International Research Training Group

Jasper Symposium

Jasper Park Lodge

Jasper, Alberta, Canada

August 27-30, 2012

IRTG MEMBRANE BIOLOGY

RTG 1830 GERMAN CANADIAN

COMPLEX MEMBRANE PROTEINS IN CELLULAR DEVELOPMENT AND DISEASE

FUNDING AGENCIES

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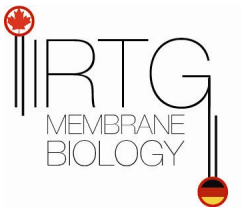

IRTG JASPER SYMPOSIUM – JASPER PARK LODGE
AUGUST 27-30, 2012

PROGRAM SCHEDULE

Monday, August 27th	
9:00am	**Travel to Jasper**
2:00pm	**Hotel Check in and Registration**
2:45pm	**Main Session – Mary Schaeffer Ballroom**
2:45pm-3:00pm	**Welcome and Opening Comments by Joe Casey & Ekkehard Neuhaus**
	SESSION I MEMBRANE TRANSPORT PROTEINS PART 1 **Mary Schaffer Ballroom** **Chair- Grant Kemp**
3:00pm-3:30pm	Maria Müller *(PhD student in the group of Ekkehard Neuhaus)* **-** Plastidic sodium transport and its effects on plant metabolism
3:30pm-4:00 pm	Chris Cheeseman – Differential regulation of human SLC2A9a and SLC2A9b isoforms in urate handling in *Xenopus laevis* oocyte expression system
4:00pm-4:30pm	Joachim Deitmer/Holger Becker The role of monocarboxylate transporters (MCT) for ketone body uptake
4:30pm-5:30 pm	**Poster set up and break**
5:30pm-6:30 pm	**Dinner – Ballroom C**

6:45pm	**SESSION I MEMBRANE TRANSPORT PROTEINS PART 2** **Mary Schaffer Ballroom** **Chair- Grant Kemp**
6:45pm-7:15 pm	Larry Fliegel Regulation of Activity of the Mammalian Na^+/H^+ Exchanger
7:15pm-7:45pm	Jan Kullmann *(for Eckhard Friauf)* Glycinergic neurotransmission and the reuptake systems GlyT2 and GlyT1
7:45pm-8:15 pm	Joe Casey Corneal Disease caused by a misbehaving membrane protein
8:15pm-9:45pm	**Poster Session I- Presenters at posters Cash Bar Ballroom**
9:45-??	Socialize with drinks
Tuesday, August 28th	
7:00am-8:30am	**Breakfast Buffet – Ballroom C**
8:30am	**SESSION II ORGANELLAR MEMBRANE PROTEINS** **Mary Schaffer Ballroom** **Chair- Steven Willows**
8:30am-9:00am	Jan Riemer Redox control in respiratory chain biogenesis
9:00am-9:30am	Shangmei Hou *(Graduate Student Tom Hobman lab)* Flavivirus infection and peroxisome biogenesis: Implications for anti-viral signaling
9:30am-10:00am	Ajay Ramesh *(PhD student in the group of Johannes Herrmann)* Molecular dissection of the mitochondrial protein import machinery
10:00am-10:30am	Emmanuelle Cordat Regulation of kidney anion exchanger 1 trafficking
10:30am-10:45am	**Break**
10:45am-11:15am	Nico Schäuble (PhD student in the group of Richard Zimmermann) Gating of the Sec61 channel by BiP, Sec63, and Calcium-Calmodulin
11:15am-11:45am	Cuc Quynh Nguyen-Truong *(PhD student in the group of Jens Rettig)* Functional analysis on CAPS2 splice variants in mouse chromaffin cells
11:45am-12:15pm	Manfred Schmitt Plasma membrane localized KDEL receptors as key in A/B toxin endocytosis and retrograde transport
12:15pm	**Pick up bag lunches in Ballroom C**

12:30pm-5:30pm	**Jasper Excursion**
6:00pm-7:00pm	**Dinner – Ballroom C**
7:15 pm	**SESSION III THE POWER OF BIOPHYSICS APPLIED TO MEMBRANE PROTEINS** **Mary Schaffer Ballroom** **Chair-Amira Fitieh**
7:15pm-7:45pm	Sandro Keller Biophysical approaches to the functional reconstitution of membrane proteins
7:45pm-8:15pm	John Githaka (PhD Student from Nicolas Touret's lab) Control of angiogenesis through clustering of CD36 receptors
8:15pm-9:45pm	**Poster Session II- Presenters at posters Cash Bar** **Mary Schaffer Ballroom**
9:45-??	Socialize with drinks
Wednesday, August 29th	
7:00am-8:30am	**Breakfast – Ballroom C**
8:30am	**SESSION IV MEMBRANE PROTEINS AND ION HOMEOSTASIS** **Mary Schaffer Ballroom** **Chair- Justin Fedor**
8:30am-9:00am	Jutta Engel Unusual properties of voltage- and Ca^{2+}-dependent big conductance K^+ (BK) channels in mammalian inner hair cells
9:00am-9:30am	Przemek Gorski *(PhD student from Howard Young's lab)* Structure and function of calcium transport regulatory complexes
9:30am-10:00am	Barbara Niemeyer/Christian Backes The Immune Synapse in Helper- and Killer cells
10:00am-10:30am	Xing-Zhen Chen Function and regulation of TRP-type channel TRPP3
10:30am-10:45am	**Break**
10:45am-11:15am	Anouar Belkacemi (PhD student in the group of Veit Flockerzi) New functions of the beta3 subunit of voltage-gated Ca channels
11:15am-11:45am	Joel Weiner A novel protein export pathway via *escherichia coli* porins

11:45am-12:15pm	Todd Alexander: Cldn14 and calcium homeostasis
12:15pm	**Pick up bag lunches in Ballroom C**
12:30pm-5:30pm	**Jasper Area Excursion**
6:00pm-7:00pm	**Dinner – Ballroom C**
7:15pm-9:15pm	**Small groups: Meetings between partner labs – See Separate Schedule**
Mtg. Spaces	**Ballroom BC** • Emerald Lounge • The Moose's Nook Northern Grill • Oka Sushi • Tent City Pub
9:15pm- ???	**Socialize with drinks**
Thursday, August 30th	
7:30am-8:30am	**Breakfast Buffet –Ballroom C**
8:45am	**SESSION V NUCLEOSIDE TRANSPORT** **Mary Schaffer Ballroom** **Chair- Gonzalo Vilas**
8:45am-9:15am	Sandra Witz *(PhD student in the group of Torsten Möhlmann)* A novel membrane protein in *Arabidopsis thaliana* involved in pyrimidine degradation and salvage – the plastidic nucleobase transporter PLUTO
9:15am-9:45am	Joanne Lemieux Overexpression of nucleoside transporters for structural studies
9:45am-10:00am	**Closing remarks**
10:15am	**Pick up bag lunches in Ballroom C**
10:30am	**Departure**

SPEAKER ABSTRACTS

SESSION I MEMBRANE TRANSPORT PROTEINS Part 1
Monday, August 27th – Afternoon Chair: Grant Kemp

3:00pm – 3:30pm Maria Müller

PLASTI DIC SODIUM TRANSPORT AND ITS EFFECTS ON PLANT METABOLISM

Maria Müller[1], Grant Kemp[2], Howard Young[2], Ilka Haferkamp[1] and H. Ekkehard Neuhaus[1].
[1] Department of Plant Physiology, Technische Universität Kaiserslautern, Germany,
[2] Department of Biochemistry, University of Alberta, Edmonton, AB, Canada T6G 2H7.

High salt concentrations in soil impose osmotic and ionic stress on plants and thus limit their growth and yield. Therefore, plants have evolved mechanisms to avoid deleterious cytosolic Na^+ concentrations, including active Na^+ extrusion out of the cell and intracellular compartmentalization - mainly to the vacuole - by Na^+/H^+ antiporters. Na^+/H^+ antiporters in the plasma membrane (SOS1 family) and the tonoplast (NHX family) have been characterized in detail so far. It is discussed, whether mitochondria and plastids (NhaD family) contribute to a certain extent to the overall subcellular compartmentalization of sodium.
NhD1 from *Arabidopsis thaliana* is located to the inner envelope membrane of plastids, as shown by GFP analysis. Expression studies revealed its involvement into salt stress response. Complementation of an *E. coli* knock-out strain (lacking the main bacterial Na^+/H^+ antiporters) and reconstitution of NhD1 into proteoliposomes, followed by uptake studies indicate the function of NhD1 as a Na^+/H^+ transporter. Analysis of NhD1 overexpressor lines reveal an increased salt sensitivity of these *Arabidopsis* mutants, displayed by a reduced fresh weight production and lower chlorophyll content. Furthermore, overexpressors show diminished photosynthetic activity and increased plastidic sodium levels indicating Na^+ uptake of chloroplasts via NhD1.
This work is financially supported by the Deutsche Forschungsgemeinschaft, DFG, GRK 845

3:30pm – 4:10pm Chris Cheeseman

DIFFERENTIAL REGULATION OF HUMAN SLC2A9A AND SLC2A9B ISOFORMS IN URATE HANDLING IN *XENOPUS LAEVIS* OOCYTE EXPRESSION SYSTEM

Chris I. Cheeseman. Department of Physiology and Membrane Protein Disease Research Group, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada.

It is now widely accepted that human GLUT9 (SLC2A9) is a high capacity urate transporter. This is backed by genome-wide association scans, which identify SNPs within SLC2A9 gene coincident with imbalance in plasma urate, overexpression studies, and GLUT9's predominant expression in liver and kidney, which are intimately involved in human plasma urate homeostasis. In humans, two splice variants of SLC2A9 exist: SLC2A9a (full length) and SLC2A9b (delta N). These two isoforms differ in their terminal region both in number and composition of the amino acid residues. Exisitng functional analysis suggested that the two isoforms are functionally identical, and that the N-terminal differences account purely for the differential targeting of SLC2A9a, to the basolateral membrane, and of SLC2A9b, to the apical membrane of polarized epithelia. However, recent findings from our lab suggest that the two isoforms display different urate transport profiles in response to extracellular and intracellular hexoses. OBJECTIVE: To investigate the underlying mechanism responsible for urate transport profile differences in response to *trans*-hexoses, focusing on the potential role of the N-terminus. METHODS: *X. laevis* oocyte expression system was used to overexpress hSLC2A9a and hSLC2A9b isoforms. Radiolabelled urate uptake and efflux was measured in presence and absence of *trans*-hexoses, PKA activators and PKC activators. Kinetic experiments and cell-surface biotinylation were performed to further characterize potential drug effects. Putative serine (Ser) phosphorylation sites within the N-terminus of SLC2A9a were mutated to alanines (Ala) using site-directed mutagenesis. RESULTS: Non-specific PKC activator, phorbol myristate acetate (PMA) affects SLC2A9a-, but not SLC2A9b-, mediated urate uptake and efflux. Kinetic experiments reveal that both affinity and capacity of the transporter are altered by PMA, suggesting that PKC-dependent modification of the transporter may be involved in regulating SLC2A9a activity

without changes in cell surface expression. Furthermore, cAMP activators (IBMX and Forskolin) altered SLC2A9b-, but not SLC2A9a-, mediated urate uptake dtermined at a concentration close to the functional Km. These observations suggest that urate reabsorption (SLC2A9a) and urate secretion (SLC2A9b) may be differentially regulated in polarized epithelia, such as the proximal convoluted tubule epithelia, in order to maintain plasma urate homeostasis. Verification of these observations in a relevant mamalian system is now being undertaken.

4:10pm-4:30pm Joachim Deitmer/Holger

H^+/HCO_3^--COUPLED MEMBRANE TRANSPORTERS AS SUPPORTED BY CARBONIC ANHYDRASES

Holger M. Becker, Michael Klier & Joachim W. Deitmer

We have studied members of two acid/base-coupled membrane transporter families, the monocarboxylate transporters (MCT, *SLC16* family) and the electrogenic sodium-bicarbonate cotransporter (NBCe1, *SLC4A4*) in astrocytes *in vitro* and *in situ*, and when expressed in *Xenopus* oocytes. We have found different modes of interaction between these membrane carriers and carbonic anhydrase (CA) isoforms. Transport activity of these carriers was measured by recording cytosolic H^+ using H^+-sensitive microelectrodes or the H^+-sensitive dye BCECF during substrate exposure. MCT1-MCT4 carry lactate, pyruvate and ketone bodies in cotransport with H^+ (1:1) across the cell membrane. Functional, isoform-specific interaction was found for MCT1, MCT2, and MCT4 with CAII and/or CAIV and CAIX. All interactions, as studied in oocytes, resulted in a robust increase of the transport of lactate by the MCTs. Augmentation of transport activity was independent of the catalytic activity of the CAs, but appeared to require binding of CA to MCT. In contrast, the enhancement of transport activity of NBCe1 by CAs required catalytic CA activity, being dependent on the fast, reversible conversion of CO_2 to H^+ and HCO_3^-, and hence presumably on the rapid availability of HCO_3^- as substrate for the NBCe1. The concept of transport metabolon, if applicable for the interactions of CAs with MCTs and NBC, comprise rather different types of interactions, which include molecular binding and may even involve chaperones as mediators between CA and carrier, at least between MCT and extracellular CA.

SESSION I MEMBRANE TRANSPORT PROTEINS Part 2
Monday, August 27tn – Evening Chair: Grant Kemp

6:45pm-7:15pm Larry Fliegel

REGULATION OF ACTIVITY OF THE MAMMALIAN Na^+/H^+ EXCHANGER BY ACCESSORY PROTEINS

Larry Fliegel, Xiuju Li, David Schrama, Benardo Alvarez, Joe R. Casey, Pratap Karki, Yongsheng Liu. Department of Biochemistry, University of Alberta, Edmonton, AB, Canada T6G 2H7.

The mammalian Na^+/H^+ exchanger isoform 1 (NHE1) is a ubiquitously expressed membrane protein that regulates intracellular pH in the myocardium. NHE1 is also important in mediating myocardial hypertrophy and NHE1 activity is important in growth and metastasis of several types of cancers. Mammalian NHE1 has a regulatory carboxyl terminal cytosolic tail of over 300 amino acids. We demonstrated that two proteins bind to the cytosolic tail and regulate NHE1 activity. Carbonic anhydrase II bound to this domain *in vitro*, and was co-imunoprecipited with NHE1. Expression of carbonic anhydrase II modified NHE1 activity and phosphorylation increased its binding. Carbonic Anhydrase II bound to the penultimate 13 amino acids of the NHE1 tail. A screen for other regulatory binding proteins revealed that the serine/threonine kinase B-Raf also binds to this domain. Immunoprecipitation of NHE1 from HeLa and HEK cells confirmed the association of B-Raf with NHE1 *in vivo*. The expressed and purified C-terminal 182 amino acids of the NHE1 protein were shown to associate with B-Raf protein *in vitro*. We examined the role of B-Raf in regulating NHE1 in malignant melanoma cells. Melanoma cells with the $B\text{-}Raf^{V600E}$ mutation demonstrated increased resting intracellular pH that was dependent on elevated NHE1 activity. NHE1 activity after an acute acid load was also elevated in these cell lines. Moreover, inhibition of B-Raf

activity by either sorafenib, PLX4720 or by siRNA reduction of B-Raf levels, abolished ERK phosphorylation and decreased NHE1 activity. These results demonstrate that B-Raf associates with, and stimulates NHE1 activity and that B-Raf^{V600E} also increases NHE1 activity which raises intracellular pH. Our studies show that the NHE1 cytosolic tail is regulated in a complicated and perhaps tissue specific manner by multiple accessory proteins. Supported by CIHR.

7:15pm-7:45pm Jan Kullmann

GLYCINERGIC NEUROTRANSMISSION AND THE REUPTAKE SYSTEMS GLYT2 AND GLYT1

Jan Kullmann and Eckhard Friauf
Animal Physiology group, University of Kaiserslautern, Germany

Neurotransmission via chemical synapses is mediated primarily by two groups of neurotransmitters, excitatory and inhibitory. The latter group comprises mainly GABA and glycine, the two major inhibitory neurotransmitters in the mammalian central nervous system. The physiology of inhibitory transmission, which is mediated via about 1/3 of all synapses, is much less understood than excitatory transmission. Inhibitory microcircuits in the auditory brainstem that are involved in sound localization are ideally suited for experimental approaches. Neurons in these microcircuits need to perform ultrafast signaling in the sub-millisecond range and at exquisitely high temporal precision. An important question is the role of two neurotransmitter transporters (GLYT2, GLYT1) that are involved in the re-uptake and recycling of the inhibitory neurotransmitter glycine. These two transporters are located in glial cells and axon terminals of presynaptic neurons, respectively, and their function is only poorly understood. Auditory brainstem neurons in general, and inhibitory projection neurons in particular, fire action potentials at very high and unprecedented rates (up to 800 Hz) and thus perform high-fidelity synaptic transmission under very strict constraints. Therefore, we postulate that efficient re-uptake and replenishing systems are established and maintained which participate in transmitter homeostasis and differ from those in other neural systems in which time precision is less crucially important. Therefor we analyzed particularly the projection from the medial nucleus of the trapezoid body to the lateral superior olive, which is very well suited for analyzing glycinergic transmission and GlyT function. Complex electrophysiological depletion and recovery protocols provided evidence regarding an important role for GlyT2 in homeostasis in the short as well as intermediate time range (subsecond - 30 mins). Analyses employed GlyT2-knockout mice and the use of specific transporter antagonists. Furthermore we will present first data of a proteomic approach to identify interaction partners of GlyT2 via pull-down assays and immunoprecipitation.

7:45pm-8:15pm Joe Casey

CORNEAL DISEASE CAUSED BY A MISBEHAVING MEMBRANE PROTEIN

Sampath Loganathan, Gonzalo Vilas and Joe Casey. Department of Biochemistry, University of Alberta, Edmonton, AB, Canada T6G 2H7.

Mutations of the gene, SLC4A11, cause three distinct conditions, all of which manifest as severe visual impairment whose only treatment is corneal transplantation. Congenital hereditary endothelial dystrophy (CHED) and Harboyan syndrome (HS) are relatively rare recessive genetic diseases whose visual symptoms appear at birth or in infancy. In addition to blindness, individuals with HS also suffer sensorineuronal deafness. Mutations of SLC4A11 also cause the dominant genetic disease, Fuchs endothelial corneal dystrophy (FECD). This illness is relatively common (4% of the population will suffer the condition), but occurs with onset in the fifth to seventh decade of life. Analysis of 50 disease alleles of SLC4A11 revealed that most share a common molecular phenotype: retention in the endoplasmic reticulum and failure to mature to the cell surface. We are testing these mutants to determine which mutants are temperature sensitive, that can be processed to the cell surface at 30 °C. If so, they are candidates for small molecule folding correctors, as a therapeutic strategy. We are also screening these mutants for their level of function, which we have found to be a novel form of water movement across the plasma membrane. Supported by Canadian Institutes of Health Research.

