



## Society for Experimental Biology Annual Main Meeting 28th June – 1st July 2009, Glasgow, UK

### A3 – PROGRESS IN ION TRANSPORTING CELL RESEARCH: STUDIES ON INVERTEBRATE AND VERTEBRATE MODELS AND THEIR REGULATION

#### A3.1

09:00 Tuesday 30th June 2009

**Phosphorylation of focal adhesion kinase at tyrosine 407 regulates CFTR anion channel and NKCC cotransporter in osmosensitive ion transporting mitochondrion rich cells of euryhaline killifish**

William S. Marshall (St. Francis Xavier Univ.)

$\text{Na}^+, \text{K}^+, 2\text{Cl}^-$  cotransporter (NKCC1) is the regulated basolateral entry for NaCl and Cystic Fibrosis Transmembrane conductance Regulator (CFTR) anion channels are the regulated exit pathway in  $\text{Cl}^-$  secretion by teleost mitochondrion rich salt secreting (MR) cells of the gill and opercular epithelia of euryhaline teleosts. By confocal light immunocytochemistry and immunogold TEM, using regular and immunopurified phospho-antibodies directed against conserved sites, we found that killifish CFTR (kfCFTR) and the tyrosine kinase Focal Adhesion Kinase (FAK) phosphorylated at Y407 (FAK pY407) are colocalized in the apical membrane and in subjacent membrane vesicles of MR cells. Similarly, NKCC colocalizes with FAK pY407 in the basolateral surface. FAKpY407, unlike other FAK phosphorylation sites (Y397, Y576, Y577, and Y863), is osmosensitive and dephosphorylates during hypotonic shock of epithelial cells and rephosphorylates with isotonic restoration. FAKpY576 is present in apical membrane only and is insensitive to osmolality. Hypotonic shock and the  $\alpha$ -2 adrenergic agonist clonidine (neither of which affects cAMP levels) rapidly and reversibly inhibit  $\text{Cl}^-$  secretion by isolated opercular membranes, simultaneous with dephosphorylation of FAKpY407, located in the basolateral and apical membranes. FAKpY407 is re-phosphorylated, especially in the apical membrane, and  $\text{Cl}^-$  secretion rapidly restored by hypertonic shock as well as by forskolin and isoproterenol that operate via cAMP and PKA. Western analysis confirms the dephosphorylation/rephosphorylation of FAKpY407. FAK colocalizes also with integrin B1, a well known mechanosensor protein. We conclude that hormone mediated, cAMP dependent and osmosensor integrin/FAK mediated, cAMP independent pathways converge on a mechanism to activate CFTR and NKCC simultaneous with  $\text{Cl}^-$  secretion. Supported by NSERC.

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#### A3.2

09:40 Tuesday 30th June 2009

**Ontogeny of salinity tolerance and osmoregulation in the sea-bream *Sparus aurata***

Charlotte Bodinier, Elliott Sucre, Laura Lecurieux-Belfond, Guy Charmantier (University of Montpellier 2)

The gilthead sea bream, *Sparus aurata*, is a euryhaline teleost hatching in the open sea. The larvae drift to the coast and juveniles migrate into estuaries and lagoons where salinity varies from brackish to hyper-salinity levels. The ontogeny of osmoregulation in *Sparus aurata* was studied at successive stages, from day 2 (D2) post-hatch to late juveniles (D300), exposed to different salinities ranging from fresh water to 45.1 ‰, at 18 °C. Salinity tolerance ranged between 5.1–39.1 ‰ at D3, and between 1.0–45.1 ‰ from D75. The fish were hyper-hypo-osmotic regulators, in all studied stages. The osmoregulatory capacity appeared size-dependent and reached its maximum after D96. The acquisition of the full ability to hypo- and hyper-regulate occurred in four steps, with particularly marked increases at mouth opening and metamorphosis. The localization of the ionocytes in the integument and gills was followed in the same stages. The main site of osmoregulation shifted from the integument to the gills from D30 to D70, with a corresponding sharp increase in the osmoregulatory ability. Our results suggest that the early development of osmoregulatory ability, and thus of salinity tolerance, may provide an advantageous flexibility for the migration of *Sparus aurata* between sea and estuaries and lagoons.

