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The First World Congress on Water Channel Proteins (Aquaporins and Relatives) Celebrating the 25th Anniversary of the Discovery of the First Water Channel Protein (Later Called Aquaporin 1), Cluj-Napoca, Romania, October 27-29, 2011

PROGRAM

Thursday October 27, 2011

8:00 – 19:00 : Registration (Foyer of "Gheorghe Dima" Music Academy, Street Ion I.C. Brătianu no. 25, Cluj-Napoca)

All SESSIONS will take place in Sala Studio, "Gheorghe Dima" Music Academy (Street Ion I.C. Brătianu no. 25, Cluj-Napoca)

10:00 -11:00: Opening Ceremony

SESSION 1

Chairs: Philip W. KUCHEL (Singapore);
Vasile V. MORARIU (Cluj-Napoca, Romania);
Octavian POPESCU (Cluj-Napoca, Romania)

11:00 – 11:45: Inaugural Plenary Lecture:
Key-note speaker: Gheorghe BENGA (Cluj-Napoca, Romania): Twenty five years since the discovery in Cluj-Napoca, Romania, of the first water channel protein (later called aquaporin 1)

11:45 -12:30: Plenary Lecture:
Key-note speaker: Philip W. KUCHEL (Singapore): Water exchange in red blood cells: historical comment and NMR studies

12:30 – 14:30: Lunch Break

SESSION 2

Chairs: Mihai COCULESCU (Bucharest, Romania);
Ross P. HOLMES (Winston-Salem, USA);
Andrea YOOL (Adelaide, Australia)

14:30 -15:15: Plenary Lecture:

Key-note speaker: Andrea J. YOOL, Ewan M. CAMPBELL **(Adelaide, Australia): Resolving a controversy: molecular gating of aquaporin-1 as a dual water and ion channel**

15:15 -15:45: Plenary Lecture:

Olivia L. BURTA, Diana C. PELEA, Ovidiu P. BURTA **(Oradea, Romania): Hemolytic disease of the newborn marked by aquaporins**

15:45 -16:00: Alina TEHANIUC, Gheorghe BENGA **(Suceava & Cluj-Napoca, Romania): Red blood cell water permeability in elderly people and in patients with cardiovascular diseases**

16:00 – 16:30: Coffee Break

SESSION 3

Chairs: Angela BORDA (Tg. Mures, Romania)
Olivia L. BURTA (Oradea, Romania)
Mihai CRUCE (Craiova, Romania);
Victor I. POP (Cluj-Napoca, Romania);

16:30 – 17:00: Plenary Lecture:

Key-note speaker: Ross HOLMES (Winston-Salem, USA): Water channel proteins in the kidney in health and disease

17:00 – 17:15: Mihai COCULESCU, Corin BADIU, S. RADIAN **(Bucharest, Romania): Familial hypothalamic diabetes insipidus by Phe53Ser mutation, in a decade**

17:15 – 17:45: Gheorghe BUMBU, **Olivia L. BURTA,** Adrian G. BUMBU **(Oradea, Romania): Cryotherapy-induced expression of water channel proteins in prostate cancer**

17:45 - 18:00: George C. PRIBAC, Simona DAMIAN, Aurelia COVACI, Constatin CRĂCIUN, Corina ROSIORU, Constantin PUICĂ, Endre MATHE, Maria CZAPAR, Liana MOS, Coralia COTORACI, Aurel ARDELEAN: **Ultrastructural studies on the effect of seeds of *Trigonella graecum* on alcoholized rats kidney**

18:00 - 18:15: Plenary Lecture:

Key-note speaker: Helga STAN-LOTTER (Salzburg, Austria): Water channel proteins in Archaea

18:15- 18:25: Animation: Horatiu I. BURTA, Bogdan LEAHU, Olivia L. BURTA **(Oradea, Romania): An "inside view" of the ADH renal mechanism of action and AQP 2 synthesis**

19:00 – 20:00: RECITAL presented by The "Gheorghe Dima" Music Academy for participants in the Congress and invited persons

AUREL MARC (Oboe) OCTAVIA MARC (Piano)

Johann Sebastian Bach: "Jesu, joy of Man's Desiring" from Cantata BWV 147

Johann Wenzel Kaliwoda: "Morceau de salon" op. 228 for oboe and piano

Sigismund Toduță: "...de băsmuit" from the Six pieces for solo oboe cycle

Amilcare Ponchielli: "Capricio" op. 80

20:30 – 22:30: Welcome Reception (in the Restaurant of The Academic College, "Babes-Bolyai" University Cluj-Napoca)

Friday October 28, 2011

8:00 – 19:00 : Registration (Foyer of "Gheorghe Dima" Music Academy)

SESSION 4

Chairs: Karina ALLEVA (Buenos Aires, Argentina)
Aurel ARDELEAN (Arad, Romania)
Christophe MAUREL (Montpellier, France)
Francisc MIXICH (Craiova, Romania)

9:30 – 10:15: Plenary Lecture:
Key-note speaker: Christophe MAUREL, Véronique SANTONI, Doan – Trung LUU, Lionel VERDOUCQ, Colette TOURNAIRE-ROUX, Moira SUTKA, Guowei LI,
Yann BOURSIAC, Michael WUDICK, Olivier POSTAIRE **(Montpellier, France)** :
Aquaporins and the responses of plants to their challenging environment
10:15 – 10:45: Gerd P. BIENERT, Manuela D. BIENERT, François CHAUMONT **(Louvain–la–Neuve, Belgium): Molecular and functional characterisation of a recently identified class of plant aquaporins: the X intrinsic proteins**
10:45 – 11:00: Adrien CHEVALIER, Gerd P. BIENERT, François CHAUMONT **(Louvain–la–Neuve, Belgium) : Role of transmembrane domain 3 in the trafficking of plant aquaporins to the plasma membrane**

11:00 – 11:30: Coffee break

SESSION 5

Chairs: Gerd P. BIENERT (Louvain la Neuve, Belgium);
Mihaela CORNEANU (Timisoara, Romania)
Coralia COTORACI (Arad, Romania);
Constantin CRĂCIUN (Cluj-Napoca, Romania)

11:30 – 11:45: Cintia JOZEFKOWICZ, Pablo ROSI, Gabriela AMODEO**, Karina ALLEVA (Buenos Aires, Argentina): Interaction PIP1-PIP2: involvement of loop A?**

11:45 – 12:00: Mercedes MARQUEZ, **Karina ALLEVA,** Gabriela AMODEO **(Buenos Aires, Argentina): PIP aquaporins and ripening in strawberry**

12:00 – 12:15: Dorin D. CAMEN, Carmen G. BEINSAN, Laura BIGYILAN, Radu L. SUMALAN **(Timisoara, Romania): Research regarding physiological responses of Banat's common bean landraces (*Phaseolus vulgaris* L.) seedlings to hydric stress**

12:15 – 12:30: Ioan SARAC, Gallia BUTNARU **(Timisoara, Romania): Water quality evaluation using citological methods**

12:30 – 14:30: Lunch Break

SESSION 6

Chairs: Tsutomu NAKADA (Niigata, Japan);
Hartwig WOLBURG (Tübingen, Germany);
Masato YASUI (Tokyo, Japan);

14:30 - 15:15: Key-note speaker :Tsutomu NAKADA (Niigata, Japan): Brain science of water molecules: a salute to professor Linus Carl Pauling

15:15 – 15:45: Key-note speaker : Hartwig WOLBURG, Susan NOELL, Petra FALLIER-BECKER, Andreas F. MACK, Karen WOLBURG-BUCHHOLZ **(Tübingen, Germany): The management of the blood-brain barrier in health and disease**

15:45– 16:15: Key-note speaker : Masato YASUI, Hiroko IKESHIMA, Yoshinori YUKUTAKE, Jungo KATO, Kimiko TASUMI **(Tokyo, Japan): Roles of aquaporin-4 in brain disorders**

16:15– 17:00: Key-note speaker: Vincent J. HUBER (Niigata, Japan): Development of biologically relevant aquaporin 4 ligands

17:00–17:30: Coffee Break

SESSION 7

Chairs: Vincent J. HUBER (Niigata, Japan) ;
Norikazu MAEDA (Osaka, Japan);
Liana MOS (Arad, Romania);
Corneliu TARBA (Cluj-Napoca, Romania)

