y^+LAT1-4F2hc function as obligatory exchangers and thus do not directly contribute to the net amino acid (re)absorption. The aromatic amino acid transporter TAT1 (Slc16a10) is known to function as a low affinity facilitated diffusion pathway and we have previously shown in the *Xenopus* oocytes expression system that it can complement the vectorial transport function of LAT2-4F2hc by recycling exchange substrates. Based on these functional data and on the localization of TAT1 in the basolateral membrane of proximal kidney tubule and small intestine, we hypothesize that it plays an important role for the transepithelial amino acid (re)absorption. However, TAT1 is also expressed in several other organs, for instance also in the liver where it localizes to the sinusoidal membranes of perivenous hepatocytes. Homozygous *tat1* knock out mice (Ingenium Pharmaceuticals AG) are viable and show no gross phenotype. When kept in metabolic cages for 24 hours with free access to food and water, they exhibit specific aminoaciduria, their urine containing higher amounts of L-aromatic amino acids, of L-aspartic acid and L-lysine. However, the amino acid analysis of plasma showed increased levels of L-tyrosine and L-tryptophan and an almost normal fractional reabsorbtion of most amino acid in the kidney. This suggests that, unlike hypothesized, there is no major deficit in the basolateral efflux and that at least one additional amino acid transporter capable of recycling exchange substrates of LAT2- and y^+LAT1-4F2hc is or can be expressed at a sufficiently high level in the same basolateral membrane. We postulate that the increase in plasma aromatic amino acid concentration is due to a defect in their transport into metabolizing cells, in particular of the liver. Further analysis of the phenotype of *tat1* null mice aiming at understanding the role of this transporter in vivo will focus on the function of other TAT1 expressing tissues and on the expression of other basolateral amino acid transporters the (re)absorptive epithelia of kidney and small intestine.

T2-05
Analysis of accessible sites of the extracellular loop of acid-sensing ion channel 1A (ASIC1A

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ASICs are non-voltage-gated neuronal sodium channels activated by protons. They belong to the Epithelium Sodium channel/Degenerin family of ion channels. Activation of ASICs induces a cell depolarization and there is evidence that ASICs contribute to long term potentiation, the expression of fear, the termination of epileptic seizures, nociception and damages after ischemia. These functions make ASICs potential drug targets.
Functional ASICs are homo- or heterotrimeric assemblies of homologous subunits. The crystal structure of the chicken ASIC1 at low pH has recently been determined at 1.9Å resolution. Each subunit has two transmembrane domains and a large extracellular loop, the N- and C-termini being cytoplasmic. The extracellular loop (ECL) is the sensor of the surrounding acidity and the target of several modulators as for example toxins and divalent cations.
We introduced cysteine (cys) residues at selected sites of the ECL of hASIC1a with the aim to identify and study the accessible sites of the channel protein. Cys residues were introduced by site-directed mutagenesis at sites known from the literature to be modulatory sites, or part of the binding site of PcTx1, an inhibitor of

ASIC1a. The mutant proteins were expressed in Xenopus oocytes. All engineered mutant channels were functional and there was no significant difference between their pH dependence and that of wild type (wt) ASIC1a. MTSEA-biotinylation was used to identify channels that contain cys residues accessible from the extracellular solution. This analysis showed solvent-accessible cys residues in the mutants E97C, K133C, Y342C, D351C, Q358C, E359C, N368C and N395C, but not in wt hASIC1a. To determine the functional relevance of the mutated residues we incubated Xenopus oocytes expressing hASIC1a with sulfhydryl reagents and analyzed the changes in ASIC function by two-electrode voltage-clamp. Extracellular exposure to charged methanethiosulfonate reagents affected the pH dependence of the mutants E97C, Y342C, D351C and Q358C. For the mutants E97C, D351C and Q358 the positively charged sulfhydryl reagent MTSET and the negatively charged MTSES had similar effects on the pH dependence of activation, suggesting that the sign of the introduced charge was not critical at these positions, and that the changed pH dependence was rather due to the increased polarity or to a steric effect. MTSET and MTSES had opposed effects on the pH dependence of the mutant Y342C, suggesting that the introduction of a charge at this position interfered with electrostatic interactions that are critical for channel activation. The exposure to either of these MTS reagents decreased the maximal peak current of Y342C and Q358C while only the negatively charged reagent decreased the peak current amplitude of D351C and N368C.
Our analysis illustrates the functional importance of some selected residues in the ECL of ASIC1a and will be the basis for further analyses addressing the mechanisms of ASIC gating.

T2-06

ACE2 and Collectrin are tissue-specific associated proteins of Hartnup amino acid transporter B^0AT1

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Background and aims: Hartnup amino acid transporter B^0AT1 (SLC6A19) is the major luminal sodium-dependent neutral amino acid transporter of kidney proximal tubule and small intestine. The expression of B^0AT1 in kidney proximal tubule was recently shown to depend on its association with Collectrin (Tmem27), a homologous of angiotensin converting enzyme 2 (ACE2). Since Collectrin is almost absent from small intestine, we tested the hypothesis that its homolog ACE2 interacts with B^0AT1 in enterocytes. Furthermore, since B^0AT1 expression depends on the presence of an associated protein, we tested the differential impact of Hartnup-causing mutations on B^0AT1 interaction with kidney and intestinal accessory proteins. **Results:** Immunofluorescence and western blot experiments in collectrin and *ace2* null mice demonstrate that expression of B^0AT1 in small intestine critically depends on ACE2. In contrast ACE2 is not required for the expression of B^0AT1 in kidney where Collectrin is the accessory protein. Co-expression of four newly and five previously identified Hartnup disorder-causing missense mutations of B^0AT1 with Collectrin or ACE2 in *Xenopus laevis* oocytes reveals differential interactions. For instance, we show that the function of the high frequency D173N and of the newly identified P265L mutant B^0AT1 transporters is increased in the presence of ACE2 but not of Collectrin. **Conclusion:** Our data show that ACE2 and Collectrin are tissue-specific partner proteins of the amino acid transporter B^0AT1 in intestine and kidney, respectively. Differential functional association of mutant B^0AT1 transporters with tissue-specific proteins is suggested to participate to the phenotypic heterogeneity of human Hartnup disorder.

T2-07

Towards a cell-free vesicular P-Glycoprotein transport assay: Incorporation of possible drug sensors by liposome fusion

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P-Glycoprotein (P-gp) is an ATP-dependent membrane transporter which effluxes from the cell several structurally unrelated compounds, influencing absorption and tissue distribution of many drugs (1).
We are developing a cell-free P-gp transport assay to identify P-gp substrates, modulators and inhibitors. Methods are evaluated to detect inward transport of drugs by their accumulation within the vesicle lumen or inner lipid leaflet after addition of ATP to the vesicles. To incorporate possible sensors for drug accumulation we aim to fuse the P-gp vesicles with liposomes containing soluble or lipid-linked sensor. Here we show the successful fusion of membrane vesicles from P-gp over-expressing cells (P388/ADR) with liposomes containing fluorescent-labelled phospholipids.
P388/ADR membrane vesicles were catalytically active in hydrolysing ATP and 50% of the activity originated from P-gp. A significant transport of the fluorescent P-gp substrate Hoechst 33342 was observed. Incubation of these vesicles with liposomes (40% negatively charged phosphatidylserine, 0.25% NBD- and 0.25% rhodamine-phosphatidylethanolamine) and Ca^{2+} resulted in an increase of the initially quenched fluorescent signal, indicating fusion of the vesicles with the liposomes. The fused vesicles showed high ATPase activity and are now further characterized.
Various soluble and lipid-linked probes will be tested with this system in order to find an appropriate sensor for drug accumulation.

Acknowlegment: We are grateful for the financial support from OPO Foundation, Switzerland.

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T2-08

Monitoring integration of proteins into free-standing lipid bilayers

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Artificial bilayer lipid membranes (BLMs) have extensively been investigated as model systems for biological membranes. They offer several advantages for functional studies of isolated membrane proteins: (I) no interference from cellular processes, (II) high freedom in the variation of the system composition (pH, buffer, protein density, lipid composition) and (III) the possibility to study membrane proteins from intracellular organelles. The main drawback of free-standing BLMs in small apertures is the fact that they are usually fragile and transmembrane events can be monitored only for a limited time. We use silicon nitride membranes with regularly arranged nanopores to increase the bilayer stability but offering although a high total bilayer area. [1] Depending on the pore diameter and the lipid composition such free-standing bilayers are stable for days. The function of integrated channel proteins is determined as a change in the resistance of the lipid bilayer. We have monitored the spontaneous insertion of the pore forming protein alpha-hemolysin (aHLY) using chronoamperometry. The time required for the formation of single pores depends on the aHLY concentration and on the chip pore size, reflecting the time used for the self assembly of the heptamer. Sodium diffusion across BLMs with different amounts of incorporated aHLY was measured using ion selective electrodes (ISE). We are currently investigating the voltage-gated sodium channel from Bacillus halodurans (NaChBac). Stable free-standing lipid bilayers with integrated NaChBac were formed by fusing proteoliposomes with preformed BLMs using the nystatin / ergosterol method. Single channel current of NaChBac was

recorded and the sensitivity to the channel blocker nimodipine has been shown. However, a high protein density in the bilayer can hardly be obtained by this method needed to investigate channels and transporters using ISE. A much simpler way to form a lipid bilayer would be the direct fusion of proteoliposomes to the nanoporous support and the protein density will be controlled by the predermined protein to lipid ratio.

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T2-09

Amino Acid Transport Across The Murine Blood-Brain Barrier

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The brain microvascular endothelial cells (BMECs) which compose the blood-brain barrier (BBB) provide a diffusion barrier between the blood and the brain interstitial fluid. The role of the BBB by protecting the brain from changes in the extracellular fluid includes maintaining an asymmetry in the amino acid (AA) composition and concentration between blood and brain. The aim of the work is to understand how the AA equilibrium between brain and blood is achieved by the different AA transporters expressed in the polarized mouse BMECs in a physiological context. To examine the mRNA expression of BBB AA transporters, microarray analyses of differentially treated primary mouse BMECs (non-cultured, single-cultured and co-cultured with glial cells in non-contact) were done and compared with each other. 30 out of a total of 50 AA transporter mRNAs were expressed in non-cultured and/or cultured BMECs. In non-cultured BMECs, 4F2hc mRNA was most prominently expressed, followed by Lat1, Taut, Snat2, Snat5 and Eaat3. The levels of 22 of these AA transporter mRNAs (73%) was altered by culture. Real-Time RT-PCR of 18 AA transporters confirmed their down-regulation during culture (Eaat2, Eaat1, Taut, 4F2hc, Cat-1, Cat-3, Lat1, Pat1, Snat3, Snat5). In contrast, y^+Lat2, xCT and Lat3 were up-regulated in all three culture conditions. Additionally, the protein expression and the localization of some transporters at the luminal and/or abluminal side of the polarized BMECs is being examined in mouse brain tissue sections using immunofluorescence. Preliminary data shows that 4F2hc, Eaat3 and Snat1 are expressed at the BBB. In summary, mRNA and protein expression of AA transporters at the BBB are investigated to obtain a better understanding of their physiological and pathophysiological role in this structure.

T2-10

Human imino acid transporter SIT1 (SLC6A20) function is modulated by accessory proteins Collectrin and ACE2.

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The sodium dependent imino transporter 1 (SIT1, Slc6a20) is a Na+- and Cl- dependent co-transporter of imino- and neutral amino acids that localizes at the luminal membrane of renal proximal tubule cells and small intestine enterocytes. We have previously shown that the expression of the mouse ortholog of SIT1 is decreased in the kidney of Collectrin knock out mice. Collectrin was shown previously to be necessary for the expression of B^0AT1 (Slc6a19) in kidney proximal tubule and to increase the function of B0AT1 when co-expressed in Xenopus oocytes. The type I transmembrane glycoprotein Collectrin is expressed mostly in the kidney, and structurally it is homologuous to the membrane anchor region of Angiotensin Converting Enzyme 2 (ACE2), an important player of the Renin-Angiotensin-Aldosteron-System (RAAS). In contrast to the situation observed in kidney, it is not Collectrin but ACE2 that was shown to be necessary for the expression of B0AT1 in small intestine, where Collectrin is almost absent. The aim of the present study is to characterize the function of the human ortholog of SIT1 and to analyze its interaction with the tissue-specific accessory proteins Collectrin and ACE2. Co-expression of human Collectrin or ACE2 with human SIT1 in Xenopus laevis oocytes induced a transport of the imino acid proline that was 2 - 3-fold higher, when compared to the oocytes expressing the transporter alone. This increased uptake is probably due to a higher number and/or transport rate of SIT1 expressed at the cell surface, as suggested by analogy to observations made for B^0AT1 and also by the fact that the maximal transport rate of L-Pro was increased (V_{max} hSIT1 alone: 4.3 +- 1.6 pmol/h/oocyte, V_{max} hSIT1 + hColl: 32.6 +- 10.6 pmol/h/oocyte, V_{max} hSIT1 + hACE2: 15.7 +- 3.2 pmol/h/oocyte), whereas the apparent affinity.for L-Pro was not significantly changed ($K_{0.5}$ hSIT1 alone: 0.16 +- 0.13 mM, $K_{0.5}$ hSIT1 + hColl: 0.17 +- 0.12 mM, $K_{0.5}$ hSIT1 + hACE2: 0.08 +- 0.04 mM). These preliminary results suggest that Collectrin and ACE2 are also accessory proteins of the human imino acid transporter, hSIT1, in kidney and small intestine, respectively.

T2-11

Regulation of voltage-dependent calcium channels by Nedd4-1

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Background: Calcium entry into excitable cells can be regulated by controlling both the activity of calcium channels and the amount of channels available at the plasma membrane. In recent years, emphasis has been placed on studying the trafficking of these channels from the ER/Golgi to the plasma membrane. By contrast little is known about their internalisation and degradation. One post-translational modification shown to be involved in membrane protein internalisation and subsequent degradation is the attachment of ubiquitin moieties by ubiquitin ligases. Previously, it has been shown that cardiac ion channels such as Nav1.5 and KCNQ1 are down-regulated by ubiquitin ligases of the Nedd4 family. These regulations involve the interaction between the PY motif of the target channels and the WW motif of the ubiquitin ligases. Despite the absence of such PY motif in the cardiac voltage-gated calcium channel Cav1.2, we investigated whether this channel could by regulated in the same manner than other cardiac channels as previously described.

Aim of the study: The aim of this study was to investigate the regulation of cardiac calcium channels by Nedd4 ubiquitin ligases

Methods: We co-expressed, in HEK293 cells, L-type calcium channels (Cav1.2) and its two regulatory subunits Cavbeta and Cavalpha2delta1 together with the ubiquitin ligases Nedd4-1, Nedd4-2, and WWP2, and examined by voltage-clamp whole-cell recordings the calcium current. We next determined by western blot and surface biotinylation assays the availability of the different subunits of calcium channels in total HEK293 lysates, and at the cell surface. Levels of ubiquitylation of the different subunits were assessed by pull-down GST-S5A and immunoprecipitation of Cav1.2 and its subunits.

Results: We found that co-expressing the ubiquitin ligase Nedd4-1 significantly reduced Cav currents, and decreased Cav and its subunit protein levels. This effect was Nedd4-1 specific since none of the other members of the Nedd4 family we tested, Nedd4-2 and WWP2, produced a similar effect. Moreover, with Nedd4-1-CS, a catalytic inactive form of Nedd4-1, we did not observe any effect neither on calcium current nor on protein level. We also found that the effect of Nedd4-1 was dependent on the co-expression of the Cavbeta subunit. No Nedd4-1-dependent increase in ubiquitylation of the Cav protein was found; and unexpectedly, the two regulating subunits Cavbeta and Cavalpha2delta1 were detected

to be deubiquitylated upon Nedd4-1 co-expression.

Conclusions: Our data suggest that Nedd4-1regulates the expression of Cav channels and its subunits by Nedd4-1 via an indirect mechanism constituting a new regulatory pathway to be determined. In may be speculated that either degradation or sequestration of the channels into intracellular complexes can dynamically regulate the availability of Cav channels at the plasma membrane and therefore calcium entry.

T2-12

The leak mode of electrogenic sodium coupled P_i cotransporters (SLC34A1, 2).

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Reabsorption of inorganic phosphate (P_i) in renal proximal tubules is mediated by Na^+/P_i cotransporters, members of the SLC34 family (NaPi-IIa, NaPi-IIc). The electrogenic NaPi-IIa and the electroneutral NaPi-IIc prefer divalent phosphate (P_i) and show strict stoichiometries of 3:1 and 2:1, respectively. For NaPi-IIa one net charge is translocated per cotansport cycle.
Under voltage clamp, in the absence of P_i, a leak current is associated with the expression of NaPi-II in *Xenopus laevis* oocytes. The P_i transport inhibitor, phosphonoformic acid (PFA) blocks this component of the membrane current (I_{PFA}) and as its magnitude correlates with the cotransport activity, it appears to be intrinsic to NaPi-IIa. The leak current is approximately 10% of the P_i-induced current under saturating concentrations of the substrate. Moreover, a feature of this current is that its reversal potential (E_r) is in the range -10 to -30 mV, close to what we would predict for a chloride conductance.
To better define ionic components of the leak current, we replaced 90% of external chloride with other anions. I_{PFA} remained unchanged by anion substitution except for gluconate, whereby I_{PFA} was increased and P_i-induced current (I_{Pi}) was concomitantly smaller. Moreover, E_r was shifted towards positive potentials indicating that there was a partial suppression of inward chloride flux. A comparison with the responses of non-injected oocytes indicated that PFA suppressed an endogenous oocyte Cl^- conductance. Because of the small magnitude of I_{PFA} (typically < 20 nA) we investigated its properties using a double cys mutant construct that displays a 6-fold higher I_{PFA} when one of the engineered cys residues is modified by methanethiosulfonate (MTS) reagents. Our data suggest that I_{PFA}, like the wild type, is Na^+-dependent, as indicated by a Nernstian shift of E_r with changing Na^+.
Our data are consistent with a kinetic model in which the leak and the cotransport modes are mutually exclusive and share common partial reactions. The leak originates from a uniport carrier mode, which involves the translocation of the 1st Na^+ ion that interacts electrogenically with the empty carrier before P_i binding. In the electroneutral isoform (NaPi-IIc), this electrogenic step is absent and this is consistent with the absence of a PFA inhibitable leak current.

T2-13

Involvement of the sodium calcium exchanger, working in the reverse mode, during histamine-induced calcium entry in endothelial cells

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Ca^{2+} entry in endothelial cells (EC) is a key signaling event required for the majority of cellular functions. Among the diversity of mechanisms proposed that leads to Ca^{2+} entry, two major pathways have emerged. One pathway requires the presence of an agonist that, upon receptor activation, produces second messenger(s) that subsequently opens a Ca^{2+} permeable ion channel. Based on its proposed mechanism of activation, this Ca2+ entry is referred to as receptor-activated Ca^{2+} entry (RACE). The other pathway is directly linked to the Ca^{2+} filling state of the endoplasmic reticulum (ER), and is called capacitative Ca2+ entry or store-operated Ca^{2+} entry (SOCE). We previously showed that on endothelial cells, histamine and thapsigargin (TG) induced an important Ca^{2+} entry. However, this Ca^{2+} entry is associated with a pronounced store depletion in the case of TG, while not in case of histamine, suggesting that both pathways involved different signaling pathways and probably different Ca^{2+} entry channels. It remains that a precise characterization of agonist- versus store depletion-induced Ca^{2+} entry pathways in endothelial cells was not performed.
The aim of this study is the electrophysiological characterization and comparison of the currents activated by an histamine versus store depletion. The patch clamp method in whole-cell configuration (perforated patch) was used on endothelial cells derived from human umbilical vein (Ea.hy926). In addition, cytosolic Ca^{2+} imaging with Fura-2 was used to determine the impact of gene invalidation on Ca^{2+} signalling.
In presence of 10mM extracellular Ca^{2+}, the whole-cell current activated by agonist showed a strong outward rectification with a reversal potential around -25mV. This current is blocked by 10µM KB-R7943, an inhibitor of the Na^+/Ca^{2+} exchanger (NCX), which leads us postulating the involvement of NCX working in the reverse mode as being part of the Ca^{2+} entry process activated by histamine. On the contrary, in presence of 10mM extracellular Ba2+ (with 2mM Ca^{2+}), the histamine-activated current is inwardly rectifying with a reversal potential around 25mV. On the other hand, passive depletion of the ER by thapsigargin activated an inwardly rectifying current with a positive reversal potential (around 25mV) both in presence of 10mM Ca^{2+} as well as 10mM Ba^{2+}, the current being larger in Ba^{2+} medium. By measuring the cytosolic Ca^{2+} concentration, we showed that the downregulation of NCX3 as well as the addition of Ni^{2+} (a non-specific blocker of NCX) had an inhibitory effect on histamine-stimulated calcium entry but not TG-induced calcium entry.
In conclusion, histamine and TG activated different currents when recorded in 10mM Ca^{2+}, while in presence of 10mM Ba^{2+} both currents are difficult to differenciate. The reason for this discrepancy remains to be investigated, but points to the existence of different currents supporting RACE and SOCE in endothelial cells. Moreover it seems that reverse mode of the NCX3 is involved in the RACE but not in the SOCE pathway.

T2-14

Male Fertility and Copper Import: The Role of Drosophila Ctr1C

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The strong functional conservation of important systems that control copper levels makes Drosophila melanogaster an excellent model system for the study of copper metabolism. Similar to the situation in mammals, Ctr1 proteins serve as the main copper importers in the fly. Of the three identified Ctr1 homologs, the Ctr1A and Ctr1B proteins have been studied in detail. While Ctr1B has been shown to take up copper from the intestine (its levels being adjusted to Cu demand by the metal responsive transcription factor dMTF-1, thus allowing survival under conditions of sparse or excess copper), Ctr1A constitutes an ubiquitously and apparently constitutively expressed „house-keeping“ importer.
Here, we focus on the function of a third Ctr1 homolog, the Ctr1C protein. Using gain- and loss-of-function analysis, we demonstrate that Ctr1C functions as a testis-specific copper importer that plays a role in male fertility.
Ectopic overexpression of Ctr1C (ubiquitously or eye-specifically) elicits copper-dependent phenotypes that are very similar to the ones observed with Ctr1B, showing that Ctr1C can function as a copper importer. Currently we are examining the subcellular localization of the Ctr1C protein in clonal overexpression systems.
Loss of Ctr1C leads to a specific phenotype: In flies that are mutant for Ctr1B, i.e. have low intra-organismal copper levels, Ctr1C mutant males are almost completely sterile. Taken together with the specific expression of Ctr1C during spermatogenesis and

the fact that the sterility can be rescued by copper supplementation, these data hint at an important role of copper in male fertility in Drosophila.
We will also present a hypothesis about the evolutionary origin of this Drosophilid-specific third Ctr1 homolog.

T2-15

How many Na+ ions interact with the renal electroneutral Pi transporter, NaPi-IIc?

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In mammals, phosphate is essential for structural, metabolic, synthesis and signaling processes. To control the circulating levels of inorganic phosphate (Pi) in the blood, the kidneys play a central role by reabsorbing Pi from the glomerular filtrate. This is mediated primarily by solute carriers of the SLC34 gene family. They catalyse uphill Pi transport across the brush border membrane by using the free energy of the Na+ electrochemical gradient.
SLC34 gene products includes the electrogenc NaPi-IIa/b that cotransport with a 3:1 Na^+:Pi stoichiometry and the electroneutral NaPi-IIc, that displays a 2:1 stoichiometry, which suggests that it lacks one of the 3 Na+ interaction sites proposed for the electrogenic isoforms. Mutations in NaPi-IIc are associated with a rare genetic disease, Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH). As NaPi-IIc shows no transport-related electrogenic activity, its kinetics have until now only been studied using radio-labelled isotopes. To provide a more direct readout of substrate interactions, and offer a new tool to study NaPi-IIc mutations, we developed a fluorometry-based system that employs site-specific labelling with a fluorophore to characterize substrate-induced conformational changes when NaPi-IIc is expressed in Xenopus oocytes.
We introduced a novel Cys in the third extracellular loop (S437C) of the mouse NaPi-IIc isoform. Labelling of Cys-437 with MTS-TAMRA resulted in complete suppression of cotransport, but changes in fluorescence were detected in response to changes in extracellular Na^+ concentration in the absence of Pi and in response to changes in extracellular Pi concentration in presence of Na^+. These data, combined with information obtained from 32P uptake, indicate that Na^+ is the first ion to interact with the transporter and the substrate binding order is Na^+-Pi,-Na^+. The apparent substrate affinities for Na+ and Pi agreed with those obtained by uptake demonstrating the applicability of the fluorometric technique for kinetic studies on electroneutral cotransport system. Analysis of our fluorescence data also shows that like the electrogenic NaPi-IIb, 2 Na^+ ions interact with NaPi-IIc before Pi binding but only one Na+ ion is translocated. This result provides compelling evidence that SLC34 proteins share common motifs for substrate interaction.