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**A3.3****10:30 Tuesday 30th June 2009****Aquaporin 8 (AQP8) intestinal mRNA expression increases in response to salinity acclimation in yellow and silver European eels (*Anguilla anguilla*)**

Christopher P. Cutler (Georgia Southern University), Clare Philips (University of St Andrews), Neil Hazon (University of St Andrews), Gordon Cramb (University of St Andrews)

Work in this lab and elsewhere has shown that when euryhaline eels are acclimated from freshwater to seawater, the expression of (the water-selective) aquaporin 1 (AQP1) water channel mRNA and protein is upregulated (up to 25-fold; Martinez et al., *Am.J.Physiol.*288: R1733–43 2005). Similar up-regulation can also be induced (up to 50-fold) by infusion of the 'seawater' acting hormone, cortisol, into freshwater fish. Immunohistochemical studies show that the main location of AQP1 expression in surface cell epithelia, is in the rectal region, and the posterior intestine surrounding the sphincter that delineates the intestine from the rectum. These data suggested a potential osmoregulatory role for aquaporins in trans-epithelial water uptake from the intestinal lumen following seawater acclimation, however, for the transcellular water uptake pathway to be playing a significant role in intestinal water absorption, further water transport proteins, such as other aquaporins, have to be involved. Studies have shown that Aquaporin 8 (AQP8) is one such candidate. Data show 1) that AQP8 is only expressed at a significant level in the intestine, that 2) its mRNA expression increases in response to salinity acclimation and 3) preliminary data suggest that unlike AQP1, the increase in AQP8 mRNA expression is not under the control of the hormone, cortisol.

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**A3.4****11:00 Tuesday 30th June 2009****Intestinal expression of Aquaporins in the European eel (*Anguilla anguilla*): Effects of SW acclimation on isoform expression**

Gordon Cramb (University of St Andrews), Brian Powell (University of St Andrews), Caroline J. Osborne (University of St Andrews), Anne-Sophie Martinez (Université de Caen), Christopher P. Cutler (Georgia Southern University), Svetlana Kalujnaia (University of St Andrews)

Five aquaporin (AQP) isoforms, comprising two homologues of mammalian aquaporin 1 (called AQP1 and AQP1 duplicate), a novel aqua-glyceroporin called AQPe and two homologues of the "unorthodox" or "atypical" mammalian aquaporin 8 (called AQP8a and AQP8b) and are expressed within the intestine of the European eel (*Anguilla anguilla*). All isoforms are expressed within anterior, mid and posterior sections of the intestine, although AQP1 expression predominates within the posterior/rectal segment. Expression of AQP1, AQP8a and AQP8b isoforms increased in all intestinal segments two days after transfer of fish from freshwater (FW) to sea water (SW). No consistent changes in intestinal expression were found with AQPe or AQP1 duplicate with the latter exhibiting highly variable levels of expression between animals within the same experimental groups. Although single mRNA species were identified for most isoforms, Northern blot analysis revealed three and possibly four different mRNA transcripts for AQP8b,

ranging from 1.6 to 2.6 kb in length. The different transcripts appeared to be expressed in a random manner between fish in each experimental group although all were observed to up-regulate when fish were acclimated to SW. Immunohistochemistry identified AQP1 to be present within the endothelial cells of blood vessels within the intestine and also to the apical brush-border membrane of epithelial cells within the posterior and rectal segments. Antisera for AQP8b identified specific staining of intracellular vesicles/mitochondria in the perinuclear regions of epithelial cells along the whole length of the intestine. The possible physiological roles of these AQP isoforms within the GI tract will be discussed.

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**A3.5****11:20 Tuesday 30th June 2009****New molecular insights in calcium and magnesium (re)absorption**

Joost Hoenderop (Radboud University Nijmegen)

The kidney and small intestine constitute the influx pathways into the extracellular pool and play, therefore, a primary role in  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  homeostasis. The kidney determines the final excretion of these divalents into the urine and is a prime target for regulating the  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentration in the extracellular compartment. Although  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  transport in kidney occurs throughout the whole length of the renal tubule, the fine regulation takes place primarily in the distal part of the nephron. A major breakthrough in completing the molecular details of the abovementioned  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  pathways was the identification of a family of epithelial  $\text{Ca}^{2+}$  channels (TRPV5 and TRPV6) and  $\text{Mg}^{2+}$  channels (TRPM6 and TRPM7). Defective TRPV5 or TRPV6 function could ultimately impair the  $\text{Ca}^{2+}$  conserving capacity of the body and could, therefore, contribute to hypercalciuria and serious age-related bone disorders. Ablation of the TRPV5 gene in mice seriously disturbs renal  $\text{Ca}^{2+}$  handling resulting in compensatory intestinal hyperabsorption and bone abnormalities. Likewise, we have recently extended our studies to the  $\text{Mg}^{2+}$  selective channels, TRPM6 and TRPM7 to obtain insight in the molecular regulation of active  $\text{Mg}^{2+}$  (re)absorption. These studies offer for the first time a realistic approach to investigate the functional and regulatory aspects of the epithelial  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  channels that are prime targets for hormonal control of the active  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  flux from the intestinal lumen or urine space to the blood compartment.