17:30 – 18:00: Key-note speaker: Norikazu MAEDA (Osaka, Japan): Metabolic impact of water channel proteins

18:00 – 18:30: Sabina JELEN, Sören WACKER, Camilo APONTE-SANTAMARIA, Martin SKOTT, Aleksandra ROJEK, Urban JOHANSON, Per KJELLBOM, **Søren NIELSEN**, Bert de GROOT, **Michael RÜTZLER (Aarhus, Denmark) : Aquaporin-9 is the primary route of hepatocyte glycerol uptake for glycerol gluconeogenesis**

18:30 – 18:45 Corneliu TARBA (Cluj-Napoca, Romania) : Water channels in mitochondria

18:45 – 19:00: Gabriel CORNEANU, Mihaela CORNEANU: Carbon nanotubes as water channel

CLOSING SESSION

Chairs: Gabriel CORNEANU (Arad & Timisoara, Romania) ;
Zoltan PAVAI (Tg. Mures, Romania);
Helga STAN-LOTTER (Salzburg, Austria)

19:00 – 19:15: Gheorghe BENGA (Cluj-Napoca, Romania): The OUTNOBEL Foundation

19:15 – 19:30: Constantin CRĂCIUN (Cluj-Napoca, Romania): Impressions from attending in November 2003 in Detroit, USA, the first "George Emil Palade" Lecture and award of the "George Emil Palade" Gold Medal to Günter Blobel (1999 Nobel Laureate in physiology or medicine)

19:30: Closing Ceremony
Including Presentation of the Award of The "Octavian Fodor" Foundation for the best presentation of a young researcher

20:30: Gala Dinner

Saturday October 29, 2011

9:00 – 19:00: Tour of Cluj-Napoca

ABSTRACTS OF ORAL PRESENTATIONS

(IN ALPHABETICAL ORDER OF THE CORRESPONDING AUTHOR)

INTERACTION PIP1-PIP2: INVOLVEMENT OF LOOP A?

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Introduction: The *Beta vulgaris* plasma membrane (PM) expresses aquaporins that as far as from now have shown to have apparent different transport activity in Xenopus oocytes: *Bv*PIP1;1, *Bv*PIP2;1 and *Bv*PIP2;2. Both *Bv*PIP1;1 and *Bv*PIP2;1 gives low oocyte PM osmotic water permeability (P_f) while *Bv*PIP2;2 gives high P_f. In the literature, the present accepted paradigm is that PIP1 aquaporins have low water transport capacity in comparison with PIP2, which show high water transport; many reports as well show that PIP1-PIP2 co-expression present much higher P_f than the expected for PIP2 alone. This aspect of PIP1 functionality and/or trafficking is still under investigation, and the purpose of our work points to elucidate red beet PIPs behavior considering the above-mentioned framework.

Materials and Methods. We assayed *Bv*PIP and mutants functionality by means of heterologous expression in Xenopus oocytes. P_f was calculated after measuring the rate of oocyte swelling induced by transferring oocytes to ND96 diluted five-fold.

Results and Discussion: In order to test if the low P_f detected for oocytes injected with *Bv*PIP1;1 or *Bv*PIP2;1 was a consequence of cRNA degradation, we synthetized both cRNA with a polyA tailing and ARCA capping. Under these conditions P_f of oocyte PM expressing *Bv*PIP2;1 was compatible with water transport through active aquaporins; no changes where found for *Bv*PIP1;1. PIP1-PIP2 coexpression experiments show that while *Bv*PIP2;2 was able to interact with *Bv*PIP1;1, *Bv*PIP2;1 shows no functional interaction. *Bv*PIP2;1 amino acid sequence showed differences in the loopA in comparison with the same loop of most PIP2. In order to investigate the influence of this motif in PIP1-PIP2 interaction we tested two mutants. Functional studies showed that both are able to transport water and, interestingly, preliminary results indicates these two mutated channels would affect P_f when co-expressed with *Bv*PIP1;1 in oocytes.

Conclusion: *Bv*PIP2;1 is an active aquaporin with limitations to functionally interact with *Bv*PIP1;1. Mutations in *Bv*PIP2;1 loop A reverse this situation suggesting the involvement of this loop in the PIP2-PIP1 interaction.

Keywords: Plant aquaporins, PIP interaction.

PIP AQUAPORINS AND RIPENING IN STRAWBERRY

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Introduction: In plants, aquaporins known as PIPs are usually expressed at the level of the first membrane barrier: the plasmalemma; however their involvement or association in specific physiological processes is not clear. In previous work, we characterized two strawberry fruit-specific aquaporins,FaPIP1;1 and FaPIP2;1 showing its expression profile associated with ripening (Alleva et al., 2010). Both PIPs have also been studied in terms of their water permeability (P_f). In this work we analyze water-related parameters at the fruit level, PIP1/PIP2 expression profiles at different ripening stages and the pH regulation of both water channels in order to gain insight into role of the water transport components in this framework.

Materials and Methods: Water-related parameters at the fruit level such as relative water content and growth rate in a daily pattern measurement, where analyzed for six different ripening stages in greenhouse grown strawberry plants -large green (LG), white (W), 25%, 50%,75%,and 100% red-FaPIP aquaporin water transport activity was studied by means of cRNA injection in an heterologous system. Xenopus oocytes were injected with cRNA of FaPIP1 and/or FaPIP2. P_f was determined by measuring the rate of oocyte swelling induced by a hypo-osmotic shock (Zhang and Verkman, 1991). Oocyte internal (cytosolic) pH was modified pre-incubation for 15 min in different solutions (pH=5.8 - 7.6).

Results and Discussion: At the fruit level: To reach 100%R, strawberry ripening process involves a daily course distribution mainly dominated by the LG and W stages, 41% and 22% of the days respectively. Growth rate duplicates from LG to W and later decrease constantly. Also, the larger variation in relative water content is observed from LG to W stage. Interestingly, the aquaporin profile reflects changes inbetween LG to W (both FaPIP increasing) and 100% red (FaPIP1 high, FaPIP2 low). At the molecular level: while there is a relationship between the quantity of FaPIP2 cRNA injected in the oocytes with water permeability, the co-expression of different ratios of FaPIP2/FaPIP1 cRNA do not impact in P_f variations. Regarding pH regulation, EC50 for FaPIP2;1 is 6.0, while FaPIP1;1-FaPIP2;1 is 6.4, showing a different sensing of pH by PIP co-expression. EC50 of the dose-response curves for oocytes co-expressing FaPIP1/FaPIP2 in different ratios is constant, accompanying P_f results.

Conclusion: Awareness of transcelular water movements during ripening due to outbreak of aquaporin learning is discussed. Water relations at a fruit level are complex, and aquaporin role in ripening is still unknown, our results contribute to organize some pieces of the process both at whole fruit and at molecular level.

Keywords: plant aquaporins, fruit ripenin

TWENTY FIVE YEARS SINCE THE DISCOVERY IN CLUJ-NAPOCA, ROMANIA, OF THE FIRST WATER CHANNEL PROTEIN (LATER CALLED AQUAPORIN 1)

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Water channels or water channel proteins (WCPs) are transmembrane proteins that have a specific three-dimensional structure with a pore that can be permeated by water molecules. WCPs are a large family (over 450 members) that are present in all kingdoms of life. The first WCP was discovered in the human red blood cell membrane in 1985 in Cluj-Napoca, Romania, by the group of Benga, reported in two landmark papers (Benga Gh., Popescu O., Pop V.I., Holmes R.P. p-Chloromercuribenzene-sulfonate binding by membrane proteins and the inhibition of water transport in human erythrocytes. *Biochemistry.* **25**, 1535-1538, 1986; Benga, Gh., Popescu, O., Borza, Victoria, Pop, V.I., Mureşan, A., Mocsy, I., Brain, A., and Wrigglesworth, J. Water permeability of human erythrocytes. Identification of membrane proteins involved in water transport. *Eur. J. Cell. Biol.* **41**, 252-262, 1986).

