

SA1

Glomerular permeability regulation by the podocyte foot processes

T. Benzing

Department of Medicine IV, University of Cologne, Koeln, Germany

Recent studies have emphasized the critical role of podocytes for the size-selective filtration barrier of the kidney and revealed novel aspects of the mechanisms leading to proteinuria, both in inherited and acquired diseases. It has been shown that a specialized cell junction, the slit diaphragm, connecting neighboring podocytes, is of critical importance to the development and the integrity of the glomerular filter. Several critical structural protein components of the slit diaphragm have been identified. In addition to their structural functions, these proteins participate in common signaling pathways. This talk will focus on what is known about the importance of the podocyte for the function of the glomerular filter of the kidney. It will provide a snapshot of our current understanding of the signaling properties of slit diaphragm proteins and project a framework for further studies necessary to delineate the function and dynamics of the slit diaphragm protein complex and the pathogenesis of nephrotic syndrome.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

SA2

How cation entry into renal glomerular epithelial cells can regulate podocyte function

M.P. Winn

Medicine, Duke University Medical Center, Durham, NC, USA

Focal segmental glomerulosclerosis (FSGS) has been previously characterized as having primary (idiopathic), secondary and familial forms. In the latter category, both autosomal recessive and dominant inheritance patterns have been reported. Reports of familial forms of FSGS date back as far as 1956, with the observation of an autosomal recessive disease primarily within the Finnish population. The disease process is characterized by massive proteinuria *in utero*, with up to 20 to 30 grams of protein loss per day. *NPHS1* encodes a gene product termed 'nephrin', within which numerous mutations including deletions, insertions, nonsense, missense and splicing errors have been described. Nephrin localizes to lipid 'rafts' within the slit diaphragm of the podocyte. Steroid-resistant nephrotic syndrome (SRNS) is another human disorder that is characterized by autosomal recessive nephrotic syndrome. This disorder manifests between 3 months and 5 years of age, rapid progression to ESRD, and with few cases of recurrence after renal transplantation. The gene product is podocin (*NPHS2*), located on 1q25-31. Podocin most likely functions in the structural organization of the slit diaphragm and regulation of its filtration function. It has been shown to interact *in vivo* with both nephrin and CD2-associated protein (*CD2AP*), a cytoplasmic binding partner of nephrin. Mutations in the alpha-actinin 4 gene (*ACTN4*), which localizes to chromosome 19q13, have been associated with autosomal

dominant FSGS, characterized by adult onset disease of variable severity and rate of progression to ESRD. Fractions of the mutant protein have been shown to form large aggregates within podocytes ultimately compromising the function of the normal actin cytoskeleton, both through its abnormal function and toxic accumulation.

Recently, a disease-causing mutation for hereditary FSGS has been localized to chromosome 11q 21-22, with the subsequent identification of transient receptor potential cation channel, subfamily C, member 6 (*TRPC6*) as the disease causing gene. The missense mutation causes a highly conserved proline in the first ankyrin repeat of *TRPC6* to become a glutamine at position 112 (P112Q). Additional work as reported by Reiser et al. has corroborated findings implicating *TRPC6* in the pathogenesis of familial FSGS. The *TRPC6*^{P112Q} mutation is highly conserved and causes increased and prolonged calcium transients in transfected cells. The mutant channel also significantly enhances cation signals triggered by AT1 receptor activation. Biotinylation and immunostaining studies reveal that the mutation also appears to cause mislocalization of the ion channel to the cell surface. *TRPC6*-related FSGS suggests an additional mechanism for renal disease pathogenesis. Knowledge of *TRPC6*-mediated calcium entry into cells may offer unique insights into therapeutic options for glomerular diseases. Calcium as a second messenger affects many cellular functions such as contraction, apoptosis, vasoregulation and mechanosensation to name a few. We suggest that the exaggerated calcium signalling conferred by the *TRPC6*^{P112Q} mutation disrupts glomerular cell function or may cause apoptosis. We further speculate that the mutant protein may amplify injurious signals triggered by ligands such as angiotensin II that promote kidney injury and proteinuria. Clinical manifestations of renal disease do not appear until the 3rd decade in individuals with the *TRPC6*^{P112Q} mutation. This is in contrast to individuals with Finnish nephropathy and steroid-resistant nephrotic syndrome who typically develop proteinuria *in utero* or at birth. This delay may reflect the difference between these recessive disorders and the autosomal dominant mechanism of inheritance in our family, as such the presence of one normal *TRPC6* allele may postpone the onset of kidney injury. Patients with autosomal dominant FSGS due to mutations in the *ACTN4* gene also have a delayed onset of kidney disease. Because channels tend to be amenable to pharmacological manipulation, our study raises the possibility that *TRPC6* may be a useful therapeutic target in chronic kidney disease.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

SA3

Regulation of podocyte function by intracellular signalling

M. Saleem

Academic Renal Unit, University of Bristol, Bristol, UK

In diseases resulting in proteinuria, the delicate architecture of the podocyte is remodelled in response to as yet unknown signals. Crucial to understanding this has been the discovery of podocyte molecules that are mutated in hereditary proteinuric diseases, and which are functionally linked to the actin cytoskeleton. These molecules include nephrin, podocin and *TRPC6*,

which are localised to the podocyte slit diaphragm. It is becoming increasingly clear that the slit diaphragm is a signalling complex, crucial to morphological integrity, though regulation of this process is little understood.