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**A3.6****11:50 Tuesday 30th June 2009****Effect of elevated dietary copper on Cu-transport protein expression and localisation and metal status of zebrafish**

David Boyle (NIFES), Andreas Nordgreen (NIFES), Nicolas R. Bury (King's College London), Anne-Katrine Lundebye (NIFES)

Copper is an essential micronutrient which acts as a cofactor for a number of key proteins. At elevated concentrations, however, Cu is toxic and Cu uptake and excretion are tightly regulated to meet functional requirements but avoid excess. In this study, we investigated the role of metal transport proteins in the response to elevated

dietary Cu in zebrafish. Fish were fed diets containing 25 (control), 100, 250 and 500 mg Cu kg<sup>-1</sup> for 28 days. At time-points during the exposure, intestines and livers were excised for mRNA transcript analysis and measurement of tissue metal concentrations. Expression of apical Cu-import proteins, copper transport protein 1 (CTR1) and divalent metal transport 1 (DMT1), the metal binding protein, metallothionein (mt2) and the intestinal basolateral Cu export protein ATP7a are presented. The plasticity of Cu-transport protein expression along the length of the intestine in response to altered dietary Cu was analysed with *in situ* hybridisation with gene specific probes. Data are discussed in terms of Cu homeostasis in zebrafish and the implications for the assimilation of other essential and non-essential metals via shared transport pathways.

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### A3.7

13:30 Tuesday 30th June 2009

#### ***Drosophila* as a model for renal function in insects and humans**

Julian A. Dow (University of Glasgow), Shireen-Anne Davies (University of Glasgow)

The ion and fluid-transporting capabilities of the insect (Malpighian) tubule are remarkable, but recent work has shown that this is only a fraction of their contribution to the organism. Tubules ramify throughout the haemocoel of the insect, and so are well-placed to sense, and respond to, a range of insults. In particular, insect tubules are autonomous immune tissues, capable of mounting an effective killing response to invading bacteria. They are also highly active in metabolism and detoxification, and provide defence against xenobiotics. This is particularly important, because insecticide resistance is becoming widespread.

Although *Drosophila* diverged from the mammalian lineage over 400 M year ago, it has proved to be a valuable model for a whole range of human processes – notably development. We have shown that *Drosophila* tubules faithfully replicate the salient phenotype of several human renal genetic diseases. This opens the possibility of faster, cheaper and more humane modelling of human disease in a simple model organism.

Chintapalli, V.R., Wang, J., Davies, S.A. and Dow, J.A.T. (2007). Using FlyAtlas to identify better *Drosophila* models of human disease. *Nature Genetics* 39, 715–720.

Dow, J.A.T. (2009). Insights into the Malpighian tubule from functional Genomics. *Journal of Experimental Biology* 212, 435–45.

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### A3.8

14:10 Tuesday 30th June 2009

#### **Novel phenotypes for the mitochondrial *sesB* gene in *Drosophila melanogaster***

Selim Terhzaz (University of Glasgow), Pablo Cabrero (University of Glasgow), Shireen A. Davies (University of Glasgow), Julian A. Dow (University of Glasgow)

Mitochondria are essential organelles for the function of the cell, as they produce ATP, buffer cytosolic calcium and sequester apoptotic factors. Impairment of mitochondrial function results in metabolic

insufficiency, oxidative damage and cell death, and can contribute to severe tissue pathologies and diseases. The mitochondrial adenine nucleotide translocase (ANT) is a crucial component in the maintenance of cellular energy homeostasis, as well as in the formation of the mitochondrial permeability transition (MPT) pores. ANT catalyzes ADP/ATP exchange across the mitochondrial inner membrane. A great advantage of working with *Drosophila* is the availability of mutants for pathways of interest. We show here that mutants of the gene encoding mitochondrial ADP/ATP translocase, *sesB*, which from our published microarray data is enriched in tubule, impact on mitochondrial function. Based on the interaction between the putative MPT pore component *sesB* and Ca<sup>2+</sup> signalling we aimed to characterize the role of the *sesB* gene in cytosolic and mitochondrial Ca<sup>2+</sup> signalling. In parallel, using confocal microscopy we assessed changes in mitochondrial biogenesis in *sesB*<sup>1</sup> allele mutant and in flies in which RNAi against *sesB* is expressed specifically in tubule principal cells. Our studies of these mutants revealed defects in mitochondrial morphology and distribution, cytosolic ATP depletion, impairment of fluid secretion and sensitivity to oxidative stress.