In 1990's other WCPs were discovered in plants, microorganisms, various animals and humans and it became obvious that the WCPs belong to the superfamily of major intrinsic proteins (MIPs, over 800 members). WCPs include three subfamilies: a) aquaporins (AQPs), which are water specific (or selective water channels); b) aquaglyceroporins (and glycerol facilitators), which are permeable to water and/or other small molecules; c) "superaquaporins" or subcellular AQPs. The physiological roles of WCPs in kidney, gastrointestinal system, respiratory apparatus, central nervous system, eye, adipose tissue, skin have been extensively studied and important implications of WCPs in various diseases have been found. Benga's group has the world priority in describing the involvement of WCPs in epilepsy (Benga Gh., Morariu V. V., Membrane defect affecting water permeability in human epilepsy. *Nature* **265**, 636-638, 1977) and Duchenne muscular dystrophy (Şerbu A.-M., Marian A., Popescu O., Pop V. I., Borza V., Benga I. and Benga Gh., Decreased water permeability of erythrocyte membranes in patients with Duchenne muscular dystrophy. *Muscle&Nerve* **9**, 243-247, 1986; Benga Gh., Popescu O., Pop V. I., Borza V., Hodârnău A., Popescu M., Şerbu A.-M. and Benga I., Studies on water permeability and protein erythrocyte membranes in patients with Duchenne muscular dystrophy. *Muscle&Nerve* **12**, 294-301, 1989), reviewed by Benga Ileana (Priorities in the discovery of the implications of water channels in epilepsy and Duchenne muscular dystrophy. *Cell. Mol. Biol.* **52**, 46-50, 2005).

The whole WCP field became in the last 20 years a very hot area of research in biochemistry and molecular cell biology, with wide and increasing implications in

laboratory medicine. An important direction of research is represented by studies leading to new methods for diagnosis and therapy of diseases in veterinary and human medicine.

MOLECULAR AND FUNCTIONAL CHARACTERISATION OF A RECENTLY IDENTIFIED CLASS OF PLANT AQUAPORINS: THE X INTRINSIC PROTEINS

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Introduction. In plants, the movement of water and small neutral solutes across diverse membranes is reliant on the aquaporin (AQP) protein family. AQPs fulfil crucial roles in a variety of physiologically important processes, like water conductance, gas and nutrient uptake and translocation, metalloid homeostasis and signal transduction. Recently, a novel, phylogenetically distinct AQP subfamily, the X intrinsic proteins (XIPs) has been discovered *in silico* in the genomes of moss and a wide variety of eudicots but not in Arabidopsis and monocots. Surprisingly, XIPs were also identified in several fungi and a protozoan species and are therefore, in contrast to all other plant AQP subfamilies, not plant specific. Being just discovered, little is known about XIPs from the different organisms.

Materials and Methods. Various XIPs from plants and fungi were cloned and analyzed on the genomic, transcriptional and phylogenetic level. Transgenic *NtXIP1;1*-promoter-*GUS* and YFP protein-tagged NtXIP1;1α and NtXIP1;1β tobacco plants were generated and analyzed by (confocal-) microscopy. Transport, complementation and survival assays with the heterologously expressed XIPs in *Xenopus laevis* oocytes and various *Saccharomyces cerevisiae* yeast mutants were performed.

Results and Discussion. *XIP* cDNA and gDNA were cloned from tobacco, potato, tomato, and morning glory. A conserved sequence motif in the first intron of *Solanaceae XIPs* initiates an RNA-processing mechanism that results in two splice variants (α and β). When transiently or stably expressed in tobacco plants, YFP protein-tagged NtXIP1;1α and NtXIP1;1β were both localized in the plasma membrane. Transgenic tobacco lines expressing *NtXIP1;1*-promoter-*GUS* constructs and RT-PCR studies showed that *NtXIP1;1* was expressed in all organs. The *NtXIP1;1* promoter was mainly active in cell layers facing the environment in all above-ground tissues. Using an XIP antibody and splice-variant-specific qPCR primers, we are currently generating a detailed expression map. Heterologous expression of XIPs demonstrated that these isoforms facilitate the transport of bulky solutes, such as glycerol, urea, and boric acid. In contrast, permeability for small solutes, such as ammonia and water, was very low or undetectable. The important role of conserved amino acid residues in XIPs in the substrate specificity was demonstrated. Interestingly, *Fusarium oxysporum*, a fungal pathogen infecting the *Solanaceae* species, is also encoding for a XIP gene, which we are characterizing at the moment.

Conclusions. Our data suggest that XIPs function in the transport of uncharged solutes across the cell plasma membrane in specific plant tissues, including at the interface between the environment and external cell layers.

Keywords: X intrinsic protein, solute transport, substrate selectivity, alternative splicing

CRYOTHERAPY-INDUCED EXPRESSION OF WATER CHANNEL PROTEINS IN PROSTATE CANCER

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Introduction. Prostate cancer is recognized as one of the most common non-dermatological male cancers wide world, being placed among the first 3 causes of cancer death. Among applied therapy, radical prostatectomy and external beam radiotherapy remain the main procedures used for prostate cancer with reasonable results. Another option is prostate cryotherapy, but limited to certain types of prostate cancer: localized or locally advanced, when was confirmed to be a primary and salvage treatment. Cryotherapy is considered a minimally invasive procedure, by applying locally a freezing temperature resulting *in situ* tissue ablation, in a selective destroying manner of affected tissue with preservation of vital structures around the prostate, such as urinary bladder and rectum. Aquaporins are cellular membrane constituents that control the permeability of endothelial and epithelial barriers and function as selective pores allowing water, glycerol and other small solutes to pass through the cell membrane, due to these effects, being actively involved in cryotherapy outcomes.

Discussions. The cryotherapy is based on "cellular cold injury" which occurs when temperature falls below 0^0C, when the temperature ranges between −7 and −20°C starts to be formed extracellular ice, and when the values are lower than −40°C will induce a complete eradication of the affected tissue. Ice formation will create a hyperosmolar extracellular environment and expose cells to osmotic stress. Lower temperatures (<−15°C) are associated with intracellular ice formation, which is almost always lethal to the cells. Many studies (immunohistochemistry, RT-PCR, Western blot) proved that cryotherapy induce an increased expression of *AQP3* in prostate cancer cells: in the immediate post-freeze period there are 5- and 50-fold increases in AQP3 expression in DU145 and PC-3 cells, respectively, followed by a regression to the untreated level. This response can be interpreted as an important adaptive mechanism used by the cells to face the osmotic stress associated with cryoinjury. Researches are focused on finding inhibitors of AQPs, such as mercuric chloride, which has been used routinely to test *AQP* function in animal and plants cells.

Conclusions. Inhibiting the AQPs, the main responsible for controlling water transport across the plasma membrane, will induce an intracellular water retention with subsequent increase of critical temperature at which intracellular ice will form, thus *AQP* inhibition

by pharmacological blockers might represent a future therapeutic approach for making more sensitive the human prostate cancer cells to cryotherapy.

Keywords: Aquaporins, prostate cancer, cryotherapy

HEMOLYTIC DISEASE OF THE NEWBORN MARKED BY AQUAPORINS

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Introduction. The paper aims to emphasize the involvement of blood-groups active molecules in human red blood cells function in immune diseases; this is based on the fact that certain antigenic structure, part of different erythrocyte systems are carried on by highly expressed protein molecules (firstly described by Professor G. Benga) all together members of the *AQP* family, some high selective for the passage of water across the plasma membrane, and aquaglyceroporins which allow the transport of small non-ionic molecules.

Discussions. The first aquaporin, originally called CHIP28, was accidentally discovered during an investigation of Rh system, one of most complex erythrocyte system recognized as the second in importance (after ABO system) in the field of transfusion medicine, but in the obstetric field represents the primary importance, being the main cause of hemolytic disease of the newborn/HDN. The relation between rhesus haemolytic disease and alloimmunisation was recognized in 1953. Another blood group system "Colton" involved in immuno-hematology is composed of three antigens: Co(a), Co(b), and Co3; Colton antigens were found to be located on the channel forming integral protein (CHIP-1) being a product of the AQPI (*Aquaporin-1*). The CHIP-1 protein is located on different surfaces, such as: epithelia, endothelium, descending tubules and apical surfaces of proximal tubules in addition to red blood cells. When extremely rare in Colton system the Co3 antigen lacks, the individuals are identified as Colton "null" phenotype, referred as Co(a-b-), missing actually the water channel AQP1 that carries the Colton antigens therefore the cells have reduced water permeability. In Colton "null" phenotype, the individuals can develop severe reactions in certain circumstances (as pregnancy: incompatibility in Colton system: mother - foetus) such as hydrops fetalis, or mild/moderate posttransfusion hemolysis (incompatibility in Colton system: donor-recipient) based on development of antiCo(a) antibodies (allo-antibodies).