SESSION II ORGANELLAR MEMBRANE PROTEINS
Tuesday, August 28th – Morning Chair: Steven Willows

8:30am-9:00am Jan Riemer

REDOX PROCESSES IN COMPLEX I ASSEMBLY

Alican Erdogan and Jan Riemer
Cellular Biochemistry, Department of Biology, University of Kaiserslautern, Kaiserslautern, Germany

Complex I of the respiratory chain of mitochondria is crucial for cellular energy production. It is also a major source of reactive oxygen species, and dysfunctions of the complex have been implicated in the pathogenesis of a variety of neurodegenerative disorders. Dysfunctions often occur as a result of an impaired assembly, but so far only little is known about the biogenesis and maintenance of Complex I in mammalian cells. Seven of its subunits are encoded in the mitochondrial genome, while the remaining 38 subunits have to be imported from the cytosol. We aim to characterize the function of five proteins in the assembly/maintenance of Complex I that are linked to redox pathways. (1) The four uncharacterized nuclear-encoded Complex I subunits NDUFS5, NDUFB7, NDUFB10 and NDUFA8 lack typical mitochondrial import signals but instead contain conserved cysteine residues. The proteins are likely imported and trapped in the intermembrane space in a redox-dependent manner (twin-Cx_9C proteins). This process of oxidative folding is facilitated by the oxidoreductase Mia40 and is coupled to the activity of the respiratory chain. It is unknown how this redox pathway affects the assembly and maintenance of Complex I and whether the coupling to the respiratory chain provides a feedback control for respiratory chain assembly. We will therefore study the import and the function of these proteins as well as the role of Mia40 in Complex I biogenesis and maintenance on the molecular level. (2) We will also detail the function of the uncharacterized mitochondrial flavoprotein, FoxRed1. Homozygous mutations in this protein have been linked to isolated Complex I deficiencies. The cells of the patients expressed FoxRed1 mutants, and the levels of Complex I were strongly reduced. The molecular function of FoxRed1, however, remains unclear. Here, we will present initial data on the characterization of redox processes in Complex I assembly and introduce the project strategy.

9:00am-9:30am Shangmei Hou

EXPRESSION OF FLAVIVIRUS CAPSID PROTEINS AFFECTS PEROXISOME BIOGENESIS AND POTENTIALLY ANTIVIRAL SIGNALING

Shangmei Hou, Jae Hwan You and Tom C. Hobman. Department of Cell Biology, University of Alberta, Edmonton, AB, Canada, T6G 2H7

Flaviviruses comprise a large family of positive sense, single-stranded RNA viruses that are transmitted mainly through arthropods. Every year they infect hundreds of millions of people resulting in enormous economic and health burden. Unfortunately, there are very few vaccine and therapeutic options for these viruses. Our laboratory is studying virus-host interactions at the cellular level with the goal of identifying novel anti-viral targets. Much of our research has focused on flavivirus capsid proteins. The capsid protein is a small structural component of the virus, whose major function is to protect the viral genome. Interestingly, it has been shown to possess nonstructural functions. A proteomic screen identified the peroxisome biogenesis factor, Pex19p as a host protein that interacts with the capsid proteins of West Nile virus (WNV) and Dengue virus (DENV). Given the evidence that peroxisomes are platforms for innate antiviral defense, we hypothesize that the alteration in this membranous organelle may be of significance for virus survival and efficient replication. Several lines of evidence support this idea. First, infection of cells with WNV or DENV leads to ~ 40% reduction in the number of peroxisomes. Second, the subcellular distributions of two peroxisomal membrane proteins, Pex3p and Pex16p, which require the function of Pex19p, are altered in the presence of WNV/DENV capsid proteins. Finally, microarray analyses revealed that capsid expression leads to ~5-fold increase in mRNA of two other peroxisome proliferation factors, Pex11βp and dynamin-related protein. Altogether, it suggests that infection of these flaviviruses and/or expression of the capsid proteins could lead to changes in peroxisome biogenesis, possibly as a countermeasure against the host cell antiviral signaling. Supported by CIHR, AIHS, NSERC, and Canada Research Chairs.

9:30am-10:00am Ajay Ramesh

MOLECULAR DISSECTION OF THE MITOCHONDRIAL PROTEIN IMPORT MACHINERY

Ajay Ramesh and Johannes Herrmann. Cell Biology, University of Kaiserslautern, Germany.

A majority of the mitochondrial proteins are encoded by the nuclear genome. Upon synthesis in the cytosol, these proteins have to be translocated to their respective destinations within the mitochondria. The translocase of the outer membrane (TOM) and the translocase of the inner membrane (TIM) complexes facilitate the translocation of preproteins across the outer and inner membranes of the mitochondria, respectively. The TIM23 complex mediates the translocation of precursor proteins that are targeted to the mitochondrial matrix or to the mitochondrial inner membrane. Tim17 is an integral component of the TIM23 translocase containing four transmembrane domains. The sequence of Tim17 is highly conserved amongst fungi, plants and animals including humans. All Tim17 proteins contain two conserved cysteine residues that are located directly adjacent to the first and second transmembrane domains facing the intermembrane space. The relevance of these cysteine residues is not known. Various functions such as regulation and sorting during preprotein translocation, recruiting of the presequence assisted motor (PAM) machinery, regulation of the protein-conducting channel, and prevention of mitochondrial DNA loss have been assigned to Tim17. However, the actual role of Tim17 still remains unknown. Growth tests and *in vitro* import experiments revealed that one of these cysteine residues was vital for the viability of the cell under stress conditions as well as for the efficient import of specific matrix proteins. The results point at a critical mechanistic or regulatory role of cysteine residues in Tim17 for the import of mitochondrial preproteins. Further work will be presented during the talk. Supported by DFG (IRTG 1830).

10:00am-10:30am Emmanuelle Cordat

ADAPTOR PROTEIN 1 COMPLEXES REGULATE INTRACELLULAR TRAFFICKING OF THE KIDNEY ANION EXCHANGER 1 IN EPITHELIAL CELLS

Ensaf Y. Almomani*[1], Jennifer C. King*[1],Janjuree Netsawang†, Pa-thai Yenchitsomanus†, Prida Malasit†, Thawornchai Limjindaporn†, R. Todd Alexander* and Emmanuelle Cordat. * Department of Physiology, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada; †Division of Medical Molecular Biology and BIOTEC-Medical Biotechnology Unit, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Distal renal tubular acidosis (dRTA) can be caused by mutations in the gene encoding the anion exchanger 1 (AE1) and is characterized by defective urinary acidification, metabolic acidosis and renal stones. AE1 is expressed at the basolateral membrane of type A intercalated cells in the renal cortical collecting duct (kAE1). Two dRTA mutations result in the carboxyl-terminal truncation of kAE1; in one case, the protein trafficked in a non-polarized way in epithelial cells. A recent yeast two-hybrid assay showed that the carboxyl-terminal cytosolic domain of AE1 interacts with adaptor protein complex 1 (AP-1A) subunit □1A (mu-1A) (27). Here, we show the interaction between kAE1 and mu-1A and B *in vitro* by reciprocal co-immunoprecipitation in epithelial cells and *in vivo* by co-immunoprecipitation from mouse kidney extract. When endogenous mu-1A (and to a lesser extent mu-1B) was reduced, kAE1 protein was unable to traffic to the plasma membrane and was rapidly degraded via a lysosomal pathway. Expression of either siRNA resistant mu-1A or mu-1B stabilized kAE1 in these cells. We also show that newly synthesized kAE1 does not traffic through recycling endosomes to the plasma membrane, suggesting that AP-1B, located in recycling endosomes, is not primarily involved in trafficking of newly synthesized kAE1 when AP-1A is present in the cells. Finally, siRNA resistant AP-1A rescued cell surface trafficking of newly synthesized kAE1 in cells knocked down for endogenous AP-1A/B. Our data demonstrate that AP-1A regulates processing of the basolateral, polytopic membrane protein kAE1 to the cell surface and that both AP-1A and B adaptor complexes are required for normal kAE1 trafficking. Supported by CIHR and Kidney Foundation of Canada.

10:45am-11:15am Nico Schäuble

GATING OF THE HUMAN SEC61 CHANNEL BY BIP, SEC63, AND CALCIUM-CALMODULIN

Nico Schäuble, Sven Lang, Martin Jung, Johanna Dudek, Adolfo Cavalié and Richard Zimmermann. Medical Biochemistry and Molecular Biology, Saarland University, D-66421 Homburg, Germany.

Two major functions of the mammalian endoplasmic reticulum (ER) are the biogenesis of secretory and membrane proteins as well as serving as an calcium (Ca^{2+}) reservoir for cellular signaling. ER import of polypeptide precursors carrying a N-terminal signal-peptide is strictly dependent on an ER-resident protein translocase with the Sec61 complex as central component. Hitherto, an additional role of the ER-lumenal Hsp70 chaperone BiP and its Hsp40 co-chaperone Sec63 for protein translocation is well characterized in yeast. We depleted the levels of Sec63 and BiP using siRNA or a specific toxin, and tested whether reduction of these proteins in human cells affects Sec61 dependent protein translocation into the ER of mammalian cells. Reductions of both, Sec63 and BiP, revealed overlapping, precursor specific involvements in the initial phase of co-translational protein translocation into the ER of semi-permeabilized cells. Moreover, when we replaced Sec61α with diabetes-linked Sec61αY344H we obtained a comparable phenotype suggesting a potential BiP binding site in ER luminal loop 7 in the vicinity of tyrosine 344. Previous work also characterized the Sec61 channel as a potential Ca^{2+} leak channel. In accordance with the transport results we observed a similar channel gating behavior with respect to Ca^{2+} permeability. In Ca^{2+} imaging experiments, reduction of cellular BiP by different means resulted in a significantly increased Ca^{2+} leakage from the ER. However, additional knockdown of Sec61α strongly inhibited this effect. Moreover, Sec61αY344H phenocopied the BiP-depletion effect and was also not longer affected by BiP reduction. Preceding experiments also identified Ca^{2+} calmodulin as limiting Ca^{2+} leakage through Sec61 by binding to a cytosolic IQ-motif in the N-terminus of Sec61α in Ca^{2+} dependent manner. Thus, gating of Sec61 channels has to be tightly regulated to ensure proper protein transport and, simultaneously, sufficient sealing of the translocation pore and involves cytosolic as well as ER luminal factors.

11:15am-11:45am Cuc Quynh Nguyen-Truong

FUNCTION OF SPLICE VARIANTS OF CAPS2 (CA^{2+} DEPENDENT ACTIVATOR PROTEIN FOR SECRETION) IN MOUSE CHROMAFFIN CELLS

Cuc Quynh Nguyen-Truong, Varsha Pattu, Claudia Schirra, Mahantappa Halimani, David Stevens, Jens Rettig. Department of Physiology, University of Saarland, 66424 Homburg, Germany

The Ca^{2+} dependent activator protein for secretion (CAPS) is involved in priming synaptic vesicles and large dense core vesicles (LDCVs) for exocytosis. The two CAPS isoforms, CAPS1 and CAPS2, preferentially prime the Readily Releasable Pool (RRP) in mouse chromaffin cells. We have shown that rat CAPS1 and CAPS2 rescue the secretion deficit in mouse chromaffin cells lacking both CAPS1 and CAPS2 (CAPS DKO). Six CAPS2 splice variants have been detected in the mouse cerebellum. Using RT-PCR, we show that all splice variants (CAPS2a - CAPS2f) are expressed at different developmental stages in mouse chromaffin cells. In rescue experiments, CAPS2b, CAPS2d or CAPS2e were expressed in CAPS DKO chromaffin cells lacking CAPS1 and all CAPS2 splice variants. Secretion in these cells was compared to secretion in CAPS DKO cells. To this end we performed capacitance measurements in whole cell patch clamp and simultaneous amperometric recordings to monitor catecholamine release in CAPS DKO cells. Secretion was elicited by flash photolysis of np-EGTA caged Ca^{2+}. Expression of mouse CAPS2b, which has a short deletion in the Munc13 Homology Domain (MHD, the putative priming domain), rescued the CAPS DKO secretion defect, enhancing the RRP and sustained secretion. CAPS2d, with a C-terminal deletion of much of the priming domain failed to rescue. Surprisingly, expression of CAPS2e, with a C-terminal truncation which includes all of the MHD, also enhanced secretion in DKO cells. Our results indicate that in addition to an interaction with syntaxin mediated by the C-terminal MHD domain, N-terminal domains in CAPS can promote exocytosis, likely via the Plekstrin homology and C2 domains. Thus, it is likely that even the strongly truncated splice variant CAPS2e modulates exocytosis. Supported by the DFG.

11:45am-12:15pm Manfred Schmitt

PLASMA MEMBRANE LOCALIZED KDEL-RECEPTORS AS KEY IN A/B TOXIN ENDOCYTOSIS AND RETROGRADE TRANSPORT

Esther Gießelmann, Julia Dausend and Manfred J. Schmitt.
Molecular & Cell Biology, Department of Biosciences (FR 8.3), Saarland University, D-66041 Saarbruecken, Germany

Microbial and plant A/B toxins such as cholera and Shiga toxin, ricin and the yeast (*S. cerevisiae*) viral A/B toxin K28 represent secreted heteromeric protein toxins which enter eukaryotic target cells by receptor-mediated endocytosis. After retrograde transport through the secretory pathway *via* early endosomes, Golgi and the ER, the toxins translocate into the cytosol to kill their host [1]. Here we demonstrate that the essential cellular K/HDEL receptor Erd2p represents the key component in retrograde toxin transport by interacting with the unmasked ER retention motif HDEL at the C-terminus of the toxin's β-subunit [2, 3]. Furthermore, this motif can likewise facilitate uptake and retrograde transport of H/KDEL carrying proteins into yeast cell spheroplasts, indicating that a minor portion of the cellular H/KDEL receptor also colocalizes in the plasma membrane. This important observation was confirmed by confocal laser scanning fluorescence microscopy as well as reporter assays using *in vivo* cell-growth as read-out. Precise Erd2p topology prediction was an essential prerequisite to further characterize the endocytosis of the toxin/receptor complex K28/Erd2p. Therefore, a set of reporter fusions was established to determine the *in vivo* topology of the yeast KDEL-receptor Erd2p. Targeted toxin receptor mutagenesis uncovered endocytic motifs within cytosolic receptor domains affecting toxin sensitivity. In conjunction with TIRF-based mobility tracking of fluorescently labelled toxin/receptor complexes and biochemical analysis the Erd2p, receptor endocytosis was shown to rely on multiple mechanisms, including ubiquitylation, Pan1 complex components such as Sla1p as well as the adapter protein complex AP2 which so far had been described to be exclusively involved in endocytosis in mammalian cells [4].

1. M.J. Schmitt and F. Breinig, Nat. Rev. Microbiol. 4 (2006), p. 212.
2. K. Eisfeld et al., Mol. Microbiol. 37 (2000), p. 926.
3. F. Riffer et al., Microbiology 148 (2002), p. 1317.
4. S.Y. Carroll et al., Dev. Cell 17 (2009), p. 552.

SESSION lll THE POWER OF BIOPHYSICS APPLIED TO MEMBRANE PROTEINS
Tuesday, August 28th – Evening Chair: Amira Fitieh

7:15pm-7:45pm Sandro Keller

BIOPHYSICAL APPROACHES TO THE FUNCTIONAL RECONSTITUTION OF MEMBRANE PROTEINS

Sandro Keller,[a] Carolyn Vargas,[a] Nadin Jahnke,[b] and Oxana Krylova[b]

[a]Molecular Biophysics, University of Kaiserslautern, Kaiserslautern, Germany
[b]Biophysics of Membrane Proteins, Leibniz Institute of Molecular Pharmacology FMP, Berlin, Germany

Membrane proteins make up roughly 30% of all proteins encoded by the human genome and represent about 50% of drug targets in the human body. They fulfil vital functions as receptors and signal transducers, channels and transporters, motors and anchors. Many of these functions are amenable to biochemical and biophysical investigation only after the membrane protein of interest has been extracted, purified, and reconstituted into artificial liposomes. Extraction from the host-cell membrane and chromatographic purification are usually performed with the aid of detergents. However, detergent micelles do not allow the study of vectorial functions such as solute transport or signal transduction. Therefore, numerous membrane proteins need to be reconstituted from a purified, detergent-solubilised state into liposomes in order to regain their native structures and activities. Unfortunately, functional reconstitution

has remained one of the main bottlenecks in the handling of membrane proteins. In particular, gauging the success of reconstitution experiments has thus far been limited to trial-and-error approaches. To address this problem, we have established high-sensitivity isothermal titration calorimetry (ITC) as a powerful method for monitoring the reconstitution of membrane proteins into liposomes. ITC has previously been employed for characterising liposome solubilisation and reconstitution in the absence of protein.[1,2] Recent work in our laboratory has demonstrated that ITC is also excellently suited for tracking the complex process of membrane-protein reconstitution in a non-invasive and fully automated manner. The approach is exemplified for the prokaryotic potassium ion channel KcsA, which was functionally reconstituted into stable proteoliposomes at high protein densities. Electrophysiological experiments confirmed that KcsA regained its functional activity upon ITC-guided reconstitution.

[1] H. Heerklotz, A. D. Tsamaloukas, and S. Keller. *Nat. Protoc.* **2009**, *4*, 686–697.
[2] O. O. Krylova, N. Jahnke, and S. Keller. *Biophys. Chem.* **2010**, *150*, 105–111.

7:45pm-8:15pm John Githaka

SINGLE MOLECULE STUDY OF THROMBOSPONDIN-1 RECEPTOR, CD36, IN THE ENDOTHELIAL CELL PLASMA MEMBRANE

John M. Githaka[1], Anindya Chanda[2], Gaudenz Danuser[2], Khuloud Jaqaman[3] and Nicolas Touret[1]
[1]Department of Biochemistry, University of Alberta, Edmonton, Canada; [2]Department of Cell Biology, Harvard Medical School, Boston, USA; [3]Department of Systems Biology, Harvard Medical School, Boston, USA.