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### A3.9

14:30 Tuesday 30th June 2009

#### **Differential neuronal expression of three *Drosophila* ion transport peptide splice forms indicate multiple functions of peptidergic neurons**

Heinrich Dirksen (Stockholm University Dept. of Zoology), Aditya Mandali (Stockholm University), Taishi Yoshii (University of Regensburg), Johannes Strauss (Stockholm University), Charlotte Helfrich-Förster (University of Regensburg), Dick R. Nässel (Stockholm University)

We identified previously two long (DrmlTPL1 and -L2) and one amidated short isoform (DrmlITP) of insect ion transport peptides (ITPs) as products derived by alternatively splicing from the *Drosophila itp*-gene (CG13586). The peptides are members of a large family of arthropod neuropeptides incl. crustacean hyperglycemic hormones (CHH/ITP-family), but similar ITPs are only known in locusts to have antidiuretic bioactivity on the hindgut. We localised the peptides by *in situ* hybridisation and immunocytochemistry with isoform-specific antibodies in the nervous system of larval (L3) and adult *Drosophila melanogaster* and screened Gal4-lines specific for peptidergic cells. Four neurosecretory cells in brain-corpora cardiaca/allata putatively release DrmlITP as a hormone in all stages. DrmlITP also occurs in interneurons in the brain/ventral ganglia and in neurons efferent towards the hindgut. Some interneurons are identical to well-known circadian clock neurons for which the effector molecules were elusive but are responsible for the evening bouts of locomotor activity in flies. DrmlTPL1 and -L2 were found only in peripheral lateral bipolar and putative sensory neurons which are likely to play a role in the control of growth, hindgut ion transport and heart beat. With DrmlITP identified in brain neurosecretory cells, hindgut-innervating neurons in the abdominal ganglia and one pair in the abdomen close to the larval anal organ or innervating the adult rectal pads, both chloride-transporting organs, we are facing an enormous complexity in multiple functions of differentially expressed ITP/Ls derived from a single gene. Preliminary results using Gal4-driven RNAi in distinct peptidergic neurons look promising to find deficiency phenotypes.

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**A3.10****14:50 Tuesday 30th June 2009****FlyAtlas.org: a comprehensive gene expression database of *Drosophila melanogaster* tissues**

Venkateswara R. Chintapalli, Jing Wang, Pawel Herzyk, Julian Dow (University of Glasgow)

A vital link in functional genomics is finding where a novel gene is expressed, as this determines the phenotypes that are likely to be informative. Although it is in principle possible for an individual investigator to study expression of a gene comprehensively, it would make great sense to do this job once, properly, as a service to the community. Generated as part of the UK BBSRC's Investigating Gene Function initiative, the FlyAtlas online database (<http://flyatlas.org>) provides authoritative gene expression levels for 18500 transcripts initially for 11 adult *Drosophila* tissues and 2 larval tissues (see website for tissues), with comparison of their enrichments relative to whole fly. As the continuous development of the resource it has so far been extended to 5 adult and 5 larval tissues.

The meta-analysis of the data shows:

- Most adult tissues express 30–40% of the computed *Drosophila* genome.

- For each tissue, there are several hundred genes which are expressed in that tissue but not detectably anywhere else. So, to provide useful functional information for these genes, it is necessary to work on these tissues.

- It is possible to calculate the relative contribution of each tissue to the whole-fly array signal, and so form an "equation of the fly". The results show that whole-fly arrays are only capable of detecting large changes in widely expressed genes, and their use should thus be deprecated. Funded by BBSRC, UK.

Chintapalli, V.R., Wang, J. and Dow, J.A.T. (2007). *Nature Genetics* 39: 715–720.

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**A3.11****15:40 Tuesday 30th June 2009****What's new with intestinal transport processes in marine teleosts: osmoregulation?**

Martin Grosell (University of Miami), Josi R. Taylor (University of Miami), Edward M. Mager (University of Miami), Cameron Williams (University of Miami), Janet Genz (University of Miami), Steve F. Perry (University of Ottawa), Katie M. Gilmour (University of Ottawa)

Anion exchange contributes significantly to intestinal  $\text{Cl}^-$  absorption in marine teleost fish and is thus vital for successful osmoregulation. The  $\text{HCO}_3^-$  secreted into the intestinal fluid by this mechanism results in concentrations of up to ~100 mM and high pH, leading to the formation of  $\text{CaCO}_3$  precipitates. Recent advances in our understanding of the transport processes involved in intestinal anion exchange in marine and euryhaline teleost fish include the demonstration, via pharmacological tools, molecular biology and immunohistochemistry, of a role for the  $\text{H}^+$ -pump (V-ATPase) in apical  $\text{H}^+$  extrusion. In addition, the presence of an electrogenic ( $\text{nHCO}_3^-/\text{Cl}^-$ ) exchange protein (SLC26a6) has now been documented in both a euryhaline and marine teleost fish. The  $\text{H}^+$  pump defends against cellular acidification which might otherwise occur as a consequence of the high rates of base secretion fueled, in part, by endogenous epithelial  $\text{CO}_2$  hydration. In addition, apical  $\text{H}^+$  extrusion likely maintains  $\text{HCO}_3^-$  concentrations lower in the unstirred layer at the