Conclusion. Proper monitoring during pregnancy, antenatal and post-natal is important to avoid the HDN. The management is differentiated according to the moment: "in utero" as soon as the blood samples confirm anaemia, transfusion should be performed with group "O" negative packed cells cross-matched with maternal blood; future therapy should involve selective modulation of the maternal immune system, making the need for intrauterine transfusions a rarity; and "after delivery" phototherapy, transfusion. Is

mentioned that individuals Co(a-b-) do not appear to have any health problems related to the missing of CHIP-1.

Keywords: Aquaporins, Colton antigens, allo-antibodies

AN INSIDE VIEW OF THE ADH RENAL MECHANISM OF ACTION AND AQP2 SYNTHESIS

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Introduction: It is the only aquaporin regulated by vasopressin. The basic job of aquaporin 2 is to reabsorb water from the urine while its being removed from the blood by the kidney.
Arginine vasopressin (AVP), also known as vasopressin, or antidiuretic hormone (ADH), is a neurohypophysial hormone found in most mammals, including humans. Vasopressin is a peptide hormone that controls the reabsorption of molecules in the tubules of the kidneys by affecting the tissue's permeability, like any molecule in the human body,

Material and method. The ADH has a spectacular dynamics, that is why we tried to approach this phenomenon in a different manner, in an innovative approach aided by a fascinating journey through a world seen only by the lenses of the optic and electronic microscopes. This journey was developed by us, in Maxon Cinema 4d and Adobe After Effects.

Discussions. The purpose is a better understanding of the ADH mechanism of action at the renal level on V2 receptors which are G protein-coupled receptors on the basolateral plasma membrane of the epithelial cells, couple to the heterotrimeric G-protein Gs, which activates adenylyl cyclases III and VI to convert ATP into cAMP, plus 2 inorganic phosphates, the insertion of aquaporin-2 water channels by exocytosis of intracellular vesicles, an alternative for thousands of words, an illustration of an unseen phenomenon.

Conclusion. We succeeded to develop a 3d scientific animation that explains the entire chain of ADH secretion, using a very modern and attractive tool.

Keywords: AVP, Maxon Cinema 4d, aquaporin-2

RESEARCH REGARDING PHYSIOLOGICAL RESPONSES OF BANAT'S COMMON BEAN LANDRACES (*PHASEOLUS VULGARIS* L.) SEEDLINGS TO HYDRIC STRESS

Dorin D. CAMEN, Carmen G. BEINSAN, Laura BIGYILAN and Radu L. SUMALAN

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Osmotic stress tolerance in plants is a complex phenomenon that involve morphological and developmental changes as well as physiological and biochemical processes. Two components have been identified as the probable cause of salinity toxicity, osmotic stress and ion toxicity.

Salinity is considered a significant factor affecting crop production and agricultural sustainability in arid and semi-arid region of the world, reducing the value and productivity of the affected land (Gama et al., 2007). The identification of tolerant genotypes that may sustain a reasonable yield on salt affected soils has been a strategy adopted by scientists to overcome salinity (Lee Dilly et al.,1994).

The experimental results achieved made evident the existence of some bean genotypes with a good tolerance to osmotic stress during germination (Berini, Bocsa Romana, Dudestii Noi, Sudrias urcatoare, Sacu, Sudrias pitica, Ciresu, Santana, Tincova, Comoraste).

The biological material used in our study consists of 6 *Phaseolus vulgaris* local landraces: Berini, Bocsa Romana, Dudestii Noi, Sacu, Ciresu and Soceni. The experimental variants are were: V_0 – control (distillated water), V_1 – PEG -1 Mpa, V_2 – PEG – 2 Mpa, V_3 – PEG- 4 Mpa). During seed germination we have determined the seed germinating rate, and free proline content (mg/g f.w.). The germination seed rate was determined by counting germinated seeds and it was repeatedly done during the experimental period (Bewley and Black, 1994). The proline accumulation is a common metabolic response of superior plants affected by water deficit and osmotic stress condition. This subject was intensely debated in the scientific world in the last 20 years (Sumalan and Dobrei Carmen, 2002). Transpiration rate was calculated by the method of successive weighing using bean leaf, the results was related to foliar surface.

From the results obtained regarding the germination potential measured at various time intervals, it has been noticed that Dudestii Noi local land race showed the higher germination rate on V_3, but this result can be inconclusive because of the fact that this land race have origin unknown. Regarding the free proline accumulation the results are not very different in the V0 and V1 but the differences are evident in V_2 and V_3

confirming the theory that the proline is an osmoprotectant component. The best tolerant genotype was Dudestii Noi with 3.247 mg/g f.w.

Generally, transpiration rate tended to decline with increasing salinity. This may be due to the fact that lowered water potentials in the root can trigger a signal from root to shoot (such as abscisic acid, which has been suggested to be the operating mechanism.

Keywords: proline, stress, phaseolus, transpiration

FAMILIAL HYPOTHALAMIC DIABETES INSIPIDUS BY Phe53Ser, IN A DECADE

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Background. Familial hypothalamic *diabetes insipidus* (FHDI) is a rare genetic disease, characterized by abnormal synthesis / release of arginine-vasopressin (AVP) leading to impair renal water reabsorbtion, positive water free clearance and excessive water intake.
Aim. We followed-up along 10 years a family with FHDI, in which a new mutation Phe53Ser of arginin vasopressin gene was previously identified by our group*.
Case history. Five cases in three generation developed diabetes insipidus. History reveals that the clinical polyuria-polydipsia syndrome developed gradually in the first 5 years of life. The family history confirms familial aggregation: father (1st generation), children, a boy and a girl (2 nd generation) and 2 of three children (3rd generation).
All family members were evaluated by water free clearance several times during last decade. The dehydration test, followed by desmopressin administration confirmed the persistence of positive water free clearance along dehydration period and a negative water free clearance after administration of antidiuretic hormone. The urine osmolalities started from around 100 mOsm/L and did not changed significantly after dehydration. A negative water free clearance was obtained only after desmopressin administration and established the diagnosis of central hypothalamic *diabetes insipidus*. Computed tomography, did not shown any anatomical changes of hypothalamo-pituitary region. MRI showed the absence of enhanced signal in pituitary posterior lobe, an image characteristic for the central *diabetes insipidus*.
All patients with FHDI were treated with desmopressin – (Minirin intranasal or Minirin_ melt 120 mcg/tb. Along years, the necessary dose of desmopressin increased slightly, reaching a complete substitution only with desmopressin 120 mcg/tb, t.i.d.
By sequencing AVP exons the same Phe53Ser mutation was identified in four family members, which all expressed clinical *diabetes insipidus* and was not found in the other members of family without *diabetes insipidus*
In conclusion, the hypothalamic familial *diabetes insipidus* produced by Phe 53Ser mutation, show a complete penetrance in both sexes, and is clinically expressed since 5 years, progressimg in time

Key words: familial hypothalamic *diabetes insipidus*, Phe53Ser Mutation, Water free clearance