T2-16

Regulation of the cardiac sodium channel $Na_v1.5$ by a member of MAGUK proteins: SAP97

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BACKGROUND:
$Na_v1.5$, the main voltage-gated sodium channel expressed in the heart, plays a major role in the initiation of the cardiac action potential and electrical conduction. A precise regulation and localisation of $Na_v1.5$ channels in cardiomyocytes is thus necessary for correct heart function. The three last amino-acids of $Na_v1.5$ C-terminus (SIV) constitute a PDZ-domain binding motif that was shown to interact with the syntrophin-dystrophin complex in cardiomyocytes (Gavillet & al, Circ. Res 2006). This motif is also known to interact with PDZ domains such as the ones found in proteins of the MAGUK (Membrane Associated GUanylate Kinase) family. Among their multiple roles, MAGUK proteins can cluster proteins and localize them at the plasma membrane.
AIM:
In this study, we investigated the interaction between SAP97, one cardiac MAGUK protein, and $Na_v1.5$. We postulated that this interaction may be implicated in correct localisation, anchoring, turn-over and/or regulation of biophysical properties of $Na_v1.5$.
RESULTS:
Pull-down experiments were performed using $Na_v1.5$ C-terminus fusion proteins and human or mouse heart protein extracts. These assays revealed that the association between SAP97 and $Na_v1.5$ depends on the PDZ-domain binding motif of Nav1.5. This interaction was specific for SAP97 and $Na_v1.5$ as no pull-down could be detected with PSD95 or ZO-1, two MAGUK proteins also expressed in human heart. The functional consequences of this interaction were studied via patch-clamp experiments. Upon silencing of SAP97 in HEK293 cells stably expressing $Na_v1.5$, a 56% reduction in the whole-cell sodium current was observed without any decrease in the total $Na_v1.5$ protein amount. In control HEK293 cells, the sodium current measured after transient transfection of plasmid coding for $Na_v1.5$-ΔSIV channels (lacking the three last amino-acids) was reduced compared to wild-type channels. Immunostainings on frozen mouse heart slices were also performed. These assays confirmed the presence of $Na_v1.5$ at the intercalated discs as well as at lateral membranes. Colocalisation of $Na_v1.5$ and dystrophin specifically at lateral membranes was also demonstrated. The possible colocalisation of $Na_v1.5$ and SAP97 at the level of intercalated disks is currently investigated. This would support the hypothesis of the presence of two pools of $Na_v1.5$ channels: one targeted at lateral membranes by the syntrophin-dystrophin complex, and another one targeted at intercalated discs by SAP97.
CONCLUSIONS:
These findings strongly support the existence of an interaction between $Na_v1.5$ and SAP97 in cardiac tissue. This interaction also depends on the presence of $Na_v1.5$ PDZ-domain binding motif and may play a role in determining the channel density at the plasma membrane. Additional biochemistry, cytochemistry and biophysical experiments will allow us to further address this question.

T2-17

Plasma membrane-anchored Annexin A6 reduces Ca^{2+} entry by stabilizing the cortical actin cytoskeleton

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The annexins are a family of Ca^{2+} - and phospholipid-binding proteins, which transiently interact with membranes upon increase of $[Ca^{2+}]_i$ or during cytoplasmic acidification. To address the function of annexins at the plasma membrane (PM), we fused fluorescent protein-tagged annexins A6 and A1 with H- and K-Ras membrane anchors. Stable PM localization of membrane-anchored annexin A6 significantly decreased the store-operated Ca^{2+} entry (SOCE), but did not influence the rates of Ca^{2+} extrusion. This attenuation was specific for annexin A6 as PM-anchored annexin A1 did not alter SOCE. Membrane association of annexin A6 was necessary for a measurable decrease of SOCE, since cytoplasmic annexin A6 had no effect. Constitutive PM localization of annexin A6 resulted in the rearrangement and accumulation of F-actin at the PM, indicating a stabilized cortical cytoskeleton. Disruption of the actin cytoskeleton using latrunculin A abolished the inhibitory effect of PM-anchored annexin A6 on SOCE. Taken together, our results implicate annexin A6 in the actin-dependent regulation of Ca^{2+} entry.

T2-18

Effects of mineralocorticoid and K^+ concentration on K^+ secretion and ROMK channel expression in a mouse cortical collecting duct cell line

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The cortical collecting duct (CCD) plays a key role in the regulated K^+ secretion, which is mediated mainly through ROMK channels located in the apical membrane. However, the mechanisms of the regulation of urinary K^+ excretion with regards to K^+ balance are not well known. We took advantage of a recently established mouse CCD cell line (mCCDcl1) to investigate the regulation of K^+ secretion by mineralocorticoid and K^+ concentration. We first verified the role of ROMK in K^+ secretion in this cell line by showing that tertiapin-Q, a ROMK-specific inhibitory peptide, inhibited the barium-sensitive current with an apparent affinity of 6 nM. Overnight exposure to 100 nM aldosterone did not significantly change the K^+ conductance, while it increased the amiloride-sensitive Na^+ transport. Aldosterone also induced an increase of the transepithelial K^+ gradient. Overnight exposure to a high K^+ (7 mM) concentration produced a small but significant increase in the apical membrane barium-sensitive K^+ conductance. The mRNA levels of all ROMK isoforms measured by qRT-PCR were not changed by altering the basolateral K^+ concentration, but were decreased by 15% to 45% upon treatment with aldosterone (0.3 or 300 nM for 1 and 3 hours). In conclusion, mCCDcl1 cells demonstrate a significant ROMK-mediated K^+ secretion. The ROMK-mediated K^+ conductance is regulated according to the basolateral K^+ concentration in absence of detectable changes in ROMK mRNA expression. Aldosterone stimulates K^+ secretion through its effect on Na+ transport in spite of a small decrease in ROMK expression.

T2-19

Dose-dependant effect of ClC-2 disruption in mice

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The chloride channel ClC-2 is one of the plasma membrane channels of the CLC family which comprises chloride channels as well as chloride transporters. ClC-2 is expressed almost ubiquitously with a particular high level of expression in the brain, the testis and the colon. This channel is activated by hyperpolarisation, cell swelling, and extracellular acidification and is also modulated by intracellular chloride concentration. Its physiological function has not yet been clarified in detail. The disruption of ClC-2 in the mouse leads to a widespread vacuolisation of the white matter of the brain and an early degeneration and later loss of photoreceptors and male germ cells, suggesting a primordial role of ClC-2 in the brain, the eye and the testis.

In order to further elucidate the physiological function of ClC-2, a tissue-specific knock-out mouse has been generated. Unfortunately, the resulting mouse possesses an additional exon in the N-terminal part of the ClC-2 gene. This leads to a strong reduction of the amount of ClC-2 protein as shown by Western Blot on testis and brain. Interestingly, this mutant mouse has an intermediate phenotype between the one of the full knock-out and the wild type mouse. Indeed, this low ClC-2-expressing mouse shown a degeneration of the retina, as observed in the full ClC-2 KO mouse. On the other hand, the male are fertile, which indicates that the germ cells are still viable. So, it seems that the phenotype observed in the full ClC-2 KO mice is dependent on the amount of the ClC-2 protein. This also indicates that the retina is more sensitive to a reduction of ClC-2 activity than the testis. This fact could involve different mechanisms impair with the disruption of ClC-2 in these two organs.

T2-20

mRNA expression of selected lipid transporters and modulators in canine intestinal tissues during chronic enteropathies: effect of supplementation with omega-3 and -6 polyunsaturated fatty acids?

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Inflammatory bowel disease (IBD) and food responsive diarrhoea (FRD) frequently occur in dogs, but the pathogenesis of these diseases is still unclear. IBD is associated with the dysregulated immune response or lack of tolerance to normal gut bacterial flora, whereas FRD is linked to the hypersensitivity to particular food components. Fatty acids (FA) enter cells predominantly through an active transport. They may act as signal molecules regulating nuclear transduction and transcription. Integral membrane fatty acid translocase (FAT), fatty acid transport proteins (FATP), and caveolin (Cav)-1 are potential facilitators of FA uptake. Long-chain acyl coenzyme A synthetase (Ascl) and FA binding proteins (FABP) are involved in intracellular FA activation and trafficking, whereas peroxisome proliferator-activator receptors (PPAR) function as regulators of lipid metabolism. Furthermore, PPAR are strongly linked to inflammatory reaction. We tested if the supplementation with polyunsaturated fatty acids [PUFA, (n-3 and n-6; omega)] in addition to routinely proposed means for the therapy of IBD and FRD significantly alters the duodenal mRNA expression of FA transporters and regulators. Using quantitative RT-PCR we measured the mRNA levels of FAT, Cav-1, FATP-1, 2, 3, 4, 6, liver (L) and intestinal (I)-FABP, Ascl-1, 4, 5, 6, and of PPARγ in duodenal biopsies from dogs suffering from spontaneous FRD (n=14) and IBD (n=7), before (FRDbefore and IBDbefore) and after (FRDafter and IBDafter) the supplementation with PUFA for 4 weeks. Duodenal samples from healthy dogs euthanized for unrelated reasons served as controls [healthy controls (HC); n=14]. The mRNA expression of FAT was similar in FRDbefore and FRDafter, but was higher than in HC ($P < 0.05$). In contrast, the expression in IBD (before and after) remained comparable to HC. The mRNA expression of Cav-1 did not vary among FRD (before and after) and HC, while expression in IBDafter was lower ($P < 0.05$) than in IBDbefore and HC. FATP-4 and FATP-6 were most abundant amongst all tested FATP isoforms. The expression of FATP-4 and FATP-6 was similar in FRDbefore and FRDafter, but was higher ($P < 0.05$) than in HC; however in IBD (before and before) their levels were similar to HC. The mRNA expression of L-FABP and of I-FABP was comparable in FRDbefore and FRDafter, but was higher ($P < 0.05$) than in HC. In contrast, the mRNA expression of L-FABP and I-FABP in IBDafter was higher ($P < 0.05$) than in HC, but was comparable to IBDbefore. The transcripts of Ascl-5 were the most abundant Ascl isoforms. The mRNA expression of Ascl-5 was similar in FRDbefore and FRDafter, but was higher ($P < 0.05$) than in HC; in contrast gene expression in IBD (before and after) was comparable to HC. The mRNA expression of PPAR was unaltered in FRDbefore and FRDafter, but was higher ($P < 0.05$) than in HC; gene expression in IBD (before and after) was similar to HC. Our results demonstrated the lack of effects associated with 4 weeks supplementation with PUFA on the mRNA expression of genes involved in fatty acid uptake, activation and trafficking in the duodenum of FRD and IBD (except for Cav-1). Equally high mRNA levels of PPARγ a key nuclear receptor involved in lipid metabolism as well as associated with inflammatory reactions, in FRDbefore and FRDafter may suggest the continuous presence of inflammatory reactions in duodenal tissues, rendering likely a possible interference of inflammatory mediators with gene expression levels.

T2-21

Hyperglycaemia correlates with ATP-binding cassette transporter A1 (ABCA1) gene expression and function in humans - a potential new mechanism for cardiovascular disease

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Type 2 diabetes (DM) and impaired glucose tolerance (IGT) are common conditions associated with insulin resistance and premature coronary heart disease. Epidemiological studies indicate a continuous relationship between glycaemia and cardiovascular disease. The ATP-binding cassette Transporter A1 (ABCA1) is responsible for cholesterol export which initiates reverse cholesterol transport. Decreased ABCA1 function has been associated with premature cardiovascular disease. We have previously demonstrated leukocyte ABCA1 gene expression to negatively correlate with fasting plasma glucose concentration in healthy men. We hypothesised that leukocyte ABCA1 expression and function negatively correlate with glycaemic indices in men with impaired glucose regulation and drug naive DM.
Leukocyte ABCA1 gene expression was measured in individuals by quantitative PCR. Cholesterol efflux was assessed in a cohort of patients using primary skin fibroblasts cultured from 20 participants, tritiated cholesterol and apolipoprotein A1.
Results demonstrate a significant negative correlation between leukocyte ABCA1 gene expression and FPG (n=25, rs=-0.57, p=0.03) as well as HbA1c (rs=0.59-p=0.0024). Gene expression was lower in hyperglycaemic patients than healthy participants (data Log transformed, t=4.259, p<0.001). Liver X receptor (LXR) alpha and peroxisome proliferator-activated receptor (PPAR) gamma gene expression did not show a significant relationship with glucose levels. ABCA1 mediated cholesterol efflux negatively correlated with fasting glucose (n=20, r=-0.43).
Our data suggest that glycaemic indices negatively associate with measures of ABCA1 gene expression and function in patients with IGT and DM. This observation is not explained by regulation of upstream nuclear ligands. Our findings may help to explain the continuous relationship between hyperglycaemia and cardiovascular disease.

T2-22

Differential expression and localization of lipid transporters and their regulators in relationship to blood serum metabolites in the mammary gland during pregnancy, involution and lactation

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The mammary gland (MG) undergoes morphological and physiological changes during pregnancy, lactation and involution. The transport of lipophilic compounds across mammary gland epithelial cells (MEC) determines milk lipid content and composition. ATP-binding cassette (ABC) transporters are known to play a pivotal role in cellular lipid efflux and are associated with hereditary diseases. As only scarce information is available about the transfer of lipids and cholesterol in the MG, we investigated the expression and localization of lipid transporters and regulators of lipid homeostasis in the bovine MG during different physiological stages.
Repeated MG biopsies and blood samples were taken from ten dairy cows during involution pregnancy and throughout lactation. mRNA levels of candidate lipid transporters and their regulatory genes were determined by quantitative RT-PCR. In parallel to mRNA profiles we also analyzed blood serum metabolite profiles to reveal potential relationships with transporter gene expression. In addition, ABCA1 and ABCG1 proteins were localized in mammary tissues of the same animals by immunohistochemistry.
Expression of the lipid efflux transporters ABCA1 and ABCA7 were significantly elevated during involution as compared to lactation (P=0.0197 and <0.0001, resp.) and down regulated post partum (P=0.0443 and 0.0003, resp.). ABCG1 showed a similar trend without reaching statistical significance. ABCG2, a drug transporter that also influences milk yield and milk composition, was significantly increased post partum and throughout lactation as compared to the nonlactating state (P<0.0001). The intracellular cholesterol transporter NPC1 as well as the regulators LXRα, PPARγ, SREBP1, SREBP2 were increased post partum as compared to lactation (P=0.0003, 0.0271, <0.0001, 0.038 resp.). Correlation analysis of ABCA1, ABCA7 and ABCG1 mRNA profiles with blood serum cholesterol levels revealed significant inverse relationships (r=-0.39, -0.51, -0.29, resp; P<0.05). ABCA1 and ABCG1 localized in MEC and stromal cells and showed differential activity in MEC during involution/pregnancy compared to lactation. In contrast to MEC the activity of ABCA1 and ABCG1 in stromal cells was unaltered. The mRNA levels of ABCA1 and ABCG1 corresponded to the protein expression pattern evaluated by semi quantitative analysis of the immunohistochemical sections.
This study demonstrates that lipid transporters and regulatory genes are differentially expressed and localized in the MG throughout the pregnancy-lactation cycle. Our results indicate that ABCA1 and ABCG1 might play a role in the removal of cholesterol from MEC during involution and pregnancy thereby preventing damage of the epithelium by intracellular cholesterol accumulation. These transporters may also be involved in the transfer of cholesterol into milk during lactation. ABCA7 is likely to be implicated in phagocytosis of apoptotic MEC during involution. Our results furthermore suggest that NPC1 could be involved in the cellular redistribution of cholesterol taken up during lactation. Regulation of lipid transporters in the MG is only partially associated with transcription factors that control lipid homeostasis because the induction of lactation is triggered by lactogenic hormones that may interfere with regulators of lipid homeostasis.

T2-23

Flecainide testing of patients suffering from LQT3 syndrome, induced by an L1786Q Nav1.5 mutation, unmasked Brugada syndrome

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Background: Mutations in SCN5A, encoding the cardiac sodium channel Nav1.5, can result in both long QT syndrome (LQT3) and Brugada Syndrome (BrS). Several SCN5A mutations have been found to underlie LQT3 and BrS, respectively. However, so far only 4 mutations have shown an overlapping phenotype. The pivotal criterion for a Long QT diagnosis is a prolonged QT interval on the surface electrocardiogram (ECG). In contrast, a prerequisite for a BrS diagnosis is an ST elevation in the precordial leads on the ECG. These ST elevations are normally dynamic and may only appear after sodium channel blockage. Hence, diagnosing BrS patients can be difficult.
Results: In a Danish family suffering from Long QT syndrome a mutation in SCN5A, changing an leucine into and glutamine at position 1786 (L1786Q), was found. The proband presented with an aborted cardiac arrest, and his mother died suddenly unexpected at the age of 65. Electrophysiological investigations of the mutant in CHO cells revealed a reduced peak current, a negative shift in inactivation properties and a positive shift in activation properties, compatible with Brugada Syndrome. Furthermore, blocking the sodium current with TTX revealed a drastic increase in the sustained (INa,late), confirming that this mutation induces LQT syndrome.
Conclusion: Even though the ECG's of the patients only showed QT prolongation and no ST elevation, flecainide revealed coved ST elevation in all gene carriers. This was confirmed in vitro, as electrophysiological experiments of the mutant revealed functional

characteristics explaining the two different phenotypes. Based on this study we hypotheses that some of the LQTS patients carrying SCN5A mutations actually may have BrS too.

T2-24

Regulation of NaPi-IIa in a GABARAP-deficient murine model

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The Na/Pi-cotransporter NaPi-IIa (SLC34A1) is a major regulator of phosphate (Pi) homeostasis. Expression of NaPi-IIa in the brush border membrane (BBM) of renal proximal tubules is controlled via association with several partners, among them the GABA-Receptor Associated Protein (GABARAP). GABARAP knock-out mice show reduced urinary excretion of Pi and increased expression of NaPi-IIa in BBM. In this study we analyze the effect of GABARAP-deficiency on the dietary and hormonal regulation of NaPi-IIa.

Wildtype and GABARAP knock-out mice were fed 5 days with either low (LPD; 0.1%) or high (HPD; 1.2%) Pi diets. When fed with HPD, expression levels of NaPi-IIa, urinary Pi excretion and uptake of 32Pi into BBMV were similar in wildtype and GABARAP knock-out mice. LPD led to an increased abundance of NaPi-IIa in BBM in both groups, but expression of NaPi-IIa was greater in BBM of GABARAP knock-out than in wildtype mice. Urinary Pi excretion of GABARAP knock-out mice was reduced and 32Pi-uptake into their BBMV tended to be higher than in wildtype mice. To study the hormonal regulation of NaPi-IIa in the absence of GABARAP, mice were injected intraperitoneally with either vehicle or parathyroid hormone (PTH; 0.5 µg/g body weight). PTH administration negatively regulated NaPi-IIa abundance in BBM of both groups. Despite the higher basal levels of NaPi-IIa in GABARAP knock-out mice, the residual 32Pi-uptake and NaPi-IIa expression in BBM upon PTH injection was comparable in GABARAP knock-out and wildtype mice.

In summary, we found that phosphaturic factors (HPD, PTH) reduce the expression of NaPi-IIa to comparable levels in GABARAP knock-out and wildtype mice, whereas in response to Pi-conserving factors (LPD) upregulation of NaPi-IIa is greater in GABARAP knock-out than in wildtype mice

T2-25

The relative contribution of acid-sensing ion channels (ASICs) and TRPV1 to pH sensing at body temperature

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Acid-sensing ion channels (ASICs) are cation-selective ion channels opened by a drop in extracellular pH. ASICs are expressed in the central and the peripheral nervous system. There is evidence for an involvement of ASICs in nociception, fear, synaptic long term potentiation, termination of seizures and neurodegeneration after ischemic stroke. All known ASICs isoforms (1a, 1b, 2a, 2b, 3 and 4) are expressed in the peripheral nervous system. Homotrimeric channels formed by ASIC1a, -1b, -2a or -3 are opened by a drop in extracellular pH. Asic2b, even though it is not opened by acidic pH, is involved in the formation of heteromeric channels with other isoforms, yielding channels with different properties compared to homomeric assembly. ASIC4 is not opened by protons and might regulate the membrane availability of other isoforms. TRPV1, the capsaicin receptor, is opened by pH, capsaicin, anandamide and heat. It is involved in pepper taste, heat evoked nociception and inflammatory thermal hyperalgesia. So far, almost all the biophysical data for ASICs were obtained at laboratory ambient temperatures (20-25˚C). Since ASICs and TRPV1 are both pH sensors in the peripheral nervous system and TRPV1 is proton-gated and is modulated by temperature, we are interested in studying the relative importance of ASICs and TRPV1 in sensory neurons at body temperature. To obtain information on the temperature dependence of the biophysical properties of cloned ASICs and TRPV1, we performed whole-cell patch-clamp experiments in mammalian cell lines stably expressing ASIC1a, ASIC1b, ASIC2a, ASIC3 or TRPV1. No significant shifts in pH dependence of activation, pH dependence of steady-state inactivation or the sustained current fraction were measured for ASIC1a, ASIC1b, ASIC2a and ASIC3. An acceleration of the kinetics of desensitization with increasing temperature was measured for all four isoforms. For TRPV1, we confirmed that under our experimental conditions, the heat response is modified by the pH. TRPV1 activation curves were not different between room temperature (25˚C) and body temperature (35˚C). Dorsal root ganglia (DRG) from rats express all isoforms of ASICs. We found in a previous study three types of ASIC-mediated proton-gated currents in small diameter rat DRG neurons. Two of these three types of currents are mediated by heteromeric assemblies of ASICs subunits. Heteromeric channels might be modulated by heat. In our previous study in DRG neurons, performed at laboratory temperature, ASICs were found to contribute more to pH sensing than TRPV1 at pH ≥ 6. We investigate currently the temperature dependence of proton-gated currents in DRG neurons in order to assess the contributions to pH sensing of ASICs and TRPV1 at body temperature. Knowing the relative importance of ASICs and TRPV1 channels is important to be able to choose an appropriate pharmacological tool to relieve pain in patients with peripheral inflammation.

T2-26

Oxygen-induced regulation of the Na,K-ATPase in rat heart.

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The present work was designed to study the impact of deoxygenation of the heart tissue on the Na,K-ATPase structure and activity.

Na,K-ATPase plays a critical role in heart survival under conditions of limited oxygen supply. It is the major ion gradient keeping enzyme and one of the main ATP consumers. In the myocardial tissue α2 isoform is expressed along with the housekeeping α1 isoform of catalytic subunit of Na,K-ATPase and the obligatory β1 and β2 regulatory subunits. Cardiac-specific regulatory FXYD protein phospholemman (PLM) is known to be involved in regulation of the activity of the enzyme. Decrease in oxygen availability associated e.g. with ischemic conditions was reported to cause inhibition of the pump activity. Mechanisms of hypoxia-induced deactivation of the ATPase have never been assessed and ATP deprivation has been accepted as a major trigger of the Na,K-ATPase deactivation, however it is still under discussion.

Isolated blood-perfused rat heart model was used to assess oxygen-induced responses of the hydrolytic activity of the Na,K-ATPase and its structural/allosteric modifications. Hearts were isolated and perfused with blood of the same animal equilibrated with gas phase containing 21, 15, 10, 5 and 3% O2. Ventricular tissue was harvested 1 h after perfusion and crude homogenate as well as sarcolemmal fractions prepared and used for hydrolytic activity measurements, WB and IP.

Deoxygenation resulted in a dose-dependent suppression of the Na,K-ATPase activity (up to 5-fold) in crude homogenate with Ki for the ATPase observed at ~ 12% O2 in gas phase corresponding to the hemoglobin oxygen saturation of 90,2%. In sarcolemmal fraction on the contrary ouabain-sensitive ATP cleavage was increasing with deoxygenation. The same phenomenon was reported earlier for the ex vivo model of myocardial ischemia. This suggests that hypoxia is a major factor in control of the Na,K-ATPase activity in ischemic heart. Hypoxic responses of the enzyme both in crude homogenate and in the sarcolemmal fraction were abolished in the hearts treated with the inhibitor of NO synthases, L-NAME. In L-NAME-treated ventricular tissue activity of the Na,K ATPase was retained at the lowest level which was

equal to hypoxic in homogenate and lower than in normoxic basal level in the sarcolemmal fraction.
Opposing effects of deoxygenation on the Na,K-ATPase in membrane fraction and crude homogenate imply that at least two oxygen-sensitive factors are in control of its function. Hypoxia-induced stimulation of the ATPase is most likely achieved by the phosphorylation and the following dissociation of the PLM. Indeed, similar to that observed in ischemic heart, we have found an increase in phosphorylated PLM in hypoxic myocardium. In addition, nitration of tyrosine in α1-subunit of the pump during hypoxia increased almost 1.5 times, whereas application of the L-NAME abolished nitration.
Hypoxia-induced inhibition of the pump activity in the homogenate probably is connected to the release of the soluble inhibitor of the pump. Our data shows that the inhibitor is inactivated by the thermal denaturation, so it can have the protein nature. Furthermore, application of the hypoxic homogenate or plasma, gatherd from hypoxic blood, to sarcolemmal fraction inhibits Na,K-ATPase activity on 20%, and 40% respectively. This suggests that the inhibitor presents not only in the homogenate of the hypoxic heart tissue, but also is released in the bloodstream.

T2-27

Involvement of acid-sensing ion channels (ASICs) in neuropeptide secretion from sensory neurons

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Acid-sensing ion channels (ASICs) are non-voltage-gated sodium channels activated by an extracellular acidification. They are widely expressed in neurons of the central and peripheral nervous system. Four genes code for six different homologous subunits (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, ASIC4). A functional ASIC channel is formed by three identical or different subunits. In the brain, ASICs are involved in different processes such as memorization, fear and neurodegeneration after cerebral ischemia. ASICs of the peripheral nervous system are likely sensors for extracellular acidifications occurring in pathological conditions such as inflammation, ischemia and lesions. In such situations, ASICs may therefore mediate the sensation of pain. We are interested in the role of ASICs in sensory neurons. These neurons of the peripheral nervous system are activated by sensory input such as touch, temperature and chemical stimulation and send projections into the central nervous system. The small diameter sensory neurons can be biochemically classified into two populations depending on their ability to bind Isolectin B4 (IB4). IB4-negative neurons (i.e. not binding IB4) are able to release neuropeptides such as calcitonin gene-related peptide (CGRP) or substance P (SP). These peptides can induce, maintain or increase neuroinflammation by promoting vasodilatation of capillaries and by plasma extravasation. Our laboratory has previously shown that calcium-permeant homomeric ASIC1a channels are present in a majority of IB4 negative – i.e. peptidergic – small sensory neurons. We hypothesized that a local acidification can produce an ASIC-mediated calcium-dependant neuropeptide secretion. To test the hypothesis we have first verified the co-expression of ASIC subunits and CGRP by using immunochemistry on adult rat dissociated primary sensory neurons. This analysis showed that most CGRP-positive neurons, which represent about half of the small diameter neurons express ASIC1 subunits. Preliminary results from SP immunostainings indicate similar proportions of SP-ASIC1 co-expression. Calcium imaging experiments from sensory neurons with fura2-AM dye showed that an acidification can induce an increase of intracellular Ca^{2+} concentration, which is essential for secretion. Acid mediated calcium increase was seen in the presence of the TRPV1 inhibitor capsazepine, suggesting that ASICs may mediate directly or indirectly (by depolarization) the entry of calcium. Preliminary secretion assay results showed that CGRP secretion can be induced by extracellular acidification in cultured rat sensory neurons. Simultaneous application of tetrodotoxin and lidocain decreased CGRP secretion, indicating that voltage-gated Na channels are involved in this process. Psalmotoxin 1, an ASIC1a-specific inhibitor, reduced the acidification-induced CGRP secretion. Taken together we show that ASICs are involved in neuropeptide secretion and provide information on some of the underlying mechanisms.

