protein aquaporin-2, a process critical to regulation of water excretion. However, whether the abundances of other proteins undergo parallel changes is unknown and mechanisms of abundance changes are unexplored. We carried out large-scale measurements of protein half-lives ($t_{½}$) and relative translation rates for natively expressed proteins in cultured renal mpkCCD(clone 11) cells (±dDAVP, 1 nM) using metabolic labeling with stable isotopes (SILAC) coupled with protein mass spectrometry (LC-MS/MS). A mass-balance model, assuming first-order kinetics of degradation, allowed calculation of $t_{½}$ and the relative translation rate for each identified protein. Initial experiments (n=3) have provided $t_{½}$ measurements for over 1,000 proteins, a small fraction of which (<1%) was found to change significantly in response to vasopressin, including a 66% decrease in the $t_{½}$ for the adhesion protein B-CAM, whose stability is believed to be regulated by SUMOylation via UBC9. The $t_{½}$ of aquaporin-2 was not significantly changed (mean 12.6 hr). Translation-rate measurements show that among all proteins quantified (>500), 55 changed significantly at least 1.5-fold. Among these were two additional proteins involved in cell adhesion and epithelial polarity, viz. afadin and β-catenin-like protein-1. Aquaporin-2 showed the largest increase in translation rate in response to dDAVP (>16-fold). Many of the proteins showing changes in abundance in response to vasopressin did not have corresponding changes in mRNA levels (Affymetrix arrays). Further studies are underway to define the mechanism of mRNA-independent changes in translation rates in response to vasopressin.

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F-PO1620

Mice Lacking TRPV4 Show Abnormal Thirst Regulation in Absence of Renal Defects Sylvie Janas,[1] Sara Terryn,[1] Olivier R. M. Schakman,[2] Johannes Loffing,[3] Philippe Gailly,[2] Olivier Devuyst.[1] *[1]Nephrology, UCL; [2]Cellular Physiology, UCL; [3]Anatomy, University of Zürich.*

TRPV4 is a polymodal cation channel of the transient receptor potential (TRP) family. It is expressed in hypothalamic neurons including the subfornical organ, median preoptic area, and the organum vasculosum of the lamina terminalis, where it could play a role in osmotic sensing. TRPV4 has also been evidenced in the thick ascending limb (TAL) of the rat nephron, where its role remains unclear. To further analyze the role of TRPV4 in osmoregulation, we investigated its distribution pattern and the functional consequences of its disruption in mouse. Using qPCR on microdissected segments and immunohistochemistry, we found that TRPV4 is abundantly expressed in the proximal tubules (apical and basolateral), late distal convoluted tubules and throughout the connecting tubules and collecting ducts (basolateral), including principal and intercalated cells. By contrast, TRPV4 was undetectable in glomeruli and TAL, and weakly abundant in early DCT. Metabolic studies in 12 weeks old TRPV4 knock-out (KO) and wild-type (WT) mice revealed that disruption of TRPV4 does not influence renal function and urinary concentration at baseline. The KO mice did not exhibit anomalies in activity, rearing, food and water intake. Moreover, both WT and KO mice showed a similar renal NaCl and water excretion in response to furosemide injection, acute water loading (100 µl/g BW i.p.), overnight water deprivation, and chronic dietary NaCl restriction (17 days, 0.01% NaCl). However, acute administration of hypertonic saline solution (0.5 M NaCl) resulted in a significantly lower water intake in the TRPV4 KO vs WT mice, suggesting a central defect in osmoregulation. These results demonstrate that TRPV4 is widely expressed in the mouse kidney, but not in the TAL. Disruption of TRPV4 is not reflected by significant alterations in the renal handling of NaCl and water. However, TRPV4 KO mice show a decreased thirst response to hypertonic NaCl, which substantiates the major role of TRPV4 in the central regulation of osmolality.

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F-PO1621

Membrane Topology of Mouse Urea Transporter B Defined Using Epitope Tags Bryce MacIver, John Mathai, Mark L. Zeidel.

Urea fluxes via urea transporters (UTs) help regulate urine osmolality. Hydropathy analysis of many mammalian and bacterial UT sequences show a common motif of a highly hydrophobic region of 5 transmembrane helical passes. UT-B comprises 2 such regions (here called TM1 and TM2) linked by a hydrophilic domain. To assess the validity of the predicted topology map we placed hemagglutinin (HA) tags at various positions in the protein predicted to be intracellular or extracellular loops (fig. 1). We expressed these tagged proteins in *Xenopus* oocytes and assayed for UT function by isotope uptake. We then examined the accessibility of the HA tag to its antibody in whole oocytes. Intracellular tags only become accessible when the oocyte is permeabilized by detergent. All tagged positions in fig. 1 show urea uptake 5-15 times that of control except TM1-1, which appears to be non-functional and TM2-1, which shows ~2 fold increase. To date, antibody localization of TM1-2 and G1 tags corroborate their predicted position, while G2 tag differs in that it appears accessible with detergent permeabilization.