apical surface than in the bulk luminal fluids and thus facilitates continued anion exchange. Furthermore,  $\text{H}^+$  pump activity hyperpolarizes the apical membrane potential which provides the driving force for apical electrogenic  $\text{nHCO}_3^-/\text{Cl}^-$  exchange to occur against both  $\text{Cl}^-$  and  $\text{HCO}_3^-$  gradients. We propose that similar coupling of apical  $\text{H}^+$  extrusion and  $\text{nHCO}_3^-/\text{Cl}^-$  exchange accounts for  $\text{Cl}^-$  uptake in freshwater fish and amphibians against very steep  $\text{Cl}^-$  gradients. Supported by NSF (0416440, 0714024, and 0743903).

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**A3.12****16:10 Tuesday 30th June 2009****Acid-base regulatory capacity and associated proton extrusion mechanisms in marine invertebrates: An overview**

Frank Melzner (IFM-EOMAR), Magdalena A. Gutowska (CAU Kiel), Marian Hu (IFM-EOMAR), Meike Stumpp (IFM-EOMAR)

Anthropogenic  $\text{CO}_2$  emissions will lead to an increased average ocean  $\text{pCO}_2$  of potentially 1900 ppm (ca. 0.2 kPa) by the year 2300 (Caldeira and Wickett 2003). Associated shifts in carbonate system speciation will cause ocean pH to fall by a maximum of 0.5–0.8 units. Most existing studies suggest that organisms/taxa with high standard metabolic rates, highly sophisticated convection systems and efficient gas exchange organs are coping best with elevated ocean  $\text{pCO}_2$ . Typically, these taxa (decapod crustaceans, and cephalopods) are able to rapidly compensate extracellular pH by accumulating large amounts of bicarbonate. The underlying molecular machinery for this accumulatory response is still largely unknown for most marine invertebrate taxa. Thus, we will briefly review (a) acid-base regulatory responses in a range of marine invertebrates (bivalves, echinoderms, crustacea, and cephalopoda) and then (b) highlight and discuss their main ion-regulatory organs and potential acid-base regulatory proteins. In addition, we will present results from ongoing gene expression studies that specifically target transcripts relevant for ion- and acid-base regulation in the gill of the cephalopod *Sepia officinalis*, the crustacean *Carcinus maenas* and pluteus larvae of the sea urchin *Strongylocentrotus purpuratus* in response to environmental hypercapnia.

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**A3.13****16:30 Tuesday 30th June 2009****Regulation of the paracellular path in the salmonid gill: Molecular and cellular aspects of claudin-10e and claudin-30 expression**

Christian K. Tipsmark (University of Southern Denmark), Steffen S. Madsen (University of Southern Denmark)

The paracellular permeability condition in fish gill epithelia is critical for establishing the appropriate conditions for uptake vs. secretion of sodium chloride in freshwater (FW) and seawater (SW) environments, respectively. While many fish are stenohaline and experience only FW or SW, euryhaline species are able to acclimate to either environment and thus present a very attractive model for examining molecular and cellular plasticity in the epithelia. In the current study the expression and localization of gill specific claudin-

10e and claudin-30 and the general tight junction protein occludin were examined in Atlantic salmon exposed to salinity changes. Specific antibodies and primers were developed to claudin-10e and claudin-30. While claudin-10e mRNA and protein expression is elevated strongly in response to SW, claudin-30 expression is depressed suggesting a particular significance in SW and FW, respectively. Data for alteration in abundance and localization during smoltification and after treatment with hormones will also be presented.

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### A3.14

17:00 Tuesday 30th June 2009

#### Osmolytes and osmoregulation in the euryhaline European eel, *Anguilla anguilla*

Svetlana Kalujnaia (University of St Andrews), Caroline J. Osborne (University of St Andrews), Gordon Cramb (University of St Andrews)

The life cycle of a euryhaline teleost such as the European eel (*Anguilla anguilla*) involves migration between freshwater (FW) and marine seawater (SW) environments with only minor changes in the osmolality of body fluids. A microarray technique used to screen for differential gene expression following transfer from FW to SW highlighted a group of genes related to the synthesis and transport of solutes known to act as osmolytes in mammalian cells. One gene involved in inositol production, is myo-inositol monophosphatase (IMPA1). QPCR, Western blotting and immunohistochemical analyses were used to compare the expression of IMPA1 in the gill and kidney as fish move between FW and SW environments. The results confirmed the initial microarray data where significant increases in expression were observed at 2 days and up to 5 months after transfer of eels to SW. Immunohistological studies have revealed that IMPA1 is expressed in most cells within the gill, but especially within chondrocytes found within the branchial arch and primary filaments. In the kidney, cells expressing IMPA1 were found mainly within clumps of hematopoietic tissue surrounding the tubules and glomeruli throughout the organ. In FW acclimated eels immunoreactivity predominated towards the periphery of the kidney and immunofluorescence were less abundant. No difference in the low levels of IMPA1 expression was found in the intestine, as related studies indicate that other osmolytes including taurine may have osmoprotective roles in this tissue. These studies have indicated a potential role for IMPA1 and the osmolyte inositol in salinity adaptation in the European eel.