Thrombospondin-1 (TSP-1), an endogenous extracellular matrix anti-angiogenic factor, is down-regulated in many tumors. CD36 is the main receptor mediating TSP-1's anti-angiogenic activity through signaling cascades that induce endothelial cell apoptosis. The binding of a multivalent ligand to CD36, anti-CD36 immunoglobulin M (IgM), similarly induces endothelial cells apoptosis while divalent anti-CD36 immunoglobulin G (IgG) does not suggesting that CD36 clustering is important in this apoptotic signal. Not much is known about the dynamic organization of CD36 into multimeric complexes upon TSP-1 binding, and whether this is aided by β_1-integrin, another TSP-1 receptor that inhibit endothelial cell migration. In this study, we used single molecule and super-resolution imaging to assess the spatial organization of CD36 in the plasma membrane of human dermal microvascular endothelial cells. We compared CD36 dynamics and organization in unstimulated and TSP-1 stimulated cells. Treatment with 10 nM TSP-1, a dose that activated apoptosis in the endothelial cells, resulted in a significant increase in CD36 directed mobility in an actin guided pattern as revealed by single particle tracking. TSP-1 also increased activatory phosphorylation of Src Family Kinase, Fyn, at focal adhesions (where most of β_1-integrin is found) and along actin filaments in a CD36-dependent manner. Based on our data, we propose that TSP-1 binding results in a change in CD36 dynamics, potentially mediated by actin and β_1-integrin, which facilitates CD36 clustering and subsequent downstream signaling.

SESSION IV MEMBRANE PROTEINS AND ION HOMEOSTASIS
Wednesday, August 29th – Morning Chair: Justin Fedor

8:30am-9:00am Jutta Engel

CA2+- AND VOLTAGE-DEPENDENT BIG CONDUCTANCE POTASSIUM (BK) CHANNELS IN SENSORY HAIR CELLS – ESSENTIAL FOR PROPER HEARING BUT STILL A MYSTERY

Jutta Engel, Barbara Fell, Niels Brandt, Stefan Münkner. Department of Biophysics, Medical Faculty, Saarland University, 66424 Homburg/Saar, Germany.

The mammalian BK channel is a widely expressed ion channel that serves many functions, e.g. controlling smooth muscle tone and neuronal excitability. In cochlear inner hair cells (IHCs), BK channels are expressed from the onset of hearing (P12 in mice) onwards and underlie the fast-activating K^+ outward current $I_{K,f}$. They are responsible for fast repolarization of the receptor potential and for the small time constant of the IHC. Due to their large chord conductance, BK channels moreover extend the dynamic range of sound IHCs can respond to. Activation of BK channels in most cells not only requires depolarization but also a local rise in intracellular Ca^{2+} to shift the activation range from > 0 mV towards negative voltages. Mammalian IHC BK channels are localized at the neck and are therefore spatially segregated from presynaptic $Ca_v1.3$ L-type Ca^{2+} channels, which are located at the IHC basolateral pole. As IHC BK channels open around -50 mV, they either need alternative Ca^{2+} sources for opening or the interaction with proteins that uncouple channel gating from Ca^{2+} (e.g. LRRC26, Yan and Aldrich, 2010). Paradoxically, 90 % of the whole-cell BK current of IHCs is unaffected by omission of extracellular Ca^{2+} or block of $Ca_v1.3$ channels (Marcotti et al., 2004) whereas in contrast, inside-out patches containing BK channels are still very sensitive to applied Ca^{2+} (Thurm et al., 2005). We hypothesize different pools of BK channels in IHCs: A larger pool, which is insensitive to Ca^{2+} influx, is clustered at the IHC neck and may sense Ca^{2+} release from ER or interact with gating modifiers. A much smaller pool of BK channels may be localized close to $Ca_v1.3$ channels. We aim to test these hypotheses using transcript analysis, patch-clamp recordings, immunohistochemistry at light and electron microscopy level and life imaging techniques. Supported by DFG.

9:00am-9:30am Przemek Gorski

STRUCTURE AND FUNCTION OF SERCA CALCIUM TRANSPORT REGULATORY COMPLEXES.

Przemek A. Gorski[1], John Paul Glaves[1, 2], Howard S. Young[1, 2]
[1]Department of Biochemistry and [2]National Institute for Nanotechnology, University of Alberta

The sarcoplasmic reticulum (SR) is a calcium storage organelle in muscle cells that contains a calcium pump (SERCA) required for the reuptake of calcium into the SR for muscle relaxation. The activity of SERCA is tightly regulated through reversible interactions with the short integral membrane proteins, phospholamban (PLB) and sarcolipin (SLN). Defects in the regulation of SERCA are a central determinant in end-stage heart failure. Consequently, the regulatory mechanisms imposed by PLB and SLN could have clinical implications for heart and skeletal muscle diseases. There is significant sequence homology in the transmembrane regions of PLB and SLN which suggests a similar mode of binding to SERCA; however, PLB and SLN differ in their unique N- and C-terminal domains. PLB has a longer cytoplasmic N-terminus (28 residues in PLB versus 7 residues in SLN) that is important in the physiological regulation of SERCA, while the unique luminal C-terminus of SLN (^{27}RSYQY^{31}) appears to encode much of the inhibitory function of this protein. The structural differences in these domains could be responsible for the differences in their regulation of SERCA. Amongst our experimental approaches, we functionally characterize mutants of PLB and SLN using co-reconstituted proteoliposomes with SERCA that mimic the SR membrane. These same proteoliposomes can be used for two-dimensional crystallization and structural studies by cryo-electron microscopy. An overview of research in the Young laboratory will be presented, as well as some recent unpublished insights into the luminal domain of SLN. Our results reveal that the C-terminal tail of SLN is a distinct, essential domain in the regulation of SERCA from the luminal side of the membrane, and that the functional properties of the SLN tail can be transferred to other transmembrane peptides including PLB. Supported by the Canadian Institutes of Health Research and Alberta Innovates – Technology Futures.

9:30am-10:00am Barbara Niemeyer / Christian Backes

THE IMMUNE SYNAPSE IN T HELPER- AND KILLER CELLS

Barbara A. Niemeyer, Christian Backes, Helene Lyrmann, Bin Qu, Markus Hoth.
Department of Biophysics, Medical Faculty, Saarland University, 66424 Homburg/Saar, Germany.

Cell polarization is a key feature of T-cell function. Analogous to neuronal synapses, the immunological synapse (IS) between T cells and antigen-presenting cells provides a highly polarized signalling port to ensure specific information exchange, which in the case of cytotoxic T cells or natural killer cells can lead to the release of cytotoxic granule content into the synaptic cleft. A key step for T-cell activation is a rise in the cytoplasmic calcium concentration. Here, Ca^{2+}-sensor molecules (stromal interaction molecule, STIM1), localized in the membranes of the ER sense the T cell receptor (TCR) induced increase in IP_3 by sensing the IP_3 induced decrease in ER calcium. Clustered STIM molecules within the ER then localize in close proximity to the IS plasmamembrane and activate Orai channels to provide Ca^{2+} influx. In the first part of the presentation, we will provide an overview of the current projects that aim at understanding mechanisms of T cell polarization and modulation of calcium signalling by the extracellular environment. In one project we show that the major isoform of Orai channels in naïve T helper cells, Orai1, can be inhibited by irreversible oxidation of an extracellular localized cysteine, which is lacking in its close homolog Orai3. Upregulation of Orai3 and formation of heteromeric channel complexes in stimulated T helper cells thus provides a mechanism for immune cells to adapt to oxidative environments during inflammation. The second part of the presentation will focus on analyzing the function of natural killer (NK) cells, introducing a new tool to measure NK mediated cytotoxicity.
Supported by the DFG and by Saarland University HOMFOR funds.

10:00am-10:30am Xing-Zhen Chen

FUNCTION AND REGULATION OF TRP-TYPE CHANNEL TRPP3

Xing-Zhen Chen, JungWoo Yang, Wang Zheng, Qian Wang, and Shaimaa Hussein. Membrane Protein Disease Research Group, Department of Physiology, University of Alberta, Edmonton, Alberta, Canada.

Transient receptor potential polycystin isoform 3 (TRPP3) is a homolog of TRPP2 that is mutated in 10-15% of autosomal dominant polycystic kidney disease (ADPKD) but itself is not implicated in ADPKD. TRPP3 is present in multiple tissues, such as retina, brain, tongue and kidney. TRPP3 possesses a similar membrane topology as other TRP channels and voltage-gated cation channels, and was first reported in 1999 to be a non-selective cation channel activated by Ca. Since 2006, TRPP3 has known to be present bipolar cells of the tongue and activated by protons in an off-response way in the presence of an interacting partner protein called PKD1L3. However, still little is known about its channel function and regulation, and there are controversies regarding its involvement in acid sensing. Our laboratory focuses on studying function and regulation of TRPP2 and TRPP3. Here we present some of our recent progresses on TRPP3. We found that scaffolding protein RACK1 (receptor for activated C kinase-1) inhibits TRPP3 channel function through binding to its N-terminal domain. We also found that the N-terminal residue cysteine 38 is critical for its channel function and dimerization, likely through forming a disulfide bond between two cysteine 38 residues. We found that TRPP3 expressed in oocytes alone is sufficient to induce the off-response. Further, we found that acid sensing ion channel (ASIC) physically interacts with TRPP3 and increases its off-response. Our data are important in elucidating the channel function/regulation and physiological function of TRPP3. Supported by CIHR and AIHS.

10:45am-11:15am Anouar Belkacemi

ALTERED FIBROBLAST MOTILITY AND SKIN WOUND HEALING IN MICE DEFICIENT IN THE β3 SUBUNIT OF VOLTAGE-ACTIVATED CALCIUM CHANNELS

Anouar Belkacemi[1], Matthias W. Laschke[2], Claudia Scheuer[2], Petra Weissgerber[1], Ulrich Wissenbach[1], Michael D. Menger[2], Stephan E. Philipp[1], Andreas Beck[1] and Veit Flockerzi[1]. [1]Institut für Experimentelle und Klinische Pharmakologie und Toxikologie and [2]Klinisch-Experimentelle Chirurgie, Universität des Saarlandes, 66421 Homburg, Germany

$Ca_V\beta$ subunits of voltage-activated Ca^{2+} channels (VACCs) are required for trafficking of the pore-forming $Ca_V\alpha1$ subunit to the plasma membrane and modulation of Ca^{2+} current kinetics. We identified $Ca_V\beta2$ and $Ca_V\beta3$ protein expression in primary mouse embryonic fibroblasts (MEFs), skin fibroblasts and cardiac fibroblasts, cells typically lacking voltage-activated Ca^{2+} influx. Apparently, $Ca_V\beta$ subunits serve functions in fibroblasts unrelated to VACCs. Co-expressed in COS-7 cells, $Ca_V\beta3$ immunoprecipitates with IP_3R type 1, 2 or 3, and vice versa. Radio-receptor assays and Fura-2 measurements revealed, that basal IP_3 production and agonist (lysophosphatidic acid or bradykinin)-induced IP_3-dependent Ca^{2+} release, respectively, were significantly increased in MEFs isolated from β3-deficient mice compared to wild-type, whereas thapsigargin-induced Ca^{2+} release was unaffected. In addition, overexpression of $Ca_V\beta3$ in HEK-293 cells significantly reduced Ca^{2+} release after stimulation of endogenous Gq-coupled muscarinic receptors by carbachol. Apparently, 3 acts as a brake on IP_3-dependent Ca^{2+} release either by direct interaction with IP_3Rs or by interfering with pathways generating or metabolizing IP_3. Fibroblasts are migrating cells and involved in various physiological and pathophysiological processes. We found no apparent difference in angiogenesis, cell survival and cell proliferation between wild-type and β3-deficient MEFs, but migration (scratch assay) and chemotaxis (transwell chamber) were significantly affected. Non-muscle Myosin IIA and F-actin serve diverse functions in cell contractility, cytokinesis and locomotion, but expression levels of Myosin IIA or F-to-G-actin ratio were apparently unaffected. However, collagen secretion, essentially contributing to wound healing, was significantly enhanced in β3-deficient MEFs. Thus, skin wound closure happened significantly faster in β3-deficient mice compared to wild-type. In summary, we identified Ca_V β3proteins in fibroblasts, their interaction with IP_3 receptors and/or IP_3-generating/metabolizing pathways and we showed that in MEFs IP_3-dependent Ca^{2+} release, migration, chemotaxis and collagen secretion is significantly changed in the absence of $Ca_V\beta3$, and that $Ca_V\beta3$is a critical determinant of skin wound closure. Supported by the Deutsche Forschungsgemeinschaft DFG (IRTG 1830 and SFB 894).

11:15am-11:45am Joel Weiner

NOVEL PROTEIN EXPORT PATHWAY VIA *ESCHERICHIA COLI* PORINS

Gerd Prehna[1,2], Guijin Zhang[3], Xiandi Gong[4], Marek Duszyk[4], Mark Okon[1,5,6], Lawrence P. McIntosh[1,5,6], Joel H. Weiner[3*], and Natalie C.J. Strynadka[1]

[1]Department of Biochemistry, Life Sciences Center, 4350 Health Sciences Mall, University of British Columbia, Vancouver, BC, V6G 1Z3, [2]Center for Blood Research, Life Sciences Center, 4350 Health Sciences Mall, University of British Columbia, Vancouver, BC, V6G 1Z3, [3]Membrane Protein Disease Research Group, Department of Biochemistry, 474 Medical Sciences Building, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada, [4]Department of Physiology, 746 Medical Sciences Building, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada, [5]Department of Chemistry, 2036 Main Mall, University of British Columbia, Vancouver BC, V6T 1Z3, Canada, [6]Michael Smith Laboratories, University of British Columbia, Vancouver BC, V6T 1Z3, Canada

Escherichia coli export the protein YebF into the extracellular medium and recombinant proteins linked to C-terminus of YebF can be secreted as passenger proteins. Secretion is mediated by a two-step process involving *sec* translocon-mediated export across the cytoplasmic membrane. As no outer membrane protein secretion system has been reported in common strains of *E. coli*, the mechanism of export across the outer membrane has remained unclear. We expressed YebF in *E. coli* strains with mutations of outer membrane protein genes leading to the identification of the porins OmpF, OmpC and the outer membrane protein OmpX as central to the export mechanism. Deletion of OmpF or OmpX blocked YebF secretion, and deletion of OmpC reduced secretion. Additionally, purified YebF could occlude the channel conductance of OmpF and OmpC in an OmpX dependent manner. We then solved the NMR structural ensemble of YebF revealing a cystatin-like fold consisting of a structured core and an extended

dynamic surface in a state of conformational exchange. This surface is conserved throughout *Enterobacteriaceae* including *E. coli* and *Salmonella* and likely serves to facilitate YebF transport as mutation of several of the dynamic residues inhibit secretion to the extracellular medium. Our results suggest that the porins OmpF and OmpC not only operate as a potential import pathway for ions and large protein toxins as previously shown, but also in conjunction with OmpX operate as a conserved bacterial protein export pathway. Supported by NSERC, CIHR, HHMI, HFSP, MSFHR and CFC.

11:45am-12:15pm Todd Alexander

ACTIVATION OF THE CA^{2+}-SENSING RECEPTOR INCREASES RENAL CLAUDIN-14 EXPRESSION AND URINARY CA^{2+} EXCRETION

R. Todd Alexander, Henrik Dimke, Prajakta Desai, Jelena Borovac, Alyssa Lau and Wanling Pan
Department of Physiology, Pediatrics & Membrane Protein Disease Research Group, University of Alberta, Edmonton, Canada

Kidney stones are a prevalent clinical condition imposing a large economic burden to the healthcare system. Hypercalciuria remains the major risk factor for development of a Ca^{2+}-containing stone. The kidney's ability to alter Ca^{2+} excretion in response to changes in serum Ca^{2+} is in part mediated by the Ca^{2+}-sensing receptor (CaSR). Recent studies revealed renal claudin-14 (Cldn14) expression localized to the thick ascending limb (TAL) and its expression to be regulated via the CaSR. We find that Cldn14 expression is increased by high dietary Ca^{2+} intake and by elevated serum Ca^{2+} levels induced by prolonged 1,25-dihydroxyvitamin D_3 administration. Consistent with this, activation of the CaSR *in vivo* via administration of the calcimimetic cinacalcet hydrochloride, led to a 40-fold increase in Cldn 14 mRNA. Moreover, overexpression of Cldn14 in a cell culture model decreased paracellular Ca^{2+} flux by preferentially decreasing cation permeability, thereby increasing transepithelial resistance. These data support the existence of a mechanism whereby activation of the CaSR in the TAL increases Cldn14 expression, which in turn blocks the paracellular reabsorption of Ca^{2+}. This molecular mechanism likely facilitates renal Ca^{2+} losses in response to elevated serum Ca^{2+}. Moreover, dysregulation of the newly described CaSR-Cldn14 axis likely contributes to the development of hypercalciuria and kidney stones. Funded by CIHR and the Kidney Foundation of Canada

SESSION V NUCLEOSIDE TRANSPORT
Thursday, August 30th – Morning Chair: Gonzalo Vilas

8:45am-9:15am Sandra Witz

A NOVEL MEMBRANE PROTEIN IN ARABIDOPSIS THALIANA INVOLVED IN PYRIMIDINE DEGRADATION AND SALVAGE – THE PLASTIDIC NUCLEOBASE TRANSPORTER PLUTO

Sandra Witz, Markus Schober, Johannes Deppe and Torsten Möhlmann. Department of Plant Physiology, Technische Universität Kaiserslautern, Germany.

Nucleotide metabolism is of great importance for plant growth and development and can be divided into nucleotide *de novo* synthesis, salvage pathway and degradation. In plants, the two initial enzymatic reactions of the *de novo* pyrimidine synthesis occur in plastids, whereas further reactions are localized in the cytosol and mitochondria. Pyrimidine catabolism as well as salvage pathway reactions occur in plastids while the main enzyme releasing the nucleobase uracil, NSH1, is localized in the cytosol. Based on these findings a nucleobase import system must be localized in the plastid envelope providing substrates for the essential salvage pathway. PLUTO (for plastidic nucleobase transporter) was identified as the sole member of the Nucleobase:Cation-Symporter1 protein family in *Arabidopsis thaliana* and shares 23% sequence identity to FUR4, a uracil permease in *Saccharomyces cerevisiae*. A detailed biochemical characterization will be shown after heterologous expression in an *Escherichia coli* mutant lacking the endogenous uracil importer uraA. PLUTO transports uracil, adenine and guanine with apparent affinities of 16.4, 0.4 and 6.3 µM, respectively. Transport was markedly inhibited by low concentrations of a proton uncoupler, indicating that PLUTO functions as a proton-substrate symporter. The subcellular localization of PLUTO in the plastid envelope will be presented with GFP-studies in Arabidopsis leaf protoplasts. Thus, PLUTO represents a protein for the absolutely required import of pyrimidine nucleobases into plastids. Furthermore, a first amino acid residue critical for PLUTO substrate specificity was identified. This work is financially supported by the Deutsche Forschungsgemeinschaft, DFG.