CARBON NANOTUBES AS WATER CHANNEL

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Carbon nanotubes (CNTs) are allotropes of carbon with a cylindrical nanostructure, having with length to diameter ratio of up to 132,000 : 1. These cylindrical carbon molecules have unusual properties, which are valuable for nanotechnology, medicine, electronics, optics, a/o. Hummer et al. (2001), in an experiment performed with a single-walled nanotube of 13.4 Å long and with a diameter of 8.1 Å, demonstrate the possibility as the water molecules to penetrate into and conducted through the nanotube. Based on these observations, they suggest that "carnbon nanotuybes, with their rigid nonpolar structures, might be exploited as unique molecular channels for water and protons, with the channel occupancy and conductivity tunable be changes in the local channel polarity and solvent conditions". Yulin Yang et al. (2010), established that the capacity to control in the artificial water channel of carbon nanotubes (CNTs) is controlled by reducing or intensifying interaction energy between water molecules ant he wall of the CNTs channel. For the first time, Liu et al. (2009) used double-walled carbon nanotubes (DWCNTs) as artificial water channel proteins. They consider that synthetic transmembrane channels based on DWCNTs would be preferred when the functional inner tube needs to be isolated from biomembrane environment. Also, they established that the bilayer structure of DWCNTs is advantageous for carbon nanotube based transmembrane channels. This novel design could promote more sophisticated nanobiodevices which could function in a bioenvironment with high biocompatibility. Another experiment, regarding to use of carbon nanotubes as water channel, was performed by Titov et al. (2010). They use the molecular dynamics simulations combined with iterative screening to test if one can design mechanically controllable and selective molecular pores. The first model pore was formed by two stacked carbon nanocones connected by aliphatic chains at their open tips, in analogy to aquaporins. It turns out that when one nanocone is gradually rotated with respect to the other, the molecular chains alter the size of the nanopore formed at the cone tips and control the flow rates of liquid pentane through it. The second model pore was formed by two carbon nanotubes joined by a cylindrical structure of antiparallel peptides. By application of a torque to one of the nanotubes, while holding the other, they can reversibly fold the peptides into forward or backward-twisted barrels. Zuo et al. (2010), analyzed the single-file transport through a biomimic water channel consisting of a (6.6 Å) carbon nanotube (CNT) with different types of external point charges is studied using molecular dynamics simulations. They demonstrated that, as in the aquaporins, asymmetrically positioned charges cannot generate robust unidirectional water flow in the CNT. Thermal fluctuation in bulk water competes with charge affinity to steer the water transport, resulting in nonmonotonic flow

with intermittent reversal of transport direction. The energetic analysis suggests that the water-water interaction, determined by dipole orientation configuration, influences the transport rate significantly. They considered that these findings can provide correct biomimic understanding of water transport properties and will benefit the design of efficient functional nanofluidic devices. Recent, Xinghua Sun et al. (2011), investigated the electrophoretic transport of proteins (lysozyme) across electrochemically oxidized multi-walled carbon nanotube (MWCNT) membranes. Also, were tested other nanotubes type in nanobiotechnology (Yilmgaz et al., 2011, a/o).

WATER CHANNELS IN MITOCHONDRIA

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Introduction. Researches of the last two decades have revealed a major involvement of the mitochondrion in a series of cell processes far beyond its traditional bioenergetic role. It is now clear that mitochondria have a decissive impact in cell signalling and regulation, especially in cell proliferation and cell death. A major event which takes place at the level of the inner mitochondrial membrane, the formation of the so-called permeability transition pore (PTP), is ubiquitously involved in the above-mentioned processes. The induction of PTP, on the other hand, is generally triggered by the action of various internal and external stressors, their most obvious effect being a swelling of the mitochondrial matrix that closely preceeds PTP formation. Although the phenomenon of mitochondrial swelling, both *in vivo* and especially *in vitro*, has been observed long ago, its mechanism and significance has only recently begun to be understood. Due to a very large surface-to-volume ratio of the inner mitochondrial membrane, it has been assumed that the most important factor underlying the swelling is the permeability of the lipid bilayer, i.e. a simple diffusion process of water. Gradually, however, as the structural and functional complexity of this membrane has been unravelled, it was realized that different ion and substrate carriers or channels might also facilitate water permeation.

Main content. Despite the discovery of aquaporins (AQPs) in different types of cells and tissues almost 3 decades ago, it was not generally assumed that mitochondria posses such special water channels until about 10 years ago, when the presence of AQP 8 was demonstrated, most notably, in kidney mitochondria (Elkjaer *et al,* 2001). It is the purpose of this communication to review the presence of AQPs in mitochondria of different types of tissues and to discuss the main evidence for their presence and role in mitochondria. Soon after the presence of AQP 8 was observed in mitochondria of kidneys and gastrointestinal tract, its presence was also demonstrated in rat liver mitochondria (Ferri *et al.,* 2003) and murine CNS stem cells (La Porta *et al.,* 2006). Also, a shorter isomorph of AQP 9 was discovered in brain mitochondria (Amiry Moghaddam *et al.*, 2005). Lee and Thévenod (2006) discuss the possible role of mitochondrial AQPs in cellular life-and-death decisions. A series of papers published by Calamita's group between 2005-2009 must also be mentioned. The one published in 2006 is probably the most relevant. It represents a complex biochemical, immunological and biophysical study on intact mitochondria, mitoplasts, inner membrane vesicles, outer membrane vesicles and liposomes composed of lipids extracted from the inner membrane. It clearly shows the contribution of AQP water channels for water permeation in mitochondria, although additional patways of water permeation are also suggested.

Selected references. Amiry-Moghaddam *et al.* (2005), FASEB J. **19**, 1459-1467; Calamita *et al.* (2005), J. Biol. Chem. **280** (17), 17149-59; Calamita *et al.* (2006), Biochim. Biophys. Acta

WATER CHANNEL PROTEINS IN THE KIDNEY IN HEALTH AND DISEASE

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Introduction. One of the main functions of the kidney is to maintain fluid balance by reabsorbing water. Water channel proteins play a pivotal role in this process and the expression of a number of aquaporins has been reported in the kidney. Information on how these proteins participate in renal function and the onset of disease is emerging but significant gaps in our knowledge still exist. The aim of this presentation is to identify these gaps and to examine how this information may be ascertained.

Results and Discussion. Seven aquaporins have reported to be expressed in the kidney and experimental studies have revealed that while they play a major role in regulating fluid retention they also may have functional roles in glycerol and anion transport, cell proliferation and cell migration. Water retention results from the transport activities of aquaporins in the proximal tubule, the thin limbs of Henle and the collecting duct. Valuable insights have been obtained into the vasopressin-induced regulation of aquaporin-2 activity in the collecting duct, and the trafficking of membrane vesicles, but some steps are still not well characterized. The precise roles and functional mechanisms associated with the other water channel proteins also require further resolution. The loss of aquaporin-2 is associated with nephrogenic diabetes insipidus, but the relationship of the other aquaporins to renal malfunction is uncertain. It is likely that they play a role in renal fibrosis. Studies with genetically modified mice that lack specific aquaporins have revealed changes in renal function, suggesting that their precise roles in human disease may remain uncovered.

Conclusions. Water channel proteins are vital to renal function. Vasopressin stimulated water retention is the best characterized function. The precise functional roles and the mechanisms of action of these proteins in health and disease require further resolution.

Keywords. Kidney, water homeostasis, vasopressin,

DEVELOPMENT OF BIOLOGICALLY RELEVANT AQUAPORIN 4 LIGANDS

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Abstract. Aquaporin 4 (AQP4) is the principle water transporter in the central nervous system, and is believed to be essential for maintaining physiological water balance. It is also distributed throughout the body with significant expression levels in the eyes, lungs, kidneys and skeletal muscles. Alterations to AQP4 distribution and expression levels have been linked to a number of disorders, which include cerebral edema, epilepsy, neuromyelitis optica, Duchene muscular dystrophy and brain tumors. Modulation of AQP4 function may be therapeutically beneficial; however, at a more basic level, its role in disease progression remains poorly understood.

The physiological roles of AQP4 have, for the most part, been studied using knock out (KO) animals lacking the AQP4 gene, as well as by pathohistological techniques. However, AQP4 ligands that can be used *in vivo* to modulate its function, or act as tracers in studying changes to its localization are valuable for gaining a more complete understanding of its various roles. From these ligands, new therapeutic agents and diagnostic methods can be envisioned.

Our laboratory has recently reported the small molecule AQP4 inhibitor TGN-020. This compound was selected based on conserved physicochemical features with other AQP4 modulators, and was found to inhibit AQP4 water flux in a Xenopus oocyte *in vitro* assay. Subsequent studies demonstrated the efficacy of single dose TGN-020 administration in reducing rodent brain swelling following an ischemic insult. Our laboratory has also developed a ^{11}C-labeled version of TGN-020 that can be used to visualize the real-time distribution of physiological AQP4 by positron emission tomography imaging.

This presentation will focus on the development of TGN-020, and the results of recent biological studies involving it.