T2-28

Regulation of Voltage Gated Sodium Channel Nav 1.7 by ubiquitin ligases and beta auxiliary subunits

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BACKGROUND: Neuropathic pain (NP) is a disabling disorder occurring as a consequence of lesion or dysfunction of the nervous system. NP causes a positive shift toward hyperexcitability of peripheral nervous system. This peripheral activity is mainly carried by voltage-gated sodium channels (VGSC), where Nav1.7 isoform seems to be an important player since loss of function mutations reported in its gene are linked to congenital inability to experience pain. VGSC contain an α-subunit (Nav1.x), the pore of the channel, and β-subunits responsible for the regulation of channel density at the cell membrane. Ubiquitin ligases of the Nedd4 family are also known to regulate the channel density at the cell membrane. The aim of this study was to investigate the molecular and cellular mechanisms involved in the regulation of the membrane density of Nav1.7 that may be altered in NP.

METHODS: In vitro experiments were carried out to investigate whether Nedd4-2 and different β-subunits are involved in modulating the density of Nav1.7 channels at the cell surface. Whole-cell patch on HEK293 cells transfected with Nav1.7 allowed the recording of sodium currents I_{Na}. If β-subunits or Nedd4-2 have any modulatory effects, co-transfection of these regulatory proteins should modify the amplitude of INa. The interaction between Nedd4-2 and Nav isoforms was previously reported to depend on a consensus sequence xPPxYx (PY-motif) in the c-terminus. Mutations of the PY-motifs allow us to demonstrate that this sequence is important for interacting with Nedd4-2. Biochemistry experiments were performed to study this interaction. GST-fusion proteins composed of the 66 last amino acids of the c-terminal of Nav1.7 (WT or PY mutated) and GST were used to pull-down Nedd4-2 from HEK293 lysates.

RESULTS: Co-transfection experiments with Nedd4-2 decreased the current amplitude by ~80% (n = 36, p<0.001), without modifying the biophysical properties of INa. This effect was dependent on the PY-motif since mutations in this sequence abolished the down-regulatory effect of Nedd4-2. The importance of this motif was further confirmed by pull down experiments since the PA and YA mutants completely abolished the interaction with Nedd4-2. The INa of PY-motif mutant channels recorded 3 d after transfection were 2 to 3-fold larger than with WT, suggesting a defective internalisation process. Co-transfection of β-subunits showed an overall opposite effect since β1, β2, and β3 increased the I_{Na}: for β1 by ~100% (n=22, p<0.001); for β2 by ~ 70% (n=22, p<0.01), and β3 of ~200% (n=17, p<0.001). Interestingly, the β-dependent INa up-regulation did not depend on the integrity of the PY-motif. β4 did not modify the amplitude of INa. β1 and β2 subunits also caused a positive shift in the voltage-dependence of both activation and inactivation.

CONCLUSION: In HEK293 cells, Nedd4-2 and β subunits have opposite effects in terms of regulating the density of Nav1.7 at the cell surface. The xPPxYx motif is important because its disruption abolished Nedd4-2 effect by decreasing the affinity between these two proteins.

PERPSPECTIVES: We need to confirm in vivo these results combining experimental pain animal models together with knock-out mice for these two genes in order to correlate the cell surface modulation of Nav1.7 with differences in pain sensitivity.

T2-29

Expression of the neutral amino acid transporter B^0AT1 (Slc6a19) and its specific renal accessory protein Collectrin in MDCK cell line

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Backgroung and aim: The broad range amino acid transporter B^0AT1 (Slc6a19) interacts with two specific tissue accessory proteins, Collectrin in the kidney and its homolog the angiotensin converting enzyme 2(ACE2) in small intestine. A strong down regulation of B^0AT1 expression in the kidney and small intestine was observed in the *collectrin* and *ace2* knock-out animals respectively. Additionally, a significant increase of the transporter function was observed when B^0AT1 was co-expressed with Collectrin or ACE2 in oocytes of *Xenopus laevis*. To better understand the interaction between B^0AT1 and Collectrin, as well as its impact on B^0AT1 expression and stability we overexpressed B^0AT1 alone or in the presence of the kidney specific accessory protein Collectrin in MDCK cells. **Results:**Stable MDCK cells lines expressing B^0AT1, Collectrin, or B^0AT1 and Collectrin were first generated using a retroviral system. The integrity of monolayers formed by stably transfected cells was tested by measuring mannitol fluxes. The expression of the transporter or the accessory protein was assayed by immunofluorescence and the transport function was assayed by uptakes of radiolabeled amino acid. Interestingly, the MDCK cell line expressing B^0AT1 and Collectrin did not form tight monolayers. B^0AT1 as well as Collectrin localization appeared to be intracellular exhibiting a paranuclear staining pattern that evokes endoplasmatic and Golgi localization. However functional tests demonstrated a significant higher L-Ile uptake in B^0AT1-Collectrin co-expressing cell lines than in wild type (wt) and in cell lines expressing separately B^0AT1 or Collectrin. Taken together, these results imply that the expression of Collectrin plus B^0AT1 interferes with normal cell differentiation and growth, suggesting the possibility that the induced uptake of amino acids and Na^+ is toxic for these cells. To circumvent this problem, we utilized an inducible lentivirus system to express B^0AT1 in the MDCK cells. We first produced a stable MDCK cell line expressing inducibly the repressor protein (KRAB). This cell line was used as acceptor for our gene of interest, B^0AT1 expressed under the control of the KRAB transrepressor. We analyzed B^0AT1 expression by q-PCR and immunofluorescence. Our preliminary results suggest that B0AT1 is expressed in the MDCK-KRAB cells only after induction. The electrical resistance and the phenotype of these cells were like the wild type. **Conclusion:** The MDCK cells line inducibly expressing B^0AT1 can now be used to study the interaction of this amino acid transport with its kidney specific accessory protein Collectrin without disturbing the homeostasis of the cells.

T2-30

Phenotypical characterization of GILZ-deficient mice

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The glucocorticoid-induced leucine zipper (GILZ) is a X-linked transcription factor and was originally described as a gene induced by dexamethasone, a glucocorticoid receptor agonist, from a mouse thymocyte subtraction cDNA library (D'Adamio et al., 1997). It is ubiquitously expressed and may therefore play a role in different functions, for example, adipogenesis, renal sodium reabsorption, immunity or fertility. GILZ might interact with PPARγ2, C/EBPδ, Notch and other genes to regulate these functions.

Using the Cre-Lox recombination technique, we generated mice constitutively lacking the vital region of the GILZ gene, thus abolishing the function of this gene in the whole mouse. Knockout mice are viable and no obvious phenotype can be observed. The molecular analysis shows in most of the tissues analyzed from knockouts, GILZ mRNA transcripts expression is abolished. We are currently studying the function of this gene in various organs and tissues. The first results will be discussed.

T2-31

Dynamic redistribution of calcium sensitive potassium channels (hKCa3.1) in migrating cells

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Calcium sensitive potassium channels (KCa3.1) are expressed in virtually all migrating cells. Their activity is required for optimal cell migration so that their blockade leads to a reduction of the migratory speed. KCa3.1 channels must be inserted into the plasma membrane in order to elicit their physiological function. However, the plasma membrane of migrating cells is subject to rapid recycling by means of endo- and exocytosis. We had shown previously that single hKCa3.1 channel molecules are moving in a freely diffusive manner once inserted into the plasma membrane. Here we focussed on the endocytic internalization and the intracellular transport of the human isoform hKCa3.1. A hKCa3.1 channel construct with an HA-tag in the extracellularly located S3-S4 linker was transfected into migrating transformed renal epithelial MDCK-F cells. Channel internalization was visualized and quantified with immunofluorescence and a cell-based ELISA. Movement of hKCa3.1 channel containing vesicles as well as migration of MDCK-F cells were monitored by means of time lapse video microscopy. hKCa3.1 channels are endocytosed during migration. Most of the hKCa3.1 channel containing vesicles are moving with a speed of up to 2 µm/s in a microtubule-dependent manner towards the front of MDCK-F cells. Our experiments indicate that endocytosis of hKCa3.1 channels does not occur as rapidly as described for the plasma membrane. At least part of it is clathrin-dependent since hKCa3.1 channel proteins are colocalized with clathrin adaptors and since it is impaired when a C-terminal dileucine motif is mutated. The C-terminal dileucine motif is also important for the subcellular localization of hKCa3.1 channels in migrating cells. Mutated channels are no longer concentrated at the leading edge.

T2-32

Electrophysiological studies of native mitochondrial transporters, respiratory chain proteins, and oxidative phosphorylation

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Transporters, channels and pumps of the inner mitochondrial membranes are involved in all major mitochondrial functions such as ATP synthesis, regulation of apoptosis, control of cellular calcium, thermogenesis, and in balancing production of reactive oxygen species. Due to their central role, dysfunctions of mitochondrial transport proteins can result in a variety of pathologies including diabetes, obesity, aging, cancer, and neurodegenerative diseases. However, functional investigation of mitochondrial transport proteins in their native surroundings is tedious. Here, we describe the use of the SURFE2R technology for cell-free electrical measurement of transporters, respiratory chain proteins, and ATP-synthesis in native inner mitochondrial membranes. The membranes were isolated from pig heart mitochondria and adsorbed on gold electrodes coated with solid-supported membranes. The resulting biosensors were measured with the standard SURFE2R equipment. Following transport proteins and electrical processes were characterized: the ADP/ATP exchanger (ANT), the respiratory chain complexes I, III, IV and V (NADH dehydrogenase, bc1 complex, cytochrome c oxidase, and F-type ATPase), and the oxidative phosphorylation.

The ANT was activated by ATP or ADP concentration jumps. Complex I and III were activated by NADH, whereas complex IV was studied by means of rapid perfusion of the reduced cytochrome c. The F-type ATPase (complex V) was studied in the reverse mode (ATP hydrolysis) as well as forward mode (ATP synthesis). All tested proteins were pharmacologically characterized using specific substrates and inhibitors. Additionally, oxidative phosphorylation (ATP synthesis) was measured by ADP concentration jumps in presence of NADH-generated proton gradients. These protocols allowed detection of uncouplers and respiratory chain inhibitors in highly sensitive manner. Moreover, application of specific inhibitors and decylubiquinone revealed a tight functional interplay between the complexes I and III. In summary, the presented results demonstrate a new, easy and reliable approach for functional studies of mitochondrial transporters and pumps in their native surroundings, and help to understand the complex molecular mechanisms of different mitochondrial functions. Besides, the technology platform can be used to perform screens for mitochondria-targeted drugs and to test for mitochondrial toxicity of pharmacologically relevant compounds.

T2-33

Cell-free electrophysiology of the viral proton channel M2

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The A-M2 protein is a proton-selective ion channel in the viral envelope of the influenza A virus. The influenza virus enters the host cell by endocytotic pathway. In the endosomes, the A-M2 channel is essential for the fusion of the viral envelope with the host endosomal membrane, and thus for the release of the viral genome into the cytosol. Therefore, the inhibition of A-M2 by drugs amantadine and rimantadine prevents the virus from taking over the host cell. In the past decade, a rapid increase of the virus resistance to amantadine and rimantadine was recorded. Hence, new needs for high-quality higher-throughput methods for functional studies of A-M2 and for the development of M2-targeted drugs have emerged. Here, we describe the use of the SURFE2R technology platform with a 96-biosensor array for cell-free electrical measurement of the A-M2 channel activity. A-M2 was investigated either in CHO cells or after the reconstitution in proteoliposomes. For CHO-cells, the wild-type A-M2 and the amantadine-resistant S31N mutant were transiently expressed, and the corresponding cell membranes were purified by a sucrose gradient centrifugation. The membranes were adsorbed onto the lipid-coated gold electrode of the SURFE2R sensors. The A-M2-mediated conductance was activated by pH jumps through a rapid exchange of the „non-activating“ solution of pH 7 by the „activating“ solution of pH 6. Electrical currents detected with the wild-type A-M2 were inhibited by amantadine. In contrast, the activity of the amantadine-resistant S31N mutant was not inhibited at the corresponding amantadine concentrations, and was only partially inhibited using higher amantadine levels. The assay was highly sensitive with an amantadine IC50 of 0.6 µM for the wild-type A-M2. The amantadine inhibition was fully reversible with a time constant in range of 1-2 min. As expected, both, the wild-type A-M2 and the S31N mutant were inhibited by copper. The data demonstrate the feasibility of the technology for high-content functional and pharmacological studies of the A-M2 activity in a 96-array format.

Poster Topic 3 – Cell-Cell Interactions

Poster Topic 4 – Membrane Receptors

T4-01

Mature erythrocytes as a target for erythropoietin

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Erythropoietin (Epo) is the major regulator of the red blood cells (RBCs) production and important determinant for the adequate oxygen delivery in the tissues. Epo suppresses the apoptosis and promotes cell survival and proliferation of hematopoietic cells, increasing the numbers of erythroid progenitors in the bone marrow. The expression of Epo receptor (EpoR) decreases gradually during the late stages of erythroid development. Therefore, reticulocytes and mature RBCs were suggested to lack EpoR and were considered Epo insensitive. However, Epo binding to mature erythrocytes was reported and Epo-induced responses in mammalian RBCs were described. The nature of Epo binding sites and the signaling cascades involved in the reported Epo-induced regulation of ion transport and redox state remained unknown.

The aim of the present study was to characterize the Epo binding to mouse erythrocytes and to elucidate what are the signaling pathways and the downstream targets affected by the cytokine.

We found that mouse reticulocytes and mature erythrocytes can specifically bind Epo. The dissociation constant, sensitivity to EpoR-antibodies, and adjacent downstream signal-transduction pathway strongly suggest that Epo binding sites represent classical erythropoietin receptors. Epo binding sites are not equally distributed within RBC population. Reticulocytes and young erythrocytes have markedly higher ability to bind Epo in comparison to adult and old erythrocytes. Our results suggest that all RBCs have a potential to be Epo-sensitive, but the range of the cellular response should be age-dependent. Further, investigation revealed that Epo treatment of mouse erythrocytes activates PI3K/Akt pathway leading to phosphorylation of endothelial type nitric oxide synthase in the cells (RBC-eNOS) at Ser-1177. Epo-induced RBC-eNOS phosphorylation resulted in increase of the enzyme activity and production of either nitric oxide (NO) or superoxide radicals, depending on substrate (L-arginine) availability. Both RBC-eNOS products affected directly cellular redox state by shifting it to more reduced or oxidized respectively. Therefore the regulation of RBC-eNOS by Epo may protect from oxidation or promote oxidative stress in mouse erythrocytes. The putative outcomes of Epo-induced regulation of RBCs NO production and cellular redox state on erythrocyte properties and function will be discussed.

T4-02

Postsynaptic density protein PSD-95, and NMDA receptor levels are markers for cognitive failure.

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Aging is known to impair memory processes, but it does not affect everyone to the same extent. Previously, we assessed and classified individual performance in cognitive tests (place learning, retention and working memory) of senescent rats in relation to PSD-95 protein levels. Two subgroups were established, one severely impaired across all tests and the opposed group being unimpaired. Given the relation of PSD-95 and NMDA receptors, we extended our study, included young rats and additional Western blots, as well as immunohistochemical analysis of frontal cortex and hippocampal tissue. Gradient electrophoresis gels (4%-15% SDS-PAGE) were either stained with Coomassie blue or transferred to nitrocellulose and immunostained with antibodies for PSD-95 and NMDA receptor subunits NR1, NR2A, NR2B, followed

by densitometric quantification. Immunohistochemical quantification of the same proteins was performed in parallel. Western blots analyses emphasized a significant increase in PSD-95 and NR2B in the frontal cortex whereas a tendency to decrease was observed in the hippocampus of the cognitively impaired rats. Immunohistochemical analyses confirmed the PSD-95 increase in the frontal cortex of cognitively impaired rats and showed similar trends for NR2B. On the contrary, no significant differences between groups were observed in the NR1 and NR2A subunit levels. Our results suggest that the levels of PSD-95 and NMDA-R2B correlate with cognitive performance and that PSD-95 could not only link to the NMDA receptor complex but also influence its function.

T4-03

The role of NMDA receptors in myocardial responses to hypoxia

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The expression of a functional NMDA receptor in the heart has been reported, but its physiological role remains unclear.

Glutamate levels in blood plasma (~70 µM) and heart interstitium

(10-35 µM) are much lower the receptor E50 of 500 µM. Levels of homocysteine (HC) are, on the contrary, within the receptor activation range (E50 14 µM) varying from 8.5 µM in healthy subjects to 400 µM in patients with hyperhomocysteinemia. When above the threshold of 12-15 µM, HC becomes a risk factor for elderly people and patients with chronic cardiac failure.
The purpose of our ongoing study is to assess myocardial responses to the high levels of HC and its oxidative product, homocysteic acid (HCA), as well as the impact of these potential risk factors into vulnerability of the young and senescent heart to hypoxic insult.
Isolated blood-perfused rat heart model that we have used allowed the precise control of the blood oxygenation levels (pO2 of 7,48±0,2 kPa was chosen as normoxia and 2,60±0,21 kPa in hypoxia), the perfusion rate and the concentrations of the added substances as well as the detection of functional parameters. Hypoxic responses of the hearts of young rats (303±4 g, 3 months) were compared with those of old animal group (471±35g, 12 months).

In senescent hearts hypoxia caused oxidation assessed as a shift in half-cell redox potential for the reduced to oxidized glutathione couple (Ehc ~ -log([GSH]2/[GSSG]) from -125.7±5.4 mV under normoxic conditions to -114.0±3.2 mV in hypoxic hearts. Deoxygenation also resulted in sodium accumulation in the ventricular tissue and bradycardia followed by stunning. Young hearts were more resistant to hypoxia showing only minor bradycardic response with the sodium levels preserved and heart rate remaining stable over 1 hour of hypoxic treatment.
Heart rate was sensitive to the presence of agonists (300 µM HCA or NMDA) or inhibitor (100 µM MK-801) of the NMDA receptors. Presence of the agonists in the circulating blood caused a 10-15% increase whereas the blocker caused a 40% reduction in the heart rate with no changes in glutathione and ATP levels observed.
Accumulation of HC in plasma occurs gradually with aging. Interestingly, exposure of young hearts to HC when hypoxic caused a dramatic decrease in the heart rate along with oxidation (Ehc shifting from -140.2±6.3 to -128.7±8.4 mV). This response to deoxygenation resembled that of the senescent hearts and suggested that inhibition of the NMDA receptors could rescue the aged hearts from the hypoxia-induced stunning. Indeed, pre-treatment of the senescent hearts with MK-801 prevented stunning and heart rate was maintained at the levels 20% lower than in the basal normoxic ones. Oxidation observed in the aged hearts upon deoxygenation was also prevented by MK-801.

These observations indicate that the activation state of the NMDA receptors is an important factor in control of the myocardial redox state and sensitivity of the heart to hypoxia. Thereby, HC may indeed be an important risk factor especially in the elderly subjects and pregnant women.

T4-04

Directed Evolution of a GPCR – an Exhaustive Mutagenesis Study to Improve Expression Level and Stability of rNTR1

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The family of G-protein coupled receptors (GPCRs) is the largest and most diverse group of membrane proteins. GPCRs play a key role in various physiological processes and thus represent a valuable medical and pharmaceutical target.
Structural and functional data of GPCRs are indispensible for their characterization as well as for future drug development. Great effort is made to obtain crystal structures but so far, crystal structures could only be solved for a few either naturally abundant (rhodopsin) or stability-engineered GPCRs (for example β2-adrenergic receptor). The main reasons for this are the low overexpression level and the intrinsic instability of GPCRs solubilized in detergent. In order to make GPCRs more amenable for biochemical and structural analysis, the factors limiting expression level and stability have to be determined. Previous data suggested that the low expression level and instability of GPCRs might be attributed to the poor biophysical properties, i. e. the amino acid sequence, and not the general topology of the GPCRs.
In order to analyse this hypothesis, we perform an exhaustive and position-specific mutational analysis of a GPCR, rNTR1-D03. We use 380 DNA libraries in which each single position of rNTR-D03 is fully randomized. The libraries are expressed in E. coli and the best expressed and functional variants are selected by fluorescence-activated cell sorting (FACS) using a fluorescence-labelled agonist of rNTR1, BODIPY-neurotensin. From the analysis we will thus learn, for each position, which amino acids are not acceptable, acceptable and preferred. The results of this study will give insight into the influence of every single position on the expression level and biophysical stability of the receptor. This knowledge can be used to make the GPCR family more accessible to functional and structural studies.

T4-05

CXCR7 – A Novel Role as Chemokine Scavenger

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CXCR7 (RDC1) is a seven transmembrane domain receptor that by phylogenetic analysis groups into the subclass of chemokine receptors. Recent studies provide evidence that CXCR7 can bind CXCL12 (SDF-1) and CXCL11 (ITAC), although ligand-engagement fails to induce classical intracellular signaling including migration. Here, we show that in MDCK epithelial cells, CXCR7 and CXCR4 can internalize CXCL12. However, CXCR7-transfected MDCK cells were much more powerful in the uptake and degrading of the internalized chemokine. We observed pronounced CXCR7-mediated CXCL12 degradation also in primary human umbilical vein endothelial cells (HUVEC) endogenously expressing the CXCR7.
CXCR7 appeared to cycle form and to the plasma membrane even in the absence of ligand. In un-stimulated transfected MDCK cells, a large fraction of CXCR7 localized in intracellular compartments. This reservoir was capable of trafficking to the cell surface. Taken together, our results indicate constitutive cycling of CXCR7, and reveal a novel function as chemokine scavenger.

T4-06

GPCR activity during cell migration determined by FRET

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Cell migration is essential for viability of multicellular organism; it is critical for development, repair and defense processes. The binding of chemoattractants to their surface receptors represents the first step of the series of signaling events that lead to the spatial activation of the cytoskeleton and directed migration. Since cells become highly polarized when exposed to shallow gradients of chemoattractants, it implies that the external gradient is amplified inside the cell to form the distinct front and back morphology. As receptors represent the link between external guidance cue and the internal signaling machinery, it had been proposed that this amplification could arise either, from the asymmetric distribution of the receptors themselves, or from the local differences on receptor occupancy, or through intracellular inhibitory feed-back mechanisms leading to an asymmetric distribution of intracellular signal transduction proteins. Using receptors tagged with fluorescent probes it is possible to monitor the spatio-temporal activation of GPCRs in migrating cells. Recently it was shown that intramolecular FRET of CFP/YFP tagged GPCRs reliably reports the activation state with kinetics comparable to wild type molecules. We stably expressed a CFP/YFP tagged-α2-adrenergic receptor (α2-AR) in monocytic THP-1 cells. Receptor activity was assessed by measuring calcium mobilization, MAPKinase activation and in chemotaxis assays using modified Boyden chambers. The transfected THP-1 cells display a strong chemotactic activity against the small molecule agonist UK 14'304 with an efficacy comparable to the migration elicited with the chemokine MCP1 (CCL2) which acts on the endogenous receptor CCR2. The chemotactic response was also confirmed by time-laps video microscopy of cells migrating towards a chemoactractant-filled micropipette.
A consistent FRET efficiency value (35%) was detected by acceptor (YFP) bleaching method in live THP1 cells expressing the α2-AR CFP/YFP construct. Agonist-dependent variations of FRET intensities were measured in migrating cells by time-laps video microscopy. Our findings indicate that over time changes in FRET efficiency can be observed throughout the entire plasma membrane indicating that the receptors residing at the front and rear become activated to a similar degree. The observations suggest the presence of rapid feed-back signaling events presumably starting from the leading edge, which prevent the activation similar signaling cascades at the uropod. In contrast to apparent continuous activation of the receptors over the plasma membrane we find the rapid activation of PI3K at the leading edge through the observation of the recruitment of a mCherry-tagged PKB-PH-domain. On the other hand we observe a rapid reversal of the polarization axis upon repositioning of the dispoending meicropipette indicating that receptors at a uropod are competent to respond to chemoattractants.

T4-07

Functional Characterization of Stable KDR/Neuropilin-1 Complexes with VEGF

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Angiogenesis plays a crucial role in a variety of physiological and pathological conditions including cancer and cardiovascular disease. Among the major mediators of angiogenesis are vascular endothelial growth factors (VEGFs). Mammalian VEGF-A and -B and placenta growth factor (PlGF) are required for blood vessel formation, while VEGF-C and -D regulate the formation of lymphatic vessels. In addition, parapoxviruses encode a set of VEGF homologues called VEGF-E, stimulating proliferation of endothelial cells in vitro and vascularization of sheep skin in vivo. Proteolytic processing and alternative splicing results in multiple VEGF isoforms differing in receptor specificity. Their function is mediated through binding to multiple VEGF receptors. These include three types of tyrosine kinase growth factor receptors, VEGFR-1, -2 and -3 and the nonsignaling co-receptors neuropilin-1 and neuropilin-2 (Nrp-1 and Nrp-2) that modulate VEGFR activity. VEGF signaling is further modulated by heparan sulfate (HS) proteoglycans.

Nrp-1 is a type I transmembrane glycoprotein that is widely expressed in endothelial cells (EC) of developing blood vessels and in various tumor cell lines. It functions as part of receptor complexes in processes as diverse as angiogenesis, neuronal guidance and cell adhesion. In order to improve our understanding of how VEGF signaling is mediated by Nrp-1, we have biochemically and biophysically characterized the interaction with a series of VEGF splice variants as well as mutant and chimeric proteins. We determined the motifs required for binding to Nrp-1 in the presence (VEGF-A_{165}) or absence (VEGF-E) of an HS-binding sequence. We identified the carboxyterminal peptides RPPR and DKPRR as Nrp-1 binding epitopes of VEGF-E and VEGF-A, respectively. VEGFs containing an RPPR motif promoted HS-independent coreceptor complex assembly between VEGFR-2 and Nrp-1. Functional studies showed that stable coreceptor assembly by VEGF correlated with its ability to promote vessel formation in an embryoid body angiogenesis assay.