9:15-9:45am Joanne Lemieux

OPTIMISATION OF NUCLEOSIDE TRANSPORTER EXPRESSION/PURIFICATION TRIALS FOR X-RAY CRYSTALLOGRAPHY

Melissa A. Morrison, Cory L. Brooks, Maria A. Syed, and M. Joanne Lemieux.
Department of Biochemistry, University of Alberta, Edmonton, AB, Canada T6G 2H7.

Concentrative and equilibrative nucleoside transporter proteins (CNTs and ENTs) are membrane proteins known to regulate transport of nucleoside and nucleobases. This role is critical for DNA and RNA biosynthesis as well as cell signaling events. Both proteins are gradient-driven transporters with CNTs being Na^+/nucleoside co-transporters and ENTs being nucleoside antiporters. Both CNTs and ENTs are critical for uptake of many anti-cancer and anti-viral drugs. Recently, a structure of the *Vibrio cholera* CNT was solved using X-ray crystallography. With 39% similarity, there may be some overall similarities between the bacterial and human transporters. However, to reveal substrate and drug binding sites and to facilitate rational drug design, structural information on the human or higher eukaryotic transporters is needed. Membrane proteins are known to be difficult to study and overexpress, however studying these proteins has become increasingly important in recent years due to their importance as drug targets. The eukaryotic system *Pichia pastoris* is ideal for expression of membrane proteins due to its high protein processing and recombinant ability. Nucleoside transporters from several phylum including different isoforms were tagged with green fluorescent protein (GFP) and incorporated into *Pichia pastoris*. GFP will fluoresce *in-vivo*, allowing for rapid visualization of membrane proteins expressed. This enables a means of comparing successful relative expression. We have identified several candidate proteins using a plate screen methods that may provide sufficient quantities for structural studies, i.e. milligram quantities. Optimization of purification is being conducted. Once purified protein is obtained crystallization trials will ensue. Research supported by CIHR and CFI. MJL is a Canada Research Chair in Membrane Protein Structure and Function, and an AIHS Scholar. CSB is supported by CIHR and AHFMR. MAS is supported by IRTG NSERC CREATE in Membrane Training.

Schedule of Poster Sessions

Poster Session 1 – M.S. Ballroom A/B

Monday, August 27th 8:15pm – 10:00pm

Posters 1 - 20

Please submit your poster to Trish at the registration kiosk by 5:30pm on Monday. The posters will remain displayed in the ballroom following your session until noon Tuesday August 28th.

1 ADAPTOR PROTEIN 1 COMPLEXES REGULATE INTRACELLULAR TRAFFICKING OF THE KIDNEY ANION EXCHANGER 1 IN EPITHELIAL CELLS. Almomani EY, King JC, Netsawang J,Yenchitsomanus PT, Malasit P, Limjindaporn T, Alexander RT, Cordat E.	4 REGULATION OF NHE1 BY SUSTAINED ACIDOSIS IN RENAL CELLS Ayodeji Aderibigbe and Larry Fliegel.	7 THE Na^+/H^+ EXCHANGER ISOFORM 3 (NHE3) IS REQUIRED FOR ACTIVE PARACELLULAR AND TRANSCELLULAR Ca^{2+} UPTAKE FROM MOUSE CAECUM. Juraj Rievaj, Wanling Pan and Todd Alexander
2 PROTON DYNAMICS IN THE CEREBELLAR CORTEX OF MOUSE Marco D Alt and Joachim W Deitmer.	5 CARBONIC ANHYDRASE II BINDS TO AND INCREASES THE ACTIVITY OF THE EPITHELIAL SODIUM PROTON EXCHANGER, NHE3: PART OF A PROXIMAL TUBULAR METABOLON? Devishree Krishnan, Lei Liu, Emmanuelle Cordat, R. Todd Alexander	8 STRUCTURAL INVESTIGATION OF A TWO TRANSMEMBRANE HELIX OF THE HUMAN NA+/H+ EXCHANGER ISOFORM 1 USING NMR SPECTROSCOPY. Brian L. Lee, Claudia Alves, Larry Fliegel, Brian D. Sykes
3 SOS1: AN NA^+/H^+ ANTIPORTER IN THE PLASMA MEMBRANE OF *ARABIDOPSIS THALIANA* Kerstin Duscha, Maria Müller and H. Ekkehard Neuhaus.	6 EXPRESSION, PURIFICATION AND STRUCTURAL ANALYSIS OF MULTIPLE TRANSMEMBRANE SEGMENTS OF THE HUMAN Na^+/H^+ EXCHANGER ISOFORM 1 Grant Kemp. Brian L. Lee, Howard S. Young, Brian D. Sykes, and Larry Fliegel	9 RAPID EXPRESSION SCREENING OF CONCENTRATIVE AND EQUILIBRATIVE NUCLEOSIDE TRANSPORTERS IN *PICIHA PASTORIS* Melissa A. Morrison, Cory L. Brooks, Maria A. Syed, M. Joanne Lemieux

10

AMINO ACID HYDROPHOBICITY SCALE FOR α-HELICAL MEMBRANE PROTEINS

Martin Textor and Sandro Keller.

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EXAMINING THE DIMERIC INTERFACE AND TRANSPORT MECHANISM OF AE1 USING A CLC CHLORIDE CHANNEL BASED HOMOLOGY MODEL

Pamela Bonar, and Joseph R. Casey.

18

HOMOLOGY MODELLING AND DOCKING STUDIES ON PLASTIDIC NUCLEOBASE TRANSPORTER (PLUTO): VALIDATION OF THE ROLE OF SUSBTRATE BINDING RESIDUES

Pankaj Panwar[1], Sandra Witz[2], Torsten Möhlmann[2], M. Joanne Lemieux[1]

11

THREONINE 144 AND VALINE 147 CONFER SALT TOLERANCE TO SOD2, THE Na^+/ H^+ EXCHANGER OF SCHIZOSACCHAROMYCES POMBE.

Asad Ullah, Grant Kemp, Brian L Lee, Claudia Alves, Howard Young,

15

CLAUDIN EXPRESSION IS INTERDEPENDENT IN THE LOOSE EPITHELIAL CELL CULTURE MODEL, OPPOSSUM KIDNEY CELLS

Jelena Borovac[1], Reid S. Barker[1], Juraj Rievaj[1], Andrew Rasmussen[1], Wanling Pan[1], Rachel Wevrick[2] and R. Todd Alexander[1,3],

19

MOLECULAR DISSECTION OF THE MITOCHONDRIAL PROTEIN IMPORT MACHINERY

Ajay Ramesh and Johannes Herrmann

12

NON-ENZYMATIC MODULATION OF AQP1 ACTIVITY BY CAII

Gonzalo L. Vilas, Joe Casey and Todd Alexander

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UPCOMING PROJECT: SOLVING THE CRYSTAL STRUCTUE OF THE NTT-TYPE ATP/ADP TRANSPORTER FROM CHLAMYDIAE

Oliver Trentmann, Ilka Haferkamp, Benjamin Rieder and H. Ekkehard Neuhaus

20

ROLE OF THE GLYCINE TRANSPORTER GLYT2 IN SYNAPTIC DEPRESSION AT INHIBITORY AUDITORY SYNAPSES

Martin Fuhr, Florian Kramer, Dennis Bakker, Désirée Griesemer, and Eckhard Friauf

13

CA^{2+}-CALMODULIN INHIBITS TAIL-ANCHORED PROTEIN INSERTION INTO THE MAMMALIAN ENDOPLASMIC RETICULUM MEMBRANE

Sarah Haßdenteufel, Nico Schäuble, Anika Müller, Martin Jung, Richard Zimmermann.

17

SWEET17, A NOVEL ELEMENT CRITICAL FOR CONTROL OF LEAF FRUCTOSE CONTENT IN ARABIDOPSIS

Patrick A.W. Klemens, Fabien Chadon, Anne Krapp and H. E. Neuhaus

Schedule of Poster Sessions

Poster Session 2 - M.S. Ballroom A/B

Tuesday, August 28th 8:15pm – 10:00pm

Posters 21-41

Please submit your poster to Trish at the registration kiosk on Tuesday by 10:30am. Posters will remain displayed in the ballroom following your session until noon Wednesday, August 29th.

21 NK-CELL CYTOTOXICITY: A SINGLE CELL IMAGING ASSAY TO ELUCIDATE THE DEPENDENCY OF KILLING ON CALCIUM CHANNEL ACTIVITY Christian Backes, Barbara A. Niemeyer, Markus Hoth, Carsten Kummerow	24 QUANTITATIVE ANALYSIS OF CALCIUM DEPENDENT MIGRATION IN HUMAN CYTOTOXIC T CELLS Hélène Lyrmann[1], Marc Neef[2], Carsten Kummerow[1], Karsten Kruse[2], Markus Hoth[1].	27 PHYSICAL INTERACTION AND FUNCTIONAL REGULATION BETWEEN TWO ACID SENSORS, TRPP3 AND ASIC Qian Wang, Jagdeep Tuli, and Xing-Zhen Chen
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POSTER ABSTRACTS

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ADAPTOR PROTEIN 1 COMPLEXES REGULATE INTRACELLULAR TRAFFICKING OF THE KIDNEY ANION EXCHANGER 1 IN EPITHELIAL CELLS.

Almomani EY, King JC, Netsawang J, Yenchitsomanus PT, Malasit P, Limjindaporn T, Alexander RT, Cordat E. Department of physiology, University of Alberta, Edmonton, AB, Canada T6G 2H7.

Distal renal tubular acidosis (dRTA) can be caused by mutations in the gene encoding the anion exchanger 1 (AE1) and is characterized by defective urinary acidification, metabolic acidosis and renal stones. AE1 is expressed at the basolateral membrane of type A intercalated cells in the renal cortical collecting duct (kAE1). Two dRTA mutations result in the carboxyl-terminal truncation of kAE1; in one case, the protein trafficked in a non-polarized way in epithelial cells. A recent yeast two-hybrid assay showed that the carboxyl-terminal cytosolic domain of AE1 interacts with adaptor protein complex 1 (AP-1A) subunit μ1A (mu-1A) (27). Here, we show the interaction between kAE1 and mu-1A and B in vitro by reciprocal co-immunoprecipitation in epithelial cells and in vivo by co-immunoprecipitation from mouse kidney extract. When endogenous mu-1A (and to a lesser extent mu-1B) was reduced, kAE1 protein was unable to traffic to the plasma membrane and was rapidly degraded via a lysosomal pathway. Expression of either siRNA resistant mu-1A or mu-1B stabilized kAE1 in these cells. We also show that newly synthesized kAE1 does not traffic through recycling endosomes to the plasma membrane, suggesting that AP-1B, located in recycling endosomes, is not primarily involved in trafficking of newly synthesized kAE1 when AP-1A is present in the cells. Our data demonstrate that AP-1A regulates processing of the basolateral, polytopic membrane protein kAE1 to the cell surface and that both AP-1A and B adaptor complexes are required for normal kAE1 trafficking. Supported by CIHR, Kidney Foundation of Canada, and WCHRI.

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PROTON DYNAMICS IN THE CEREBELLAR CORTEX OF MOUSE

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The intra- and extracellular concentration of free protons is regulated by chemical buffering and proton transport. Minor changes in pH may affect protein function, fluidity of the membrane, and a variety of metabolic processes. We have performed calibrated *in situ* live-cell imaging with the proton-sensitive fluorescent dye BCECF to measure the intracellular proton buffer capacity. The buffer ratio indicates that only one proton in 400000 is unbound and thereby chemically active, and that about 50% of the total buffer strength is mediated by the CO_2/HCO_3^- buffer system and the other 50% by intrinsic buffers. We have investigated the role of carbonic anhydrase (CA), which catalyses the reaction from CO_2 and H_2O to HCO_3^- and H^+. We used CAII and CAIV knockout (KO) models to isolate the contribution of the different isoforms to the dynamics of the CO_2/HCO_3^- buffer system. Addition and removal of CO_2/HCO_3^- from the perfusate showed that the extracellular CAIV enhances CO_2-induced intracellular acidification, while CAII enhances CO_2-induced acidification and also the alkalinisation upon CO_2 removal. In the presence of the CA blocker 6-Ethoxy-2-benzothiazolsulfonamid (EZA, 10 μM), the rates of acidification and alkalinisation in both KO and WT were significantly reduced. The expression level of CAII and CAIV was monitored by Western blotting. A major part of the transmembrane acid/base transport is coupled to the Na^+ gradient. We were able to determine the cell type-specific contribution of the sodium bicarbonate cotransporter NBCe1 and the sodium proton exchanger NHE in $[H^+]$ regulation. The removal of sodium from the external solution caused a fast cytosolic acidification, which was to 75% HCO_3^- -mediated in WT Bergmann glia and to 60% in WT granule cells, while HCO_3^- did not contribute in NBCe1 KO mice. These results on acute neural tissue provide insights into cellular proton-coupled processes that so far have only been described in *in vitro* studies. Supported by the DFG (De 231/24-1)

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SOS1: AN NA^+/H^+ ANTIPORTER IN THE PLASMA MEMBRANE OF *ARABIDOPSIS THALIANA*

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As salinity causes a severe decrease in growth rate and yield plants have developed different mechanisms to cope especially with the predominant overabundance of sodium: to maintain Na^+ homeostasis they either extrude sodium ions from the cell or they compartmentalize sodium intracellular into the vacuole. These processes are achieved by Na^+/H^+ antiporters catalyzing sodium movement against the respective electrochemical gradient. There are three different families of these antiporters known in plants: the IT/NhaD family, whose members are localized to the plastidial envelope, and the cation/proton antiporter 1 family which can be divided into two subfamilies: the intracellular IC-NHE/NHX subfamily and the NhaP/SOS1 subfamily whose members are localized to the plasma membrane.

Previous studies *in planta* have shown that SOS1 from *Arabidopsis thaliana* is a highly specific Na^+/H^+ exchanger, catalyzing Na^+ efflux across the plasma membrane. Structural analyses of this antiporter predict 12 transmembrane domains followed by a remarkably long hydrophilic C-terminal extension facing the cytosol. This C-terminal tail is common to all SOS1 transporters while it is absent in all members of the other transporter families. As C-terminal regions of many other transporters interact with a variety of regulatory proteins, the cytosolic tail of SOS1 is expected to be part of a complex network of interacting proteins. However, corresponding biochemical analyses of the antiporter are still limited. Therefore, it is my intention to characterize the SOS1 antiporter biochemically as well as biophysically with major interest on the identification of potential binding partners interacting with the C-terminal region. Finally, reconstitution of the entire carrier into proteoliposomes as well as into oocytes is planned in order to determine the transport kinetics in detail. This work is financially supported by the Deutsche Forschungsgemeinschaft, DFG, and the IRTG 1830.

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REGULATION OF NHE1 BY SUSTAINED ACIDOSIS IN RENAL CELLS

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The Na^+/H^+ exchanger isoform 1 (NHE1) is an integral membrane glycoprotein ubiquitously expressed in mammalian cells. It primarily functions to protect cells from intracellular acidification by exchanging an intracellular proton for an extracellular Na^+. In renal cell lines, chronic metabolic acidosis has been shown to increase the mRNA levels and activity of NHE1. In this study, we sought to characterize the mechanism by which NHE1 is activated in mammalian kidney by acidosis. We expressed wild type and mutant NHE1 cDNAs in Mardin-Darby Canine Kidney (MDCK) cells. All the cDNAs had a L163F/G174S mutation, which conferred a 100-fold resistance to EMD87580, an NHE1-specific inhibitor used to inhibit endogenous NHE1 activity. This mutation was also insensitive to S3226, an inhibitor used to specifically inhibit endogenous NHE-3 activity. We then examined the activation and phosphorylation of wild type and mutant NHE1 in response to sustained intracellular acidosis (SIA). While wild type NHE1 showed significant increase in activity and phosphorylation following SIA, mutation of the following amino acids - Ser771, Ser776, Thr779 and Ser785 prevented NHE1 activation and resulted in decreased phosphorylation levels after acidosis. We also observed an increase in phosphorylation levels of extracellular-regulated kinases-1/2 (ERK1/2) and $p90^{RSK}$ following SIA, which was blocked by an ERK inhibitor, U0126. The activation of NHE1 by SIA was also blocked by U0126. These results suggest that SIA activates NHE1 in mammalian kidney cells through the ERK-dependent pathway and this is mediated by one or more of the following amino acids - Ser771, Ser776, Thr779 and Ser785. Supported by CIHR.

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CARBONIC ANHYDRASE II BINDS TO AND INCREASES THE ACTIVITY OF THE EPITHELIAL SODIUM PROTON EXCHANGER, NHE3: PART OF A PROXIMAL TUBULAR METABOLON?

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180 L of water and 1.7 kg of NaCl is filtered by the glomerulus daily. More than 2/3rds is reabsorbed by the proximal tubule. Even a slight alteration in this can cause hypertension and its consequences cardiovascular disease and renal failure. The molecular determinants of this process link the absorption of sodium to that of bicarbonate. We therefore propose that the molecules responsible for these combined fluxes (the sodium proton exchanger, isoform 3 (NHE3), and carbonic anhydrase isoform II (CAII)) physically and functionally interact to increase the rate of reabsorption of sodium and bicarbonate from the proximal tubule. The purpose of these studies is to demonstrate this interaction between NHE3 and CAII. To this end, murine renal sections were immunostained for CAII and NHE3. CAII was observed to colocalize with NHE3 in the brush border membrane. GST pull down studies confirmed a distal region of the carboxy terminus of NHE3 binds to CAII (a.a's 630-730). To assess whether a functional interaction between NHE3 and CAII exists, we stably over-expressed NHE3 with three exofacial HA tags in oppossum kidney (OK- $NHE3_{38HA3}$) cells. Immunostaining and immunoblotting for the tag demonstrated expression of the exchanger in the apical membrane. NHE3 activity was then assessed as the rate of recovery of pH induced by an acid load and measured with the fluorescent ratiometric probe BCECF-AM. The cells were acidified by switching them from bicarbonate free medium, to one containing bicarbonate and bubbled with 5% CO2. OK-$NHE3_{38HA3}$ cells demonstrate significantly greater recovery of intracellular pH than pCDNA transfected controls that is both Na^+ dependent and inhibited by 100 μM 5-N-Ethyl-N-isopropyl amiloride (EIPA). To demonstrate a functional interaction we, inhibited endogenous CAII with 100 μM acetozolamide (ATZ) and then repeated the measurement. This revealed significantly decreased NHE3 activity. To ascertain whether CAII binding *per se* activates NHE3, we over expressed $NHE3_{38HA3}$ with CAII, or the catalytically dead CAII mutant (CAII-V143Y). These studies revealed an increase of NHE3 activity induced by CAII coexpression that was absent when the catalytically dead mutant was coexpressed. To confirm that CAII activity increases NHE3 activity we repeated the assay but induced an acid load via a ammonia prepulse in the absence of bicarbonate. The presence of acetozolamide failed to inhibit NHE3 when activity was measured this way. Together these studies confirm that CAII binds to and increases NHE3 activity, likely by providing substrate for the exchanger. Further studies are aimed at determining if the physical interaction is also necessary to augment NHE3 activity.