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### A3.15

Poster Session – Tuesday 30th June 2009

#### Effect of dietary nucleotide on the osmoregulatory function of pyloric caeca in Caspian Sea salmon, *Salmo trutta caspius*

Sadegh Oulad (Tarbiat Modares University Marine Biology Group), Abdol Mohammad Abedian (Tarbiat Modares University), Saber Khodabandeh (Tarbiat Modares University Marine Biology Group)

The influence of food additive nucleotide on osmoregulatory function of pyloric caeca was studied in Caspian Sea salmon juveniles. This trial was achieved in two levels of dietary nucleotide (0.25% and 0.5% of additive nucleotide in meal-based diet) and a control group (0%). Juveniles fish ( $W=12$  g) were cultured for 8 weeks, and then, 12 samples ( $W=26$  g) from each treated group were transferred into saline water (18 ppt). Following 72 h acclimation, the samples were fixed for histological and immunohistochemical studies. Histology yields through light microscopy by using Hematoxyline–Fushin staining and immunolocalization of  $\text{Na}^+, \text{K}^+$ -ATPase was observed through fluorescent microscopy, using IgG $\alpha$ 5 (as primary antibody) and FITC (as secondary antibody). Results showed that, in all samples, immunofluorescence of  $\text{Na}^+, \text{K}^+$ -ATPase was observable in the baso-lateral parts of pyloric caeca enterocytes. In control group this fluorescence was weak compared to nucleotide received groups. A good immunostaining was found in enterocytes of two nucleotide received groups and between them the 0.5% group showed the maximum immunofluorescence of  $\text{Na}^+, \text{K}^+$ -ATPase. Alternation in  $\text{Na}^+, \text{K}^+$ -ATPase presence in pyloric caeca epithelium showed that this food additive material possesses an important function in adaptation of fish in 18 ppt salinity with enhancement of capability of osmoregulation.

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### A3.16

Poster Session – Tuesday 30th June 2009

#### Localization of a Rhesus-related ammonium transporter and gene expression patterns in gills of *Carcinus maenas* exposed to high environmental ammonia

Anne-Kathrin Blaesse (University of Osnabrueck), David W. Towle (MDIBL), Sue Edwards (Appalachian State University), Dirk Weihrauch (University of Manitoba)

Molecular studies have identified the expression of a Rhesus-related protein (RhCM) in gills of the green shore *Carcinus maenas* and since mammalian Rhesus proteins have been shown to mediate the transport of ammonia, it has been suggested that RhCM may participate also in the active ammonia excretion across the crab gill. To determine the localization of the Rhesus protein in the gills of *C. maenas* we employed an antibody which was raised against the C-terminus of RhCM. Immunolocalization studies showed dot-like apical signals in the gill lamellae of posterior gills of the crab. Double staining using the anti-RhCM antibody and an anti- $\text{Na}^+, \text{K}^+$ -ATPase antibody showed the expected basolateral localization of the  $\text{Na}^+, \text{K}^+$ -ATPase and an apical signal of the anti-RhCM antibody. RhCM is thus poised to be involved in ammonium transport across the apical membrane of gill epithelial cells. To investigate whether gene expression of RhCM and other transport proteins might respond to elevated environmental ammonia concentrations, we exposed hyper-regulating brackish water acclimated crabs to  $1 \text{ mmol} \cdot \text{l}^{-1} \text{ NH}_4 \text{Cl}$  for two h to 14 days and investigated gene expression changes using a newly developed 4462-feature oligonucleotide microarray. The microarray assays revealed 2.0- to 2.8-fold down-regulation of RhCM over the entire time of ammonia exposure compared to control animals. In contrast, transcript levels for an aquaporin and a  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$ -channel showed a 3.5- to 4.6-fold and 6.5- to 14-fold up-regulation, respectively.

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**A3.17****Poster Session – Tuesday 30th June 2009****Genetics of cell: Cell junctions in insect renal tubules**

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The integrity and controlled permeability of cell junctions are essential for epithelial function and for the functioning of the blood–brain barrier. The septate junction is the insect equivalent of the mammalian tight junction, and takes two forms; the common pleated type, and the rarer smooth septate junction. Using microarray data from flyatlas.org, we have compiled a list of known junctional genes that are expressed in *Drosophila* Malpighian (renal) tubules, and screened RNAi lines (where available) for changes in resting or stimulated fluid secretion. Interestingly, several genes show a common RNAi phenotype, of a high basal secretion rate that cannot be further elevated by treatment with neurohormones. In parallel work, we are attempting a proteomic approach to identify novel proteins associated with the smooth septate junctions of Malpighian tubules. This will provide a broader understanding of junctional function in invertebrates.