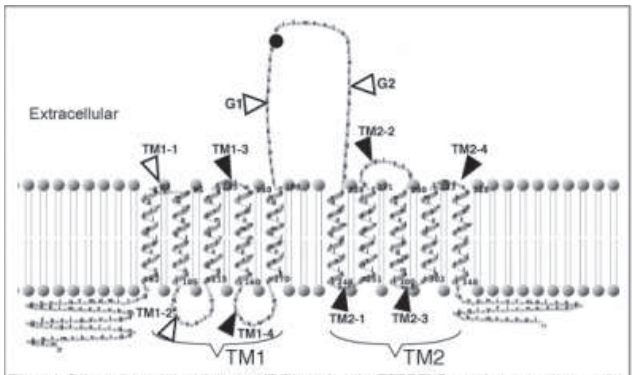

Figure 1. Schematic depiction of mouse UT-B based on the TOPCONS membrane topology prediction algorithm (Bernsel, A. *et al.* Nucleic Acids Research, 2009) and drawn with TMRPres2D (Ioannis, C. *et al.* Bioinfomatics, 2004). The topology prediction shows two regions labeled TM1 and TM2 that each comprise 5 transmembrane passes. Open triangles depict positions of HA tag insertions that have been tested for both functionality and location with respect to the membrane while closed triangles are HA tagged insertions tested for function only at this time. The closed circle represents a likely glycosylation site.

Recently a crystal structure of a bacterial UT (dvUT) was reported (Levine *et al.*, Nature, 2009). This structure supports the predicted topology showing two hemi-cylindrical domains similar to TM1 and TM2 in fig.1, but adding an additional helix to the 5 transmembrane spans from the topology prediction. However, this additional helix, termed the pore helix, does not span the membrane, instead each pore helix lies at a 50° angle to create a selectivity filter suitable for urea. Interestingly, the G2 tag falls within a region equivalent to a pore helix region of dvUT, yet is functional, but requires detergent for antibody access. In conclusion, our topology mapping corroborates closely with the dvUT structure.

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F-PO1622

Dietary Protein Affects Urea Transport across Bladder Epithelium in Rats David A. Spector, Jie Deng, Kerry J. Stewart. *Divs of Renal Medicine and Cardiology, Johns Hopkins Univ Sch of Med, Baltimore, MD.*

Mammalian bladder epithelia (urothelia) is generally considered impermeable to urinary constituents, but high levels of urea and creatinine and presence of UTB and other transporters in bladder tissue suggest that net urothelial transport (Tx) may occur. We developed an in-vivo rat model to study water and solute Tx across urothelia. To determine the effects of dietary protein on urea Tx we collected 24 hour urines from four groups of rats (n=12) provided with diets differing in protein concentrations (40%, 18%, 6%, 2%- protein deficient), instilled 0.3 ml urine via urethral catheter into bladders (isolated by ureteral ligation) of anesthetized 225 gm rats, and measured urine volume and concentrations of urea and other solutes (as "controls") in Instilled and Retrieved (after 60 min dwell) urine. Net change (= Tx) of volume and solutes across urothelium were calculated as**:** Retrieved minus Instilled, and differences within and between groups were compared.**Results**: After one hour bladder dwell, there was a mean 2% increase in retrieved urine volume ($p < .0001$) which did not differ between dietary groups. As dietary protein decreased there was a stepwise fall of Instilled UN concentration as well as net urea Tx - reflected by a decrease in change of urine urea concentration (table) and quantity (mg,not shown).

Dietary Protein	40%	18%	6 %	2%
Instilled UN concentration,mg/dl	5157*	2937*	549	247
UN concentration change,mg/dl	-583*	-270	-141	-87
percent change	- 11.1	-11.5	-25.5*	-23.2*

* $p < .0001$ vs other diets

In contrast, percent of instilled urea transported was greater in the two low dietary protein groups than in the higher dietary protein groups ($p < .0001$). Regression analysis (not shown) showed a strong direct relationship between initial urine urea concentration and resultant urea Tx. Urine creatinine and K concentrations also fell following one hour bladder dwell, but there were no differences in transport between dietary groups. **Conclusions**: Urea and other solutes are transported across rat urothelia, with magnitude of urea Tx mediated, in part, by dietary protein concentration and consequent urine urea nitrogen concentration.

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F-PO1623

Urea Is Important for the Urinary Concentration Capacity of the Isolated Blood Perfused Rabbit Kidney Paul S. Steels,[1] Jerry Toelsie,[2] Yves Cuypers,[1] Robbert Bipat.[2] *[1]University Hasselt, Belgium; [2]Anton de Kom University, Suriname.*

It is generally accepted that urea plays a critical role in the urine-concentrating mechanism in mammals, but herbivores have a different handling of urea than carnivores and omnivores (Bankir in The Kidney 5th ed. Brenner p. 595). Gunther and Rabinowitz (KI, 1980,17,205) have reported that urea did not enhance the concentrating ability in calm, unanesthetized, vasopressin-treated rabbits. In this study we used the isolated blood perfused rabbit kidney (Cuypers et al Pfluegers Arch,2000,440,634) to test whether its urinary concentrating activity is indeed independent of urea. Left kidneys of female rabbits