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EXPRESSION, PURIFICATION AND STRUCTURAL ANALYSIS OF MULTIPLE TRANSMEMBRANE SEGMENTS OF THE HUMAN Na^+/H^+ EXCHANGER ISOFORM 1

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Membrane proteins are crucial to proper cell function and both hereditary and non-hereditary diseases and disorders are associated with genetic or functional defects in these molecules. Over the past 50 years scientists have been studying membrane protein function and regulation which has led to an increased understanding of biology and what role membrane proteins play in it. One key piece in a more complete understanding of protein function comes from a protein's structure. Although structural biology has exploded in the past several decades, leading to a huge wealth of information about soluble proteins, membrane protein structure determination has remained difficult and relatively few structures have been published. One reason for this is the challenge that expressing and purifying a membrane protein presents. One way to overcome this has been to use a 'divide and conquer' approach. In the past our group has published structures of single transmembrane segments (TMS) determined by nuclear magnetic resonance (NMR) of the human sodium protein exchanger isoform 1 (NHE1). Presently we are using current structural models to direct our experimental design and we have developed a system that allows the expression of larger regions of the membrane domain (currently up to 3 contiguous TMS) of NHE1 as a soluble fusion protein. Following this we have used a method of liquid-liquid extraction to further purify the construct making it of suitable quality for NMR. These 3 TMS represent what is thought to compose the transport pore of NHE1 thus allowing us to study how the TMS helices may function in the membrane. We believe this system could be used to study the structure function relationships of other multiple TMS bundles from other membrane proteins. Supported by CIHR and AITF.

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THE Na^+/H^+ EXCHANGER ISOFORM 3 (NHE3) IS REQUIRED FOR ACTIVE PARACELLULAR AND TRANSCELLULAR Ca^{2+} UPTAKE FROM MOUSE CAECUM.

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Calcium absorption from the intestine occurs via both paracellular and transcellular routes. While the transcellular pathway has been extensively studied, the significance of paracellular absorption is not known. Unlike passive diffusion, secondarily active paracellular Ca^{2+} uptake occurs against an electrochemical gradient with the energy provided by water absorption. Because water passively follows ions movement, the absorption of sodium and calcium could be partially interlinked. NHE3 plays a crucial role in intestinal sodium absorption in mice. The phenotype of NHE3 knockout mice displays intestinal water retention, impaired calcium homeostasis and decreased duodenal calcium uptake. However, it is not clear whether the altered calcium absorption is due a defect in the paracellular or transcellular pathway and even which part of the intestine is crucial for calcium absorption in mice. Therefore we used Ussing chambers to measure calcium absorption across different segments of mouse intestine. We found that the caecum was the only segment that demonstrated net calcium uptake. qPCR revealed that caecum express NHE3, as well as all genes implicated in transcellular and paracellular Ca^{2+} absorption. Pharmacological inhibition of NHE3 by 100µM 5-(N-Ethyl-N-isopropyl) amiloride decreased Ca^{2+} flux across the caecum in the luminal to serosal direction by more than 28% and increased flux in the opposite direction. NHE3 knockout animals had the luminal to serosal flux decreased by more than 60% with respect to wild type controls. Voltage clamp experiments in Ussing chambers suggest that this is due to decreased transcellular and secondarily active paracellular absorption while qPCR showed diminished expression of Trpv6 (calcium channel implied in transcellular absorption pathway) in NHE3 knockout animals. In conclusion, our data demonstrate involvement of NHE3 in Ca^{2+} absorption from mouse caecum and support the theory that this is at least partially due to the role of NHE3 in sodium and water absorption. Supported by AIHS.

8

STRUCTURAL INVESTIGATION OF A TWO TRANSMEMBRANE HELIX OF THE HUMAN NA+/H+ EXCHANGER ISOFORM 1 USING NMR SPECTROSCOPY.

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The human sodium/hydrogen exchanger isoform 1 (NHE1) is an integral membrane protein which exchanges intracellular H^+ for extracelluar Na^+. It is involved in intracellular pH regulation in the heart, and has been implicated in ischemic heart disease and cardiac hypertrophy. There is currently no high resolution structure of the entire NHE1 protein, however we have previously used nuclear magnetic resonance spectroscopy (NMR) to determine the structures of individual transmembrane (TM) helices of NHE1 in membrane mimetics. Detailed structural information would be useful for understanding the mechanism of NHE1 and for the development of inhibitors. To gain further structural insight into the protein, including helix-helix interactions to perhaps build up a structure of NHE1, we have produced a peptide containing the TM 6-7 region of NHE1 (residues 226-274). This peptide was produced as a maltose binding protein fusion construct, and purified using TEV protease cleavage and HPLC. Both unlabeled and ^{15}N-labelled peptide has been produced. We have investigated the structure of this peptide using NMR in dodecylphosphocholine micelles. TM 6 in this peptide has a similar structure to the previously published isolated TM 6 structure, consisting of two short helical regions (residues 229-236 and 239-247) separated by an extended segment (237-238) while TM 7 (252-274) is straight compared to the kinked structure of the isolated TM 7. Interactions between TM 7 and TM 6 may account for the difference in structure of TM7 expressed with TM 6, compared to their isolated structures. While each TM helix forms a consistent structure, the interaction between them is not as well defined and is the subject of future experiments. Supported by AIHS, CIHR and HSFC.

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RAPID EXPRESSION SCREENING OF CONCENTRATIVE AND EQUILIBRATIVE NUCLEOSIDE TRANSPORTERS IN *PICIHA PASTORIS*

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The overexpression of milligram quantities of protein remains a key bottleneck in membrane protein structural biology. In order to cope with the frequently poor expression levels associated with these challenging proteins, it is often necessary to screen a large number of homologues in order to find a well expressing clone. Using the heterologous, eukaryotic expression host *Pichia pastoris,* we have developed a simple fluorescent induction plate-screening assay that allows for the rapid detection of well expressing clones of green fluorescent protein (GFP) fused eukaryotic membrane proteins. This screening system has been utilized to study the concentrative and equilibrative nucleoside transporters (CNTs and ENTs, respectively). This method has rapidly accelerated the process to find high expressing nucleoside transporters by allowing simultaneous screening of multiple isoforms from mouse, rat, and arabidopsis to list a few. This has enabled us to identify which organisms and isoforms contain prospective purification targets. Not only does the GFP enable pre-screening for optimum expression, but it also has been advantageous for purification techniques. These techniques include protein solubility tests visualized through in-gel fluorescence and protein mono-dispersity tests in detergent through fluorescence size exclusion chromatography. Using the example of the CNTs and ENTs, we will demonstrate that when a large number of clones are screened a small number of highly expressing "jackpot" clones can be isolated.

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AMINO ACID HYDROPHOBICITY SCALE FOR α-HELICAL MEMBRANE PROTEINS

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A hydrophobicity scale for amino acids (AA) is a measure of their relative affinities for hydrophobic phases and comprises their Gibbs free energies of transfer between two phases of different polarity [1]. Such scales are essential for understanding the energetics of protein-membrane interactions and the molecular forces determining membrane protein (MP) stability. They are widely used for identification of genes encoding for MPs as well as the prediction of transmembrane domains on the basis of known AA sequences. However, despite the abundance and pharmacological importance of α-helical MPs, up to now there is no experimentally derived hydrophobicity scale available that is based on the partitioning of an α-helical MP between the aqueous phase and a phospholipid membrane.
Hence, this project aims at establishing an AA hydrophobicity scale by usage of the α-helical MP Mistic [2] from *B. subtilis*. Mistic inserts autonomously into lipid bilayers but can be unfolded completely and reversibly due to its uncommonly hydrophilic surface. On the basis of the known Mistic NMR structure as well as topology assessment by way of linear dichroism spectroscopy, a mutation site exposed to the hydrophobic membrane core will be chosen. This residue will be substituted by the 19 non-wild type AAs. After expression and purification, the mutants will be reconstituted in large unilamellar lipid vesicles and subjected to unfolding/refolding experiments. During titrations of proteoliposomes with urea, the protein unfolding equilibrium is followed by multidimensional spectroscopy, that is, combined circular dichroism and fluorescence spectroscopy. Analysis of such data provides a difference Gibbs free energy of transfer $\Delta\Delta G°_{w}$ for each point mutant. The latter reflects the energetic contribution of the respective AA to overall MP stability in comparison with the wild type, which is expected to correlate with AA hydrophobicity. Supported by DFG.

[1] J.L. MacCallum and D.P. Tieleman. *Trends Biochem. Sci.* **2011**, *36,* 653–662.
[2] T.P. Roosild, J. Greenwald, M. Vega, S. Castronovo, R. Riek and S. Choe. *Sci.* **2005**, *307*, 1317–1321.

11

THREONINE 144 AND VALINE 147 CONFER SALT TOLERANCE TO SOD2, THE Na^+/H^+ EXCHANGER OF SCHIZOSACCHAROMYCES POMBE.

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Sod2 is the plasma membrane Na^+/H^+ exchanger of fission yeast *S. pombe.* It plays an important role in providing salt tolerance to *S. pombe* by removing excess intracellular sodium (or lithium) in exchange for protons. We examined the role of amino acids residues of transmembrane segment IV (TMIV) (^{126}FPQINFLGSLLIAGCITSTDPVLSALI^{152}) in activity of this protein using alanine scanning mutagenesis, and examining LiCl or NaCl tolerance in sod2-deficient *S. pombe*. We identified two amino acids, T144 and V147, critical for the function of Sod2. The mutations T144A and V147A resulted in a defective sod2 protein that did not confer salt tolerance when re-introduced into *S. pombe*. Sod2 protein with other mutations in TMIV, exported either sodium or lithium as efficiently as the wild type Sod2 or had intermediate effects on salt tolerance of *S. pombe*. When we mutated T144 to aspartic acid or lysine the mutant proteins were impaired in their ability to allow growth in both lithium and sodium containing media, comparable to the knock out sod2 yeast (sod2::ura4). However, the protein was apparently functional with replacement of threonine residue with a serine residue to remove intracellular lithium but not sodium. Western blot analysis showed that mutants T144A and V147A were expressed at comparable levels as the wild type sod2. To study the structure of TMIV, we expressed and purified TMIV (amino acids 125-154) as a fusion protein with maltose binding protein. The TMIV peptide was released by cleaving the fusion protein with tobacco etch virus (TEV) and purified by organic extraction. The organic extraction was of sufficient purity for direct use in NMR experiments yielding data of ample quality to construct a reasonable structural model. The model indicates a helical region (amino acids 128-142) followed by an extended kinked region (amino acids 143- 146) and ended with a helical region (amino acids 147-154). Supported by Natural Sciences and Engineering Research Council of Canada.

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NON-ENZYMATIC MODULATION OF AQP1 ACTIVITY BY CAII

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Aquaporins are a family of membrane proteins that function as water channels, facilitating the movement of water molecules across the plasma membrane, down a concentration gradient. The first family member AQP1, is predominantly expressed in red blood cells and the kidney. The basic structural and functional unit of the kidney is the nephron where AQP1 is expressed in both the proximal tubule and thin descending limb. Its presence there is essential for the reabsorption of 2/3rds of the 180L of water filtered by the glomerulus daily. Carbonic anhydrase II (CAII) is ubiquitously expressed. It catalyzes the reversible hydration of carbon dioxide. Apart from playing a pivotal role in acid-base balance, CAII also modulates the activities of other proteins through direct protein-protein interaction. Specifically CAII binds to and enhances the activity of the sodium/hydrogen exchanger NHE1, sodium bicarbonate cotransporter NBC1, monocarboxylate transporter MCT1 and the chloride/bicarbonate exchanger AE1. Sequence analysis of human AQP1 revealed the presence of two C-terminal contiguous motifs that match the CAII binding sites found in AE1. Moreover, osmotic swelling assays showed that the water flux of membrane-enriched cortical kidney fractions from $CAII^{-/-}$ mice was approximately 50% lower than wild-type mice. These results lead us to hypothesize that water flux through AQP1 was enhanced by an interaction with CAII. To test this possibility we performed osmotic swelling assays on *Xenopus laevis* oocytes and HEK293 cells transiently expressing different combinations of AQP1, CAII and the catalytically inactive CAII isoform, V143Y. We found that CAII enhances the water channel activity of AQP1 regardless of catalytic activity, in both model systems. Future studies are aimed at clearly delineating the site of interaction between CAII and AQP1. Regardless this interaction is likely highly relevant to both respiratory and renal physiology.

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CA^{2+}-CALMODULIN INHIBITS TAIL-ANCHORED PROTEIN INSERTION INTO THE MAMMALIAN ENDOPLASMIC RETICULUM MEMBRANE

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Tail-anchored (TA) proteins of the mammalian endoplasmic reticulum (ER) membrane are involved in a variety of biological processes, including signal sequence-dependent protein transport into the ER, ER calcium leakage, ER-associated protein degradation, apoptosis, and vesicular trafficking. Recently, cytosolic components and pathways, involved in the posttranslational insertion of TA proteins into the ER membrane of yeast and mammals, have been identified. Searching for regulatory mechanisms of TA protein biogenesis, we carried out *in vitro* protein transport experiments. For this purpose precursors of model TA proteins were synthesized in reticulocyte lysate in the presence of [^{35}S]methionine and after inhibition of protein synthesis, protein insertion was provided for 30 min by supplementation with microsomal membranes, in presence or absence of Ca^{2+}-calmodulin (CaM) (0.7 mM, 0.26 mg/ml) or 200 µM TFP. TA Proteins were partially extended at their carboxyl termini via an opsin-derived N-glycosylation site to create a reliable assay for membrane insertion, beyond carbonate resistance. We found that Ca^{2+}-CaM inhibits the insertion of TA proteins into ER membranes, and that this inhibition was prevented by trifluoperazine, a CaM antagonist that interferes with substrate binding of Ca^{2+}-CaM. Taking into account that this generally inhibition was irrespective of the cytosolic pathway, we tested the effect of Ca^{2+}-CaM on the spontaneous membrane insertion of Cytochrome b5 (Cytb5) in buffer. Indeed inhibitory action of Ca^{2+}-CaM was observed even in the absence of cytosolic factors, why we propose that Ca^{2+}-CaM binds directly to the TA proteins. To demonstrate directly interaction, we carried out chemical cross-links with EDC in the presence of cytosolic factors. The prominent cross-link of Synaptobrevin 2 (Syb2) to TRC40, the main targeting factor, was replaced by a CaM-Syb2 cross-link after CaM addition. Thus, CaM appears to regulate TA insertion into the ER membrane in a Ca^{2+} dependent manner by displacing targeting factors. Supported by Grants from the Deutsche Forschungsgemeinschaft (FOR 967 and GRK 1326).

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EXAMINING THE DIMERIC INTERFACE AND TRANSPORT MECHANISM OF AE1 USING A CLC CHLORIDE CHANNEL BASED HOMOLOGY MODEL

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AE1 is a member of the SLC4 subfamily of electroneutral Cl^-/HCO_3^- exchangers. AE1 is highly expressed in erythrocytes (eAE1), and a truncated variant (kAE1) is expressed in the renal collecting duct. There is still no high-resolution structure of the AE1 membrane domain, which alone is required for AE1's transport activity. A recent electron microscopy structure of the AE1 membrane domain was proposed to have a similar protein fold to ClC chloride channels. We developed a 3D homology model of the AE1 membrane domain using the *E. coli* ClC channel structure as a template. This model agrees well with a list of biochemical spatial constraints for AE1, which include cysteine scanning mutagenesis, proteolytic cleavage and N-linked glycosylation insertion data. Using the homology model of AE1, we designed mutagenic studies to examine the dimer interface and transport mechanism of AE1. Tryptophan point mutations along the dimer interface within *E. coli* ClC were found to disrupt the ClC dimer. In a similar fashion, we created tryptophan point mutations along the ends of the helices that form the dimer interface in the AE1 homology model. The ability of these mutants to form dimers was assessed by chemical crosslinking. To investigate the transport mechanism of AE1, we created alanine mutations in regions of the AE1 homology model that correspond to residues involved in the transport mechanism of ClC proteins. The transport activity of these mutants was assessed by Cl^-/HCO_3^- exchange assays in HEK293 cells. Mutants that had a transport rate significantly different from wild-type AE1 were further investigated by assessing the transport activity of additional amino acid substitutions at that position. These studies will not only allow us to confirm the validity of the AE1 homology model, but also provide valuable insights into the structure and transport mechanism of AE1. Supported by CIHR and AIHS.

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CLAUDIN EXPRESSION IS INTERDEPENDENT IN THE LOOSE EPITHELIAL CELL CULTURE MODEL, OPPOSSUM KIDNEY CELLS

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The effect of claudins on paracellular fluxes has been predominantly studied in either MDCK or LLCPK cells. Neither of these model systems demonstrates the very low transepithelial resistance (TER) associated with leaky epithelia such as the renal proximal tubule. Moreover, results from one model system are not always consistent with another. Opossum kidney (OK) cells form tight junctions, yet have a very low TER. We therefore set out to characterize the paracellular transport properties of this cell culture model. Ussing chamber dilution potential measurements revealed that OK cells exhibit a very low TER (11.7 ± 1.4 ohm cm^2) and slight cation selectivity ($P_{Na}/P_{Cl} = 1.081 \pm 0.002$) similar to the proximal tubule *in vivo*. Bi-ionic potential measurements revealed that OK cells display Eisenmann permeability sequence IV, with the permeability of monovalent cations ranking $K^+ > Cs^+ > Rb^+ > Na^+ > Li^+$. This is the same sequence displayed by the proximal tubule *in vivo*. Quantitative real-time PCR studies found that OK cells express claudin-4 > -1 > -6 > -20 > -9 > -12 > -11 > -15. Overexpression of claudin-4 significantly increased TER, proportionately decreased Na^+ and Cl^- permeability and increased levels of claudin-1, -6, and -9 mRNA. Knockdown of claudin-4 in the over-expressing cells significantly decreased TER without altering claudin expression; suggesting that claudin-4 forms a barrier in OK cell tight junctions. However, knockdown of endogenous claudin-4 decreased claudin-1, -9 and -12 expression, without significantly affecting TER. Claudin-2 over-expression decreased TER, significantly increased sodium and chloride permeability and decreased claudin-12 expression. Together our results suggest that claudin expression is tightly coupled in OK cells. Supported by the Kidney Foundation of Canada.