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**A3.18****Poster Session – Tuesday 30th June 2009****Luminal and contraluminal nutrients and their influence on transepithelial voltage and strong alkalization in the anterior midgut of larval yellow fever mosquitoes (*Aedes aegypti*)**

Horst Onken (Wagner College), Sejmir Izeirovski (Wagner College), Stacia B. Moffett (Washington State University), David F. Moffett (Washington State University)

Anterior midguts of larval *Aedes aegypti* were isolated, bathed in aerated mosquito saline containing serotonin (0.2 μM), and perfused with NaCl (100 mM). The lumen negative transepithelial voltage ( $V_{te}$ ) was measured and luminal alkalization was determined through the color change of luminal m-cresol purple from yellow to purple after luminal perfusion stops. Luminal addition of 10 mM amino acids (arginine, glutamine, histidine or proline) or dicarboxylic acids (malate or succinate) resulted in more negative  $V_{te}$  values, whereas luminal addition of glucose was without effect. In the presence of TRIS chloride as luminal perfusate, addition of amino acids or dicarboxylic acids did not change  $V_{te}$ . These results are consistent with Na<sup>+</sup>-dependent absorption of amino acids and dicarboxylic acids. Effects of serotonin withdrawal indicated that nutrient absorption is stimulated by this hormone. Strong luminal alkalization was observed with mosquito saline containing serotonin on the hemolymph-side and 100 mM NaCl in the lumen, indicating that alkalization does not depend on luminal nutrients. Omission of glucose or dicarboxylic acids from the hemolymph-side solution had no effect on luminal alkalization, whereas omission of amino acids significantly decelerated it. Re-addition of amino acids restored alkalization. These results indicate that amino acid catabolism is a major source of energy for the cells and/or that it provides base equivalents necessary for luminal alkalization. Funded by the NIH (RO1AI063463).

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**A3.19****Poster Session – Tuesday 30th June 2009****Cellular mechanisms of acid secretion in the posterior midgut of larval mosquito (*Aedes aegypti*)**

Urmila Jagadeshwaran (Washington State University), Mathew Hardy (Washington State University), Horst Onken (Wagner College NY), David F. Moffett (Washington State University), Stacia B. Moffett (Washington State University)

The posterior midgut was studied for the cellular mechanisms of the reacidification, using an isolated, perfused gut preparation. We measured the transepithelial voltage ( $V_{te}$ ) and the rate of acid secretion as indicated by the color change of m-cresol purple during intervals of perfusion stop. The lumen-positive voltage and reacidification were stimulated in the presence of serotonin (0.2 μmol l<sup>-1</sup>). The V-type H<sup>+</sup> ATPase inhibitor concanamycin A, inhibited acidification and decreased  $V_{te}$ . The carbonic anhydrase inhibitor, acetazolamide, on hemolymph-side almost abolished  $V_{te}$ , but had no effect on acidification. Similarly DIDS, the inhibitor of anion transporters, Ouabain, an inhibitor of Na<sup>+</sup>-K<sup>+</sup> ATPase, and Ba<sup>2+</sup> ion, an inhibitor of K<sup>+</sup> channels, all decreased  $V_{te}$  but did not slow or inhibit acid secretion. Luminal- and hemolymph-side DPC, also an inhibitor of anion transporters, reduced  $V_{te}$ . Unilateral substitution of gluconate for Cl<sup>-</sup> affected  $V_{te}$  in a way consistent with a greater permeability for Cl<sup>-</sup> versus Na<sup>+</sup>. Cl<sup>-</sup> substitution in the lumen decreased  $V_{te}$  but when applied on both side of the tissue simultaneously,  $V_{te}$  returned to the control values. Hemolymph- and luminal-side Na<sup>+</sup> substitution or addition of the Na<sup>+</sup>/H<sup>+</sup> exchange inhibitor, amiloride, reduced  $V_{te}$  but not luminal acid secretion. Amino acid removal on the luminal side resulted in significant decrease in  $V_{te}$  but not acidification. These results indicate that changes in voltage and reacidification are independent processes. Acidification requires only the apical proton pump and Cl<sup>-</sup> selective paracellular pathway. However the chemical source of secreted H<sup>+</sup> and the fate of the resulting co-ions are still unknown.