Currently we are using X-ray crystallography, electron microscopy and small angle X-ray scattering to obtain information on the Nrp-1 / VEGF interaction on a structural level at high and low resolution. A detailed knowledge of the protein-protein interfaces may offer opportunities for the development of biologically active substances for diagnostic or therapeutic applications.

T4-08

Activation of receptor tyrosine kinases: Ligand-induced structural rearrangement of the extracellular domain of VEGF receptor 2

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Vascular endothelial growth factors constitute a family of six polypeptides, VEGF-A, -B, -C, -D, -E and PlGF, that regulate blood and lymphatic vessel development. VEGFs specifically bind to three types of receptor tyrosine kinases, VEGF receptors -1, -2 and -3. Some VEGF isoforms also bind to neuropilins and heparansulfate glycosaminoglycans resulting in increased signaling by VEGF receptors. Receptor specificity of VEGFs is determined by flexible loops that shape the receptor binding domain as shown recently by structural and biochemical data.
VEGF receptor tyrosine kinases are activated upon ligand-induced dimerization. We separately study the role of the extracellular, (ECD) ligand binding, and the transmembrane domain in receptor dimerization and activation. Using negative stain electron microscopy on recombinant VEGFR-2 ECD proteins we could show earlier that the monomeric ECD is highly flexible in the absence of ligand (Ruch et al., 2007). Binding of VEGF to membrane-distal Ig-like domains causes receptor dimerization and promotes structural rearrangement of ECD monomers. VEGF binding promotes further interaction between ECD monomers through the membrane-proximal Ig-like domain 7. In related receptor tyrosine kinases such as Kit and PDGF receptors it was shown that an interaction of membrane proximal Ig-like domains is indispensable for receptor dimerization and activation.
We investigated the role of Ig-like domain 7 in VEGFR-2 dimerization and activation using a series of receptor mutants. Preliminary biochemical and structural data point to a crucial role of the membrane proximal domain of VEGFR-2 in receptor activation. Hence, we will present data for recombinant receptor proteins expressed in mammalian cells and discuss a mechanism for receptor activation.

T4-09

Structure/function analysis of VEGF receptor 2; from ligand binding to receptor activation

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Vascular endothelial growth factors constitute a family of six polypeptides, VEGF-A, -B, -C, -D, -E and PlGF, that regulate blood and lymphatic vessel development. VEGFs specifically bind to three types of receptor tyrosine kinases, VEGF receptors -1, -2 and -3. VEGF receptors are activated upon ligand-induced dimerization followed by conformational changes that reorient the cytoplasmic kinase domains. This leads to stimulation of kinase activity and phosphorylation of specific tyrosine residues in the cytoplasmic part of the receptor that instigate signaling. Proper orientation of the cytoplasmic domains and concomitant kinase activation of receptor dimers depends on the structure of the juxtamembrane domain, the rotational angle under which the hydrophobic transmembrane domains associate with eachother, and the ligand-induced reorientatiuon of the extracellular domain. In order to elucidate the role of dimerization in VEGFR-2 activation we studied the activity of membrane-bound receptor kinase dimers in vitro and in live cells and designed a library of VEGFR-2 mutants lacking the extracellular domain and carrying a dimerization motif in the transmembrane helix. These mutants differ in the rotational angle at which the kinase domains associate with each other. Our results show that only protein dimers with distinct orientations of the receptor molecules show constitutive kinase activity, suggesting that dimerization is necessary, but not sufficient, for receptor activation.
By means of coimmunoprecipitation assays with differently tagged receptor proteins we also investigated the role of the ECD, the transmembrane (TMD) and the kinase domain in the activation of full length receptors. We could show that dimerization-promoting TMDs or deletion of a specific subdomain (Ig-like domain 4) in the ECD lead to ligand-independent receptor activation while mutating Ig-like domain 7 rendered the receptor inactive. In addition, extracellularly truncated receptor mutants carrying the native transmembrane domain formed dimers, while full length receptors showed only ligand-dependent dimerization. This suggests a propensity of the transmembrane domain and/or additional intracellular domains to promote receptor dimerization. In the absence of ligand, the extracellular domain apparently prevents spontaneous receptor dimerization maintaining low kinase activity. Together with our earlier structural data of the extracellular domain of VEGFR-2 we propose a mechanism for receptor activation linking structural changes initiated by ligand binding in the extracellular domain to the intracellular kinase domain.

T4-10

A novel CD44-mediated death pathway in neutrophils

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CD44 is a transmembrane glycoprotein involved in cell-cell and cell-matrix interactions. It is expressed in multiple cell types and implicated in a wide range of physiological and pathological processes, such as morphogenesis, organogenesis, haematopoiesis, leukocyte activation, lymphocyte homing, wound healing, cell migration, tumour growth and metastasis. Ligation of CD44 with the monoclonal antibody A3D8 has been shown to induce leukeamic cell death and initial studies on neutrophils showed that GM-CSF increases the CD44 mRNA level. Thus, we hypothesized that CD44 might induce cell death in neutrophils.
In a concentration-dependent manner and upon priming with the pro-inflammatory cytokine GM-CSF, ligation of CD44 resulted in the induction of neutrophil death. Similarly to GM-CSF, additional pro-inflammatory cytokines (e.g. IL-1 and IL-6) as well as the chemotactic peptide fMLP were also able to enhance CD44-mediated cell death in neutrophils. Interestingly, the pan-caspase inhibitor, z-VAD, could not delay this process suggesting that CD44 ligation and GM-CSF priming are mainly launching caspase-independent neutrophil death. Moreover, the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor, diphenyleneiodonium chloride (DPI), strongly blocked CD44-mediated neutrophil death upon GM-CSF priming indicating a possible involvement of reactive oxygen species (ROS) in this signalling cascade. By investigating neutrophils from chronic granulomatous disease (CGD) patients, which have a genetic mutation in the NADPH oxidase impeding the generation of ROS, we could confirm that ROS are crucial players in CD44-mediated neutrophil death. Morphologic characterizations of this process showed an aberrant morphology with cytoplasmic vacuolizations. The chromatin of neutrophils appeared to be more disintegrated and less condensed as compared to classical apoptotic neutrophils, suggesting an autophagic-like process under these conditions. Finally, neutrophils derived form patients suffering of different inflammatory diseases such as sepsis, rheumatoid arthritis and cystic fibrosis, demonstrated increased CD44-mediated death. Additional priming with GM-CSF did not further increase neutrophil death, indicating that neutrophils were already primed in vivo.
Taken together, these data suggest that a ROS-dependent and caspase-independent death pathway is initiated by CD44 ligation in neutrophils following GM-CSF priming.

T4-11

"C-terminal splicing of β1 integrins control morphology of focal adhesions by differential integrin adaptor recruitment"

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Integrins mediate the mechanical link between the extracellular matrix and the actin cytoskeleton. They play an important role in cell adhesion and migration during development as well as for tissue integrity in the adult organism. Extracellular ligand and adaptor proteins binding to the cytoplasmic tails are required for its function. Alternative splicing of the cytoplasmic tail of β1-integrins during muscle differentiation suggest a role in the modification of the mechanical properties of integrins.
In order to analyse differences of integrin dynamics in respect to the recruitment of specific intracellular adaptors or splice variants, we decided to fluorescently tag β1-integrins. We introduced GFP molecules at the c-terminus of the cytoplasmic tail as well as into the extracellular domain, at sites allowing the reversible association with extracellular matrix ligands.

When expressed in mouse C2C12 myoblasts the c-terminally tagged β1-integrins were highly expressed at the cell surface, with only a minor contribution to focal and fibrillar adhesion. In contrast, non tagged β1-integrins revealed by antibody staining and extracellularly tagged β1-integrins localized efficiently to focal adhesions in the cell periphery and to streak like fibrillar adhesions in the center of the cell.

When comparing extracellularly tagged β1A and the muscle specific β1D constructs, we observed differences in the morphology of the integrin contacts underneath the center of the cell. While the β1A contacts were thin, long and parallel to each other, β1D contacts were shorter and distributed centripetally. We measured the morphology of these contacts and confirmed the variation in the length of the contacts. We also analyzed the composition of the contacts and observed paxilin and vinculin, two adaptors characteristic of focal adhesions, in the β1D contacts but only traces in the long streak like adhesions formed with β1A. These findings suggest that the integrin β1D stabilizes focal adhesions all over the cell surface, recruiting paxilin and vinculin to establish force transmission and signalling while β1A forms fibrillar adhesions required for extracellular matrix organisation.

Concerning the strong cell surface expression of c-terminally tagged integrins, we analyzed the possibility that the GFP tag altered focal adhesions recruitment and integrin activation, by blocking critical adaptor proteins. The extracellularly tagged β1

integrins permitted us to study the role of the c-terminus in integrin recruitment to focal adhesions. We created different mutations to reveal the important residues required for normal integrin function. While the removal of the last two aminoacids was tolerated, a further deletion caused the accumulation of extracellular tagged integrins at the cell surface, suggesting that the c-terminus of β1A integrins is an important site for integrin recruitment into focal adhesions.

In conclusion the differences between the two sequences of the cytoplasmic tails of β1A and β1D create different environments for adaptor protein binding that modifies the formation and function of integrin-dependent adhesions.

Supported by the Swiss National Foundation and the FSRMM(Fondation Suisse de Recherche sur les Maladies Musculaires)

T4-12

Modulation of angiogenesis by Notch-signalling inhibition in the chick area vasculosa

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Angiogenesis is a tightly regulated process which plays a crucial role in many pathological conditions such as cancer and cardiovascular disorders. Complex cellular interactions determine vascular patterning and Notch appears to be a key regulator of angiogenesis. DLL4-Notch signalling is an important, highly conserved, cell-cell communication pathway. This is a promising target for novel approaches in anti-angiogenic therapy. In this study the chick area vasculosa was used as an angiogenesis assay for investigating the effect of Notch inhibition on vessel development. Blocking of proteolytic processing of Notch by γ-secretase inhibitor (GSI) was evaluated in dose-, stage- and application- dependent manner. There was down-regulation of Notch-2, its ligand DLL4, its target Hes-5 and the arterial marker Ephrin-B2, to the same extent and in a strong temporal manner, depending on the number of applications of similar dosage GSI. Notch inhibition was associated with an evident regression of vascular length and vessel branching points. The data were correlated with the variation in cleaved Notch protein levels and immunoreactivity for tip cells (indicating sprouting), apoptosis and vascular pericytes coverage. These results elucidate the regulation of Notch-related genes and the mediating role of Notch signalling in the complex process of angiogenesis.

T4-13

Growth factor-induced cell spreading: a critical role for integrins in sensitization to substrate.

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Cell adhesion to and migration on extracellular matrix is involved in normal and pathological processes such as embryogenesis, immune defence, tissue homeostasis, but also cancer-induced angiogenesis. Cell adhesion and migration depend on the heterodimeric cell surface receptors of the integrin family, and signaling through growth factors and chemokine receptors. The integrins provide the physical link between the extracellular matrix and the actin cytoskeleton while receptor tyrosine kinases integrate and modulate cell adhesion and migration by responding to external growth factor cues present in the cell environment. A synergy between these two receptor systems has been proposed, requiring integrins to bind extracellular ligands and to link actin cytoskeleton, but this mechanism is completely unknown. A recent study has shown the importance of the adaptor protein talin in mediating matrix-integrin-actin linkage and sustained cell spreading and adhesion, postulating a critical role of the talin-integrin connection in the cellular processes stimulated by growth factor signaling such as cell spreading and migration. Because of the physiological importance of such synergies between integrin and growth factor receptors to promote cell spreading and migration during angiogenesis or wound-healing, we wanted to further investigate the signaling pathways involved. For this, we developed a cellular system that allows the expression of wildtype or mutated GFP-tagged β3 integrins in cells responding to Stem Cell Factor (SCF) stimulation. The spreading behaviour of these cells was studied in response to different substrate concentrations in the presence or absence of SCF treatment, in order to quantify the degree of synergy created. Our data confirm the critical role of integrin-ligand recognition for cell spreading since the expression of a mutant integrin that fails to recognize the extracellular substrate reduces cell spreading, while a dominant active integrin with facilitated ligand binding increased substrate responsiveness when compared to wildtype. In contrast, a mutated integrin lacking a functional NPLY motif required for talin recognition has a dominant negative effect on cell spreading with a reduced sensitivity to substrate. This dominant negativity was surprising since this NPLY mutant should not compete with endogenous integrins for the talin-dependent signaling machinery of the cell. However, when we analyzed the substrate-dependent spreading in response to SCF, this mutant also blocked the synergy response normally observed in cells expressing WT or activated integrin. When combined with the mutation which prevents the substrate binding, this double mutant looses its dominant negative effect in the growth factor induced spreading assays. These results show that the integrin synergies with SCF require a functional NPLY motif and substrate-anchored integrins, providing evidence for an additional talin-independent function of the NLY motif.

Supported by the Swiss National Foundation and the Nevus Outreach (The association of large Nevi and Related Disorders)

T4-14

Changes of EC-coupling and RyR calcium sensitivity in dystrophic mdx mouse cardiomyocytes.

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Duchenne muscular dystrophy represents a severe inherited disease of striated muscle. It is caused by a mutation in the dystrophin gene and characterized by a progressive loss of skeletal muscle function. Most patients also develop a dystrophic cardiomyopathy resulting in dilated hypertrophy and heart failure. On the cellular level, absence of dystrophin affects mechanical membrane stability and intracellular Ca signaling in cardiomyocytes. However, the cellular mechanisms that lead to deterioration of cardiac function, remain elusive. Here, we tested the hypothesis that defective excitation-contraction (EC) coupling contributes to impaired cardiac performance.

The EC-coupling gain, a measure for the effectiveness of a cardiomyocyte to amplify the trigger Ca signal by Ca release from the sarcoplasmic reticulum (SR), was determined from control and dystrophin-deficient mdx hearts. Ca currents were measured with the whole-cell patch-clamp technique, while Ca transients were simultaneously recorded with confocal imaging of fluo-3.

Initial findings indicated subtle problems of EC-coupling in mdx cells despite matched Ca loading of the SR. However, lowering the extracellular Ca concentration, a maneuver used to unmask latent EC-coupling problems, was surprisingly much better tolerated by mdx myocytes. Normalized to control conditions, the EC-coupling gain in mdx cells reached 112% compared with 31% in control myocytes, suggesting a hypersensitive EC-coupling mechanism. Further investigation of this apparent increase in Ca sensitivity by inducing slow elevations of the intracellular Ca concentration resulted in Ca oscillations after a much shorter delay in mdx cells. This observation is consistent with an enhanced Ca sensitivity of the SR Ca release channels (ryanodine receptors, RyRs). Indications of elevated cellular reactive oxygen species (ROS) generation in dystrophy point toward possible redox-modifications

on the RyR, thereby enhancing its Ca sensitivity. Preincubation of the mdx cells with a scavenger for ROS could normalize this hypersensitivity of the EC-coupling gain from 112% back to 26%, which is comparable with the gain value in control cardiomyocytes. Our data suggest that in dystrophin-deficient cardiomyocytes, EC-coupling mechanisms are critically altered, partly due to potentially arrhythmogenic changes in the Ca sensitivity of redox-modified RyRs.

Poster Topic 5 – Membrane Trafficking and Dynamics

T5-01

Regulation of beta cell function by phosphatidylinositol-4,5-bisphosphate (PIP_2)

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Background/Introduction:
PIP_2 is a critical secondary messenger that regulates diverse cellular activities such as vesicle trafficking, actin cytoskeleton remodeling and apoptosis. Although PIP_2 has been involved in the past in regulated exocytosis, its role in beta cell function has not been clearly defined. The objective of this study was to examine the role of PIP_2 in beta cell survival and insulin secretion.
Methods:
The well-differentiated transformed mouse pancreatic beta cell line MIN6B1 was used. TUNEL and caspase-3 activity experiments were performed to examine cell survival. Human growth hormone secretion (used as a surrogate marker for insulin secretion from transfected cells) was analysed by ELISA. Cells were examined by immunofluorescence + confocal microscopy.
Results:
PIP_2 was localised to actin and gelsolin-rich domains at the plasma membrane. Blocking PIP_2 action with PH-PLC-GFP or with phosphatidylinositol 4-phosphate 5-kinase Iγ (PIP5KIγ) RNAi did not reduce the secretory response to glucose. However, both treatments resulted in increased sensitivity to induced apoptosis. Over-expression of PIP5KIγ resulted in a marked inhibition of insulin secretion and accumulation of intracellular vacuoles coated with actin and PIP_2. These vacuoles, which have also been observed in PIP5KI over-expressing adipocytes and in cells expressing constitutively active Arf6 Q67L, did not contain insulin but were positive for the endosomal marker caveolin 1, Arf6 and the insulin granule membrane protein phogrin. Expression of Arf6 Q67L also resulted in the formation of the vacuoles and in a similar pattern of inhibition of regulated secretion, which was improved by blockage of PIP_2 action. On the other hand, inhibition of the RhoA/ROCK signaling pathway improved stimulated secretion of cells over-expressing PIP5KIγ without affecting vacuole formation. Constitutively active RhoA V14 resulted in increased cortical actin polymerisation which was also reduced by blockage of PIP_2 action.
Conclusion:
PIP_2 plays a pro-survival role in MIN6B1 cells. However, excess PIP_2 produced by PIP5KIγ inhibited regulated secretion accompanied by a halt in the endocytic recycling of secretory membrane (in the case of Arf6-activated PIP5KIγ) or by the perturbation of actin cytoskeleton remodelling (due to RhoA/ROCK activation of PIP5KIγ).

T5-02

Golgi to TGN transition is signal-mediated

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A widely accepted model of traffic across the Golgi Apparatus predicts the formation of a Golgi cisterna on the cis side and its anterograde movement to the trans side of the Golgi Apparatus. Resident proteins such as glycosyltransferases would constantly be retrieved by vesicular backflow to the site where they can be detected at steady-state by immunocytochemical methods. Glycosyltransferases eventually are released by post-Golgi proteolytic cleavage implicating a Golgi to TGN transition.
Here we show that this transition is mediated by a specific signal

located on the cytoplasmic domain of a subset of trans Golgi glycosyltransferases. In HepG2 cells stably transfected with constructs encoding galactosyl- or fucosyltransferase-GFP, resp., we show by time-lapse video-microscopy that these enzymes bud from the trans Golgi into numerous compact vesicles when cells were treated with bafilomycin A2, a specific inhibitor of vacuolar ATPase. Moreover, when cells were treated with monensin, a H+/Na+ exchanger, enzymes were trapped in TGN-derived osmotically swollen vesicles. Treatment with these two agents, therefore, defines a read-out system for Golgi to TGN transition. Interestingly, both enzymes carry a motif close to the N-terminus defined as E/DP which is necessary for this transition to occur. These data have been corroborated by dual labeling with giantin, a resident Golgi protein and fractionation showing redistribution of GFP chimeras in treated cells.
A bioinformatics analysis of this motif revealed its presence on a variety of post-Golgi proteins such as carriers, proteases and trafficking related proteins.

T5-03

Passive diffusion across lipid bilayers: the influence of charge from a thermodynamic view

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The ability to predict and interpret membrane permeation coefficients is of critical importance, particularly because passive transport is crucial for the effective delivery of many pharmaceutical agents to their targets. We present a thermodynamic study of lipid bilayer permeation of 2-hydroxynicotinic acid and related aromatic carboxylic acids over a wide pH range. Membrane permeation kinetics were determined between 5 and 50°C with a liposomal luminescence assay which is based on the energy transfer of the permeant to intraliposomal terbium(III) [1].

The effect of lipophilicity and hydrogen bonding capacity was evaluated in terms of entropic and enthalpic contribution to the permeation process. Interestingly, the permeation of the neutral and the anionic species of 2-hydroxynicotinic acid revealed the same enthalpy of activation while they differed in the entropy of activation.

From our data we conclude that the lower permeation rate of the anion is due to a lower degree of freedom within the lipid bilayer as compared to the neutral species. However, the difference is small enough to allow significant permeation of the anion. The activation energy for the opening of hydrogen bonds appears similar for both species.

[1] A.V. Thomae, H. Wunderli-Allenspach, S.D. Kramer. Biophys J. 2005; 89: 1802-1811.

T5-04

Influence of Myotubularin-Related-Proteins on Endosomal Sorting

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Myotubularin-related-proteins (Mtmrs) are phosphoinositides (PI)-metabolizing enzymes which specifically dephosphorylate PI-3-P and PI-3,5-P2 at position D3. The family consist of eight active and six inactive members and is defined by the presence of a phosphatase, a Pleckstrin Homoloy-GRAM, and a coiled-coil domain. Mutations in myotubularin (*MTM1*), the founding member of the Mtmr family, lead to X-linked myotubular myopathy, which is characterized by centronuclear muscle fibres. Mutations in Myotubularin-Related-Protein 2 (*MTMR2*) and Set-Binding Factor-2 (*SBF2/MTMR13*) genes lead to Charcot-Marie-Tooth disease type 4B1 and 4B2, respectively. The main hallmarks of this severe autosomal recessive hereditary neuropathy are demyelination, focally folded myelin sheets in the peripheral nerves and reduced nerve conduction velocity.
The substrate specificity of active Mtmrs suggests a function in endocytosis, sorting and degradation of proteins. PI-3-P and PI-3,5-P2 are anchor sites on endosomal membranes for effector proteins of early and late phase endocytotic processes. In mammalian cells PI-3,5-P2 is involved in the trafficking of receptor tyrosine kinases and channels.
We want to study, how Mtmrs regulate endocytosis and membrane dynamics of different receptor tyrosine kinases. Because Mtmrs interact with PDZ domain containing proteins via their PDZ-binding motif, we identify new PDZ interacting proteins.

Methods: PDZ Array, GST-Pulldown, Immunoprecipitation

T5-05

Multigene expression in mammalian cells

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The discovery and development of fluorescent proteins as reporter tags has revolutionized work in molecular and cell biology field and enables the monitoring of biological processes under the fluorescence microscope in real time. Cotransfection of cells with several plasmids leads to various ratios of the expressed fluorescency tagged proteins complicating biological analysis. Therefore, we adapt the MultiBac system developed by Fitzgerald et al. (2006) for mammalian expression. With adaptation of the system specifically for mammalian cells called MultiLabel, this new system allows the expression of several tagged proteins from a single vector. The system is fully modular allowing the rapid cloning of new destination vectors with different regulatory elements or tags. The introduction of the cDNA of interest will be compatible with the High Through Platform located at PSI.
At the moment, our MultiLabel expresses three differently tagged proteins. It is planed to assemble up to five vectors and to include elements for the generations of stable cell lines. In our study we concentrate on internalization, signalling and degradation of receptor tyrosin kinase upon ligand stimulation. For that purpose, several endosomal markers as well as sorting proteins, involved in endosomal cargo trafficking were tagged and combined in one MultiLabel expression vector.

T5-06

PRG-5, a novel member of the Plasticity Related Gene (PRG) family induces filopodia and axon growth

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Members of the Plasticity Related Gene (PRG1-4) family are brain-specific integral membrane proteins and are implicated in cellular plasticity, such as filopodia formation and axon growth after brain lesion. Here we report on the cloning of a fifth member of the PRG family named PRG-5 with high homologies to PRG-3. PRG-5 is regulated during brain development and is exclusively distributed within the nervous system. When expressed in neurons, PRG-5 is distributed in plasma membranes and induces filopodia formation as well as long neurite growth. Domain deletion and mutation studies revealed that membrane localization is essential for PRG-5 induced axon growth. Furthermore, most axon growth activity is located within the C-terminal tail of PRG-5. Silencing PRG-5 expression by siRNAs attenuates axon growth and filopodia formation. To investigate a putative role of PRG-5 in regeneration, we facilitated collapse assay with relevant neurite growth inhibition factors. Thus, axon collapse induced by lysophosphatidic acid (LPA) and myelin associated neurite inhibitors such as Nogo-A is

blocked in the presence of PRG-5. Thus, we describe the identification of a novel member of PRG family which induces neurite growth and interferes with Nogo-A neurite inhibition signaling.

T5-07

Screening for new actors implicated in Piecemeal Microautophagy of the Nucleus (PMN) in S.cerevisae

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Autophagy is a mechanism of transferring cytosolic proteins or organelles into lysosomes/vacuoles to be recycled. This recycling process is activated under starvation conditions in all eukaryotic cells or upon rapamycin treatment. Two different forms of autophagy can be observed: macro- and microautophagy.
Few years ago, a novel form of autophagy had been discovered in yeast cells: Piecemeal Microautophagy of the Nucleus (PMN). PMN is driven by formation of a nucleus-vacuole (NV) junction via direct interaction of the nuclear membrane protein Nvj1 and the peripheral vacuolar membrane protein Vac8 (step I). NV junction formation is followed by the emergence of a bulge carrying a part of the nucleus and invaginating into the vacuole (step II). The bulge develops into a tear drop like bleb (step III). Finally, a vesicle carrying nuclear material is released into the vacuolar lumen (step IV) to be degraded (step V).
In order to discover novel actors in the PMN process, we performed a large scale screening of the complete yeast KO collection from Euroscarf. The screen was based on the observation that the nucleolus (represented by nucleolar marker Nop1-GFP) was the preferential substrate for PMN in microscopy experiments upon rapamycin treatment. We took advantage of that observation to search in the KO collection for mutants defective in Nop1-GFP transfer to the vacuole. 316 gene deletions affected PMN to different extents, and we classified them into functional families. Here we present several complexes acting on the nuclear side, vacuolar side or implicated in lipid metabolism that showed strong inhibition of PMN. We describe at which step of PMN process these complexes are needed.

T5-08

A method for purification of distinct ER-derived vesicles.

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Different populations of vesicles arising from the endoplasmic reticulum (ER) are enriched for distinct cargo populations. This has been demonstrated *in vitro* biochemically (Muniz et al., 2001) and *in vivo* microscopically (Castillon et al., 2008). The mechanism and the function of this apparent segregation are not currently understood. We present a technique allowing purification of these distinct vesicle populations in this poster. Comparative techniques, such as mass spectrometry, will be performed on the purified vesicles. Lipid profiles and protein analysis of these vesicles populations will be determined.