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UPCOMING PROJECT: SOLVING THE CRYSTAL STRUCTUE OF THE NTT-TYPE ATP/ADP TRANSPORTER FROM CHLAMYDIAE

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Nucleotides are the building blocks of DNA and RNA, act as second messenger, enzyme co-factors and energy currency in all organisms. Metabolically impaired intracellular pathogenic bacteria like chlamydiae and rickettsiae import nucleotides from the host by nucleotide transporters (NTT) to cover their energy demands. We succeeded in the purification of the ATP/ADP transporter from *Protochlamydia amoebophila* (*Pam*NTT1) and its functional reconstitution into artificial lipid vesicles. Reconstituted *Pam*NTT1 mediates a hetero-exchange of ATP against ADP plus inorganic phosphate (P_i). During translocation ADP plus P_i are co-transported in a one-to-one stoichiometry to link energy provision with the export of metabolic products and maintain cellular phosphate/charge homeostasis. Detailed analyses of mutant proteins revealed that P_i interacts with the same amino acid residue as the γ-phosphate of ATP. Additionally, in the presence of ADP, *Pam*NTT1 mediates a P_i homo-exchange indicating only one binding site involved in import and export. Heterologous expression and purification of high amounts of *Pam*NTT1 provides the basis for further detailed structure/function analyses. In this context, we would like to build up a fruitful cooperation within the IRTG to solve the 2D-or even 3D-crystal structure of *Pam*NTT1.

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SWEET17, A NOVEL ELEMENT CRITICAL FOR CONTROL OF LEAF FRUCTOSE CONTENT IN ARABIDOPSIS

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The adequate production, storage and transport of sugars between source and sink organs are essential to sustain growth and development in plants. Moreover, sugars fulfil essential roles as energy equivalents, metabolic precursors as well as signal molecules during the life cycle of higher plants. Sugar transporters constitute large gene families. In Arabidopsis, 9 disaccharide transporters (SUC1-9 , Sauer, 2007), >50 putative monosaccharide transporters (Büttner, 2007) and 17 putative glucose transporters (SWEET1-17, Chen et al., 2010) have been identified based on gene homology, but the biological function of most of them is still unknown. Recently, a new family of hexose transporters (PFAM PF03083) has been identified by Chen et al. (2010). These authors revealed that the first member of this family, AtSWEET1, functions as an uniporter/facilitator at the plasmamembrane and is thus able to support import or efflux of sugars from cells. Strong evidences have been found that in total at least six out of seventeen Arabidopsis SWEET proteins (AtSWEET1, AtSWEET4, AtSWEET5, AtSWEET7, AtSWEET7, and AtSWEET13) mediate glucose transport at the plasmelamma (Chen et al., 2010). SWEETs are small proteins predicted to form a pore from seven transmembrane helices. Further evidence support the hypothesis that the SWEET sugar transporters are involved in the supply of carbohydrates to a variety of tissues in both monocotyledonous and dicotyledonous plants. Using different physiological approaches, we have identified SWEET17, as a critical factor controlling fructose content in Arabidopsis leaves. We show that inhibited SWEET17 activity leads to fructose accumulation in Arabidopsis leaves. Subcellular localization of SWEET17-GFP fusion characterizes SWEET17 as a putative fructose transporter localized at the vacuolar membrane (tonoplast).

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HOMOLOGY MODELLING AND DOCKING STUDIES ON PLASTIDIC NUCLEOBASE TRANSPORTER (PLUTO): VALIDATION OF THE ROLE OF SUSBTRATE BINDING RESIDUES

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The plastidic nucleobase transporter (PLUTO) of *Arabidopsis thaliana* is a nucleobase-cation transporter that regulates import of purine and pyrimidine nucleobases into the plastids. In an attempt to decipher the nucleoside-binding site, homology model of the PLUTO was constructed, based on the crystal structure of sodium-hydantoin transporter Mhp1. Ligand docking experiments were employed to determine which residues are involved in substrate binding. Purine and pyrimidine nucleobases were docked into the proposed binding site of PLUTO via three different scoring functions. The proposed binding model is consistent with the available experimental data and was further validated by amino acid substitutions. Remarkably, as proposed computationally and discovered experimentally, residues E113 and G127 are found to be particularly important for PLUTO function and suggested to participate in substrate binding. Therefore, we prove that overall procedure of homology modeling and docking can be a useful tool in understanding the structural basis of substrates binding to PLUTO.

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MOLECULAR DISSECTION OF THE MITOCHONDRIAL PROTEIN IMPORT MACHINERY

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A majority of the mitochondrial proteins are encoded by the nuclear genome. Upon synthesis in the cytosol, these proteins have to be translocated to their respective destinations within the mitochondria. The translocase of the outer membrane (TOM) and the translocase of the inner membrane (TIM) complexes facilitate the translocation of preproteins across the outer and inner membranes of the mitochondria, respectively. The TIM23 complex mediates the translocation of precursor proteins that are targeted to the mitochondrial matrix or to the mitochondrial inner membrane. Tim17 is an integral component of the TIM23 translocase containing four transmembrane domains. The sequence of Tim17 is highly conserved amongst fungi, plants and animals including humans. All Tim17 proteins contain two conserved cysteine residues that are located directly adjacent to the first and second transmembrane domains facing the intermembrane space. The relevance of these cysteine residues is not known. Various functions such as regulation and sorting during preprotein translocation, recruiting of the presequence assisted motor (PAM) machinery, regulation of the protein-conducting channel, and prevention of mitochondrial DNA loss have been assigned to Tim17. However, the actual role of Tim17 still remains unknown. Growth tests and *in vitro* import experiments revealed that one of these cysteine residues was vital for the viability of the cell under stress conditions as well as for the efficient import of specific matrix proteins. The results point at a critical mechanistic or regulatory role of cysteine residues in Tim17 for the import of mitochondrial preproteins. Further work will be presented during the talk. Supported by DFG (IRTG 1830).

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ROLE OF THE GLYCINE TRANSPORTER GLYT2 IN SYNAPTIC DEPRESSION AT INHIBITORY AUDITORY SYNAPSES

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During ongoing activity, synaptic strength can undergo facilitation, depression, or a mixture of both. This short-term synaptic plasticity plays a key role in synaptic computation and has been extensively investigated for excitatory synapses. Much less is known about inhibitory synapses. In the auditory brainstem, the glycinergic projection from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO) is ideally suited to study short term plasticity at inhibitory synapses. We characterized the MNTB-LSO connection in acute brain slices of P10-12 wild type and $GlyT2^{-/-}$ mice. Focal electrical stimulation in the MNTB was combined with patch-clamp recordings of LSO neurons at 25°C or 37°C. Stimulus trains were applied at 1-800 Hz and lasted up to 10 minutes. Inhibitory postsynaptic current (IPSC) amplitudes showed a frequency-dependent depression at both temperatures and phenotypes. At 25°C and stimulation frequencies ≥50 Hz, the depression was stronger than at 37°C. These results suggest that an enzyme-based mechanism participates in the supply of glycine upon high frequency stimulation. We hypothesized that GlyT2, the neuronal glycine transporter which removes the transmitter molecules from the synaptic cleft, is involved in replenishing transmitter supply. To test this hypothesis, we recorded MNTB-LSO IPSCs in $GlyT2^{-/-}$ mice at 37°C. We did this also by pharmacologically blocking GlyT2. Stimulation with 50 Hz and higher frequencies revealed a decline in amplitude with time, which was more rapid in $GlyT2^{-/-}$ than upon pharmacological blockade and in wild-type controls. Also the recovery after stimulation was reduced in $GlyT2^{-/-}$ and in mice with pharmacologically blocked GlyT2. In summary, the inhibitory MNTB-LSO synapses display a frequency-dependent short-term depression. Concerning the role of GlyT2, it appears that its absence results in a lower glycine amount in the presynaptic MNTB vesicles. However, the GlyT2 transport activity becomes crucially important for glycine homeostasis only upon extended stimulation.

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NK-CELL CYTOTOXICITY: A SINGLE CELL IMAGING ASSAY TO ELUCIDATE THE DEPENDENCY OF KILLING ON CALCIUM CHANNEL ACTIVITY

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Immune cell function is critically linked to ion channel function. Natural Killer (NK) cells are part of innate immunity and provide a rapid but non-specific first defense against harmful targets such as pathogens or cancer cells. The goal of this project is to understand NK cell cytotoxicity and the role of ion channel (mal-) function for cytotoxicity on a single cell level.
NK cells deliver their cytotoxicity via two major mechanisms. One includes induction of target cell death via Fas receptor (CD95) and Fas ligand (CD178) binding, resulting in the activation of caspases and enzymatic destruction of cellular components (apoptosis). The other results in target cell death via cytotoxic vesicles containing perforin and granzymes. Here, the exact mode of operation is not completely resolved. Perforin either directly perforates the target cell's membrane, and/or delivers granzymes, which also specifically activate a caspase-dependent pathway.To investigate NK function on a single cell level, we use live-cell imaging of human NK cells, isolated from whole blood with negative selection. As targets, we created a Jurkat cell line stably expressing a FRET (Förster Resonance Energy Transfer)-based caspase sensor, which is a reliable indicator of apoptosis. Initially, we investigated cytotoxicity using different extracellular calcium concentrations and found not only differences in killing efficiency but also in migration (see also abstract by Lyrman et al.). To understand the efficiency of target cell killing, different killing approaches (several immune cell subtypes attacking a mutual target, etc.) have thus to be combined with a quantitative migration analysis. Further, we will distinguish the NK killing mechanisms by down regulation of gene expression using RNA interference and evaluate their dependence on calcium signaling. Using this approach, we aim to elucidate the exact contribution of the two NK killing mechanisms and their potentially differential regulation by calcium channels.

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MECHANISMS OF CHANNEL ACTIVATION OF TRPP3 EXPRESSED IN XENOPUS OOCYTES BY CA AND ACID

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TRPP3 is a non-selective cation channel belongs to the transient receptor potential (TRP) superfamily with its characteristic 6 transmembrane domains. TRPP3 (also named PKD2L1) is 70% homologous to TRPP2 (also named PKD2) which is mutated in 10-15% of autosomal dominant polycystic kidney disease (ADPKD). TRPP3 has been identified for its permeability to and activation by Ca as well as its role in acid and carbonation sensation. TRPP3 has been shown to interact with polycystic kidney disease protein 1-like 3 (PKD1L3) to form heterodimers that exhibit a unique property by responding to an acid stimulus after the removal of the stimulus itself. This property was designated as an off-response. However, the underlying mechanism of either Ca- or acid-induced TRPP3 activity is still unclear. Our experiments using two-electrode voltage clamping showed that TRPP3 alone exhibits the proton-induced off-response. We then explored the role of a TRPP3 C-terminal domain and the negatively charged residues E and D located within its transmembrane domains (TMs) in channel activity. Experiments were performed utilizing the two-electrode voltage clamp technique, site directed mutagenesis, cell surface biotinylation and immunofluorescence. Preliminary data demonstrated that TRPP3 point mutantions D349N and E356N (residues located within TM2) expressed in oocytes significantly reduce channel activation and off response, while E103Q and D113N in TM1 do not. On the other hand, mutations E369Q, D390N and D476N affected the TRPP3 total expression. Further, functional studies using TRPP3 truncated mutants E566X, S581X and T622X indicated that the C-terminal fragment S581-T622 plays a critical role in channel function but not in surface membrane targeting. We are in the process of generating deletion mutant K575-T622 as well as other point mutants with deleted potential phosphorylation sites to further document roles of residues within this domain. In summary, our existing and ongoing data gain first insights into mechanisms underlying channel activations induced by Ca or acid, which is critical in understanding the physiology and pathophysiology of TRPP3. Supported by CIHR and NSERC.

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ACTIVITY DEPENDENT GLUCOSE TRANSPORT IN ACUTE CEREBELLAR SLICES: A MULTIPHOTON STUDY

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Glucose is an essential energy source required to maintain brain metabolism. It is believed that the supply of energy is adjusted to energy demand in the brain and that different cell types may have their individual glucose handling. In a previous study we found a significant higher uptake and metabolism of the fluorescent glucose analogue 2-NBDG in cerebellar glial cells compared to adjacent neurons. Addressing this issue in the present study, we have examined the effects of the excitatory neurotransmitter glutamate on the uptake and metabolism of glucose. Measurements in cultured astrocytes using an intracellular FRET-based glucose nanosensor revealed a glutamate-induced rise in the intracellular glucose concentration and a stimulation of the glial glucose transporter. We then asked if in acute brain slices, increased glucose uptake could be also measured in neurons or glial cells. Real time experiments in cerebellar slices were carried out using two-photon imaging of the fluorescent glucose analogue 2-NBDG in GFAP-mRFP1 knock-in mice, expressing the monomeric red fluorescent protein1 under the control of the hGFAP promotor. Recording the mrfp1-signals allowed reliable identification of Bergmann glial cells. In the presence of 0.5 µM TTX, application of 100 µM glutamate caused a twofold increase in the rate of 2-NBDG uptake in Bergmann glial cells. This effect was shown to be mediated by the action of glutamate transporters as the effect was also elicited by the application of D-aspartate, a glutamate transport analogue, and was fully blocked by using TBOA, an inhibitor of glutamate transport. In contrast, there was no modulation of the 2-NBDG uptake in Purkinje cells. These results suggest that glucose transport and glycolytic rate of astrocytes in acute cerebellar slices are considerably higher than those of neurons. Since the brain is mainly fueled by glucose, and as neurons presumably consume more energy than glial cells, our results are in line with the hypothesis of the astrocyte-neuron lactate-shuttle, suggesting substantial transfer of energy-rich metabolites, as e.g. lactate or pyruvate, via monocarboxylate transporters from glial cells to neurons.

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QUANTITATIVE ANALYSIS OF CALCIUM DEPENDENT MIGRATION IN HUMAN CYTOTOXIC T CELLS

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Cytotoxic T lymphocytes (CTL) scan the human body searching for virus-infected cells or tumor cells. Once they encounter a target cell, they repolarize and form a close contact with the target cell, the so called immunological synapse. To finally kill a target cell, CTL release lytic granules at the site of the immunological synapse. For effective immune response the migration of CTL has to be optimized in order to efficiently find target cells. We use *in vitro* time-lapse microscopy to study the migration of primary human CTL in absence of target cells and at varying external calcium concentrations. The analysis of the two-dimensional trajectories of CTL shows that the cells don't migrate continuously. They rather switch between a moving and a resting state. The probability of each state as well as the velocity of the CTL depends on the external calcium concentration. In the absence of external calcium the migration of CTL is impaired but not completely inhibited.
On a short time scale (seconds/minutes), CTL show directional persistence, while migration on a long time scale (minutes/hours) is random. As Brownian motion is not sufficient to describe this phenomenon, we used a model where the motion of a single cell originates from independent protrusions that effectively pull on the cell body. In this model, each protrusion is active for a characteristic time, during which it creates a persistent force in a randomly chosen direction. Using the combination of experimental and theoretical data we aim at understanding the regulation of cellular activity in efficient and inefficient immune responses.

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GATING OF THE HUMAN SEC61 CHANNEL BY BIP, SEC63, AND CALCIUM-CALMODULIN

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Two major functions of the mammalian endoplasmic reticulum (ER) are the biogenesis of secretory and membrane proteins as well as serving as an calcium (Ca^{2+}) reservoir for cellular signaling. ER import of polypeptide precursors carrying a N-terminal signal-peptide is strictly dependent on an ER-resident protein translocase with the Sec61 complex as central component. Hitherto, an additional role of the ER-lumenal Hsp70 chaperone BiP and its Hsp40 co-chaperone Sec63 for protein translocation is well characterized in yeast. We depleted the levels of Sec63 and BiP using siRNA or a specific toxin, and tested whether reduction of these proteins in human cells affects Sec61 dependent protein translocation into the ER of mammalian cells. Reductions of both, Sec63 and BiP, revealed overlapping, precursor specific involvements in the initial phase of co-translational protein translocation into the ER of semi-permeabilized cells. Moreover, when we replaced Sec61α with diabetes-linked Sec61αY344H we obtained a comparable phenotype suggesting a potential BiP binding site in ER luminal loop 7 in the vicinity of tyrosine 344. Previous work also characterized the Sec61 channel as a potential Ca^{2+} leak channel. In accordance with the transport results we observed a similar channel gating behavior with respect to Ca^{2+} permeability. In Ca^{2+} imaging experiments, reduction of cellular BiP by different means resulted in a significantly increased Ca^{2+} leakage from the ER. However, additional knockdown of Sec61α strongly inhibited this effect. Moreover, Sec61αY344H phenocopied the BiP-depletion effect and was also not longer affected by BiP reduction. Preceding experiments also identified Ca^{2+} calmodulin as limiting Ca^{2+} leakage through Sec61 by binding to a cytosolic IQ-motif in the N-terminus of Sec61α in Ca^{2+} dependent manner. Thus, gating of Sec61 channels has to be tightly regulated to ensure proper protein transport and, simultaneously, sufficient sealing of the translocation pore and involves cytosolic as well as ER luminal factors.

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PLASMA MEMBRANE LOCALIZED KDEL-RECEPTORS AS KEY IN A/B TOXIN ENDOCYTOSIS AND RETROGRADE TRANSPORT

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Microbial and plant A/B toxins such as cholera and Shiga toxin, ricin and the yeast (*S. cerevisiae*) viral A/B toxin K28 represent secreted heteromeric protein toxins which enter eukaryotic target cells by receptor-mediated endocytosis. After retrograde transport through the secretory pathway *via* early endosomes, Golgi and the ER, the toxins translocate into the cytosol to kill their host [1]. Here we demonstrate that the essential cellular K/HDEL receptor Erd2p represents the key component in retrograde toxin transport by interacting with the unmasked ER retention motif HDEL at the C-terminus of the toxin's β-subunit [2, 3]. Furthermore, this motif can likewise facilitate uptake and retrograde transport of H/KDEL carrying proteins into yeast cell spheroplasts, indicating that a minor portion of the cellular H/KDEL receptor also colocalizes in the plasma membrane. This important observation was confirmed by confocal laser scanning fluorescence microscopy as well as reporter assays using *in vivo* cell-growth as read-out. Precise Erd2p topology prediction was an essential prerequisite to further characterize the endocytosis of the toxin/receptor complex K28/Erd2p. Therefore, a set of reporter fusions was established to determine the *in vivo* topology of the yeast KDEL-receptor Erd2p. Targeted toxin receptor mutagenesis uncovered endocytic motifs within cytosolic receptor domains affecting toxin sensitivity. In conjunction with TIRF-based mobility tracking of fluorescently labelled toxin/receptor complexes and biochemical analysis the Erd2p, receptor endocytosis was shown to rely on multiple mechanisms, including ubiquitylation, Pan1 complex components such as Sla1p as well as the adapter protein complex AP2 which so far had been described to be exclusively involved in endocytosis in mammalian cells [4]. 1. M.J. Schmitt and F. Breinig, Nat. Rev. Microbiol. 4 (2006), p. 212. 2. K. Eisfeld et al., Mol. Microbiol. 37 (2000), p. 926. 3. F. Riffer et al., Microbiology 148 (2002), p. 1317. 4. S.Y. Carroll et al., Dev. Cell 17 (2009), p. 552.