A number of observations suggest that the podocyte response to external ligands is unique. For example TRPC6 is a widely expressed cation channel, yet activating mutations only affect the glomerulus (Winn et al. 2005). In clinical practice, nephrotic syndromes such as FSGS are thought to be caused by circulating plasma 'factors' that are directly toxic to the podocyte. We have shown that the circulating plasma protease, hemopexin, causes dramatic remodelling of the podocyte actin cytoskeleton, but has no effect on glomerular endothelial cells or fibroblasts. The slit diaphragm podocyte protein nephrin is restricted mainly to the podocyte, and recent data show that nephrin specifically signals to the actin cytoskeleton via the adaptor protein Nck (Jones et al. 2006). Our own studies now show that human plasma affects the podocyte in a unique way, affecting signalling to the actin cytoskeleton, subcellular localisation of nephrin and TRPC6 activation (Coward et al. 2005). These effects are dependent on the presence of nephrin, and we have evidence of a unique, nephrin-dependent, functionality of TRPC6 which switches this molecule between a plasma membrane ion channel and an intracellular calcium release channel. Additionally, the hemopexin effect is also dependent on nephrin.

Therefore we consider that nephrin is a central player in slit diaphragm signalling, and confers specificity to the way the podocyte responds to external ligands in health and disease.

Winn MP, Conlon PJ, Lynn KL, Farrington MK, Creazzo T, Hawkins AF, Daskalakis N, Kwan SY, Ebersviller S, Burchette JL et al. (2005). *Science* 308, 1801-4.

Jones N, Blasutig IM, Eremina V, Ruston JM, Bladt F, Li H, Huang H, Larose L, Li SS, Takano T et al. (2006). *Nature* 440, 818-823.

Coward RJ, Foster RR, Patton D, Ni L, Lennon R, Bates DO, Harper SJ, Mathieson PW & Saleem MA (2005). *J Am Soc Nephrol* 16, 629-37.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

SA4

Imaging ion fluxes in renal cortex *in vivo* using two-photon microscopy

J. Peti-Peterdi

Physiology, University of Southern California, Los Angeles, CA, USA

Multi-photon excitation fluorescence microscopy is a state-of-the-art confocal imaging technique ideal for deep optical sectioning of living tissues. It is capable of performing ultra-sensitive, quantitative imaging of organ functions in health and disease with high spatial and temporal resolution that other imaging modalities cannot achieve. Since the low cytotoxicity of multi-photon excitation allows continuous imaging of living tissues, real-time imaging of the tubuloglomerular feedback (TGF) and renin release mechanisms became possible. Novel, TGF-associated

morphological findings include significant cell volume changes of the macula densa under isotonic or hypertonic conditions, the existence of bulk fluid flow in the JGA, a sphincter-like contraction of the terminal, intraglomerular afferent arteriole, and the TGF-associated contraction of not only the afferent arteriole, but the entire intraglomerular mesangium. Spreading of the TGF vasoconstrictor signal in the JGA and beyond involves an extracellular ATP-mediated purinergic calcium wave. This wave was directly visualized with confocal microscopy to propagate from the macula densa and extraglomerular mesangial area to the afferent arteriole, along the vasculature to adjacent glomeruli, and also to all cells of the glomerulus including the most distant podocytes. Propagation of the TGF calcium wave from afferent arteriole smooth muscle cells to the underlying endothelium was also observed in these studies. This phenomenon may provide negative feedback and helps to balance the TGF vasoconstriction by triggering endothelium-derived vasodilator mechanisms. These imaging studies further emphasized the roles of both gap junctional communication and extracellular ATP as integral components of TGF. In addition, these studies provided functional evidence that complementing the afferent arteriolar vasoconstriction, all cells of the glomerulus actively participate in TGF by contracting the glomerular tuft, thereby helping to reduce the rate of glomerular filtration. The unexpected finding that the calcium wave of TGF was mediated by extracellular ATP provided further support that ATP itself is directly involved in TGF and not only through its breakdown to adenosine.

Renin release is the first, and at least initially, the rate-limiting step in the activation of the renin-angiotensin system which helps to maintain body salt and water balance. Additional details of the renin release mechanism were also observed using the multi-photon imaging approach. Acidotropic fluorophores including quinacrine and LysoTracker dyes (Invitrogen) are highly membrane permeant weakly basic compounds that rapidly accumulate in acidic cellular organelles. They have been successfully used to label renin granular content both *in vitro* and *in vivo*, and even as a counter stain on histological sections. Imaging the entire granular content as opposed to labeling specific molecules of interest (renin itself) is of great advantage when studying the mechanism and regulation of renin granule exocytosis. For example, there is a renewed interest in the enzymatically inactive prorenin, which is part of the granular contents and therefore its release may also be visualized even though it cannot be detected by existing assays measuring renin activity. Renin exocytosis has been visualized in real-time and on the individual renin granule level in response to a number of physiological stimuli including beta-adrenergic activation, low perfusion pressure and the macula densa mechanism. Dimming and disappearance of the entire granular content (quantal release) was observed within 2-300 ms. A significant number of renin granules was released into the interstitial side of the JGA, in addition to the vascular lumen. Not only the degranulation process, but enzymatic activity of the released renin (angiotensin I generation) was visualized in real-time using a FRET-based renin substrate.

In vivo visualization of cellular variables like cytosolic calcium in the collecting duct, intracellular pH in the proximal tubule, cell-to-cell communication and signal propagation will be shown. Basic kidney functions that can be measured by *in vivo* quantitative multi-photon imaging include glomerular filtration and permeability, concentration, dilution, and activity of the intra-renal renin-angiotensin system.