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**A3.21****Poster Session – Tuesday 30th June 2009****Impact of short-term primary culture on RVD process in turbot, *Scophthalmus maximus*, hepatocytes**

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As most animal cells, turbot hepatocytes exposed to a hypo-osmotic challenge have the ability to regulate their volume. In previous studies, we have shown that freshly isolated turbot hepatocytes constitute a practical tool for investigation of Regulatory Volume Decrease (RVD) process. To extent our investigation field, primary culture of turbot hepatocytes would constitute an appropriate alternative. However, no information exists about the impact of primary culture on cell volume regulation process in this cell type. Consequently, this work aims to

study the impact of a short-term primary culture on RVD process in turbot hepatocytes. Our results show that no significant decrease in cell viability was observed during the 4 days of primary culture. However, cell volume regulation ability seems to be altered by the culture conditions since volume regulation capacity decreased with passing days. In the same way, significant changes were observed on cell hypo-osmotic ATP release ability: from the first day of culture, ATP release was strongly increased compared with that measured at day 0. At the opposite, time of cell culture has no effect on hypo-osmotic induced exocytic activity. In conclusion, our results demonstrated that short-term primary culture of turbot hepatocytes can serve as a system for the investigation of cell volume regulation process if cautiously used. Despite the fact that culture conditions could induce alterations of some mechanisms involved in RVD, turbot hepatocytes maintain a significant ability to perform the regulatory process during at least 4 days of culture.

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### A3.22

**Poster Session – Tuesday 30th June 2009**

#### **Taking a rosy look at the *Drosophila* metabolome by mass spectrometry**

Yahya H. Hobani (University of Glasgow), Anas Kamleh (Strathclyde University), David G. Watson (Strathclyde University), Julian A. Dow (University of Glasgow)

We have established the utility of metabolomic profiling in the study of *Drosophila* mutants, using the classical eye colour mutant, rosy (a mutation in xanthine oxidase), as an example. We used a novel method with the Orbitrap mass spectrometer, coupled to a liquid chromatographic separation, that is capable of mass accuracy better than 1 ppm, and which thus allows unambiguous resolution of many molecular structures from molecular masses alone. A huge number of metabolites were identifiable. The technique was able to identify not only changes previously reported in rosy flies (increase in xanthine and hypoxanthine, and decrease in urate and allantoin), but completely unexpected impacts osmolyte biosyntheses, arginine metabolism and pyrimidine metabolism. Rosy is an example of a molybdoenzyme, and we were able to show that mutations in maroon-like, an enzyme in the pathway for synthesis of the molybdopterin cofactor required by all molybdoenzymes, both replicates the rosy metabolomic fingerprint and extends it, reflecting an additional impact on enzymes such as aldehyde oxidase. Orbitrap-based metabolomics has thus the potential to revolutionise reverse

genetic analysis of mutations in both novel and well-known genes, and to the generation of new hypotheses for experimental testing.

Kamleh, M.A., Hobani, Y., Dow, J.A. and Watson, D.G. (2008). Metabolomic profiling of *Drosophila* using liquid chromatography Fourier transform mass spectrometry. *FEBS Letters* 582, 2916–22.

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### A3.23

**Poster Session – Tuesday 30th June 2009**

#### **Localisation of ion transporters in the colon of the Australian common brushtail possum**

Ray Bartolo (University of Otago), Mike Gill (University of Otago), Bernie McLeod (AgResearch), Grant Butt (University of Otago),

The epithelial cells lining the eutherian colon function to transport water and ions in secretion and absorption. Electrogenic and electro-neutral absorption of ions and water is the primary function of the colon, but secretion of ions is also a fundamental characteristic, and predominates over absorption during secretory diarrhoea. Electrogenic  $\text{Cl}^-$  secretion is stimulated by secretagogues that elicit an increase in intracellular cAMP, cGMP and  $\text{Ca}^{2+}$  in the eutherian colon, mediated via the dual actions of the basolateral Na-K-2Cl (NKCC1) cotransporter, and the apical cystic fibrosis conductance regulator (CFTR). Previous research in our laboratory has shown that various secretagogues do not stimulate an electrogenic secretory response in the colon of the Australian common brushtail possum, which is a metatherian mammal. While, in the small intestine of the possum, we have shown that electrogenic secretion does occur, and is due to high expression of a  $\text{Na}^+/\text{HCO}_3^-$  transporter (NBC) on the basolateral membrane. The lack of an electrogenic secretory response in the possum colon suggests that the transporters required are not expressed. Using western blots and immunohistochemistry, we were able to show that CFTR, NKCC1 and NBC are indeed expressed in the possum colon, but NBC and NKCC1 are not co-localised with CFTR. Thus, the colonic epithelial cells that express CFTR, do not appear to have a mechanism to accumulate  $\text{Cl}^-$  or  $\text{HCO}_3^-$ , that can then be secreted via CFTR. Our results show a major difference in the colonic transport properties of a metatherian mammal compared to that of eutherian mammals.

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