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PHYSICAL INTERACTION AND FUNCTIONAL REGULATION BETWEEN TWO ACID SENSORS, TRPP3 AND ASIC

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TRPP3 (also called PKD2L1) belongs to the polycystic kidney disease (PKD) family and is a Ca-activated nonselective cation channel, permeable to Na, K, and Ca. It was initially cloned from human retina expressed tag and is present in neurons of the tongue, brain and retina, but its physiological roles remain unclear. Studies after 2006 suggest that in the tongue, TRPP3 localizes to taste receptor cells together with PKD1L3, another member of PKD family, possibly involved in sour tasting. The two proteins together, but not alone, traffic to the plasma membrane of HEK cells and mediate pH-dependent cation conductance termed 'off-response', that the channel is activated only after low extracellular pH is removed. Interestingly, in the tongue of two patients with an acquired sour ageusia, ie, unresponsive to sour stimuli, the mRNA and protein signals of TRPP3, PKD1L3 and ASICs (Acid Sensing Ion Channel) were undetectable, suggesting these proteins may be candidates for sour detection. Our unpublished data show that TRPP3 expressed alone, without co-expression of PKD1L3, in *Xenopus* oocytes also exhibits a similar off-response, suggesting that TRPP3 is an acid sensor. Thus, in this study, we aim to explore the relationship of the two acid sensors, TRPP3 and ASIC. Our reciprocal co-immunoprecipitation assays revealed that TRPP3 and ASIC1a are in the same protein complex in HeLa cells. Using two-electrode voltage clamp electrophysiology, we found that in *Xenopus* oocytes with ASIC1a over-expression, the off-response activity, but not the Ca-induced activation, of TRPP3 increased. By checking Western Blotting for each individual oocyte, we observed higher TRPP3 total expression level in oocytes co-expressing ASIC1a. The next experiments are to explore the reason why the off-response of TRPP3 increased, whether it is due to an increased plasma membrane targeting or to an up-regulation of TRPP3 channel function by ASIC1a through direct binding. Supported by KFC.

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ROLE OF PHOSPHORYLATION AND MEMBRANE ANCHORAGE IN THE ANTI-APOPTOTIC FUNCTION OF THE RUBELLA VIRUS CAPSID PROTEIN

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Rubella virus (RV) is a single stranded, positive sense RNA virus usually associated with the disease Rubella, or German measles. The virus is teratogenic when contracted during the early stages of pregnancy, causing a range of birth defects collectively known as congenital rubella syndrome. Pathogenesis is thought to be due in part to viral persistence, which itself is likely connected to the anti-apoptotic activity of the RV capsid protein. Previous work in our lab has established that this protein is able to block apoptosis by binding and sequestering the pro-apoptotic protein Bax. However, recent work has found a pro-apoptotic activity when a soluble, non-phosphorylated recombinant capsid was exposed to mitochondria *in vitro*. We hypothesized two possible reasons for this: (1) the membrane anchor removed from recombinant capsid is important for its anti-apoptotic function and/or (2) capsid's phosphorylation state affects its ability to modulate apoptosis. In support of the second hypothesis, we found that capsid containing a mutation that renders it hypo-phosphorylated (S46A) reduces its anti-apoptotic potential when introduced into cells via transduction. Furthermore, incorporation of this mutation into the viral genome eliminated the ability of RV to protect from apoptosis in infected cells. This mutant also completely lost its ability to protect from apoptosis in cells transfected with poly(I:C), which stimulates the innate immune response. We found that the S46A mutant was still able to bind Bax, but lost the ability to activate it. This suggests that the ability to activate Bax is an important part of capsid's anti-apoptotic mechanism. Deletion analysis suggests that capsid's membrane anchor is also important for its apoptotic activity. Together these results suggest that proper localization and phosphorylation are important for RV capsid's anti-apoptotic activity. Funded by Canadian Institutes of Health Research, Alberta Innovates Health Solutions, Natural Sciences and Engineering Research Council

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BIDIRECTIONAL REGULATION BETWEEN TRPP2 AND CELLULAR STRESS

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Unfolded protein response (UPR) is an important mechanism to maintain cell homeostasis during cellular stresses of which endoplasmic reticulum (ER) stress is caused by accumulation of unfolded proteins in the ER lumen. During ER stress, increased phosphorylation of eukaryotic initiation factor 2α (eIF2α) by pancreatic ER eIF2α kinase (PERK), as part of UPR down-regulates global protein synthesis and up-regulates translation of selected proteins. In addition to ER stress, P-eIF2α is also induced by other cellular stresses such as viral infection, oxidative stress and amino acid starvations. Moreover, UPR is critical for improving protein folding in the ER lumen during ER stress through increasing expression of ER lumen chaperones such as BiP. Mutations in TRPP2, a Ca2+ permeable non-selective cation channel mainly present in the ER membrane, account for ~10-15% of autosomal dominant polycystic kidney disease (ADPKD). Previous reports showed that TRPP2 down-regulates cell proliferation through promoting eIF2α phosphorylation by PERK. However, the role of TRPP2 in, and how it is regulated by, UPR are not well understood. Using ER stress inducers thapsigargin and tunicamycin, oxidative stress inducer arsenite, and the mimicker of virus infection polyinosinic:polycytidylic acid (Poly IC), in HEK293T, HeLa and mouse embryonic fibroblast cells, we discovered that the up-regulation of the TRPP2 expression by these stress inducers is dependent on P-eIF2α. We also found that the TRPP2 expression is up-regulated by salubrinal, an inhibitor of protein phosphatase1. Using TRPP2-deficient mouse collecting duct (MCD) cells (TRPP2-/-) and HeLa cells with TRPP2 knock-down, we found that increased BiP expression during ER stress requires the presence of TRPP2 and that the activity of cleaved caspase 3, a critical executioner of apoptosis, is significantly increased in TRPP2-/- MCD cells. Data together indicate that TRPP2 is a critical regulator of UPR for the maintenance of cell homeostasis during cellular stresses. Supported by CIHR.

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ROLES OF N-TERMINAL CYSTEINE 38 IN THE DIMERIZATION AND CHANNEL FUNCTION OF TRPP3

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Transient receptor potential polycystic 3 (TRPP3), also called polycystic kidney disease 2-like 1 (PKD2L1) or polycystin-L, forms a Ca-activated non-selective cation channel permeable to K, Na and Ca. TRPP3 is mainly present on the ER and plasma membranes. The mechanism of regulation of TRPP3 channel has been poorly understood. We previously demonstrated that a TRPP3 N-terminal domain, D21-S42, is important for the channel activity. To further determine which residue(s) in this domain is critical for the channel activity, we generated other two truncation mutants V37 (lacking aa 1-36) and T39, and by use of electrophysiology in *Xenopus* oocytes we found that V37 exhibits similar function to WT TRPP3 while T39 completely loses channel function. We continued to search for residue(s) that plays a key role in the channel function and found that C38A mutant exhibits very low activity, suggesting the importance of C38 residue for its channel activity. We also checked other cysteine mutants with electrophysiology and found they all shows similar activity with WT TRPP3. In an effort to determine how C38 affects the TRPP3 channel function, we examined the oligomerization of the channel protein. Using over-expression in *Xenopus* oocytes and HeLa cells, and native mouse tissues, we found, using non-reducing SDS-PAGE, WT TRPP3 exhibits oligomer in these models. Further, we found that C38A mutant exhibits much reduced oligomerization in both *Xenopus* oocytes and HeLa cells. Using co-immunoprecipitation with differently tagged TRPP3, we found that interaction of Flag-TRPP3 with GFP-TRPP3-C38A is reduced compared to the Flag-TRPP3 interaction with GFP-TRPP3. These data together suggest that C38 is involved in disulfide bond formation between two TRPP3 molecules and this oligomerization of TRPP3 is necessary for its channel function. Supported by CIHR, AHFMR and KFC (to XZC).

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ER-RETAINED DISEASE MUTANTS OF SLC4A11 ARE FUNCTIONAL WHEN RESCUED TO CELL SURFACE

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Mutations in the gene encoding the membrane protein 'Solute Carrier 4 member 11' (SLC4A11) causes three different posterior corneal dystrophies (congenital hereditary endothelial corneal dystrophy, Fuchs endothelial corneal dystrophy and Harboyan syndrome). Defective SLC4A11 leads to the formation of corneal edema and significant impairment of vision. Previously, we have shown that SLC4A11, localized to the basolateral surface of the corneal endothelium, plays a role in the regulation of corneal transparency and functions as a water transporter. The majority of point mutations cause this membrane protein to be misfolded and retained in the endoplasmic reticulum (ER). We explored whether ER retained mutants of SLC4A11 can be rescued to the cell surface using multiple strategies. If rescued SLC4A11 retains water transport function, this approach could be a viable therapeutic strategy. We studied SLC4A11 E143K, as a representative CHED mutant having ER retained phenotype when expressed in HEK293 cells. We also made use of catalytically inactive CHED mutant of SLC4A11, R125H, which processes to the plasma membrane at levels similar to WT, yet does not have functional activity. Immuno-precipitation experiments show that R125H and E143K mutants form heterodimers. Co-expression of catalytically inactive R125H in HEK293 cells rescued E143K, moving it from ER to the plasma membrane. We were also able to measure significant amount of functional activity arising due to this rescued E143K. Previously we had shown that some of the ER retained mutants of the SLC4A11 can be moved to the plasma membrane surface by incubating the HEK293 cells at a low temperature of 30° C. Therapies aimed at increasing the abundance of ER retained SLC4A11 mutants at the plasma membrane could be used to treat these posterior corneal dystrophies. Funding support by CIHR and IRTG.

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THE HYDROPHOBIC RESIDUE ISOLEUCINE 335 IS A KEY DETERMINANT OF HEXOSES AND URATE TRANSPORT MEDIATED BY hSLC2A9

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Urate, also called uric acid, is one of the major end products from the breakdown of dietary food and cellular purines. Studies have implicated that high levels of serum urate, hyperuricemia, is associated with diseases like gout, diabetes and hypertension. Our recent studies characterized a human glucose transporter 9, encoded by hSLC2A9 gene, as a hexose and urate transporter, which can exchange hexose with urate. However, this exchange mechanism remains unknown. Therefore, the objective of this study is to further explore the mechanism by introducing a hydrophobic residue mutation, isolucine 335 to valine (I335V), to hSLC2A9. This I-V mutation was identified as a major determinant of fructose transport but not glucose transport in hSLC2As. Hence, we hypothesize that the fructose trans-stimulation effect on urate transport will be abolished by the I335V mutation of hSLC2A9. To test this hypothesis, we used *Xenopus Laevis* oocytes expression system to over-express both hSLC2A9 wild type (wt) and its I335V mutation. Trans-stimulation studies from both 14C labelled urate flux and efflux measurements suggested that fructose trans-stimulates urate transport mediated by wt; whereas fructose only stimulates urate efflux from the oocytes mediated by I335V mutation. Two-microelectrode voltage clamp studies suggested that membrane potential may play a major role in urate and hexose transport mediated by both isoforms. Biotinylation and Western blot analysis suggested both isoforms have similar expression level on the single oocyte membrane preparation. Overall, the results from this study suggest that the hydrophobic residue, isoleucine 335, is important for the exchange of intracellular fructose with extracelluar urate mediated by hSLC2A9. This finding advances our understanding of how fructose intake is linked with serum urate, and implicates hSLC2A9 as an important transporter that may contribute to pathophysiological conditions involving urate or fructose. Supported by CIHR

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DETERMINING THE MECHANISM OF BINDING OF GLUTATHIONE S-TRANSFERASE P1 (GSTP1) TO THE PLASMA MEMBRANE

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Glutathione-S-transferase P1 (GSTP1) is involved in the detoxification of reactive compounds, including electrophiles through conjugation with the tripeptide glutathione (γ-Glu-Cys-Gly). GSTP1 is described in the literature as a cytosolic enzyme; however, we have reported the strong association of GSTP1 with the plasma membrane. GSTP1 is not removed from the plasma membrane under harsh stripping conditions [e.g., treatment of plasma membrane sheets with potassium iodide (KI), KI-EDTA or alkaline carbonate], known to remove peripherally associated membrane proteins. In contrast, exposure to NaOH and dithiothreitol (DTT) causes GSTP1 to be released from the plasma membrane. NaOH is known to cleave thioester bonds between cysteine residues of a protein and the lipid moiety of the plasma membrane while DTT is a reducing agent. Taken together these results suggest that GSTP1 could be associated with the plasma membrane through a thioester bond. Palmitoylation is the reversible post-translational addition of a 16-C saturated fatty acid to proteins through such thioester bonds and promotes the anchoring of proteins to membranes. GSTP1 has been identified as a putative palmitoylated protein in multiple "palmitoylome profiling" screens. Therefore, we are currently applying a novel, rapid, and sensitive method to confirm that GSTP1 is palmitoylated. Progress to date will be presented. Supported by Alberta Cancer Foundation, Canadian Institutes of Health Research.

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CHARACTERIZATION, STRUCTURE AND MECHANISM OF SULFIDE:QUINONE OXIDOREDUCTASE (SQR) FROM *ACIDITHIOBACILLUS FERROOXIDANS*

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Sulfide (existing as three different forms: H_2S, HS^- and S^{2-}) is involved in a variety of biological processes. In mammals, it is considered a very toxic molecule, which inhibits mitochondrial ATP production. However, it can be used as an energy source by microbes. Recently, sulfide has been identified as a signaling molecule in the cardiovascular and nervous systems. A key enzyme in maintaining the sulfide homeostasis is sulfide:quinone oxidoreductase (SQR), an ancient flavoprotein that is present in all domains of life (except plants). SQR is a peripheral membrane protein found in the periplasm that catalyzes the oxidation of sulfide species to elemental sulfur. To understand the sulfide oxidation mechanism in SQR, we purified the soluble active His-tagged SQR after heterologous expressing the *sqr* gene from *Acidithiobacillus ferrooxidans* in *E. coli* BL21(DE3). The activity assays of SQR were designed to spectroscopically measure the reduction and oxidation steps *in vitro* based on the reduction of decyl-ubiquinone and FAD, respectively. Meanwhile, we also detected the activity *in vivo* based on the detection of H_2S produced during the growth of *E. coli* in the presence and absence of wild-type and mutant SQR enzymes. The recombinant SQR was crystallized and its X-ray molecular structure was determined to 2.3 Å resolution. The active site of SQR includes three cysteines (Cys160, Cys356 and Cys128) and the FAD cofactor. The FAD cofactor accepts electrons from a sulfide and transfers them to quinone. Based on the catalytic activity and structure characters of wilt-type SQR and variants, we proposed two alternative mechanisms: (1) Nucleophilic attack mechanism that involves Cys356–S–S^- as a nucleophile which attacks the C4A atom of FAD; (2) Radical mechanism of direct electron transfer from Cys356 disulfide to FAD. The role of Cys128 (most likely in the form of a disulfide) is confined to the release of the polysulfur product. Supported by CIHR and NSERC.

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ACTIVATION OF THE INNATE IMMUNE RECEPTOR DECTIN-1 BY CLUSTERING

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Dectin-1 is a pattern-recognition receptor that plays a key role in anti-fungal immunity. It specifically recognizes beta-glucans, polymers of glucose found on the fungal cell wall. Upon ligand binding, dectin-1 triggers signaling cascades that lead to various cellular responses, including phagocytosis and the release of pro-inflammatory mediators, which all culminate in the generation of an effective immune response and eradication of the pathogen. The molecular mechanisms underlying dectin-1 activation and signal transduction are not fully understood. The aim of this study is to elucidate such mechanisms. We propose that upon binding of larger dectin-1 ligands/beta-glucans, dectin-1 receptors cluster and assemble into multimeric complexes. In these clusters, dectin-1 becomes activated and promotes the recruitment and activation of Syk (Spleen tyrosine kinase), which in turn triggers subsequent signaling cascades. To test our hypothesis, we stably expressed human dectin-1 in RAW 264.7 macrophages. Various levels of dectin-1 clustering were induced using antibody cross-linking or beta-glucans of different molecular weights. The corresponding stimulation of dectin-1 was detected by determining the activation of several downstream effectors (Src, Syk, NF-kB). We demonstrate that antibody cross-linking and larger ligands are able to induce more Syk phosphorylation than smaller ligands. To quantify the level of dectin-1 clustering, single molecule analysis by superresolution imaging was employed. Upon antibody cross-linking or ligand binding dectin-1 formed nanodomains of about 500 nm of diameter, which were identified as the nucleation site for intracellular signaling. Additionally, the phosphorylation and recruitment of Syk to regions of the plasma membrane rich in dectin-1 clusters was observed using confocal microscopy. Together, our results suggest that receptor clustering is the mechanism by which dectin-1 is activated. Supported by CIHR.

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REDOX PROCESSES IN COMPLEX I ASSEMBLY

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Complex I of the respiratory chain of mitochondria is crucial for cellular energy production. It is also a major source of reactive oxygen species, and dysfunctions of the complex have been implicated in the pathogenesis of a variety of neurodegenerative disorders. Dysfunctions often occur as a result of an impaired assembly, but so far only little is known about the biogenesis and maintenance of Complex I in mammalian cells. Seven of its subunits are encoded in the mitochondrial genome, while the remaining 38 subunits have to be imported from the cytosol. We aim to characterize the function of five proteins in the assembly/maintenance of Complex I that are linked to redox pathways. (1) The four uncharacterized nuclear-encoded Complex I subunits NDUFS5, NDUFB7, NDUFB10 and NDUFA8 lack typical mitochondrial import signals but instead contain conserved cysteine residues. The proteins are likely imported and trapped in the intermembrane space in a redox-dependent manner (twin-Cx_9C proteins). This process of oxidative folding is facilitated by the oxidoreductase Mia40 and is coupled to the activity of the respiratory chain. It is unknown how this redox pathway affects the assembly and maintenance of Complex I and whether the coupling to the respiratory chain provides a feedback control for respiratory chain assembly. We will therefore study the import and the function of these proteins as well as the role of Mia40 in Complex I biogenesis and maintenance on the molecular level. (2) We will also detail the function of the uncharacterized mitochondrial flavoprotein, FoxRed1. Homozygous mutations in this protein have been linked to isolated Complex I deficiencies. The cells of the patients expressed FoxRed1 mutants, and the levels of Complex I were strongly reduced. The molecular function of FoxRed1, however, remains unclear. Here, we will present initial data on the characterization of redox processes in Complex I assembly and introduce the project strategy.