Muniz *et al.* Cell 104(2):313-320, 2001.
Castillon *et al.* Traffic doi 10.1111/j.1600-0854.2008.00857.x

T5-09

Contribution of the vacuolar H$^+$-ATPase to different steps of membrane fusion analyzed in vitro and in vivo

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The membrane integral V0 sector of the vacuolar H+-ATPase (V-ATPase) is necessary for membrane fusion between yeast vacuoles (lysosomes) (Peters, 2001; Bayer, 2003; Baars, 2007), for secretion of neurotransmitters and insulin, phagosome-lysosome fusion and the secretion of multivesicular bodies. (Hiesinger 2005; Peri, 2008; Liegois, 2006; Sun-Wada, 2008).
The goal of the presented project is to investigate how V0 contributes to fusion and how it interacts with SNAREs. We utilize random mutagenesis to identify sites in V0 subunits that are selectively involved in vacuole fusion but dispensable for the proton pump function of the V-ATPase. Yeast cells were subjected to random mutagenesis and screened in vivo to identify mutant variants of V0 subunits that still function in proton pumping but cause fragmented vacuoles, indicative of a fusion defect. Fusion characteristics of selected mutants were analyzed in vitro with purified vacuoles (Reese et al., 2005).
Mutants with selective fusion defects were found in the proteolipids c and c' (Vma3p and Vma11p) and the d subunit (Vma6p). In the proteolipids critical mutations are concentated in the cytosolic half of the transmembrane domains and in the periphery. Content mixing and lipid mixing assays reveal a function of the proteolipid ring in the induction of lipid flow between the membranes as well as in fusion pore opening.
The results presented here show that V0 acts in several steps of the fusion process and map these contributions at the subunit or domain level. Mutations found in this screen will be instrumental in disentangling the interactions of the V-ATPase with SNAREs and in defining their contributions to membrane fusion.

T5-10

Active zone scaffolding protein SYD-2 regulates Kinesin-3 activity

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Kinesins are microtubule-based molecular motors carrying out various intracellular transport processes. The kinesin-3 member UNC-104 (in mammals KIF1A) is essential for the transport of presynaptic vesicles from soma to synapse in the nervous system of C. elegans. In vivo, UNC-104 can move fast and processive, however in vitro the motor appears slow and non-processive in single molecule motility assays. We hypothesized that UNC-104 interacting proteins might play a role in regulating the non-processive to processive transition. One molecular player that could fulfill this role is SYD-2 (synapse defective), a scaffolding protein organizing the architecture of a synapse. Here, we show the interaction of SYD-2 an UNC-104 in vitro by pull-down assays. We measured the velocity and processivity of the motor in vitro in the presence and absence of SYD-2. Our preliminary results show that SYD-2 increases the velocity of UNC-104 in a multi-motor assay (microtubule surface gliding assay) as well as on a single molecule level (analysis by TIRF microscopy).

T5-11
Myotubularins, Intermediate Filaments and Diseases

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The myotubularin protein family consists of eight active and six inactive phosphatases specific for phosphoinositides (PI-3-P, PI-3,5-P_2) that have been shown to play an important role in endosomal sorting. Three myotubularins have been linked to human diseases: Mutated myotubularin (MTM1) is responsible for X-linked myotubular myopathy, while mutations in MTMR2 or MTMR13/SBF2 lead to Charcot-Marie-Tooth disease (CMT) type 4B. Mtmr2 has been shown to interact with neurofilament light chain (NF-L), a protein mutated in CMT type 1F and 2E. An immunoprecipitation based screen revealed further intermediate filaments as potential interactors of Mtmr2. Since intermediate filaments have been shown to be crucial for the structure and localization of cellular organelles, they are particularly promising candidates.
In contrast so far only little is known about interaction partners of Mtmr13/Sbf2. In order to gain further insights into disease-relevant protein complexes and mechanisms, we searched for interaction partners of Mtmr13/Sbf2 using an immunoprecipitation as well as a Yeast Two-Hybrid based screen. Confirmed candidates were analyzed in detail. In parallel we studied the interaction network of myotubularins and intermediate filaments.

Poster Topic 6 – Others

T6-01
Tumor recovery by angiogenic switch from sprouting to intussusceptive angiogenesis after treatment with PTK787/ZK222584 or ionizing radiation.

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Background: Radiation and inhibitors of angiogenesis induce compensatory changes in the tumor vasculature not only during, but also after the treatment cessation. To assess the response to the treatment, the tumors were analyzed immediately after cessation of therapy and during the recovery phase.
Methodology: Mammary carcinoma allografts were investigated by vascular casting, electron, light, confocal microscopy and immunoblotting after fractionated irradiation or treatment with the VEGF-receptor tyrosine kinase inhibitor, PTK787/ZK222854.
Results: Irradiation and anti-angiogenic therapy had similar effects on the tumor vasculature in the recovery phase. Both treatments reduced microvascular density, particularly in the tumor medulla. After cessation of therapy, the tumor vasculature expanded predominantly by intussusception with a plexus composed of enlarged sinusoidal-like vessels containing multiple transluminal tissue pillars. Tumor revascularization originated from preserved SMA-positive vessels in tumor cortex. Quantification revealed that recovery was characterized by an angiogenic switch from sprouting to intussusception. The upregulated SMA-expression during the recovery reflected the recruitment of SMA-positive cells for intussusception as a part of angioadaptive mechanism. Tumor recovery was associated with a dramatic decrease (by 30-40%) in the intratumoral microvascular density, probably as result of intussusceptive pruning, surprisingly with only a minimal reduction of the microvascular area density. Therefore, the vascular supply to the tumor was sufficient as corroborated by HIF-1α-immunostaining.
Conclusion: Irradiation and anti-angiogenic therapy induce a switch from sprouting to intussusceptive angiogenesis as part of a compensatory response to preserve and restore perfusion. Intussusceptive angiogenesis with an associated low endothelial proliferation rate and permeability, may represent an escape mechanism and account for the development of resistance to therapy, as well as the rapid recovery of tumor vasculature after cessation of therapy.

T6-02
Regulation of sorting nexins by the ubiquitin ligase Itch in melanoma cells

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Ubiquitin ligases play an important role in regulating intracellular protein levels. Itch (AIP4) is a member of the NEDD4 family of E3 ubiquitin ligases. Substrate proteins that are targeted for degradation bind to one of the four WW domains of Itch through their PY motifs. We have identified Sorting Nexin 9 (SNX9) as a new substrate of Itch in melanoma cells. SNX9 belongs to a family of proteins sensing membrane curvature and is known to play a role in endocytosis of cell surface receptors. Ubiquitylation by Itch targets SNX9 for degradation. Unlike known substrates, we found that binding of SNX9 to Itch is mediated by the SH3 domain of SNX9 and that the proline-rich domain of Itch is essential for their interaction. This is the first example of a protein targeted for degradation through an interaction between the substrate's SH3 domain and the proline-rich domain of an ubiquitin ligase. Sorting

Nexin 18 (SNX18), a close relative of SNX9, is also an interaction partner of Itch.
Our data indicate that proteins can be targeted for degradation through an interaction with the proline-rich domain of Itch. While SNX9 presents a substrate of this type in melanoma cells, it is likely that more SH3 domain containing proteins may be regulated by Itch in the same manner.

T6-03

Phosphoproteins in teeth and their potential use for biomimetic coating of titanium implants

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The successful integration of dental implants in bone and soft connective tissues is still afflicted with major problems. There are several studies that suggest the use of biomimetic polypeptides in the design of prosthetic implants to improve the biointegration of the synthetic material.
The organic portion of teeth consists of collagens and a number of calcium-binding phosphoproteins. Recently our group has prepared a tooth specific cDNA library with a view to characterize the proteins involved in tooth formation. This effort led to the molecular cloning of ameloblastin, amelogenin, amelotin, enamelin, dentin sialophosphoprotein, dentin matrix protein, bone sialoprotein and osteopontin.
We created a system to test the tooth-specific proteins for their ability to promote cell adhesion of osteoblastic cells in vitro. Our preliminary results show, that the ameloblast-specific proteins facilitate attachment of cells to bacterial dishes and to titanium discs. Therefore it is conceivable that protein-coated titanium surfaces will improve the biointegration of dental implants in bone and soft connective tissues.

T6-04

Control of the DEAD-box RNA helicase eIF4A by eIF4G

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Translation initiation in eukarya is usually the rate-limiting and most tightly controlled stage of polypeptide synthesis. A set of about a dozen factors (eIFs) ensures efficiency and fidelity of this process [1]. A central component is eIF4F, a complex consisting of the cap-binding protein eIF4E, the ATP-dependent DEAD-box helicase eIF4A and a scaffold protein eIF4G. The structure of the complex of eIF4A and middle domain of eIF4G has been solved recently [2]. eIF4A shows an extended conformation where eIF4G holds its crucial DEAD-box sequence motifs in a productive conformation, thus explaining the stimulation of eIF4A's RNA helicase activity. Conformational changes between eIF4A's closed and open state provide a model for RNA-helicase activity and its enhancement by the interaction with eIF4G. In the open state there are three main interaction sites, one of them involves a flexible element in the N-terminal region of eIF4G's middle domain. The key residue in this site is Trp-579, where mutation to alanine decreases eIF4G's binding affinity for eIF4A significantly. Owing to its pocket shape and small surface, this interaction site might be a good target for the development of anticancer drugs. mRNAs containing long, structured 5'-UTRs, which are also present in several proto-oncogenes and somatic growth factors, have a high requirement for eIF4F. A possible strategy for anticancer drugs is therefore the development or identification of small-molecule compounds that are able to disrupt the eIF4F complex [3].

[1] Marintchev A et al. (2004) Q Rev Biophys 37: 197-284
[2] Schuetz P et al. (2008) PNAS 105: 9564-9569
[3] Kim WJ et al. (2007) EMBO J. 26: 5020-32

T6-05

***In vitro* models to determine the impact of parvalbumin expression on mitochondrial function and biogenesis in muscle.**

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The soluble Ca^{2+}-binding protein parvalbumin (PV) is expressed at high concentrations in vertebrate fast-twitch muscle fibers. Previous investigations have revealed that PV-deficiency in transgenic mice increases the mitochondrial content in fast-twitch muscles, while their fast-twitch phenotype is maintained with respect to contractile elements. In this work, we aim to understand the Ca^{2+}-dependent processes and the involved signaling pathways leading to either muscle fiber-type specific expression of proteins and/or pathways involved in mitochondria biogenesis.
For this we produced an in vitro model consisting of C2C12 skeletal mouse cells stably transfected with PV cDNA yielding robust PV expression in several clones. Controls and clones were subjected to different treatments affecting intracellular Ca^{2+} homeostasis.
Since mitochondria are also considered as transient Ca^{2+} stores in muscle cells, we first investigated whether the mitochondrial volume and/or mass in PV-transfected cells were changed in comparison to their wild-type counterparts, using respectively Imaris software for 3D reconstruction and flow cytometry for mass determination. Biochemical experiments were then performed to observe putative changes in levels of parvalbumin and factors linked to mitochondria biogenesis or implicated in related Ca^{2+} signaling. Finally, we examined the Ca^{2+} dynamics and the PV buffering role by Ca^{2+} imaging.
Initial experiments indicate that PV expression levels are modulated by increases in intracellular [Ca^{2+}]. Upon ionophore treatment, PV-transfected myotubes elevated parvalbumin protein expression but they didn't show adaptive variation in their mitochondrial mass, whereas their wild-type counterparts displayed a significant increase to cope with the increased Ca^{2+} load.

T6-06

***Drosophila melanogaster* as a model to study human neurodegenerative diseases**

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Drosophila melanogaster have been engineered to model human neurodegenerative diseases (e.g. Alzheimer's disease, Parkinson's disease, Huntington disease). Although instructive mouse models have been generated, the *Drosophila* models offer experimental advantages (e.g. short life span, forward genetic screens) that can potentially address some of the questions regarding the molecular mechanism underlying these diseases. Using *Drosophila* models, we focus on how metal homeostasis might influence Alzheimer's disease (AD) and Parkinson's disease (PD). We have generated a model of Alzheimer's disease (AD) by expressing human amyloid-β peptide, the component of the amyloid plaques in the brains of AD patients. To investigate how metal homeostasis and oxidative stress affect Aβ toxicity in vivo, we show in the transgenic *Drosophila* model that elevated Zn and Cu concentrations exacerbate Aβ-induced phenotypes. By contrast, supplementation of metal chelators or strong expression of metal scavengers suppress the toxic effects of Aβ. To study the connection between metal homeostasis and Parkinson's disease, a mutation of the parkin gene, the homolog of human parkin, has been used. It has been shown that mutations in human parkin cause recessive Parkinson's disease. *Drosophila* Parkin null mutants develop mitochondrial pathology and apoptotic muscle degeneration. They also show male and female sterility, locomotor defects, inability to fly, increased sensitivity to multiple stresses including oxidative stress, and a reduced lifespan. We were able to rescue parkin null mutant flies from oxidative stress by maintaining

them on metal chelator supplemented food. N-acetylcysteine (a precursor of the antioxident molecule glutathione) was also able to rescue the reduced longevity. The overexpression of metal-responsive transcription factor-1 (MTF-1) increased frequency of eclosure and rescued female fertility, and expanded lifespan. In conclusion, our results support the notion that an abnormal metal homeostasis in conjunction with oxidative stress contributes to the course of neurodegerative diseases.

T6-07

A Spectroscopic Approach for Monitoring the Assembly Pathway of the Vimentin Intermediate Filaments

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Our work provides a new type of analysis of the assembly mechanism of the type III IFs using near-UV circular dichroism (CD) spectroscopy. We demonstrate that by correlating the microscopically observed assembly conformations of vimentin intermediate filaments to the measured spectroscopic changes we are able to decode various amino acid configurations that occur throughout the IF assembly process. The latter includes lateral association of the tetrameric complexes to unit length filaments (ULFs), longitudinal annealing of ULFs to elongated open filaments and finally radial compaction into mature IFs (1-5).

In particular, we find three distinct near-UV CD signals of coiled-coil structures that arise from specific associations of the individual coiled-coil subunits. The first signal displays two main negative peaks at 278 and 286 nm that are attributed to the tyrosine residues. It is obtained for all vimentin complexes that appear at the early assembly stages, i.e. dimers, tetramers and ULFs, and it closely resembles the near-UV CD signature of tropomyosin (6). We can therefore conclude that there are neither any significant conformational changes in the tertiary structure of the coiled coils, nor any new interactions among the aromatic amino acids of the adjacent coiled coils appearing as a result of the lateral annealing of the IF subunits.

Upon elongation of the ULFs, the 278-nm band disappears. The loss of this band is attributed to the fact that the tyrosines – which reside mainly in the head and coil 2B domains – become rigid due to reduced conformational freedom. Therefore, the emergence of strong interactions between the head and subsequent coil 2B domains during elongation is favoured.

The maturation of the filaments – seen in electron microscopy images as compaction that occurs ~13 min after initiation of the assembly process – displays a distinct spectroscopic signature with a strong negative band appearing at 291 nm. The rise of this band has a lifetime of ~13 min for the wt vimentin, which agrees perfectly with the microscopic results. We have thus obtained an unambiguous spectroscopic signature for IF maturation. The appearance of the 291-nm band attests the generation of phenolates, stemming from the re-localization of the tyrosines into a new, strongly charged environment. The formation of these phenolates stabilizes the intermediate filament structure and concludes the assembly process.

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T6-08

Characterization of molecular mechanisms targeting PKA to cTnI in cardiomyocytes.

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The activity of the heart is modulated by the sympathetic system through the activation of β-adrenergic receptors that downstream activate the cyclic AMP-dependent protein kinase, PKA. This is an ubiquitous, broad specificity, serine/threonine kinase, that after β-adrenergic stimulation phosphorylates a set of different substrates within the cardiomyocyte, changing the contractrile properties of the cell. Phosphorylation of cardiac troponin I (cTnI) by PKA represents a physiological mechanism for alteration of the myofilament properties, decreasing myofibrils Ca 2+ sensitivity and increasing crossbridge kinetics. Due to its inespecificity, protein kinase A must be precisely targeted in proximity to its cellular substrates. Evidence collected over the last years demonstrates that compartmentalization of the kinase is achieved through the association with A-kinase anhoring proteins (AKAPs). These scaffolding proteins share the capacity to bind the regulatory subunits of PKA inside cells and thanks to unique targeting domains contained within each AKAP, PKA is localized at specific subcellular regions. Several AKAPs have been identified in adult cardiac myocytes with roles as important as the control of calcium handling and cardiac contractility, the action potential duration or the regulation of cardiomyocyte hypertrophy. The main purpose of the present study is to elucidate the functional role AKAPs in the targeting of PKA to cTnI in cardiomyocytes. By the incubation of a primary culture of neonatal rat cardiomyocytes with molecular disruptors of the interaction between PKA and AKAPs, we could observe a decrease in the phosphorylation of cTnI after b-adrenergic stimulation, suggesting that AKAPs are crucially involved in the targeting of PKA to cTnI. Based on these findings, we next investigated whether we could detect the presence of subunits of PKA subunits as well as AKAPs in sarcomere enriched preparations. Our results indicate that regulatory and catalytic subunits of PKA as well as different potential AKAPs are physically associated with the sarcomeric fractions. Future studies are aimed at identifying which of these anchoring proteins targets PKA to cTnI.

T6-09

Characterization of sterol acetate binding proteins

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Sterol homeostasis in eukaryotic cells relies on the reciprocal interconversion of free sterols and steryl esters. Steryl esters contain a long chain fatty acid esterified to the hydroxyl group of the sterol and serve as a storage form of metabolic energy. Recently a novel sterol cycle was identified in Saccharomyces cerevisiae. The reciprocal interconversion of free sterol into sterol acetate. Radiolabelling of a yeast mutant lacking SAY1 with [14C]cholesterol revealed the presence of a novel cholesterol derivative that was identified as cholesterol acetate. Accumulation of cholesterol acetate in the say1Δ mutant depends on the presence of the acetyltransferase Atf2, indicating that Say1 and Atf2 control the cycling of sterols between free and acetylated forms. The acetylated cholesterol which is formed in the ER lumen is secreted into the culture supernatant in cells lacking SAY1 (Tiwari et al EMBO J. 2007 Dec 12;26(24):5109-19).
How the cholesterol acetate which is more hydrophobic than cholesterol itself is soluble during its export as well as in the culture supernatant is unknown. We postulate the presence of a cholesterol acetate binding protein, which may bind cholesterol acetate in the ER lumen and renders it soluble outside the cell. To

identify such a protein, a cholesterol acetate binding activity was enriched by ammonium sulfate precipitation from the supernatant of say1Δ mutant cells. This activity is lost if the culture supernatant is heated for 30min at 95°C or by treatment with proteases, consistent with the presence of a heat-labile likely proteineous binding activity. The cholesterol acetate binding activity could be further enriched by isoelectric focusing (IEF). Proteins present in a particular IEF fraction were separated by SDS-PAGE and analysed by mass spectrometry.
Candidate proteins are now being examined by determining whether deletion of the corresponding gene affects export of cholesterol acetate from say1Δ mutant cells.

T6-10

Automatic acquisition of large amounts of 3D data at the ultrastructural level, using serial block face scanning electron microscopy

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Serial block face scanning electron microscopy (SBFSEM) is a new technique designed to obtain image slices through specimens inside a scanning electron microscope (SEM) in an automated process. Biological tissue in particular, is prepared by classical en-block staining and resin embedding as used for many years for transmission electron microscopy.
Producing serial sections for TEM is well known, but the technique has been naturally restrained by the technical difficulty as well as the small volumes visualised. SBFSEM is a new method that increases the 3D resolution beyond the level of light microscopy without the restrictions and difficulties of serial sections. This is done by cutting an entire block of tissue inside a SEM in order to image each freshly exposed surface, sequentially after each cut. This technique was first developed by Denk and Horstmann in order to reconstruct neuronal tissue in three dimensions.
We show now that SBFSEM is suitable for numerous tissue samples, as long they are processed by en-block staining and embedded in epoxy. We also show that not only animal tissue, but also plant tissue and cell in cultures can be studied in 3D. Multiple examples of applications of the SBFSEM to generate image stacks from significant volumes of material are shown.
For example, a volume through the CA1 region of the hippocampus of 17x17x50 microns has been obtained overnight. Synapses can be observed and counted.
All these data require afterwards to be analysed and visualized using powerful softwares that allow to handle a large amount of data as well to generate meaningful data.
As a new technique, SBFSEM offers the opportunity to look at large volume with an electron microscope as it was not possible before.

T6-11

Direct association of lamin A with the nuclear pore protein Nup88

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The nuclear intermediate filament protein lamin A is a constituent of the nuclear lamina, which is engaged in the organization of heterochromatin and nuclear architecture and is providing a platform for protein complex assembly. Mutations in the lamin A gene are associated with a diverse array of human diseases termed laminopathies. One particular point mutation in the Ig-fold, i.e. R453W, is leading to Emery-Dreifuss muscular dystrophy (EDMD). Distinct components of the nuclear lamina are interacting with nuclear pore complexes (NPCs), which are known to mediate all macromolecular trafficking between the nucleus and cytoplasm. Here, we have identified the nucleoporin Nup88 as novel interaction partner of lamin A by GST-pull down experiments using HeLa extracts. Furthermore, solution-binding assays revealed that the N-terminus of Nup88 specifically binds to the Ig-fold of lamin A, but not of B-type lamins. The interaction between lamin A and Nup88 was confirmed in situ by immunoprecipitation assays in HeLa cells. Moreover, we found that overexpression of GFP-lamin A is masking the binding site of Nup88 antibodies in immunofluorescence assays, supporting the interaction of lamin A with Nup88 in a cellular context. We further demonstrate that the epitope masking phenotype is lost in cells overexpressing GFP-lamin A (R453W) responsible for EDMD, indicating a putative role for Nup88 in laminopathic diseases.

T6-12

Endocytosis is required for fatty acid uptake in yeast

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Saccharomyces cerevisiae is a facultative anaerobic organism. Under anaerobic conditions, cells become auxotrophic for unsaturated fatty acids and sterols because de novo synthesis of these essential lipids depends on the presence of molecular oxygen. The mechanisms that control uptake of sterols and fatty acids are not well understood. We have previously performed a systematic screen to identify genes that are required for growth under anaerobic conditions {Reiner et al., 2006, Mol Biol Cell, 17, 90-103}. In this study, we analyzed these mutants for defects in uptake of fatty acids. This analysis revealed that mutants that affect endocytosis, such as YPK1, VRP1, END3, display reduced uptake of fatty acids, indicating that endocytosis is important for uptake of fatty acids. These observations suggest that uptake of fatty acids is coupled to membrane internalization and suggest that in yeast fatty acid uptake is an active process that is not efficiently bypassed by passive diffusion or “vectorial acylation”.

T6-13

Fetal-antigen-1 administration promotes survival and /or differentiation of dopaminergic neurons in ventral mesencephalic cultures.

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Fetal antigen-1 (FA1/dlk) a member of the epidermal growth factor-like family that can exert its actions both as a soluble or a transmembrane protein. FA1/dlk has been proposed to be a growth and/or differentiation factor expressed in immature cells during development. Furthermore, FA1/dlk has been shown to be expressed in the ventral mesencephalon (VM) and has been suggested as a potential alternative marker protein in dopaminergic neurons, which is of particular interest in relation to Parkinson's disease. Other and we have previously shown that glial cell line-derived neurotrophic factor (GDNF) treatment increased density of tyrosine hydroxylase (TH) positive neurons in cultures of the rat ventral mesencephalon (VM). Importantly, we observed that GDNF administration also resulted in an increase in FA1/dlk protein levels in the culture medium as compared to controls. This latter observation let us to hypothesize that FA1/dlk may act in combination with GDNF or alone as a survival factor for cultured VM neurons. Hence, in the present study we aimed at investigating the effects of FA1/dlk administration on densities of TH-immunoreactive (-ir) and Nurr1-ir neurons. In addition, we addressed the effects of FA1/dlk exposure on the number of PCNA positive cells. For that purpose primary cultures of fetal rat VM (E14) were prepared and grown for five days in absence (controls) or presence of FA1/dlk [50, 500 and 5000ng/ml]. Our preliminary results revealed that FA1/dlk exposure at all doses investigated increased densities of Nurr1-ir neurons (by 1.3 fold) as compared to controls. Similarly, densities of the TH-ir neurons were observed

to be slightly increased after FA1/dlk administration (by 1.2 fold). Moreover, the finding of a tendency for higher numbers of PCNA-ir cell in the FA1/dlk treated cultures as compared to controls hints to the idea that cell proliferation was affected in a subpopulation of cells only. In sum, the demonstration that FA1/dlk treatment influenced dopaminergic cell densities points to an important role of this protein during midbrain development. In the same line, these findings may also have impact for the treatment of Parkinson's disease.

T6-14

Indolinone from Isatis tinctoria blocks mast cell degranulation

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Mast cells are resident in many tissues and have critical biological functions. Antigens interacting with IgE bound to high affinity Fc recetors on their surface may lead to degranulation, resulting in the release of histamine, proteoglycans and serine proteases from secretory vesicles, contributing to allergic inflammation.
At the core of the inflammatory response are the class I phosphoinositide-3 kinases (PI3Ks) that phosphorylate PtdIns(4,5)P2 to PtdIns(3,4,5)P3, which then serves as a docking site for proteins containing a pleckstrin homology domain, such as PKB/Akt (1).
Isatis tinctoria L. (woad, Brassicaceae) is an ancient European dye and medicinal plant. Several anti-inflammatory constituents have been identified so far, namely tryptanthrin, α-linoleic acid, indirubin (2), and more recently, indolinone (1,3-dihydro-3-[(4-hydroxy-3,5-dmethoxy-phenyl)methylen]-2-H-indol-2-one), that efficiently blocked histamine release in rat peritoneal mast cells (3).
To elucidate underlying signalling processes, we studied the impact of indolinone on degranulation and activation of murine bone marrow derived mast cells. Indolinone abolished IgE-triggered degranulation and blocked adenosine-induced formation of PtdIns(3,4,5)P3. Furthermore, downstream events such as phosphorylation of PKB/Akt, MEK and ERK1/2 were blocked, whereas phosphorylation of PKC remained unaffected.