Keywords: Aquaporin 4, ligand, inhibitor, positron emission tomography

WATER EXCHANGE IN RED BLOOD CELLS: HISTORICAL COMMENT AND NMR STUDIES

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Introduction. Water exchange across the membranes of erythrocytes (red blood cells; RBCs) in higher animals is rapid, on the millisecond time scale. The exchange is mediated mostly by proteins [1] and yet not all the exchange is via aquaporins. The most convenient approach for estimating the permeability coefficient is the so-called manganese doping method that has been championed by Benga et al. [e.g., 2]. However, a new NMR method exploits the differential splitting of the ^2H_2O resonance of human RBCs suspended in gelatin that is 'set' and then held stretched in a special device in the NMR probe [3]. The method actually measures 'heavy water' exchange that may be different due the higher mass of the deuterium atom, but the advantage is the lack of the paramagnetic dopant, Mn^{2+}[4]. While the selective advantage of high water permeability of the human RBC is still speculated upon [5], methods to measure the shape changes in this cell are advancing [6]. Such methods hold promise for accurately characterizing RBC shapes simultaneously with measuring water exchange thus probing the effect of cytoskeletal rearrangement on this characteristic of the cell.

Materials and Methods. NMR spectra were acquired on a Bruker (Karlsruhe, Germany) Avance III 400 MHz NMR spectrometer tuned to ^1H or ^2H frequencies. A Bruker broad-band probe was used for the stretched gel experiments; while for the diffusion diffraction experiments a high-field gradient (up to 10 T m^{-1}) probe was used. Human RBCs were obtained by venipuncture (PWK) under University of Sydney Human Ethics Committee approval. Other experimental details are in the cited references.

Results and Discussion. ^2H_2O exchange measured by a new ^2H NMR quadrupolar-splitting method [4] yielded an estimated permeability coefficient for human RBCs at 25^{o}C very similar to that from the "older" ^1H NMR method [2]**.** Fast diffusion-interference experiments using *q*-space analysis based on the ^1H_2O signal, record shape changes in human RBCs on the minute time scale. The shape changes are not directly related to the mean ATP concentration inside the cells. The effects of shape changes on water transport are yet to be reported.

Conclusions. Historically the first serious suggestion that water transport in human RBCs is substantially protein mediated is credited to Benga et al. [1]. New NMR methods of measuring water transport continue to be developed [4] thus enabling different experimental conditions in which to study correlations with characteristics such as cell shape and metabolic-energy status [6].

Keywords. ^1H NMR; ^2H NMR; quadrupolar splitting; stretched gel; water permeability.

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AQUAPORINS AND THE RESPONSES OF PLANTS TO THEIR CHALLENGING ENVIRONMENT

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Introduction. Plants have to constantly adjust their water status during development and in response to sometimes very challenging environmental conditions. Uptake of soil water by roots and its delivery from xylem vessels to inner leaf tissues are crucial for maintaining the plant water status.The present talk will discuss how the integrated function and regulation of plant aquaporins can be addressed by a combination of genetic, molecular and physiological approaches and show how aquaporins contribute to water transport throughout the whole plant body.

Results and discussion. Knock-out mutants of *Arabidopsis thaliana* for Plasma membrane Intrinsic Proteins (PIPs) were used to dissect the osmotic and/or hydrostatic modes of water uptake in roots and leaves. The variation of root hydraulic architecture and of aquaporin expression in natural accessions of *Arabidopsis* provided complementary insights into the role of specific cell layers and aquaporin isoforms in root water transport. Aquaporins are also crucial for adjusting cell and tissue hydraulics in response to various environmental stimuli. Several studies have highlighted the role of Reactive Oxygen Species (ROS)-dependent signalling paths, phosphorylation, gating and sub-cellular trafficking of aquaporins in the response of roots and leaves to water stress, anoxia or changes in irradiance. In particular, the mechanisms and routes of ROS-dependent trafficking of PIPs were recently dissected in root cortical cells. Our studies also established stress-induced quantitative changes in aquaporin phosphorylation and, for the first time, a link with plant aquaporin subcellular localization. Besides environmental stimuli, auxin was found to regulate aquaporin expression and root hydraulic conductivity, in connection with a novel role for a PIP isoform in lateral root emergence. Finally, the alteration of mesophyll conductance to CO_2 in some PIP knock-out mutants will be discussed with regard to the model that PIPs facilitate CO_2 diffusion across plant cell membranes.

Conclusion. A wide range of selectivity profiles and regulation properties allows aquaporins to be integrated in numerous functions, throughout plant development, and during adaptations to variable living conditions. In particular, the possible coupling of tissue hydraulics with growth and carbon assimilation provides unique research perspectives in plant integrative biology.

Keywords: plant, stress, tissue hydraulics

BRAIN SCIENCE OF WATER MOLECULES:

A SALUTE TO PROFESSOR LINUS CARL PAULING

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Double Nobel Laureate, Linus Carl Pauling put forth a powerful model of the molecular mechanism of general anesthesia, generally referred to as the hydrate-microcrystal (aqueous-phase) theory. This hypothesis, based on the molecular behavior of water molecules, did not receive serious attention during Pauling's life time when scientific approach towards complex systems, such as the brain, was still in its infancy. The situation has since drastically changed, and, now, in the 21st century, various scientific tools are available for examining different types of complex systems. The discovery of aquaporin-4, a subtype of water channels abundantly expressed in glial systems, further highlighted the notion that dynamics of water molecules in the cerebral cortex play an important role in physiological brain functions, including information processing.

Keywords: aqueous-phase theory, neural net, Kohonen's map, aquaporin-4, brain chip

AQUAPORIN-9 IS THE PRIMARY ROUTE OF HEPATOCYTE GLYCEROL UPTAKE FOR GLYCEROL GLUCONEOGENESIS

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Introduction: Aquaporin-9 (AQP9) is a well characterized glycerol channel expressed in hepatocytes and has been hypothesized that AQP9 promotes hepatic glycerol gluconeogenesis. In line with this argument, obese, diabetic *AQP9* -/- knockout mice suffer less from fasting hyperglycemia than controls. However, plasma glucose levels rise simultaneously in *AQP9* wildtype and knockout mice after a glycerol meal. This suggested indirect contribution of AQP9 to plasma glucose levels in obese, diabetic mice.

Methods: To resolve this question we have identified potent, specific small molecule inhibitors of AQP9 in a small molecule library screen and subsequent *in silico* molecular docking. Inhibitor specificity was determined in stable cell lines, expressing either AQP9 or homologous AQP isoforms. We have analyzed inhibitor effects on glucose production from glycerol in primary hepatocyte culture as well as in perfused mouse livers.

Results: In primary hepatocyte culture, glucose output was increased by 123% (+/- 36% SEM) in the presence of 0.5mM glycerol compared to buffer controls. This increase was completely abolished in the presence of 25µM AQP9 inhibitor. No effect of the inhibitor was observed in hepatocytes isolated from AQP9-/- mice. Furthermore, at high extracellular glycerol concentrations (5mM) hepatocyte glucose output was less dependent on AQP9. Here, glycerol diffusion through the lipid bilayer may provide partial input for glycerol gluconeogenesis. Similarly, *AQP9* gene deletion or chemical inhibition abolished glycerol enhanced glucose output in *in situ* perfused liver preparations of starved mice.
Conclusions: Our experiments establish AQP9 as a potential drug target for treating type-2 diabetes. The identified inhibitors may serve as lead compounds for designing such drugs.
Keywords: Aquaporin-9, small molecule screen, glycerol

METABOLIC IMPACT OF WATER CHANNEL PROTEINS

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Our group previously cloned a cDNA belonging to aquaglyceroporin from a human adipose tissue cDNA library, designated as aquaporin adipose (AQPap). Subsequently, other groups demonstrated that AQPap was a human homologue of AQP7, which was cloned from the rat testis. AQP7 is mainly expressed in adipose tissue, testis, heart, and kidney. AQP7 is involved in glucose and glycerol metabolism based on marked modulation of its expression by dietary conditions. We previously showed that AQP7 mRNA expression is suppressed by insulin and is elevated by PPARgamma ligands in adipocytes. However, the physiological role of AQP7 has not been elucidated.