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EVIDENCE FOR THE ELECTRONIC AND REDOX COUPLING OF THE CENTERS WITHIN *E. COLI* NITRATE REDUCTASE A

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Escherichia coli flourishes as a natural and essential component of our gut flora partly because of nitrate reductase A (NarGHI), which facilitates the use of nitrate as a terminal electron acceptor. NarGHI coordinates a bis-pyranopterin cofactor, five Fe/S clusters and two *b*-type hemes. The catalytic dimer, NarGH, is anchored to the cytoplasmic face of the plasma membrane by NarI, which coordinates two distinct EPR-visible *b*-type hemes and is the site of quinol oxidation. Redox titrations of the hemes and semiquinone of NarI, in addition to the NarI-proximal [3Fe-4S] cluster of NarH (FS4), are traditionally modelled as non-interacting centers with multiple components and non-ideal electron stoichiometry. An alternative, and less arbitrary interpretation, is the existence of anti-cooperative interactions between these redox centers. Application of a three-center redox model comprising just the hemes and FS4 yields satisfactory fits for quinone-site mutants and quinone-like inhibitor bound states. However, by extending the model to include the semiquinone redox transitions the fits are greatly improved. By considering multiple cofactors simultaneously and in the context of an interacting system, midpoint potentials different from previously reported values result. Moreover, the magnitude of the interaction potentials do not follow an inverse squared distance relationship, suggesting that non-electrostatic effects are at play. This likely implies NarGHI undergoes oxidation state-dependant conformational changes, as has been suggested for a number of other metalloproteins. Paramagnetic characterisation of the system, by measuring the ability of the individual EPR signals to saturate, provides further evidence for electronic coupling between the centers. The research presented paints a picture of nitrate reductase as a highly coupled and possibly structurally dynamic enzyme. This research is supported by CIHR and AIHS.

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DEFINING A ROLE FOR THE PYRANOPTERIN COMPONENT OF THE MONONUCLEAR MOLYBDENUM COFACTOR

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E. coli nitrate reductase (NarGHI) is an excellent model system for the study of the mononuclear molybdenum cofactor, which is present in a range of enzymes critical to global geochemical cycles, bacterial metabolic diversity, and human health. The molybdenum cofactor of NarGHI has a complex structure comprising a molybdenum atom coordinated by two pyranopterins (referred to a proximal and distal), each of which is covalently linked to a guanine moiety via a phosphodiester bond. Protein crystallography has revealed that the distal pyranopterin of NarGHI has a bicyclic rather than tricyclic structure, and this project examines the factors defining its structure within the enzyme. NarG-Ser719 and NarG-His1163 are predicted to interact with the open pyran ring via hydrogen bonds inferred from the crystal structure, and we hypothesized that mutating these residues may generate a form of the enzyme with a tricyclic distal pyranopterin. NarG-Ser719Ala, NarG-His1163Ala, and NarG-Ser719Ala/His1163Ala mutants were generated for experiments, and their structures determined by protein crystallography. Only the NarG- Ser719Ala/His1163Ala double mutant resulted in the observation of pyran ring closure by protein crystallography. The single mutants have a deleterious effect on enzyme activity, with the His1163Ala and Ser719Ala/His1163Ala mutants also significantly decreasing the molybdenum reduction potentials. These results reveal the importance of the pyranopterin binding environment in controlling molybdenum redox chemistry and catalysis. Supported by AIHS and CIHR.

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NO EVIDENCE FOR THE ROLE OF MIA40 IN THE BIOGENESIS OF DRE2

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The assembly of Fe-S-clusters in mitochondria gives the essential character to the organelles. The biogenesis of Fe-S proteins in eukaryotes is the interplay of three systems: the ISC assembly, the ISC export and the CIA machineries. Recently, a new component of the CIA machinery called Dre2 was identified in *S. cerevisiae*. It contains two Fe-S clusters and is implicated in an early step of cytosolic Fe-S-cluster synthesis. All so far identified members of CIA machinery are exclusively located to the cytosol. However, recently published studies claimed that the fraction of Dre2 was present in the IMS of mitochondria. In addition, the physiological interaction of Dre2 with the mitochondrial oxidoreductase Mia40 was proposed on the basis of *in vitro* experiments with purified proteins. It was suggested that Mia40 introduces two disulfide bonds between conserved cysteine residues of Dre2 in order to trap imported protein in the IMS. Here, we demonstrate that Dre2 is entirely localized in the cytosol associated with the cytosol-exposed surface of the mitochondrial outer membrane. This mitochondria-associated fraction further increased upon overexpression of *DRE2*, however, even then no Dre2 was found in the IMS. Dre2 is relatively resistant against proteases, at least as long as the membranes were not lysed with detergents. Moreover, upregulation or depletion of *MIA40* did not affect the level of mitochondria-associated Dre2, nor did Dre2 contain disulfide bonds *in vivo*. Taken together, we conclude that Dre2 is exclusively present in the cytosol where it contains reduced cysteine residues which coordinate Fe-S-clusters. Supported by DFG (IRTG 1830).

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Simulating the Electrophysiology of Mouse Inner Hair Cells: Phase Locking and K+ Currents

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Transformation of acoustical stimuli into neuronal signals in inner hair cells (IHCs) is known to phase lock to the stimulus up to several kHz (Palmer and Russell, 1986, Hear. Res., 24:1-15). This high temporal precision is somewhat surprising since in the IHCs alone the transduction process comprises several time-dependent steps. The IHCs are depolarized by K+ ions entering through the transduction channels which activates voltage gated Ca2+-channels. The entering Ca2+ ions finally trigger exocytosis of neurotransmitter that yields postsynaptic afferent activity. Repolarization of the IHCs is mediated by several different types of K+ currents. To investigate the influence of the composition of the different ionic currents on the ability to phase lock to the stimuli we built a computer model for mouse IHCs which includes the major known currents: Ca2+, delayed rectifier, KCNQ- and BK-type K+ currents and a model for the transduction current. The findings suggest that the large conductance Ca2+ dependent BKtype K+ currents are crucial in maintaining the phase-locking to frequencies of more than one kHz in mature IHCs.

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FUNCTION OF SPLICE VARIANTS OF CAPS2 (CA^{2+} DEPENDENT ACTIVATOR PROTEIN FOR SECRETION) IN MOUSE CHROMAFFIN CELLS

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The Ca^{2+} dependent activator protein for secretion (CAPS) is involved in priming synaptic vesicles and large dense core vesicles (LDCVs) for exocytosis. The two CAPS isoforms, CAPS1 and CAPS2, preferentially prime the Readily Releasable Pool (RRP) in mouse chromaffin cells. We have shown that rat CAPS1 and CAPS2 rescue the secretion deficit in mouse chromaffin cells lacking both CAPS1 and CAPS2 (CAPS DKO). Six CAPS2 splice variants have been detected in the mouse cerebellum. Using RT-PCR, we show that all splice variants (CAPS2a - CAPS2f) are expressed at different developmental stages in mouse chromaffin cells. In rescue experiments, CAPS2b, CAPS2d or CAPS2e were expressed in CAPS DKO chromaffin cells lacking CAPS1 and all CAPS2 splice variants. Secretion in these cells was compared to secretion in CAPS DKO cells. To this end we performed capacitance measurements in whole cell patch clamp and simultaneous amperometric recordings to monitor catecholamine release in CAPS DKO cells. Secretion was elicited by flash photolysis of np-EGTA caged Ca^{2+}. Expression of mouse CAPS2b, which has a short deletion in the Munc13 Homology Domain (MHD, the putative priming domain), rescued the CAPS DKO secretion defect, enhancing the RRP and sustained secretion. CAPS2d, with a C-terminal deletion of much of the priming domain failed to rescue. Surprisingly, expression of CAPS2e, with a C-terminal truncation which includes all of the MHD, also enhanced secretion in DKO cells. Our results indicate that in addition to an interaction with syntaxin mediated by the C-terminal MHD domain, N-terminal domains in CAPS can promote exocytosis, likely via the Plekstrin homology and C2 domains. Thus, it is likely that even the strongly truncated splice variant CAPS2e modulates exocytosis. Supported by the DFG.

Inter-lab Meeting Schedule

Wednesday August 29, 2012

Time	Place	Participants
7:15-8:00pm	Ballroom B/C	Casey, Cordat, Becker, Deitmer
	Emerald Lounge	H. Young, Engel
	Emerald Lounge	Flockerzi, Chen
	Emerald Lounge	Cheeseman, Friauf
	Ballroom B/C	Hobman Lab, Herrmann
	Ballroom B/C	Alexander, Hoth
	Emerald Lounge	Weiner, Keller
	Tent City	Niemeyer, Chen
	Tent City	Reimer, Leslie Lab
	Ballroom B/C	Schmitt, Lemieux, Touret,
	Tent City	J. Young, Möhlmann
	Tent City	Neuhaus, Fliegel
8:00-8:30pm	Ballroom B/C	Fliegel, Becker, Deitmer, Alexander
	Ballroom B/C	Cordat, Engel Flockerzi
	Ballroom B/C	Niemeyer, Cheeseman
	Emerald Lounge	Leslie Lab, Herrmann
	Ballroom B/C	M**ö**hlmann, Neuhaus, H. Young
	Emerald Lounge	Hoth, Casey
	Emerald Lounge	Lemieux, Keller
	Emerald Lounge	Reimer, Weiner
	Tent City	Rettig, Touret
	Tent City	Schmitt, Fliegel
	Tent City	Zimmermann, Chen

8:30-9:00pm	Ballroom B/C	Flockerzi, H. Young
	Ballroom B/C	Deitmer, Chen
	Emerald Lounge	H. Young, Friauf
	Emerald Lounge	Flockerzi, Alexander
	Ballroom B/C	Herrmann, Lemieux, Reimer
	Emerald Lounge	Keller, Fliegel
	Emerald Lounge	Casey, Niemeyer
	Tent City	Schmitt, Cordat
	Tent City	Zimmermann, Lemieux
	Tent City	J. Young, Neuhaus
	Tent City	Hoth, Touret
9:00-9:30	Ballroom B/C	H. Young, Keller, Deitmer
	Emerald Lounge	Engel, Chen, Friauf
	Emerald Lounge	Hoth, Hobman Lab
	Ballroom B/C	Neuhaus, Cheeseman, Casey
	Emerald Lounge	Zimmermann,Cordat
	Tent City	Schmitt, Weiner
	Tent City	Lemieux, Möhlmann
	Tent City	Rettig, Fliegel

***Note**: The locations are just suggested to make sure that people can meet up. If you want to arrange a different meeting place (pool, bar?), then that is fine if you sort this out amongst yourselves.*

IRTG JASPER SYMPOSIUM SOCIAL ACTIVITES

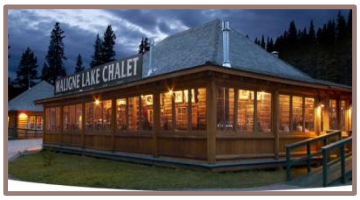
As part of the IRTG Jasper Symposium we would like to include some special times of socialization, fun and an opportunity for everyone to see and enjoy the magnificent Rocky Mountains and all the area has to offer. We have planned two full afternoon excursions on Tuesday and Wednesday from 12:30-5:30pm with bag lunches available. The tours will start from the Jasper Park Lodge and transportation will be by bus and car. Signup sheets for car- pooling will be available at the registration desk.

TUESDAY, AUGUST 28 – MALIGNE CANYON AND LAKE

"In its efforts to reach the Athabasca River, the Maligne River cut its way through the limestone terrain, carving out this steep, beautiful canyon. The level of the river changes due to underground caves which pull water away from it, but it has passed this way for more than 10,000 years. To give visitors perspective, a trail leads down along the canyon, crossing over it six times and offering incredible views of the landscape. Plaques along the way describe the area's geology, and a small building set at the upper portion of the canyon serves refreshments. Less than 10 minutes from town."

This excursion could include hiking, canoe rentals, Spirit Island Boat cruise, or afternoon tea if you would like to just sit and take in the scenery.

	**HIKING -** Maligne Lake is an exceptional hiking area with a wide variety of trails. Everything from peaceful, short-walks-in-the-woods to steep, steady uphill grinds are to be found at Maligne Lake. All routes have one thing in common however... fabulous scenery. The 44km long Skyline Trail, Jasper's most popular, highest and above treeline, multi-day hike linking Maligne Lake and the Town of Jasper.
	**CANOEING** - The Curly Phillips Boathouse on Maligne Lake, a registered historic building, rents canoes and sea kayaks for those seeking access to the backcountry shoreline camps of Maligne or just simply paddling the lakes azure waters. **Rental Rate** **Rowboats & Canoes** $30.00 per hour - minimum 2 hours $90.00 per day

	**Sea Kayak** $35.00 per hour - minimum 2 hours $100.00 per day **Deposit Requirements** A credit card imprint must be left with the boathouse attendant. All rentals must be returned to the boathouse by 6:30 pm. GST included.
	**SPIRIT ISLAND BOAT CRUISE -** This will take you on a 90 minute boat tour of Maligne Lake. **Adults $55.00**
	**AFTERNOON TEA AT THE MALIGNE LAKE CHALET** Step back in time at historic Maligne Lake Chalet this summer after your long day of touring and join in the ritual of Afternoon Tea. This Rocky Mountain Tea experience is charming and down to earth. Afternoon Tea at the Maligne Lake Chalet is offered July 1st through August 31st between 2:30pm and 4:30 pm. **Adults $32.00 Children $16.00**

WEDNESDAY, AUGUST 29 – JASPER TRAM

“Boasting some of the best views in the Rockies, this tramway delivers on its promise. You'll gaze out on lakes, mountains, glaciers, and the town itself from a vantage point of more than 7400 feet above sea level. The ride, in a 30-person car, lasts about 7 minutes and is narrated by informative guides. When you arrive at the summit, you're free to wander along the boardwalk or hike in the area, also accompanied by a guide, if you'd like. Trees don't grow this high, but there's low vegetation and an assortment of wildlife, including the small mammals (hoary marmots) known as "whistlers" for which the mountain was named. A restaurant and gift shop are available. A 10-minute drive from Jasper.” Costs of the Jasper Tram will be covered. Please email trish.graham@ualberta.ca in order to pre-book tickets.

THE ADVENTURE begins at an elevation of 1304 metres (4279 ft) above sea level in the safety of an enclosed Tram cabin. Our fully trained and knowledgeable tour guide will accompany you during the smooth seven minute trip to the Upper Station located at 2277 metres (7472 ft) above sea level. During your ascent up Whistlers Mountain our tour guide will inform you of the area,

points of interest, animal life and history as well as answering questions. More than just a pretty picture, more than just an attraction, it is a true learning experience. You will also be able to hike in the high alpine from the top of tram.

THE HIGH ALPINE is your destination and the Upper Station offers a safe and awe-inspiring view that you may enjoy for as long as you have time. Remember, you are on vacation. Stroll the boardwalks viewing the interpretive plaques and watch for wildlife. Alpine inhabitants include the Hoary Marmot (famous for its whistling alert call), White-tailed Ptarmigan, Ground Squirrels, Pikas and the occasional Bighorn Sheep. Travel off of the boardwalks onto the hiking trails to the summit is possible but done so at your own risk. Please be safe and prepared. Dress appropriately.

This trip will also include a shopping trip in the town of Jasper to check out gift and souvenir shops, sporting stores, art galleries, or historical monuments that are in and around the town.

OTHER OUTDOOR ACTIVITIES

(Car-pooling required, limited availability)
Car -pooling will be organized on-site at the registration kiosk.

HIKING TRIPS

Pyramid Lake Viewing and Hike
http://www.explorejasper.com/sights/pyramidlake.htm

Pyramid Lake is on the outskirts of Jasper, about 15 minute drive from Jasper Park Lodge. The lake has spectacular views and opportunities for short hikes.

Miette Hotsprings / Sulfur Ridge Hike
http://www.explorejasper.com/sights/miettehotsprings.htm

The Miette hotsprings are about 40 minute drive from Jasper Park Lodge. They are in a secluded location, far from main roads and nestled in the mountains. The hotsprings are warmed by volcanic activity. At the springs are hot, medium and refreshingly cold pools. The springs are adjacent to the trailhead for the Sulfur Skyline trail, which climbs 700 m vertical over 4.6 km to be rewarded by a wild Rocky Mountain view.

Tonquin Valley Hike
http://www.trailpeak.com/trail-Tonquin-Valley-near-Jasper-AB-3218

The Ramparts and Amethyst Lake as seen from a ridge of Oldhorn Mountain

"The Tonquin Valley is located in Jasper National Park, Alberta, Canada, next to the border of the provinces of Alberta and British Columbia, an area which is also the continental divide, running along the peaks of the South Jasper Range (including The Ramparts subrange) which rise above Amethyst Lake. Tonquin Creek drains Moat Lake and flows west into Mount Robson Provincial Park in British Columbia, and empties into the Fraser River. The Astoria River drains south and east into the Athabasca River."

Valley of the Five Lakes Hike

http://www.trailpeak.com/trail-Valley-of-5-Lakes-near-Jasper-AB-212

"The Valley of the Five Lakes (Long Loop) in Jasper National Park is one of the best valley bottom hikes near the town of Jasper. The lakes themselves are striking in their beauty and varied in colour. This loop takes in all five lakes and it's not as busy, for most the most part, as the short loop."

JASPER PARK LODGE ACTIVITIES

If you decide you would rather stay on site for the day there are many wonderful activities right at the Lodge itself: http://www.fairmont.com/jasper/activities-services/ -**Note some activities require pre-booking which can be done on the above website.

A list of on-site activities include:

Golf, Spa Biking, Outdoor pool, Lakes viewing, Boating (canoes, kayaks), Horseback riding, Hikes

LIST OF DELEGATES

RTG 1830 GERMAN CANADIAN
COMPLEX MEMBRANE PROTEINS IN CELLULAR DEVELOPMENT AND DISEASE

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A Special Thank you…….

To the Jasper Park Place Lodge administration and staff for all their assistance in making the IRTG Jasper Symposium a huge success!