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T6-15

Severe reduction of dendritic tree size does not protect Purkinje cells from excitotoxic death

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The development of the dendritic tree of a neuron is a complex process which is modulated by signals from afferent fibers. We have previously shown that chronic activation of metabotropic glutamate receptors (mGluR) or Protein kinase C (PKC) in organotypic cerebellar slice cultures severely inhibits the growth and development of the Purkinje cell dendritic tree. A potential physiological role of this growth inhibition by mGluR activation might be to limit the size of the Purkinje cell dendritic tree in order to reduce the number of excitatory inputs and available AMPA receptors to make the cell more resistant to excitotoxic death. We have studied whether the strong reduction of the dendritic tree size might confer an increased resistance to excitotoxic death to Purkinje cells. Purkinje cells grown in slice cultures for approximately 12 days were challenged with an exposure to 30 micromolar AMPA for 2 hours, and were left to recover for another two days. This AMPA exposure resulted in a massive excitotoxic cell death of Purkinje cells. Alternatively, Purkinje cells were grown in the presence of the mGluR agonist DHPG or of the PKC stimulator PMA for 11 days which resulted in a strong reduction of the size of the dendritic tree. These cells were allowed to recover from this treatment for 24 hrs, before they were challenged with the AMPA exposure. Despite the severe reduction of dendritic tree size these cells were equally sensitive to AMPA exposure as compared to cells from untreated control cultures. This result indicates that it is not simply the number of available AMPA receptors which determines the sensitivity of Purkinje cells to excitotoxic death.

T6-16

Structure and stability of the head-to-tail overlap in human nuclear lamins.

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The nuclear lamins constitute a special class of intermediate filaments (IFs) found in the nucleus of eukaryotic cells. They form a meshwork-like structure (nuclear lamina) that is stabilizing nuclei. Nuclear lamins are also interacting with a multitude of factors from inner nuclear membrane and chromatin. In humans three types of nuclear lamins are identified: A, B1 and B2. All types of lamins are sharing same structural futures: short unstructured head domain, dimeric coiled-coil rod domain and unstructured tail domain containing also a Ig globular domain. The first step in the nuclear lamin filaments assembly is the longitudinal head-to-tail overlap of the primary lamin dimers. To elucidate the structure of this overlap we have used minimization approach: generating small (5 heptads or 35 amino acids) C- and N-terminal fragments, to study their interaction using analytical ultracentrifugation. On the other hand we have also estimated the size of this overlap using electron transmission microscopy (TEM). In this case longer N- and C-terminal fragments were generated. We have shown also that the first 24 amino acids of the unstructured head domain have a big impact on the head-to-tail complex formation. Moreover, we have measured the stability of these complexes using surface plasmon resonance (SPR) technique. It was determined that the complexes formed between quite short N- and C-terminal fragments are quite stable. However, the deletion of the first 24 amino acids of the unstructured head domain significantly destabilizes this complex.

T6-17

Remodeling of cytoplasmic actins during Paramyxovirus infections: a process induced by the matrix protein to optimize virus particle production?

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Cytoplasmic actins have been found interacting with viral proteins and identified in virus particles. However, the role they play in the context of viral infections remains poorly studied, particularly when it comes to assessing a role for the two cytoplasmic actin isoforms, the β- and γ -actins. Taking advantage of newly produced specific antibodies, we analyzed by confocal microscopy the pattern of β- and γ -cytoplasmic actins in the course of Paramyxovirus infections. Upon Sendai virus (SeV) infection of polarized cells, we observed a spectacular remodeling of the -cytoplasmic actin accompanied by a more subtle re-organization of the γ -cytoplasmic actin. This remodeling was related to a productive viral multiplication but not to a persistent non productive infection where SeV matrix (SeV-M) protein is highly unstable. Both isoforms were associated with virus particles and their concomitant suppression

resulted in a significant decrease in virus production. Similar actin remodeling was not observed upon vesicular stomatitis virus (VSV) or with human parainfluenza virus type 5 (HPIV5) infections. Finally, SeV-M protein was found to promote drastic changes in cell morphology when transiently expressed. These effects were not observed upon expression of a non functional M mutant or of a M protein mutated in a putative actin binding domain. We propose that cytoplasmic actin remodeling is induced by the Sendai virus M protein to promote efficient virus particle production. β- and γ - cytoplasmic actins recruitment could substitute for lack of recruitment of the endosomal sorting complex required for transport (ESCRT) seen for other viruses.

T6-18

The E3 ubiquitin ligase Bre1p protects against DNA replication stress and apoptosis in *S.cerevisiae*

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Inhibitor-of-apoptosis proteins (IAPs) are able to inhibit apoptosis due to the presence of two zinc-binding motifs, i.e. baculovirus IAP repeat (BIR) and RING domains. RING domains can recruit E2-ubiquitin conjugating enzymes and catalyze the transfer of ubiquitin onto target proteins. In search for RING domain containing proteins that might be implicated in yeast apoptosis, we show here that the E3 ubiquitin ligase Bre1p is able to protect against apoptosis. Bre1p is known to be required for the ubiquitination of histone H2B on lysine 123 and only recently ubiquitination of histone H2B by Bre1p was found to be required for effective G1 checkpoint activation in response to DNA damage.
A tight G1 arrest during chronological aging, an aging process in long-term cultivated yeast cultures, which leads to physiologically induced apoptosis in yeast, protects yeast from DNA replicaton stress and extends chronological lifespan.
Here we disentangle the role of Bre1p in apoptosis and cell cycle regulation and propose a Bre1p mediated G1 checkpoint activation during chronological aging that protects against age induced apoptosis.

T6-19

Computational Dissection of Adenovirus Motion on the Cell Surface

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The earliest steps of virus host cell interactions are incompletely understood. In the case of human adenoviruses they involve primary virus attachment to the coxsackie virus B adenovirus receptor CAR, or the membrane cofactor CD46. Secondary interactions with alpha v integrins are required for clathrin and dynamin mediated endocytic uptake, which leads to infection. CAR is a type one transmembrane protein involved in cell-cell adhesions on the basolateral domain and in tight junctions. It is unknown how the virus switches from CAR to integrins and engages in endocytosis. Here we are using live fluorescence imaging at high temporal resolution in combination with single particle tracking algorithms to systematically map the movements of Adenovirus type 2 (Ad2) on human epithelial cells. We find that individual virus trajectories are of considerable heterogeneity. To unravel the information contained within these trajectories, we developed a novel trajectory segmentation protocol based on supervised support vector classifications. This approach revealed three distinct motion types, diffusion, drifts and confined motions on both filopodia and the cell body. Upon attachment, Ad2 particles were mostly randomly diffusive, before they processively drifted or were confined. Adenovirus drifts and infection required filamentous actin and myosin 2. The absence of CAR reduced Ad2 drifts whereas alpha v integrin depletion increased the drifts, suggesting a competition between CAR and integrins in viral surface motions. Fluorescence recovery after photobleaching of GFP-CAR confirmed the high surface motility of CAR. Our data suggest a model where CAR binding allows Ad2 to scan the plasma membrane while alpha v integrin engagement traps the virus at sites that can lead to endocytosis. This work shows how a combination of live fluorescence imaging and computational analyses can reveal new features of the plasma membrane organization, which are important for infectious virus uptake.

T6-20

Functional domains of the metal-responsive transcription factor 1 (MTF-1) important for nucleo-cytoplasmic distribution, transcriptional activation and dimerization

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The metal-responsive transcription factor 1 (MTF-1) is a zinc finger transcription factor that regulates its target genes in response to diverse stress signals such as metal exposure, oxidative stress or hypoxia. It is conserved from insects to mammals. Target genes include metallothioneins, encoding small cysteine-rich proteins that bind and thereby detoxify heavy metals, as well as the zinc transporter 1 (Znt1) and placental growth factor (PLGF), encoding an angiogenic protein. In non-stressed cells MTF-1 resides in the cytoplasm and the nucleus and quantitatively accumulates in the nucleus upon stress. The nucleo-cytoplasmic distribution is regulated by distinct motifs, an NES located in the acidic activation domain of MTF-1 and a sequence important for nuclear localization embedded in the DNA-binding domain. Amino acid substitutions within the NES impair both nuclear export and metal inducibility. However a refined analysis reveals that nuclear export is not a prerequisite for metal inducibility. Nuclear accumulation upon zinc, as observed for wildtype MTF-1 is impaired by deletion of a segment spanning two of the six zinc fingers. This segment fused to GFP2x confers constitutive nuclear accumulation to the otherwise equally distributed protein, suggesting an NLS is embedded within the zinc finger DNA-binding domain of MTF-1. However, this sequence does not resemble any canonical or non-canonical NLS identified so far.
A highly conserved cysteine cluster ("CQCQCAC") located in the C-terminus is necessary for homodimerization of MTF-1. A mutant protein in which all four cysteines are replaced by alanines is highly impaired in transcriptional activity, but does not show any defects in nucleo-cytoplasmic distribution nor DNA-binding activity. Using a two-hybrid experimental setup we could show that homodimerization is not further increased by metal treatment, indicating that it is not involved in metal sensing. We propose that dimerization is important to create a protein interaction surface and that the recruitment of transcriptional cofactors is impaired in the cysteine cluster mutant.

T6-21

Regulatory pathways of pregnancy-induced remission and post partum relapse of rheumatoid arthritis

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Rheumatoid Arthritis (RA) is a systemic autoimmune disease. During pregnancy there is a remission of the symptom. About 4 to 12 weeks post partum the disease relapses.
The main goal of this project is the exploration of both the remission and the relapse in order to find possibilities to prevent the relapse.
A great advantage is the fact that during pregnancy no medication is taken, which rules out the bias of influencing the results in this point.
For this study whole blood samples from pregnant healthy women and pregnant patients at different stages of their pregnancy where collected (1., 2., 3. Trimester, 6 weeks post partum oder at flare).

With methods like q-PCR and microarray analyses and most recent bioinformatic tools we aim to find targets that are responsible for remission or relapse of the disease.

T6-22

Assigning the membrane partitioning and translocation processes to lipid bilayer permeation kinetics of small aromatic carboxylic acids

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The mechanism of permeation through lipid bilayers of drug-like compounds is not resolved in detail. Being able to understand all underlying processes and how they are influenced by the physicochemical characteristics of the permeant would improve the prediction of the in vivo behaviour of substances.
We used the terbium(III) liposomal assay [1] to shed light on the permeation processes of 2-hydroxynicotinic acid, salicylic acid, chlorinated analogues of the latter and aminosalicylic acids at pH 6.5, 0.21 M ionic strength and 25°C. Egg phosphatidylcholine liposomes were loaded with terbium(III) and incubated with the test compound. On permeation through the lipid bilayer, the test compound ligates the luminal terbium(III) and a characteristic luminescence signal is detected.
Most permeation kinetics followed bi-exponential functions with rate constants between 0.2 and 4.0 s-1 for the fast phases (k_1) and between 0.02 and 0.14 s-1 for the slow phases (k_2). To assign the single rate constants to the partitioning and translocation processes, we varied the liposome size and concentration in the permeation assay. According to our model, the rate constants of the single processes depend to different extents on the ratios of inner or outer aqueous volume (V) to the barrier area (A).
The k_1 values of the bi-exponential permeation kinetics decreased linearly with increasing liposome diameters between 70 and 210 nm at a constant lipid concentration. However, the decrease was lower than expected from the relationship between the observed k, the permeation coefficient (Perm), A and V, k = Perm x A / V [1]. The k_2 values did not show such a clear size-dependence and were size-independent for some compounds. Extrapolation of k_1 to liposome sizes > 500 nm revealed $k_1 < k_2$. These data indicate that the ratio of V to A may determine whether partitioning or translocation is rate-limiting for reaching the equilibration of permeant concentration in all aqueous compartments.
Increasing the liposome concentration at a constant liposome size, i.e. reducing the ratio of outer V to A (at a ~ constant outer V), resulted in an increase in k_1. This indicates that k1 is significantly influenced by the partitioning process from the outer water phase into the outer lipid leaflet. The k_2 values increased up to a lipid concentration of 2 mg/ml and decreased at higher lipid concentrations up to 12 mg/ml. The k_1 of all compounds were ≥ 10 fold higher than the respective k_2 over the tested lipid concentration range and the kinetics were independent of the permeant concentrations.
Based on these findings and on the membrane partition coefficient of salicylic acid, we assign the slow phases to the flip-flop process between the two lipid leaflets and the fast phases to the partitioning process from the outer aqueous phase into the outer lipid leaflet. We are currently comparing the observed rate constants at different pH values to investigate the influence of permeant charge on the fast and slow rate constants.

Reference:
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T6-23

SV40- and asbestos-induced upregulation of calretinin protects mesothelial cells from cytotoxicity and may lead to mesothelioma carcinogenesis

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Malignant mesothelioma is an aggressive fatal tumor originating from mesothelial cells and is frequently associated with exposure to asbestos. Evidence is growing that SV40 virus might be involved in the etiology of mesothelioma by acting as a co-carcinogen. MeT-5A GE cells (immortalized cells of mesothelial origin), were transfected with SV40 early region genes. Only in clones with high expression levels of T large antigen (Tag), calretinin (CR) expression was considerably upregulated in comparison with the parental cell line. Incubation with asbestos fibers showed that clones with high CR expression levels had a higher survival rate. To directly assess the putative cytoprotective role of CR, cells were stably transfected with a CR expression plasmid. The survival rate was highest in clones with high CR expression levels and lower in the "low CR" and the mock-transfected control groups. Down-regulation of CR by antisense oligonucleotides or siRNA eliminated the protective effects of CR. Thus, CR appears as a major factor contributing to the resistance to asbestos toxicity.
Perturbations of signaling pathways play an important role in human cancer and the PI3K/AKT pathway is activated in many tumors including malignant mesotheliomas. Asbestos treatment of SV40-, CR-transfected and control cells resulted in approximately 50% increased pAKT levels in all clones. Adding the PI3K/AKT inhibitor PI103 to asbestos-treated cells reduced cell viability significantly in clones with high CR-expression (SV40- and CR-transfected clones), but not in the low CR-expression control group indicating that only in high CR-expressing clones the AKT pathway is involved in the protective mechanisms against crocidolite. Almost identical results were obtained with the PI3K-specific inhibitor ZSTK474. Inhibition of the mTOR signaling pathway by rapamycin reduced cell survival in all tested clones indicating that neither Tag nor CR were involved in this effect. The involvement of the ERK1/2 pathway was also investigated using the inhibitor PD98059. No effect could be detected in all groups of clones.
Elevated CR levels in mesothelial cells may be the common underlying cause leading to the increased resistance to signals (e. g. asbestos fibers) normally leading to cell death. By such a mechanism, affected mesothelial cells may escape senescence, accumulate additional mutations finally leading to a fully transformed state.

T6-25

nNOS isoforms in non-diseased and dystrophic skeletal muscles of mice and humans

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In skeletal muscles of mice and humans, neuronal nitric oxide synthase (nNOS) is present at high concentrations in the sarcolemma of skeletal muscle fibers. In comparison to non-diseased muscles, dystrophic muscles contain lower levels of nNOS. Although various isoforms of nNOS have been characterized by immunoblotting, the primary structure of these variants has not been elucidated so far. Therefore, we analysed the expression patterns of nNOS isoforms in skeletal muscles of C57BL/6 wild type (WT) and mdx mice as well as in skeletal muscles of non-diseased humans at the mRNA level.
Exon specific primer pairs were used to cover the complete nNOS sequence by RT-PCR. Most striking, three alternative splicing events were observed in mice in the nucleotide range between exon 1 and exon 4. First, a 150 bp long nucleotide sequence was

detected between exon 1 and 2, which was designated exon pi. This exon pi, which is alternatively spliced, has not been described before. Second, the original exon 2 was found to consist of two separate nucleotide sequences, a 270 bp long exon rho containing the initiation codon and a 660 bp long exon sigma. Third, the original exon 3 was designated exon tau. Exon rho, exon sigma and exon tau represent alternatively spliced exons. Due to this alternative splicing, three different mRNA variants are expressed in skeletal muscles of mice. Two of these mRNA variants contain the initiation codon and thus encode for the alpha- and beta-isoform at the protein level. The exon structure of these isoforms is: 1c-pi-rho-sigma-tau-2 and 1c-pi-rho-2. Both mRNA variants were also detected in quadriceps muscle of mdx mice, but exhibited exon 1a instead of exon 1c. In vastus lateralis muscles of non-diseased humans, an expression pattern of nNOS mRNA isoforms was found that resemble that of WT mice. Additional splicing events were found in the nucleotide sequence between exon 16 and exon 17 in mice and humans, where a 102 bp exon was inserted in some variants (exon mu). The ratio between mu- and non-mu-isoforms was about 50:50 in mice and 90:10 in humans.
Taken together, our RT-PCR analysis revealed five mRNA nNOS variants to be expressed in skeletal muscles of WT and mdx mice as well as non-diseased humans that contain the genetic information for four different proteins. The different nNOS protein isoforms might have varying functions. In further experiments, the expression pattern of nNOS isoforms in skeletal muscle of patients with Duchenne muscular dystrophy (DMD) will be analysed by RT-PCR.

T6-26

Overexpression of glutathione peroxidase downregulates ERK phosphorylation after hypoxic preconditioning / cerebral hypoxia-ischemia

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We have previously shown that overexpression of human glutathione peroxidase (GPx)1 protects the developing murine brain to hypoxia-ischemia (HI)-induced injury, indicating that hydrogen peroxide (H_2O_2) plays an important role in the pathogenesis of neonatal HI-mediated brain injury. Intriguingly however, we recently showed that overexpression of GPx1 prevents neuroprotection induced by hypoxic preconditioning, i.e. ischemic tolerance induced by a non-lethal hypoxic stimulus several hours before initiation of HI. Since this type of preconditioning protection is dependent on transcriptional changes, we speculated that overexpression of GPx inhibited signaling events involved in the altered transcriptional response, which are dependent on small, not-lethal concentrations of H_2O_2. Hypoxia-induced ischemic tolerance in the neonatal brain has previously been shown to involve enhanced signaling of the extracellular signal-regulated kinase ERK1/2. Since it is known that ERK1/2 can be activated by H_2O_2, we hypothesized that GPx overexpression reduces the activation of ERK1/2 after preconditioning hypoxia, and possibly also after HI. Indeed, while preconditioning hypoxia in wildtype animals induced a transient increase in ERK1/2 phosphorylation 30 min after returning to normoxia, ERK1/2 activation in GPx overexpressing animals was significantly dampened and fell 50% below baseline levels 24 h after preconditioning. Interestingly, induction of hypoxia-inducible factor (HIF)-1α, which has been implicated in the protective response mediated by hypoxic preconditioning, occurred before activation of ERK1/2 and did not appear to be affected by GPx overexpression. Preliminary results also suggest that GPx overexpression inhibits the preconditioning-mediated augmented activation of ERK1/2 after HI. In summary, these results strongly suggest that H_2O_2 is not only involved in HI injury, but is also an important for signaling hypoxia-induced ischemic tolerance. It is therefore important to know whether H_2O_2 produced after HI may not only have detrimental effects, but also induces endogenous brain repair processes.

Supported by National Institute of Health grant 2R01-NS33997-10A1

T6-27

Reactive oxygen species are involved in AMPK activation in pancreatic beta cells.

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Introduction : AMP-activated protein kinase (AMPK), is an intracellular energy sensor, and is stimulated by increased of cellular AMP level. However, some studies showed that AMP-kinase could be activated independently of AMP changes. AMPK is also activated during treatment of diabetes by metformin. Such an activation decreases insulin secretion and increases beta cell apoptosis. The aim of this work was to study the activation of AMPK in INS1E cells at low glucose concentration.
Material and Methods : INS1E cells were exposed during short periods (15min to 2h) to low glucose concentrations in RPMI medium. The superoxide production was evaluated using the intracellular fluorescent dye dihydroethidine, DHE. ATP, ADP and AMP levels were measured by HPLC. The phosphorylation state of AMPK, its upstream kinases (CaMKK and LKB1) and its targets (acetyl-CoA carboxylase and the substrate of mTOR, p70S6K) were studied using western blot.
Results : After less than 15min at low glucose (1mM), INS1E cells start to produce increasing levels of superoxide during at least 1h. This superoxide production was reduced by the complex I inhibitor rotenone but not by inhibition of the complex III or NADPH oxidase. The ATP/AMP ratio was altered only after 90min at low glucose while AMPK and ACC phosphorylation doubled after 15min and increased in parallel with the rate of superoxide production. The phosphorylation of p70S6K decreased when that of AMPK increased. Moreover, AMPK phosphorylation was decreased by a ROS scavenger MnTBAP, which did not alter ATP, ADP or AMP level. Low H2O2 concentrations also induced an increase of AMPK phosphorylation. The phosphorylation of AMPK was not altered by the inhibition of CaMKK, and the phosphorylation of LKB1 (Ser307 or Ser428) neither followed nor preceded that of AMPK, and was not altered by ROS scavenger.
Conclusion : In pancreatic beta cells, superoxide production by the mitochondria at low glucose concentration plays an important role in the activation of AMPK, independently of AMP level. This activation is due neither to LKB1 nor to CaMKK. The activation pathway of AMPK by radicals deserves further investigations.

T6-28

Effect of oxygen-glucose deprivation / hypoxic preconditioning on brain microvascular endothelial cells in vitro

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Dysfunction of the blood-brain barrier plays an essential role in the pathogenesis of brain injury caused by stroke (“cerebral ischemia”). Previous studies have shown that a non-lethal episode of hypoxia (“preconditioning”) protects the brain from subsequent ischemic injury. Protection is not only afforded by events that take place in the brain parenchyma (i.e. in neurons or glia), but also in cerebral microvessels. Here, we studied the effect of oxygen and glucose deprivation (OGD), which mimics ischemia in vitro, on the survival of brain microvascular endothelial cells (BMEC) in culture. The study was conducted with the purpose in mind to establish a model in which the signaling mechanisms involved in hypoxic preconditioning can be easily studied. Here, we show that OGD time-dependently induces cell death in immortalized rat brain microvascular endothelial cells. Cell death is caused, at least to some extent, by apoptosis. However, hypoxic preconditioning 24 h

before OGD had no protective effect on survival, despite induction of hypoxia-inducible factor 1-α, which has been shown to be important for preconditioning protection in neurons. Currently, we are repeating the above experiments in BMEC isolated from mouse brain, to see whether the resistance of in vitro cultured BMEC to hypoxic preconditioning is a general phenomenon.

T6-29

The protective effect of statins against late-onset Alzheimer's disease: No evidence of an alternative pathway via the induction of P-glycoprotein or alpha-secretase

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Simvastatin and other statins were reported to have a protective influence on Alzheimer's disease. This effect may be related to their therapeutic action of HMG-CoA reductase inhibition, but alternative pathways were suggested [1, 2]. A hallmark in the development of Alzheimer's disease is the deposition of amyloid-β plaques in the brain. It has been shown that the amyloid-β peptide is transported by the efflux transporter P-glycoprotein, which is expressed at the blood-brain barrier, and it is hypothesized that P-glycoprotein could be involved in the clearance of amyloid-β from the brain, reducing its toxic effect [3]. Based on this, we addressed the question whether the protective effect of simvastatin may be due to P-glycoprotein induction or alternatively, to an induction of ADAM10, an enzyme involved in α-cleavage of the amyloid precursor protein (APP). Amyloid-β generation is prevented by α-cleavage.

LS 180 cells, a human colon carcinoma cell line, were incubated for four days with different concentrations of simvastatin and fluvastatin, respectively. Rifampicin was used as a positive control for P-glycoprotein induction.

P-glycoprotein and ADAM10 expression levels were analysed by Western blot and P-glycoprotein efflux activity was estimated from flow cytometry analysis with rhodamine 123 as a substrate. The direct effect of simvastatin and fluvastatin on the ATPase activity of P-glycoprotein was tested in rafts from P388/ADR cells [4].

Neither simvastatin nor fluvastatin induced P-glycoprotein expression or enhanced rhodamine 123 efflux from the cells while the positive control rifampicin enhanced both P-glycoprotein expression and function. The statins had no influence on ADAM10 expression levels in addition. Simvastatin and fluvastatin reduced the ATPase activity of P-glycoprotein at concentrations ≥ 2 and 10 μM, respectively.

From these findings we conclude that the protective effect of simvastatin and fluvastatin against the development of Alzheimer's disease is probably neither due to P-glycoprotein induction at the blood-brain barrier nor to an induction of ADAM10.

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T6-30

The interplay of nitric oxide (NO) and reactive oxygen species (ROS) controls angiogenesis in skeletal muscle

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Although neuronal nitric oxide synthase (nNOS) plays a substantial role in skeletal muscle physiology, nNOS-knockout (KO) mice do not manifest phenotypic malfunctions in this tissue. To identify proteins that might be involved in adaptive responses, 2D-PAGE with silver-staining and subsequent tandem mass spectrometry (LC-MS/MS) was performed using extracts of extensor digitorum longus muscle (EDL) derived from KO-mice in comparison to C57Bl/6 wild-type (WT) mice. Six proteins were significantly ($p \leq 0.05$) higher expressed in EDL of KO-mice than in that of WT-mice, all of which are involved in the metabolism of reactive oxygen species (ROS). These included prohibitin (2.0-fold increase), peroxiredoxin-3 (1.9-fold in-crease), superoxide dismutase 1 (1.9-fold increase), HSP beta-1 (1.7-fold increase) and NDP kinase B (2.6-fold in-crease). Because peroxiredoxin-6 was not detected in EDL of WT-mice, the significant higher expression of peroxiredoxin-6 in the EDL of KO-mice (4.1-fold increase) was confirmed by immunoblotting. A 3.2-fold higher intrinsic hydrogen peroxidase activity ($p \leq 0.05$) was demonstrated in EDL of KO-mice than WT-mice, which was related to the presence of peroxiredoxin-6. ESR-spectrometry showed the levels of ROS to be 2.5-times higher ($p \leq 0.05$) in EDL of KO-mice than in WT-mice.