We generated and analyzed the mice lacking AQP7. The increase in plasma glycerol level in response to beta3-adrenergic agonist was severely impaired in AQP7-knockout (KO) mice. Longer starvation induced severer hypoglycemia in KO mice than in WT mice. These results indicate that AQP7 serves as an adipose glycerol channel in vivo and fat-derived glycerol determines fasting plasma glucose level. Although there was no difference in body weight between WT and KO mice until 10 weeks of age, we found that KO mice developed adult-onset obesity. To clarify the underlying mechanism for obesity in KO mice, we analyzed these mice at 6-10 weeks of age. We found the increased glycerol contents and the elevated activity of glycerol kinase (Gyk) in adipose tissue of KO mice. Gyk is a key enzyme that converts glycerol to glycerol-3-phosphate. Knockdown of AQP7 in 3T3-L1 adipocytes was associated with a significant reduction of glycerol in media and elevation of cellular glycerol content. Enzymatic activation of Gyk was observed in AQP7-knockdown adipocytes. Finally, the uptake of oleic acid significantly increased in AQP7-knockdown adipocytes.

Next, the role of cardiac AQP7 was investigated. Cardiac morphology and function in KO mice were similar to those of WT mice under basal conditions, although low glycerol and ATP content were observed in hearts of KO mice. In H9c2 cardiomyocytes, glycerol uptake and glycerol-dependent ATP elevation were decreased by AQP7-siRNA. The ex vivo heart study showed the impairment of cardiac glycerol consumption levels in KO mice. Pressure overload caused severe heart failure in KO mice.

In conclusion, a series of our data may indicate that AQP7 maintains the glycerol homeostasis in fat and heart.

Keywords: Glycerol, Aquaporin 7, Obesity, Heart Failure

ULTRASTRUCTURAL STUDIES ON THE EFFECT OF SEEDS OF *TRIGONELLA GRAECUM* ON ALCOHOLIZED RATS KIDNEY

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Introduction. Through their rich content in polyphenolic flavonoids, *Trigonella foenum graecum* seeds exert a protective antioxidant effect and membrane protector. Most researches focused on the hypoglycaemic and antidiabetic action of the seeds, with only a few reports on the use as a preventive factor in the pathology of the alcoholic kidney.

Materials and Methods. Our work was conducted on an experimental model in vivo; in which the animals were given together with the food two different amounts of grounded seeds, against the background of alcoholic intoxication. The experimental animals were adult Wistar rats, weighing 180-200g, divided into four groups: control group (M); group treated with ethanol (ER); two groups that received the same concentration of ethanol, and the food contained 5%, respectively 10% fenugreek flour. The ethanol and the flour were administered daily, for 4 weeks.

Results and Discussion. The ultrastructural analysis showed at the level of the proximal convoluted tubes, vacuolation in cytoplasm, edemas at the apical pole of the nephrocytes, rarefaction of the cytoplasmic and mitochondrial matrix, the increase in number of the lysozymes and especially deperoxisomes, as well as the congestion of blood capillaries. In the case of the groups T5R and T10R, which received Trigonella flour together with ethanol, the structural and ultra-structural changes produced by the ethylic intoxication were more attenuated, being slightly better in group T5R. The vacuolation of the cytoplasm and the number of lysozymes and peroxisomes was greatly reduced, and the aspect of the mitochondria remained in most nephrocytes similar to that of the witness animals. Most of the nuclei of the cells of the nephocytes have retained their spherical shape, being at the same time predominantly euchromatic, with little heterochromatine and evenly dispersed.

Conclusions. Our results plead in favor of using *Trigonella* seeds as a food supplement to prevent cellular deterioration and improve renal function in patients suffering from kidney and liver issues caused by excessive alcohol consumption.

Keywords: renal-protective effects, *Trigonella* seeds, rat kidney

AQUAPORIN-9 IS THE PRIMARY ROUTE OF HEPATOCYTE GLYCEROL UPTAKE FOR GLYCEROL GLUCONEOGENESIS

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Introduction: Aquaporin-9 (AQP9) is a well characterized glycerol channel expressed in hepatocytes and it has been hypothesized that AQP9 promotes hepatic glycerol gluconeogenesis. In line with this argument, model type-2 diabetic *AQP9* -/- knockout mice suffer less from fasting hyperglycemia than controls. However, plasma glucose levels rise simultaneously in *AQP9* wildtype and knockout mice after a glycerol meal. This suggested indirect contribution of AQP9 to plasma glucose levels in obese, diabetic mice.

Methods: To resolve this question we have identified potent, specific small molecule inhibitors of AQP9 in a small molecule library screen and subsequent *in silico* molecular docking analyses. Inhibitor specificity was determined in stable cell lines, expressing either AQP9 or homologous AQP isoforms. We have analyzed inhibitor effects on glucose production from glycerol in primary hepatocyte culture as well as in perfused mouse livers.
Results: In primary hepatocyte culture, glucose output was increased by 123% (+/- 36% SEM) in the presence of 0.5mM glycerol, compared to buffer controls. This increase was completely abolished by 25µM of AQP9 inhibitor (IC50~2 µM). No effect of the inhibitor was observed in hepatocytes isolated from AQP9-/- mice or in AQP9+/+ hepatocytes, when lactate and pyruvate were provided as substrates for gluconeogenesis. Furthermore, at high extracellular glycerol concentrations (5mM) hepatocyte glucose output was less dependent on AQP9. Here, glycerol diffusion through the lipid bilayer may provide partial input for glycerol gluconeogenesis. Similarly, *AQP9* gene deletion or chemical inhibition abolished glycerol enhanced glucose output in perfused liver preparations of starved mice.
Conclusions: Our experiments establish AQP9 as the primary route of hepatocyte glycerol uptake for glycerol gluconeogenesis. Thereby we provide mechanistic explanation for previous observations of alleviated model type-2 diabetes in AQP9-/- mice.
Keywords: Aquaporin-9, small molecule screen, glycerol

WATER QUALITY EVALUATION USING

CITOLOGICAL METHODES

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Introduction: Answers to the increasing problems of water are only to be found through a variety of techniques within an ecologically sound framework. The principles of the natural water and wastewater presence in different ecosystems were taken into account. Harmless waters have to be separated from harmful wastewaters to trace the sources of harmful substances.
The purposes of this work are to present original results concerning cytological characterization of some surface waters from salty and "radioactive" ecosystems.

Material and Methods: *The tested waters* were from Socodor and Bârzava different agro-ecosystems. The positive control was distilled water (DW). *The Experimental variants* were Socodor tap water (STW), ground water "Raul Morii" (SRM) and Bârzava ground water (BGW), wells situated at 50m, 100m and 150m distance from uranium pit (B100, B150, B200). In order to point out the water quality and environmental influence *Allium test* was used (Fiskesiö. 1988). *Laboratory work* 10 equal-sized cloves/4 replications were grown in individual glass jars that were filled up with 50 ml/flacons of waters. The rooting process was evaluated from 24 to 24 hours. The number and the length of root were checked after 24 hours. In the same time the root samples were collected for cytological analysis. The number of cells in mitosis was counted from 5mm region of the primary root tissue. Carr and Walker (1961) cytological protocol was used. For meristematic root cells examination the Olympus BH2-RFCA microscope was utilized. The MI% was determined by scoring more than 1,000 cells/slides. MI% was calculated as the percent of dividing cells and total number of registered cells.

Results and Discussions: The root primordia were activated and mitotic division started after 10 and 24 hours in case of DW and SRM respectively. MI% varied from 18.34±2.66 to 4.27±3.65 in DW and SRM respectively. The MI% emphasized a particular mitotic cycle depending of the waters origin. The MI% data were distributed from 4.27±3.65 to 21.22 ±1.15 in SRM and STW respectively. In comparison with Socodor, Bârzava waters pointed out high uniformity of MI% (21.35 on B100 to 24.46 on BGW). In Bârzava waters root cells were capable to perform a rapid division. Surprising no harmful

substances was highlighted by *Allium* test on Bârzava waters. In Interphase and in the mitoses phases the percent of micronuclei and misdivision was higher on roots grown in Socodor waters. It was significant higher in comparison to Bârzava (28.3%>14.9% respectively).

Conclusions. Our results pointed out the quality differences among waters originated in different locations (Socodor and Bârzava) and ecosystems.

On the contrary of our suppositions the waters collected on different distances from uranium mine were “clean” without a harmful effect. The Socodor waters drastically inhibited the number of dividing cells being more dangerously.

Keywords: Waters; environments, cell division.