We furthermore investigated whether the nNOS-deficiency is accompanied by an altered ability of the KO-mice to respond to muscle overload with capillary growth. To induce angiogenesis in EDL within 14 days, groups of WT- and KO-mice were subjected to extirpation of its functional antagonist tibialis anterior muscle (TA). Compared to the values in the contralateral EDL, this treatment raised the capillary/fiber (C/F)-ratio and capillary density within the overloaded EDL of both strains (n= 8 animals each). Strikingly, both parameters were significantly higher ($p \leq 0.05$) in KO-mice than WT-mice indicating that the extent of the angiogenic event in this experimental model is suppressed in the presence of nNOS. However, if other groups of mice were fed with a combination of ROS scavengers (vitamin C, N-acetyl-cystein, coenzyme Q) for 14 days prior TA extirpation (n= 8 animals each), significantly changed values of C/F-ratio and capillary density were noticed neither in the EDL of WT- nor KO-mice.

We suggest that the higher levels of ROS rather than NO caused the more distinct growth of the capillary network in skeletal muscle of the KO-mice than the WT-mice. Thus, angiogenesis in skeletal muscle in response to stretch is rather controlled by the interplay of NO and ROS than by one these radiacal systems alone. This consideration might be of fundamental relevance to analyze and understand the functional impact of free radicals on skeletal muscle physiology.

T6-31

Trefoil factor 1 is expressed in neurons of the postnatal and adult rat midbrain

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Trefoil factor 1 (TFF1) belongs to a peptide family (TFF1-3) particularly expressed in the gastrointestinal tract. These factors play an essential role in the function and composition of the mucosal protective barrier. TFF peptides have been reported to influence precursor cell migration and epithelial restitution. In addition, anti-apoptotic properties have been recognized for these peptides. TFF1 was first discovered in a human breast cancer cell line, and it has been shown to stimulate the migration of breast cancer cells. Recent years' research has revealed that TFFs also are neuropeptides expressed in some areas of the central nervous system but expression of TFF1 in the CNS is poorly understood so far. In the present study we investigated the expression pattern of TFF1 in the postnatal (PN) and adult rat midbrain by means of immunohistochemistry. In addition, we studied the effects of an unilateral 6-hydroxydopamine (6-OHDA) lesion on distribution of TFF1-immunoreactive (ir) cells. We detected that TFF1-ir cells were predominantly distributed in the substantia nigra pars compacta (SNC) and substantia nigra pars lateralis (SNL), whereas fewer cells were present in the ventral tegmental area (VTA). Phenotypical characterization of TFF1-ir cells in the ventral mesencephalon showed that a substantial number of TFF1-ir cells

in the SNC and SNL also expressed tyrosine hydroxylase (TH), whereas co-localization was less pronounced in the ventral tegmental area. TFF1 expression was found almost exclusively in neurons but not in glial cells based on their morphological appearance. Interestingly, we observed that the number of TFF1-ir neurons in the SNC and SNL as expressed to corresponding TH-ir cell numbers was higher in the early postnatal period as compared to the late postnatal stage (with 70% at PN7 and 35% at PN21, respectively) suggesting that TFF1 may play a role in the development and/or maturation of midbrain neurons. Moreover, preliminary data revealed that striatal number of TFF1-ir neurons was higher in the 6-OHDA lesioned side as compared to the unlesioned side (by 1.7 fold). We also detected a reduced number of TFF1-ir cells in the lesioned side of the SNC as compared to the unlesioned side hinting to the idea that a least a subpopulation of these cells are projection neurons. Identifying subpopulation of nigral neurons is of special interest in relation to Parkinson's disease, a neurodegenerative disease characterized by a progressive loss of dopaminergic neurons particularly in the SNC. In sum, our study demonstrates that distinct populations of midbrain neurons express TFF1 and that TFF1 expression pattern is altered in an animal model of Parkinson's disease.

T6-32

Dysfunction of the monogenic diabetes gene Hnf1α sensitises β-cells to endoplasmic reticulum stress by direct downregulation of the transcription factor Xbp1

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Endoplasmic reticulum (ER) stress occurs when the protein folding and processing capacity of the ER is perturbed, either by increased protein load or by interference with the protein folding machinery. The typical response of a cell to ER stress is to upregulate transcription of ER chaperones (such as BiP) and the transcription factors Atf4 and Xbp1. Pancreatic β-cells are particularly susceptible to ER stress, due to their reliance on the ER for folding and processing of insulin. Evidence for the involvement of ER stress in diabetes has been observed in multiple animal models and in human disease (Wolfram and Wolcott-Rallison syndromes). We examined whether ER stress also occurs in MODY3, caused by mutations in the transcription factor Hnf1α, using both cultured cells and diabetic mouse islets carrying a dominant-negative (DN) variant of Hnf1α. The transgenic mice express the dominant-negative gene only in the β-cells, which have been observed to have dilated ER cisternae and fewer mature secretory granules, providing ultrastructural evidence that ER function may be perturbed in this model. These mice recapitulate the MODY3 phenotype very well and display substantial loss of β-cell mass.

ER stress-associated gene expression was measured by Q-RT-PCR in INS DN Hnf1α cells with the DN gene inducible by doxycycline, and in pancreatic islets from DN Hnf1α mice relative to matched littermate controls. INS DN Hnf1α cells showed an atypical ER stress response on induction of the transgene, with increased mRNA levels of SERCA2b, the ER-associated transcription factors Atf4 and CHOP and the pro-apoptotic gene PUMA, but decreased levels of other ER stress markers including BiP, calreticulin, protein disulphide isomerase (PDI) and the transcription factor Xbp1. In cells with ER stress induced by CPA, upregulation of some of the ER stress genes (BiP, calreticulin, PDI and SERCA2b) was attenuated when DN Hnf1α was induced. In mouse islets, basal transcript levels of all chaperone genes tested (BiP, calreticulin and calnexin), as well as PDI and the SERCA calcium ATPases, were lower in the DN Hnf1α mouse islets relative to control islets, although the transcriptional response to CPA appeared less affected in the DN Hnf1α islets than in the cell line. INS DN Hnf1α cells were also more sensitive to CPA-induced cell death when the transgene was induced.

A match to the consensus Hnf1α binding site was found in the promoter of the Xbp1 gene, and binding of Hnf1α to this site in INS cells was demonstrated by chromatin immunoprecipitation. Electrophoretic mobility shift assays showed that this binding was strongly attenuated in nuclear extracts from cells overexpressing DN Hnf1α relative to control cells. This suggests that the ER stress master gene Xbp1 is a direct target of Hnf1α and is dependent on it for basal mRNA transcription. We hypothesise that in the absence of functional Hnf1α the mRNA for Xbp1 is degraded and not replaced, preventing the upregulation of Xbp1-dependent genes, especially protein folding enzymes and chaperones, in response to ER stress. This would sensitise the β-cells to ER stress-induced apoptosis and could contribute to the loss of β-cell mass in MODY3 patients.

T6-33

Dysregulation of channel activating protease 1 in mouse epidermis

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Introduction
Mutations or alterations in the expression of several serine proteases (SP) and SP inhibitors can lead to skin diseases. The channel activating protease 1 (CAP1, also known as Prostasin and Prss8) is a SP expressed in the epidermis.
The aim of this study is to investigate the importance of the regulated CAP1 expression in the skin by addressing CAP1 to the stratum basale of the epidermis.
Methods and Materials
CAP1 transgenic mice have been generated by micro-injection into fertilized mouse oocytes. The genotypes and phenotypes of the animals were assessed using standard molecular, cellular, histological means. The epidermal permeability barrier integrity was evaluated by measuring the trans epidermal water loss (TEWL).
Results
CAP1 transgenic mice show a skin phenotype characterized by scaly, un-elastic skin with less hair. At histological level the epidermis is hyperkeratotic and thicker because of an increased cell proliferation. The histology of other organs is not affected. CAP1 transgenic mice present also lower body weight and higher TEWL and an increased postnatal mortality, suggesting skin barrier defects.
Discussion
Mice with an altered CAP1 expression in the skin present impaired epidermal barrier function. This study shows that regulated CAP1 expression in the epidermis is important for the skin physiology. CAP1 transgenic mice can be used as a model for skin disorders like ichthyosis.

T6-34

Early effects of Carbachol on the motor endplate morphology of mammalian skeletal muscle fibers

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Prolonged Carbachol application for more than 30 minutes induces local contractures underneath motor endplates of skeletal muscles with localized destruction of the myofibrils and swollen mitochondria. These calcium dependent pathological changes resemble to those pathological changes which are caused by a steady calcium inward current through acetylcholine receptors at patients with the slow-channel syndrome. To get insight into the processes of the calcium homoeostasis of the motor endplate area and into the time course of possible pathological changes after disturbance of this homoeostasis, we investigated the effect of short-term Carbachol stimulation for 1 to 30 minutes to the morphology of motor endplates. Despite the isometric

experimental setup, short term applications of Carbachol induce calcium depended localized contractures of the myofibrils just below the motor endplates and a squeezing out of the endplate sarcoplasm. The squeezing results in the deformation of the beret-like shape of the endplate to a wart- to mushroom-like body, whose sarcolemma overlaps the sarcolemma of the muscle fiber. Next to these changes, we observe changes of the internal morphology of the mitochondria in form of electro-lucent plaques in the matrix. These modified mitochondria can only be found in the area of contractures and in the sarcoplasm of the motor endplate. These morphological changes of mitochondria as well as the entire motor endplates are most prominent with slow muscle fibers. The coincidence of our results concerning the fiber type specific frequency of Carbachol affected motor endplates with the available data from the literature speaks in favour of fiber type specific differences in handling of disturbances of the calcium homoeostasis in this area of the muscle fiber.

T6-35

Evaluation of AZT and Thymidine Kinase, Thymidylate Kinase and Nucleoside Diphosphate Kinase for Suicide Gene Therapy

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Background: Herpes simplex virus Thymidine Kinase (HSV1-TK) together with Gancyclovir (GCV) is well investigated as a failsafe for allogeneic haematopoietic stem cell transplantation (allo HCT). Patients who received donor T lymphocytes transduced previously with a retroviral vector coding for HSV1-TK, were successfully treated with GCV in case of Graft versus Host Disease (GvHD). Despite the demonstration of the efficacy of HSV1-TK/GCV suicide strategy, this system is limited by the viral origin of the suicide gene, which can lead to immune mediated elimination of the engineered lymphocytes.

The aim of our project, in order to improve the treatment, is the development of a new suicide gene/prodrug system with reduced immunogenicity. An engineered human enzyme, modified in its ability of activating prodrug would thus represent an interesting candidate for suicide gene therapy (SGT). Therefore we focused our study on the development of a fusion protein (fp) of the modified human cytosolic thymidine kinase 1 (hTK1) with the modified thymidylate kinase (TMK), with an enhanced ability to phosphorylate nucleoside analogue prodrugs and a reduced specifity for thymidine (dT). Furthermore we studied the efficiency of AZT phosphorylation within the cell using single (TMK) and double (TMK/ nucleoside diphosphate kinase (NDK)) transduced cells.

Methods and Results: hTK1 carrying different mutations were tested for their ability to phosphorylate AZT as well as dT. One promising candidate emerged from this screening. The substrate specificity of the hTK is shifted towards the prodrug. The modified hTK was fused with the modified TMK. 143B osteosarcoma cells lacking the hTK1 were transfected with the engineered enzyme. After treatment with AZT the cell viability was measured, revealing that the sensitivity against the prodrug for the transfected cells is around 100 fold higher then compared with cell carrying the hTK wild type. Jurkat cells were transduced with the fusion protein (lentiviral transduction). Cell viability was tested upon AZT treatment, revealing no significant difference between mock and fp transduced cells. Cells transduced with the modified TMK showed a significant decrease in cell proliferation upon AZT treatment. This effect could be significantly enhanced by the double transduction with modified NDK.

Conclusion: AZT toxicity if manifold and linked to the amount of formed AZT trisphosphate, but also to the depletion of d'NTPs caused by the competition between AZT and dT for the human TK. TMK/NDK double transduced cells together with AZT represent a novel promising suicide gene therapy strategy.

T6-36

Glatiramer acetate induces sIL-1Ra production in human monocytes through PI3Kδ/MEK1/2/GSK3β pathway(s)

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Glatiramer acetate (GA), a random mixture of peptides composed of glutamic acid, lysine, alanine and tyrosine, is an approved drug for the treatment of relapsing remitting multiple sclerosis (MS). Although the mechanism(s) of action remain elusive, it is known that it causes perturbations in T cell antigen reactivity. Recent reports indicated that GA also exerts immunomodulatory activity on cells of the monocytic lineage. IL-1β plays an important part in MS since it is a mediator of inflammation in experimental autoimmune encephalomyelitis (EAE) an MS animal model, and it is present in CNS-infiltrating macrophages and microglial cells. The secreted form of IL-1 receptor antagonist (sIL-1Ra), a naturally occurring inhibitor of IL-1β, ameliorates EAE disease course. We recently showed that GA enhanced sIL-1Ra circulating levels in both EAE mice and MS patients. This corroborates the in vitro effects of GA on the production of sIL-1Ra in isolated human monocytes. Indeed, GA induces transcription and production of sIL-1Ra but not that of IL-1β in human monocytes. Furthermore, GA diminishes the induced production of IL-1β in human monocytes. These results suggest that monocytes express a GA-specific receptor or sensor. To confirm this, we thought to determine which signaling pathways were triggered by GA. The experimental approach involved both kinase inhibitors and knockdown of kinases in human monocytes. GA-induced sIL-1Ra production was inhibited by more than 80% in the presence of UO126, an inhibitor of MEK1/2 and the same degree of inhibition was observed in the presence of PI3Kδ inhibitor. This was confirmed in monocytes knockdown for MEK2 in which GA was unable to induce sIL-1Ra production. The involvement of PI3K pathway was confirmed by the premise that inhibition of GSK3β displayed an opposite effect to PI3Kδ inhibition, enhancing sIL-1Ra production by more than 2-fold These results demonstrate that per se GA triggers cell signaling in human monocytes, further suggesting the existence of a specific receptor/sensor. Therefore the production of sIL-1Ra in human monocytes activated by GA occurred through signaling pathway(s) involving PI3Kδ, GSK3β and MEK1/2. These results demonstrate that GA directly affects monocytes by triggering a bias toward less inflammatory profile, shedding light on a new mechanism that might participate in the therapeutic effects of GA in MS.

T6-37

Investigation of melatonin in the dystrophic *mdx* mouse, a model for Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a severe X-linked muscle wasting disease causing the absence of the cytoskeletal protein dystrophin. In addition to the abnormal calcium handling, the generation of reactive oxygen species (ROS) and associated oxidative stress play crucial roles in the development of DMD. In particular, the dystrophic phenotype is associated with a reduction in cellular antioxidant defences and an increased susceptibility to oxidative stress. Accordingly, free radical injury may contribute to the loss of membrane integrity associated with the disease. In addition, *in vivo* and *in vitro* studies have shown positive effects of antioxidants on muscle dystrophies as in the *mdx* muscle, the most

common animal model for DMD. Melatonin (*N*-acetyl-5-methoxytryptamine), the main hormone from the pineal gland, is known for its antioxidant properties that range from scavenging of a variety of free radicals and reactive species to the regulation of a number of processes improving the cellular antioxidant capacity to balance excessive ROS.
In our current study, 17/18 day-old *mdx*^{5Cv} mice were treated by melatonin by daily intraperitoneal (i.p.) injection (30 mg/kg body weight) or by subcutaneous (s.c.) implant(s) (18 or 54 mg melatonin as Melovine® implants) for 12 days. This period has been reported as the onset of muscle necrosis in *mdx* mice. Isometric force recordings of the triceps surae (comprising soleus, plantaris and gastrocnemius muscles) showed that *mdx*^{5Cv} mice receiving 30 mg/kg i.p. treatment presented a significant increase of the phasic twitch tension (Pt) compared to untreated *mdx*^{5Cv} mice. This i.p. treatment restored about 43% of the force deficit of *mdx*^{5Cv} mice compared to normal mice. Treatment with 18 mg s.c. and 30 mg/kg i.p. significantly increased the maximal tetanic tension (Po): the force deficit was ameliorated by 28% and 42% respectively. As a result of these effects of 30 mg/kg melatonin i.p. on Pt and Po, the phasic-to-tetanic ratio was corrected to near-normal values. In addition, the kinetics of contraction and relaxation were shorter in melatonin-treated than in untreated *mdx*^{5Cv} mice. Moreover, the force-frequency relationship was shifted to the right with melatonin treatment in dystrophic mice approaching values of the normal mice. This latter result suggests a change in the calcium homeostasis or in the distribution of fibres toward a faster phenotype. The resistance to mechanical stress was not changed by melatonin treatment. Finally, plasma creatine activity, a well known marker for muscle injury, was decreased by the treatment in *mdx*^{5Cv} mice to near-normal values.

T6-38

Melatonin prevents oxidative-stress mediated mitochondrial permeability transition and death via enhancement of reduced pyridine nucleotides and glutathione in mouse skeletal muscle cells

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Oxidative stress-induced mitochondrial dysfunction has been shown to play a crucial role in the pathogenesis of a wide range of diseases including muscle disorders. Protecting mitochondrial function, therefore, is vital for cells to survive.
In this study, we demonstrate that melatonin, the main secretory product of the pineal gland, readily rescued mitochondria from oxidative stress-induced dysfunction and effectively prevented subsequent apoptotic/necrotic events and death in C57BL/6J myotubes. In particular, melatonin potently prevented myotube death induced by tert-butylhydroperoxide (t-BHP) in a concentration-dependent manner (10^{-4}-10^{-6} M). This protective effect was more potent than that of N-acetyl-L-cystein, a well known antioxidant that increases cellular pools of free-radical scavengers. Moreover, melatonin maintained plasma membrane integrity after t-BHP exposure and prevented t-BHP-induced fissions of the long mitochondrial filaments and mitochondrial swelling. To determine if the mitochondrial protection provided by melatonin was due to the inhibition of the formation of reactive oxygen species (ROS), intracellular ROS levels were measured using fluorescence imaging. Application of t-BHP produced a rapid and significant increase in ROS generation in myotubes. This effect was concentration dependently prevented by pretreatment of the myotubes with melatonin. Considering that t-BHP cytotoxicity was also prevented by cyclosporin A, a mitochondrial permeability transition pore (mPTP) inhibitor, we investigated the effect of melatonin on mPTP. Melatonin prevented t-BHP-induced mitochondrial depolarization and protected the pyridine nucleotides and glutathione (two regulators of mPTP opening under conditions of oxidative stress) against t-BHP-induced stress. Using isolated mitochondria, we found that melatonin (10^{-8}-10^{-6} M) desensitized the mPTP to Ca^{2+} and prevented t-BHP-induced mitochondrial swelling, pyridine nucleotide and glutathione oxidation, and enhanced mitochondrial function.
In conclusion, our findings suggest that inhibition of the mPTP may essentially contribute to the protective effect of melatonin against oxidative stress in myotubes.

T6-39

Neurobiological Studies on Cultured Neuronal and Glial Networks Using Micro Electrode Arrays (MEAs)

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Semiconductor silicon chips, i.e., MEAs with their ability to measure complex spatio-temporal neural activity, are poised to become an important and fundamental tool in neuroscience research.
Conventional MEAs consist of 64 to 128 electrodes integrated in a planar substrate, suitable for cell and tissue culture, and offer an alternative to classical electrophysiological in vitro techniques (i.e. the patch clamp). By using MEA technology, electrical signals can be extracellularly recorded from electrogenic cells and tissues (i.e. neurons, heart cells, and muscle cells). Such recordings are performed at multiple sites in a non-invasive manner over extended periods of time. Additionally, single or multiple electrodes can be used for electrical stimulation.
We have developed a CMOS-based MEA featuring a high-density-electrode array, i.e., 11`016 metal electrodes and 126 on-chip channels, each of which includes recording and stimulation electronics for bidirectional communication with electrogenic cells. This CMOS-based device provides a spatial resolution of 17µm (electrode pitch) and sampling rates ranging from 8kHz/channel up to 120kHz/channel.
Extracellular recording requires a tight coupling between the chip surface and the cells cultured on the chip. Different biocompatibility issues have to be overcome to enable long-term culturing. In order to obtain networks of neuronal cells on MEA-chip that are electrically active over extended time, we have developed different cell biology protocols for culturing dissociated mammalian neurons on these MEAs, in particular since the broad range of applications of MEAs in biomedical research is evident.
Moreover, we are developing protocols (electrical stimulation vs. biological protocols) to use the MEAs as a platform for electrical stimulation of mammalian brain cells and for monitoring electrically-induced differentiation procedures by using different biological methods. This research is relevant as a clear understanding of cell fate regulation during differentiation is important in, e.g., employing stem cells in biomedical applications. With our arrays we use mild electrical stimulation of primary rat oligodendrocytes and monitor how this stimulation modulates the course of cellular differentiation. Different factors, including trans-membrane ion distribution, influence the developmental processes of neurons. A next step will be to use MEA-recordings for an electrophysiological classification of differentiated neurons.

T6-40

Neutrophil extracellular trap formation does not require cell death

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Besides intracellular killing, neutrophils are able to exert antibacterial activities in the extracellular space by forming so-called neutrophil extracellular traps (NETs). Recently, it has been suggested that the formation of NETs require a special form of neutrophil death. In this study, we searched for potential other molecular mechanisms leading to NET formation. We meticulously analyzed both neutrophil death and apoptosis, including time-lapse video microscopy. In contrast to earlier published work, we demonstrate that NET can be generated following short-term

activation of neutrophils via Toll-like or complement receptors. Cytokine priming but not cell death is required under these conditions. Moreover, also in contrast to earlier reports, NETs contain mitochondrial but no nuclear DNA as long as cell death is prevented. We further observed that the stimulation of reactive oxygen production is required for mitochondrial DNA release and NET formation. Taken together, we report that NET formation represents a functional response following neutrophil activation in the absence of cell death. The molecular mechanisms and the cellular consequences of mitochondrial DNA release by neutrophils require additional experimentation.

T6-41

Pathogen specific response of the bovine mammary gland to lipopolysaccharide from E. coli and lipoteichoic acid from S. aureus

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Escherichia coli and Staphylococcus aureus are common causes of clinical mastitis. Infection of the mammary gland with E.coli elicits mostly an acute mastitis, whereas an infection with S.aureus causes usually a chronic and subclinical course of disease. The two bacterial species represent the two groups of gram-negative and gram-positive bacteria. Different toll-like receptor (TLR) types of the innate immune system recognize pathogen associated molecular patterns (PAMPs) towards specific cell wall components of different bacterial types. Thus, TLR-2 recognizes lipoteichoic acid (LTA) from S.aureus and other gram-positive bacteria, whereas TLR-4 recognizes lipopolysaccharide (LPS) from E. coli and other gram-negative bacteria, both characteristic PAMPs of the respective types of bacteria. In this study, we have investigated if intramammary LPS and LTA administration induces a differential immune response in vivo in dairy cows. The challenge was performed with 10µg LTA and 10µg LPS both diluted in 10ml of 0.9% saline in one quarter of 8 and 5 cows, resp. The PAMPs are derived from bacterial stems which have caused mastitis in dairy cows before. A second quarter of each experimental cow served as control (10ml of 0.9% saline). At three time points of PAMP challenge biopsy samples of the mammary gland were taken for RNA extraction and measurement of mRNA expression by real-time RT-PCR of tumor necrosis factor alpha (TNFα), lactoferrin (LF) and interleukin-8 (IL-8) and ubiquitin as a housekeeping gene. Rectal temperature was measured hourly during the experiment. The mRNA expression of TNFα was increased ($p<0.05$) after 6 h (rel. mRNA expression ΔΔCT 3.1±0.44) and 12 h (rel. mRNA expression ΔΔCT 2.1±0.42) in the LPS treated quarter but not in the LTA treated quarter. A similar reaction pattern was observed for the expression of LF. However, the mRNA expression of IL-8 was increased ($p<0.05$) in LPS treated quarters and in LTA treated quarters after 6 and 12 h, but the increment was more pronounced in response to LPS than to LTA (rel. mRNA expression ΔΔCT 8.7± 0.62 and 8.2±1.00 vs. 3.51±0.85 and 3.26±0.44 after 6 and 12h, resp.). Also in control quarters the mRNA expression of IL-8 was increased. The increase in control quarters could be explained due to the biopsy procedure. Obviously the responses to the 2 different PAMPs are regulated via different pathways. The present results are consistent with earlier investigations which showed a reduced and slower reaction of TNF-α, LF and IL-8 towards bacterial infection with S. aureus than with E.coli.

T6-42

Pathways of Abnormal Stress-Induced Calcium Influx into Dystrophic mdx Cardiomyocytes

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In Duchenne muscular dystrophy, deficiency of the cytoskeletal protein dystrophin leads to well-described defects in skeletal muscle, but also to dilated cardiomyopathy, which accounts for about 20% of the mortality. However, the precise mechanisms leading to cardiomyocyte cell death and dilated cardiomyopathy are not well understood. One hypothesis to explain the dystrophic muscle phenotype suggests that the lack of dystrophin leads to membrane instability during mechanical stress and to the activation of not yet identified calcium (Ca^{2+}) influx pathways. In the present study, potential Ca^{2+} entry pathways initiating damaging intracellular signals were explored with confocal imaging and pharmacological tools. Modest osmotic shocks were applied to isolated *mdx* cardiac myocytes, which are an established model for dystrophy. Osmotic shocks mimic some characteristics of stress encountered by the cells *in vivo*. Our results confirm that stretch-activated channels (SACs) and sarcolemmal microruptures play an important role in the initial Ca^{2+} entry, with the latter pathway also permeable for the dye FM1-43. Interestingly, our findings also suggest that Ca^{2+} influx pathways which are more prominent in cardiac than in skeletal muscle synergistically contribute to the observed Ca^{2+} responses (e.g. the L-type Ca^{2+} channels or the Na^+-Ca^{2+} exchange (NCX) importing Ca^{2+} subsequent to some Na^+ entry via the aforementioned primary pathways). This additional complexity needs to be considered when targeting abnormal Ca^{2+} influx as a treatment option for dystrophy. Supported by SNF, MDA & SSEM.

T6-43

Phosphoinositide 3-kinases mediate TNFα-induced neutrophil cell death

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Innate immunity is essential for multicellular organisms as the first line of defence against invading pathogens. Key effector cells of the human innate immune system are neutrophils. Upon cellular stimulation, neutrophils migrate to sites of inflammation where they phagocytose infectious agents and release toxic mediators. Tight regulation of granulocyte function is critical for the removal of pathogens, and strict control of cell death is important for an efficient resolution of inflammation.

TNF (tumor necrosis factor) receptor engagement in neutrophils results in either survival or cell death depending on TNFα concentrations and co-stimulatory signals. In order to gain insight into the signalling mechanisms leading to apoptosis downstream of TNF receptor, we treated neutrophils with high TNFα concentrations in combination with small-molecule inhibitors against JAK/STAT, MAPK and PI3K (phosphoinositide 3-kinase) pathways.

When PI3K activity was blocked by broad-spectrum and class IA isoform-selective PI3K inhibitors, neutrophil viability was *increased* upon TNF receptor ligation compared to control cells. These results are surprising since PI3K inhibition induces death in most cell types. The increase in viability upon PI3K inhibition of TNFα-stimulated neutrophils was due to diminished generation of reactive oxygen species (ROS) by the NADPH oxidase. Reduced ROS levels under these conditions resulted in both reduced caspase activity and increased mitochondrial transmembrane potential compared to control neutrophils. In line with these findings, neutrophils from CGD patients (who lack a functional NADPH oxidase) did not produce ROS and where therefore long-lived when treated with TNFα.

In summary, class IA PI3Ks mediate neutrophil apoptosis following

TNFα stimulation by inducing ROS production, which leads to increased caspase activity and decreased mitochondrial integrity.

T6-44

Targeting phosphoinositide 3-kinases as a new potential therapeutic strategy for the treatment of medulloblastoma

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Medulloblastoma is the most common malignant brain tumor in childhood and represents the main cause of cancer-related death in this age group. The phosphoinositol 3-kinase (PI3K) pathway, targeted by different genetic alterations in many human cancers, has been shown to play an important role in the regulation of cell survival and proliferation in medulloblastoma. Analysis of the expression pattern of the class IA PI3K isoforms demonstrated that the catalytic isoform p110α is overexpressed in medulloblastoma cell lines and tumor samples. Effects on cell survival and downstream signalling were analysed following downregulation of p110α in medulloblastoma cells by means of RNA interference. Downregulation of the expression of p110α in DAOY cells by RNAi resulted in a decrease in cell growth and cell proliferation. To gain further insight into the downstream pathways mediating p110α signals, we performed a DNA microarray analysis. Here, we screened the changes in the expression of DAOY cells caused by RNAi-mediated downregulation of p110α on the Affymetrix Gene Chip HG U133 Plus 2. We identified a group of genes involved in important cellular events, such as cell growth and apoptosis, to be affected by the downregulation of p110α. The expression of AKT2, CDK6 and BCL6 was up-regulated upon PIK3CA silencing, while IGF1R and LIFR were down-regulated. These genes might be important factors mediating p110α signals in medulloblastoma cell responses and represent new interesting target molecules for further studies.

T6-45

Suprising effect of TIP47 suppression on Sendai virus particle production

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Sendai virus (SeV) is an enveloped virus that buds from the plasma membrane of infected cells. The efficient release of viral particles involves the coalescence of both soluble viral proteins and viral glycoproteins expressed at the plasma membrane. The viral components required for the viral assembly process have been under study for many years.
Here, we investigate the role of the cellular protein TIP47 (tail-interacting protein of 47kDa) in the production of SeV particles. The suppression of TIP47 from Hela cells using a siRNA approach leads to a 5 to 30 fold increase in SeV viral production. In contrast, no significant effect on hPIV5 or VSV production was observed under the same conditions. We then show that viral protein synthesis rate was increased in cells depleted from TIP47. The mechanism responsible for the observed effects is under investigation and will be discussed.

T6-46

Excitotoxicity-induced endocytosis provides selective drug targeting of TAT peptides in cerebral ischemia through a JNK-dependent mechanism

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Enhanced endocytosis occurs in several cases of excitotoxic neuronal death and has been well characterized using FITC-dextran or HRP in dissociated cortical neurons exposed to NMDA (Vaslin et al., 2007). Using this same culture model we now report that this phenomenon is enhanced by 50 times in the case of TAT peptides, due to their electrostatic interactions with heparan sulfate proteoglycans. The NMDA-induced endocytosis was insensitive to fluid-phase inhibitors, but sensitive to inhibitors of clathrin/dynamin-mediated endocytosis. We then investigated the same phenomenon in vivo in a model of focal cerebral ischemia. Highly endocytic cells were observed in the lesion after an icv injection of FITC-dextran or HRP or TAT peptides in P12 rats exposed to cerebral ischemia. The labelled neurons displayed strongly increased endosomal markers including EEA1, and clathrin, but not classical cell death markers.

We therefore investigated whether excitotoxicity-induced endocytosis contributed to cell-entry by a powerfully neuroprotective TAT-peptide, the JNK inhibitor D-JNKI1, which comprises retro-inverso forms of the TAT transporter sequence and a 20-amino-acid sequence from the JNK-binding domain of JIP1/IB-1. In NMDA-treated cultures inhibition of clathrin/dynamin-mediated endocytosis reduced the neuroprotective effects D-JNKI1. D-JNKI1 also inhibited its own uptake, as predicted from previous experiments. We also showed that reducing endocytosis is not in itself neuroprotective. In cerebral ischemia D-JNKI1 was strongly protective over a wide dose range when administered 6h after the ischemia and reduced EEA1 labeling in the ischemic area.

In conclusion, we have shown that excitotoxicity-enhanced endocytosis occurs before cell death and provides an entry route by which neuroprotective peptides enter preferentially into the cells that need them. Moreover the fact that D-JNKI1 inhibits its own excitotoxicity-induced endocytosis may explain its wide neuroprotective dose range in cerebral ischemia, since it autoregulates its own entry preventing excessive uptake.

T6-47

IFNβ induces the production of secreted IL-1 receptor antagonist in human monocytes through a MEK2/PI3Kδ-dependent, ERK1/2-independent pathway

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Imbalance in cytokine homeostasis plays an important part in the pathogenesis of chronic inflammatory diseases including multiple sclerosis (MS). In MS, the pro-inflammatory cytokine interleukin-1β (IL-1β) is present in central nervous system infiltrating macrophages and microglial cells. In human monocytes, IFNβ, which is an approved treatment for MS, triggers the production of the secreted form of IL-1 receptor antagonist (sIl-1Ra), a specific inhibitor of IL-1β. IFNβ induces sIL-1Ra production in monocytes through a phosphatidylinositol-3-phosphate kinase (PI3K) pathway that does not involve signal transducers and activators of transcription 1 (STAT1), i.e., the canonical IFNβ signaling pathway. In this study we address the question of the involvement of mitogen-activated protein kinase kinases MEK1, MEK2, and PI3K isoforms, as well as their respective downstream elements in the IFNβ-induction of sIL-1Ra production in human monocytes. IFNβ-induced sIL-1Ra production was inhibited in the presence of UO126, an inhibitor of both MEK1 and MEK2, and in MEK2-knockdowned monocytes but not in the presence of PD98059, a MEK1 specific inhibitor, or in MEK1-knockdowned cells. This

demonstrates that MEK2 but not MEK1 controlled sIL-1Ra production in IFNβ-activated monocytes. The production of sIL-1Ra was inhibited in the presence of a PI3Kδ specific inhibitor, but the simultaneous inhibition of MEK2 and PI3Kδ did not display additional effects. This suggests that MEK2 and PI3Kδ were elements of the same signaling pathway. Interestingly, UO126 inhibited the phosphorylation of Akt (a classical PI3K downstream element) while PI3Kδ specific inhibitor had not effect on ERK1/2 phosphorylation, i.e., the canonical substrates of MEK1 and MEK2, suggesting that MEK2 was upstream PI3Kδ. Moreover, the inhibition of sIL-1Ra production was independent of the phosphorylation/activation of ERK1/2. Together, these results demonstrate that IFNβ induces sIL-1Ra production in human monocytes through a MEK2/PI3Kδ-dependent, ERK1/2-independent pathway.

T6-48

Calretinin: An important factor in designing drug therapy in colon cancer?

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Colon cancer is second leading death-causing cancer in Western countries. In colon cancer, several proteins including transcription factors are either up- regulated or down-regulated, which are linked to tumor progression. One of the proteins up-regulated in poorly differentiated colon cancers is the calcium-binding protein (CaBP) calretinin (CR), which is not expressed in normal colon epithelial cells. CR is a CaBP of the EF- hand family, which is normally expressed in the central and peripheral nervous system, but also in mesothelioma of the epithelial and mixed type. CR was found to be up-regulated in vitro in 5-fluorouracil (5-FU) treated colon cancer cells, which was discussed as a factor in making cancer cells more resistant to 5-FU treatment. We first checked for cytotoxic effects of 5-FU and an inducer of differentiation, sodium butyrate (NaBt) in the colon cancer cell lines HT-29, WiDr (both CR-positive) and CaCo-2 (CR-negative). Compared to CaCo-2 cells, CR-positive cells were more resistant to either 5-FU or NaBt treatment. Furthermore HT-29 cells expressing the highest CR levels were more resistant to a combination treatment consisting both, 5-FU and NaBt in comparison to WiDr cells. Also transient increase in CR expression was more pronounced in HT-29 cells than in WiDr cells after combination treatment. Our results support the hypothesis that CR plays a role in the resistance mechanism to 5-FU and NaBt treatment. Moreover, the morphological effects of different anti-cancer drugs suggest that each cell line responds differently to different drugs. Our data indicate that CR may be an important factor to be considered in developing new drugs or designing drug therapies in colon cancer treatment.

T6-49

Effect of aldosterone-induced proteins ATF3 and Grem2 on distal nephron transport activity and differentiation

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Aldosterone regulates Na^+ reabsorption across epithelia of the aldosterone sensitive distal nephron (ASDN) and therefore plays an important role in the maintenance of blood pressure. Previous work identified early aldosterone-regulated mRNAs in mouse ASDN by microarray analysis. Gremlin2 (Grem2), a bone morphogenic protein (BMP) antagonist and the activating transcription factor 3 (ATF3) were highly induced within 1 hour. To study the effect of Grem2 and ATF3 on distal nephron differentiation and epithelial transport, clonal mouse collecting duct cell lines (mpkCCD) inducibly expressing ATF3 or Grem2 were generated. First mpkCCD cells were transduced with a tTRKRAB construct, which allows the cells to express a fusion protein consisting of the tetracycline responsive tTR transrepressor and the bacterial protein KRAB (Krüppel associated box) which epigenetically silences expression of genes. A second construct containing the sequence of ATF3 or Grem2 and a Tet-operator were transduced into the KRAB cell lines. This way we achieved doxycycline (tetracycline) inducible expression of the two aldosterone regulated proteins. Functional experiments using transepithelial voltage-clamp to analyze potential effects on transepithelial Na^+ and K^+ transport in these cell lines are in progress. Confocal microscopy will be used to locate Grem2 and ATF3 expression to subcellular compartments. Since ATF3 and Grem2 act via transcriptional regulation, we will identify downstream effectors in mpkCCD cells by microarray. Together, these results will contribute to characterize the role of ATF3 and Grem2 in the context of transport and differentiation response to aldosterone.

T6-50

History of Wädenswiler Wine Yeasts – a Survey

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Wädenswiler wine yeast selections are used worldwide for the production of grape variety specific wines from high quality.

The wine yeast Lalvin W27 or in the past in Switzerland better known as HK4 was selected by Dr. Kurt Mayer (1928-1996) from a spontaneous fermented Pinot Noir (Blauburgunder, Spätburgunder or Clevner) grape juice from Jenins (Bündner Herrschaft). In 1974 this yeast was produced as the first Dry Yeast Product worldwide by Lallemand Inc.,.

The wine yeast Lalvin W46, in the past in Switzerland better known as HK8, was selected by Dr. Martin Schütz 1993 from a spontaneous fermented Blauburgunder grape juice from the vineyard Sternenhalde in Stäfa. Lalvin W46 has a very similar genetical background as Lalvin W27. However, Lalvin W46 has a faster fermentation start and also show better production behaviour than Lalvin W27. Both yeasts strains are hybrids between *Saccharomyces cerevisiae* x *Saccharomyces kudriavzeveii* as shown by (González et al., FEMS, 2006). *Saccharomyces kudriavzeveii* is known for the production of Japanese rice wine also known as Saki. Both yeasts can be used for fermentations of grape juices below 15 °C (cold fermentations) without producing undesired flavour compounds in the wines.

The wine yeast Lalvin W15 was selected by Dr. Martin Schütz 1993 from a spontaneous fermented Müller-Thurgau, Riesling-Sylvaner or Riesling x Madeleine Royale grape juice from the vineyard Schlossberg in Wädenswil. Lalvin W15 has a great international reputation (5.5 tons per year). Wines fermented with this yeast have increased levels of glycerol, low increase of volatile acidity, high concentrations on succinate acid which lead to desired decreases in pH-values, no increases in off-flavours etc.

Wädenswiler wine yeast 1895 was isolated from a bottle Räuschling from the year 1895. The yeast was still viable and has produced already from harvest 2008 a lot of very good and promising wines.

More details of selected Wädenswiler Wine Yeasts will be shown on the poster.

T6-51

Detection of the spoilage bacteria *Alicyclobacillus acidoterrestris* and their influence on the aromatic profile of fruit juices

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To assure high quality of fruit juices, wine and plant-derived food, it is essential to early detect spoiling yeasts and bacteria during production processes. We have established a specific and sensitive system based on real-time PCR in order to detect these microorganisms at species level qualitatively and quantitatively and before the formation of undesirable metabolites occurs.

Currently, we are working on the detection of *Alicyclobacillus acidoterrestris* in fruit juices. *Alicyclobacillus acidoterrestris* is an emerging food spoilage organism in fruit juice, fruit juice products and acidified vegetables manifesting a medicinal off-flavour which is caused by guaiacol and halogenated phenols produced by these bacteria (Murray et al., 2007, Borlinghaus and Engel, 1997). Alicyclobacilli are spore-forming, Gram-positive, thermo-acidophilic bacteria (Wisotzeky et al 1992) and possess unique fatty acids (ω-cyclohexane, ω-cycloheptane) and hopanoids in the cellular membrane (Walls and Chuyate, 1998).

We are investigating the growth of *Alicyclobacillus acidoterrestris* on different substrates (BAT media, apple juices, concentrates, Coca Cola) and under different cultivation conditions (temperature, CO_2). Cell numbers are determined using real-time PCR with specific primers and probe.
Aromatic profiles of the samples are recorded with the SmartNose® equipment and a rapid screening for the off-flavour-causing metabolites (guaiacol, 2,6-dichlorophenol, 2,6-dibromophenol) is carried out using MALDI-TOF/TOF mass spectrometry.

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T6-52

Effects of temperature and growth-medium on glycerol formation and fructophilic properties of wine yeasts

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Since 18 years we are interested in gene regulation of yeasts during alcoholic fermentation in wine-making. Our projects were focussed on the formation of desired and undesired metabolic substances during alcoholic fermentation.
We have observed that gene regulation on synthetic medium, grape juice and wine lead to different results. Our first studies were on the effects of temperature on the formation of glycerol during alcoholic fermentation. We have chosen 10 °C, 15 °C, 20 °C and 25 °C as fermentation temperature. We have observed that the glycerol formation on synthetic medium was more or less random, that means we could not detect an increase on glycerol formation with increased fermentation temperature. However, alcoholic fermentation performed on grape juice led to an absolute correlation of glycerol formation and increased fermentation temperature; the glycerol formation increased with increased fermentation temperatures (Diploma thesis Klaus Sütterlin, Albert-Ludwigs-Universität Freiburg im Breisgau Deutschland, 2001, "Regulation der Glycerinbildung von *Saccharomyces cerevisiae*").

We have a project on the occurrence of stuck fermentation in winemaking which occurred because of the glucophilic properties of wine yeasts. We could show that as soon as the ratio of glucose to fructose is below 0.1 we have a stuck fermentation. This fermentation stop could never be overcome by the glucophilic wine yeasts. We could add some glucose for the ongoing alcoholic fermentation. However the addition of glucose to wine is worldwide forbidden. We started to select wine yeasts with fructophilic properties and we were successful. We have selected fructophilic wine yeasts which are commercially produced. We have checked the fructophilic character of these yeasts on synthetic medium. This selection was not successful because we could show that every type of wine yeast (with glucophilic or fructophilic character) was able to ferment both fructose and glucose to dryness on synthetic media (Monika Volkan, Technical Assistance, personal communication). We also tried hard to introduce stuck fermentations in well going alcoholic fermentation but on synthetic media we could never get a stuck fermentation (Diploma thesis Yvo Dürr, Department für Lebensmittelwissenschaften ETHZ, 2001, "Kurieren von Gärstockungen in der Weinbereitung").

There are several scientific articles which are based on experiments on synthetic media on gene regulation. Most of them are wrong because experiments (controls) on natural substrates are missing. In this work, the impact of experiments on synthetic media versus natural substrates (grape juice) will be tested.

T6-53

Proteomic Analysis in Aortic Media of Patients with Marfan Syndrome Reveals Increased Activity of Calpain 2 in Aortic Aneurysms

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Background̃Marfan syndrome (MFS) is a heritable disorder of connective tissue, affecting principally skeletal, ocular and cardiovascular systems. The most life-threatening manifestations are aortic aneurysm and dissection. We investigated changes in the proteome of aortic media in patients with and without MFS to gain insight into molecular mechanisms leading to aortic dilatation.

Methods and Results: Aortic samples were collected from 46 patients. Twenty-two patients suffered from MFS, 9 patients had bicuspid aortic valve (BAV) and 15 patients without connective tissue disorder served as controls. Aortic media was isolated and its proteome analysed in 12 patients using two dimensional difference gel electrophoresis and mass spectrometry. We found higher amounts of filamin A C-terminal fragment, calponin 1, vinculin, microfibril associated glycoprotein 4 and myosin-10 heavy chain in aortic media of MFS aneurysm than in controls. Regulation of filamin A C-terminal fragmentation was validated in all patient samples by immunoblotting. Cleavage of filamin A and of the calpain substrate spectrin were increased in the MFS and BAV group. Extent of cleavage correlated positively with calpain 2 expression and negatively with the expression of its endogenous inhibitor calpastatin.
Conclusion: Our observation demonstrates for the first time up-regulation of the C-terminal fragment of filamin A in dilated aortic media of MFS and BAV patients. In addition our results present evidence that the cleavage of filamin A is highly likely the result of the protease calpain. Increased calpain activity might explain, at least in part, histological alterations in dilated aorta.

T6-54

Eukaryotic Elongation Factor 1A: Carrier of a Rare Ethanolamine Modification

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Eukaryotic elongation factor 1A (eEF1A) is a well-characterized cytosolic protein and much is known about its structure and functions. Apart from its pivotal role during polypeptide synthesis, eEF1A is involved in other cellular processes such as mitochondrial tRNA import, regulation of the actin cytoskeleton and cell morphology.
Several post-translational modifications have been reported, yet their roles remain unknown. Besides methylation, phosphorylation, myristoylation and acetylation, eEF1A is modified by ethanolamine phosphoglycerol (EPG) attached to two glutamate residues that are conserved between mammals and plants. Recently, we found that EPG is also present in eEF1A of the parasitic protozoa *Trypanosoma brucei*. However, in contrast to mammalian and plant eEF1A, *T. brucei* eEF1A contains a single EPG modification only. Furthermore, our group showed that the phospholipid, phosphatidylethanolamine, is the donor of the EPG moiety. The function of EPG in eEF1A has not been studied.
To define the structure and sequence requirements for EPG attachment, we introduced a series of amino acid point mutations in *T. brucei* eEF1A at, or around, the conserved modification site glu^{362}. The presence of the EPG moiety was analyzed by *in vivo* labeling of *T. brucei* eEF1A with [^{3}H]-ethanolamine, and by mass spectrometric analyses of the tryptic peptides containing the EPG modification site. We found that the substitution of glu^{362} by alanine, glutamine or aspartate abolished EPG attachment. In contrast, several point mutations around glu^{362} did not affect EPG linkage.
Moreover, to determine a minimal structural motif required for EPG attachment, we constructed a set of truncated versions of eEF1A and expressed them in *T. brucei* procyclic forms. Deletion of domains I or II and truncations in domain III of the full length protein revealed that an 80 amino acid peptide is sufficient for the attachment of the EPG moiety.

T6-55

Expression Profiling of METS and MARGS in Renal Allograft Biopsies: Differentiation between Normal, Acute Rejection and Interstitial Fibrosis/Tubular Atrophy

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Acute rejection (AR) and interstitial fibrosis/tubular atrophy (IF/TA) remain crucial complications after renal transplantation worsening long term allograft function.

Metzincins, including matrix metalloproteases (MMP), are tissue-remodelling proteases and contribute to inflammatory and fibrotic processes in the kidney. Morphological damage in AR and IF/TA are associated to qualitative and quantitative changes of the extracellular matrix (ECM). Thus, we hypothesized the involvement of metzincins and related genes in AR and IF/TA and investigated their expression in renal allograft biopsies and patient sera.

Microarray analysis of RNA from kidney biopsies with normal histology (N; n=7), AR (n=15), and IF/TA without specific etiology (n=22) focused on two transcript-sets METS (metzincins) and MARGS (metzincins and related genes); MARGS extend METS by metzincin substrates, -regulators and -inhibitors.
Potential marker genes were analyzed in an additional biopsy-set by qRT-PCR of laser captured-microdissected glomeruli and tubuli, and by immunohistochemistry (IHC). Patient sera were examined by ELISA.

Our first results (Roedder et al., AJT, 2008, accepted for publication) illustrated deregulation of METS and MARGS in IF/TA including overexpression of MMP-7 and thrombospondin 2 (THBS2).
Based on MARGS expression profiles, IF/TA and N biopsies of our and two other microarray studies were correctly classified. Furthermore, we demonstrated the involvement of epithelial to mesenchymal-transition and the wingless-type pathway in ongoing IF/TA.
Differential expression of METS and MARGS were also observed in AR compared to IF/TA and N.
METS discriminated IF/TA patients into prior AR episode positive or negative; the expression of METS was increased in IF/TA patients having formerly experienced AR.
Unlike during IF/TA, mRNA levels of MMP7 and THBS2 did not considerably alter during AR ($p>0.05$). However MMP9 showed particular AR overexpression in microdissected glomeruli and proximal tubuli, confirmed by IHC.

The identification of differential METS- and MARGS- expression in IF/TA and AR may provide a step towards the establishment of a diagnostic and prognostic marker set, and the identification of potential therapeutic targets.

T6-56

PPARβ in astrocyte maturation and functions

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PPARβ is highly expressed in the embryonic and adult brain, where it is expressed in astrocytes and neurons. Whereas the role of PPARβ in metabolic regulation as been extensively explored, up to today very little is known on its activity in the brain.
To tackle this question we prepared primary astrocyte cultures from PPARβ WT or PPARβ KO animals. We first observed an impaired stellation of PPARβ KO astrocytes when challenged either through exposure to MnCl2 or in neuron/astrocyte cocultures. This was accompanied by a different actin organization. While these observations demonstrate that PPARβ is active in astrocyte, astrocyte stellation is mainly observed in vitro

and cannot be identified to a specific function in vivo. To pinpoint the physiological role of PPARβ, we explored the main astrocyte functions that include metabolism, proliferation, migration and glutamate uptake. We now show that PPARβ KO astrocytes have an impaired glutamate uptake: PPARβ KO astrocytes show a 3 fold reduction compared to the WT control in acute uptake experiments (5min.). We are now evaluating using FRAP approaches as well as biochemical assays to further characterize this dramatic alteration and identify its prime cause.

T6-57

From the membrane to locomotion – when muscle turns slow

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Signalling propagation along myelinated muscle axons (motoneurons) controls movement by inducing the contraction of innervated muscle fibers. The frequency of electric signal propagation along motoneurons is the essential variable of this process as it dictates the speed of contraction by recruiting qualitatively different fiber types.

Here we point out that muscle activity controls the differentiation of muscle fibers via a feedback mechanism involving the assembly of myelin membrane proteins. This is shown with a multilevel approach in a mouse model for Charcot-Marie-Tooth disease. This neuromuscular disorder arises from mutations of the peripheral myelin protein 22 (PMP22). Transgenic PMP22 mice with aberrant myelin assembly show a shift towards a slow contractile muscle phenotype which does not demonstrate the typical plasticity of soleus muscle to reductions in muscle use. Muscle fiber-directed overexpression of the mechano-transducer focal adhesion kinase in the same anti-gravitational muscle of rats implies that PMP22 expression is under direct control of muscle activity.

The findings imply that not only central nervous excitation but also peripheral input governs the speed of muscle fiber contraction and possibly motoneuron excitation.

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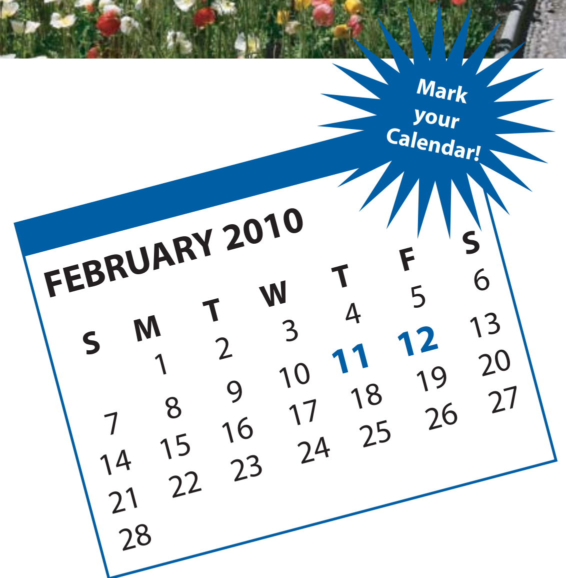

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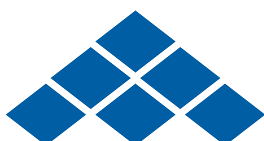

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