WATER CHANNEL PROTEINS IN ARCHAEA

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Water channel proteins (aquaporins) are present in all three domains of life - Archaea, Bacteria and Eukarya, suggesting deep evolutionary roots for these important membrane proteins. On the basis of sequence similarity the MIP (major intrinsic proteins) superfamily can be divided into two major groups: aquaporins (AQPs) and glycerol uptake facilitators (GlpFs). This talk will briefly review the current knowledge of water channel proteins and related members of the MIP family in Archaea. Typical properties of many Archaea are high resistances to environmental extremes. Maintaining water balance under conditions of high temperatures or extreme pH suggests an essential function for aquaporins, but their physiological role in Archaea has not been clarified. Aquaporin AqpM, which exhibits similarity to mammalian AQP1, was identified in *Methanothermobacter marburgensis,* which grows anaerobically at 65^{o}C. Its structure was resolved to 1.86 Å and its channel architecture was determined in detail. *Archaeoglobus fulgidus*, an archaeon growing up to 95^{o}C, possesses an evolutionary related protein (AfAqp), which was recently used for probing the facilitation of gaseous H_2S transport, and contains also a GlpF. AQPs were identified from sequences of *Methanosarcina barkeri* (MbAqp), *Methanosaeta concilii* (AqpM), *Sulfolobus acidocaldarius* (SaAqp), *Methanococcus maripaludis* (MmAqp); sequences related to MIP family proteins were found in *Methanococcus vannielii*, *Methanococcus voltae* and *Methanobacterium* sp. However, some Archaea and other microorganisms appear to lack aquaporins. Putative aquaporin-like genes have been found only in about half of the currently sequenced microbial genomes. The presence of other types of water channel proteins, which do not belong to the MIP family, cannot be excluded at this time.

THE MANAGEMENT OF THE BLOOD-BRAIN BARRIER IN HEALTH AND DISEASE

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Introduction: The blood-brain barrier (BBB) is located in the brain endothelial cell tight junctions (TJs). However, the BBB-TJs are not expressed independently, but under the control of the microenvironment of the brain. The basal lamina component agrin binds to the dystrophin-dystroglycan complex (DDC), including aquaporin-4 (AQP4). AQP4 is highly polarized over the astroglial cell surface. Under pathological conditions such as trauma, inflammation or tumour, the polarity is reduced leading to loss of water transport directionality, thus to edemas.

Materials and Methods: Human gliomas, cell culture of astrocytes or glioma cells, electron microscopy including freeze-fracturing, laser scanning immunohistochemistry, immunogold labeling, western blotting and PCR.

Results and Discussion: In agrin- and dystroglycan knockout mice, the distribution of AQP4 in astrocytes is disturbed. AQP4 is no more restricted to the superficial or perivascular astrocyte endfeet membranes. The same is found in human glioblastoma tissue. Here, we see an upregulation of the matrix metalloproteinases MMP3 and MMP2/9, which is concomitant with a loss of agrin and dystroglycan suggesting degradation of agrin by MMP3 and of dystroglycan by MMP2/9. This in turn may lead to a redistribution of AQP4 due to a failure of the binding between agrin and the DDC/AQP4-complex.

Conclusions: Agrin is capable of restricting the localization of AQP4 to the astroglial endfoot membrane. Pathological conditions may increase MMPs which cleave agrin and the DDC, leading to redistribution of AQP4 with consecutive edema formation.

Keywords: Blood-Brain Barrier, Glioma, Aquaporin-4, Freeze-Fracturing.

ROLES OF AQUAPORIN-4 IN BRAIN DISORDERS

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Aquaporin-4 (AQP4) is the main water channel in the brain and is distributed with highest density in the perivascular and subpial astrocyte end-feet. AQP4 is a critical component of an integrated water and potassium homeostasis. Indeed, AQP4 has been implicated in several neurologic conditions, such as brain edema, seizure and even mood disorders. Expression and regulation of AQP4 have been studied to understand the roles of AQP4 in physiological and pathological conditions. Here we discuss about the mechanisms how AQP4 is dynamically regulated at different levels; channel gating, subcellular distribution, phosphorylation, protein-protein interactions and orthogonal array formation. We found that AQP4 is rapidly and reversibly regulated by Zinc on a dose-dependent manner. Immunological function of AQP4 has been demonstrated against inflammatory response in brain disease models. Interestingly, AQP4 was identified as a target antigen of autoimmune attack in neuromyelitis optica (NMO). We evaluated putative epitopes on AQP4 for NMO-IgG binding. We also studied Drosophila Big Brain (Bib), since Bib has high sequence homology to AQP4, and play an important role for Drosophila neurogenesis. AQP4 may be a potential therapeutic target in several neurologic conditions. Further studies from different aspects are required to develop new drugs against AQP4.

RESOLVING A CONTROVERSY: MOLECULAR GATING OF AQUAPORIN-1 AS A DUAL WATER AND ION CHANNEL

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The family of aquaporins (AQPs) includes a broad array of channels that are differentially expressed in tissues, gated by signaling mechanisms, and capable of mediating fluxes of diverse substrates. This multifunctional capacity indicates AQPs are involved in physiological processes beyond simple water transport. Reminiscent of K^+ ion channels in their general structure, AQPs are organized as tetramers of subunits, each with six transmembrane domains and an individual water pore. A subset of the AQP family have been shown to have ion channel activity, including lens AQP0, nodulin, *Drosophila* Big Brain, AQP1 and AQP6. However, variability in responsiveness between different experimental preparations has led to controversies in the field.

Ongoing work with AQP1 has moved towards resolving one of the standing controversies, showing that variability is due to the presence of additional regulatory mechanisms that serve as "master switches" governing the availability of AQP1 to be activated as ion channels. When activated by intracellular cGMP, AQP1 carries a non-selective monovalent cation conductance (permeability sequence $K^+ \approx Cs^+ > Na^+ > TEA^+$), with no appreciable conduction of Cl^-, protons, or divalent cations Ca^{2+} or Mg^{2+}. Under physiological saline conditions, the single channel conductance is approximately 150 pS in excised patches from *Xenopus* oocytes. Molecular dynamic simulation and electrophysiological analyses indicated that cations permeate AQP1 via the pore formed at the center of the tetrameric subunits, and that a conserved intracellular loop between the 4th and 5th transmembrane domains is required for cGMP-dependent gating. Domains involved in gating and ion permeation show conserved amino acid sequences across species. Recent studies using site-directed mutagenesis and functional assays have confirmed that the AQP1 tetrameric central pore is the pathway for ion permeation, and that tyrosine phosphorylation in the carboxyl terminus regulates the responsiveness of ion channel to cGMP stimulation.

Site-directed mutant constructs of human AQP1 expressed in *Xenopus* oocytes were characterized by two-electrode voltage clamp and optical osmotic swelling analyses. Quadruple mutation of barrier hydrophobic residues (V50, L54, L170, L174) to alanines in the central pore induced inward rectification of the ionic current and shifted reversal potential approximately +10 mV, indicating increased permeability of tetraethylammonium ion. Introduction of cysteine at lysine 51 in the central pore (K51C) in a cysteine-less template created new sensitivity to block of the conductance by mercuric ion. These data show the AQP1 central tetrameric pore is the ionic pathway. Mutations of candidate consensus sites and pharmacological manipulation of serine and threonine phosphorylation did not alter cGMP-dependent responses; however, mutation of tyrosine Y253C or pharmacological dephosphorylation prevented ion channel

activation. Modification of Y253C by covalent addition of a negatively charged molecule (MTSES) rescued the cGMP-activated conductance response, an effect reversed by dithiothreitol. These data show that tyrosine phosphorylation is one of the factors that regulates the availability of the AQP1 ion channels to be gated by cGMP.

The concept of master switches regulating the availability of aquaporin ion channels moves towards resolving a standing controversy and expanding our understanding of AQPs as a multifunctional regulated channels that have potentially complex multiple levels of gating. The physiological role of a dual water and ion channel capability in AQP1 is a subject of ongoing research. Possible roles for the parallel pathways for water and ions in AQP1 include contributions to slow signaling processes and regulation of cell volume. The identification of selective pharmacological blockers of the AQP1 ion pore (which do not block the separate adjacent water pores) will be the next step for discovery and analysis of the physiological functions of AQP1 ion channels.

Keywords: AQP1; tyrosine kinase; phosphorylation; cyclic GMP; cation channel; site-directed mutagenesis; voltage clamp.