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Nephrin AKTs on actin: The slit diaphragm–actin cytoskeleton signaling network expands

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Regulation and maintenance of the actin cytoskeleton of podocytes has emerged as a critical process for preserving glomerular permselectivity. Signaling through nephrin, a crucial component of the slit diaphragm, can lead to rearrangement of the actin cytoskeleton. Zhu *et al.* identify phosphoinositide 3-kinase-dependent activation of Akt and Rac as mediators of nephrin-induced actin reorganization, expanding the signaling network linking two of the podocyte's unique structures, its actin cytoskeleton and the slit diaphragm.

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Podocytes, also known as glomerular epithelial cells, possess a unique cellular architecture, intertwining to cover the glomerular capillary loops with countless interdigitating foot processes. Alteration of this architecture through a simplification of the processes, often termed foot process effacement or fusion, appears to be a common pathologic response in proteinuric diseases. There has therefore been considerable scientific interest in how podocytes establish and maintain foot processes, and what factors could lead to their degeneration. The slit diaphragm and the podocyte's actin cytoskeleton have emerged as key contributors to the podocyte's function, and both are disrupted during foot process effacement.

The slit diaphragm is a specialized cell–cell adhesion structure that connects adjacent foot processes and is thought, together with the fenestrated endothelium and the capillary basement membrane, to maintain the permselectivity of the glomerulus (reviewed by Tryggvason *et al.*[1]). Nephrin was the first protein to be identified as an integral, and necessary, member of the slit diaphragm; mutations in nephrin are responsible for congenital nephrotic syndrome of the Finnish type.[2] Since that seminal discovery, the slit diaphragm has become a desirable neighborhood, and the list of proteins localized to this structure has steadily grown.[1] In addition to transmembrane proteins, multiple intracellular adaptor proteins, structural proteins, and signaling molecules have been reported to reside on the cytoplasmic face of the structure (reviewed by Benzing[3] and Faul *et al.*[4]). This has led to the realization that besides representing a specialized cell–cell adhesion, the slit diaphragm also plays two additional critical roles: as a signaling platform, and as an interface for the actin cytoskeleton.

Each foot process contains a central actin bundle in addition to a cortical network of short, branched actin fibers. These structures are disrupted during foot process effacement. In addition to connecting to the basement membrane via focal contacts, actin filaments also appear to link to the slit diaphragm through multiple adaptor proteins, including α-actinin, CD2AP, synaptopodin, and ZO-1 (reviewed by Faul *et al.*[4]) (Figure 1a). Mutations in several of these adaptors are known to lead to podocyte abnormalities and proteinuric kidney disease in humans and mice. The demonstration that the slit diaphragm protocadherin FAT1 binds to Ena/VASP proteins and can thereby induce actin polymerization[5] hinted that the slit diaphragm not only bound to the actin cytoskeleton but might regulate its assembly.

Evidence that nephrin itself played a critical role in regulating actin dynamics was presented in a series of papers.[6–8] These studies demonstrated that clustering of nephrin leads to phosphorylation of its cytoplasmic domain by the Src-family tyrosine kinase Fyn, which had been previously described, generating binding sites for the SH2 domain of Nck1 and Nck2. Nck1 and Nck2 are both adaptor proteins characterized by a single SH2 domain and several SH3 domains, which are capable of recruiting various regulators of the actin cytoskeleton, such as N-WASP and Pak (Figure 1b). The importance of the Nck proteins in podocyte function is underscored by the phenotype of mice lacking both nck genes in podocytes: they fail to develop foot processes and suffer from proteinuria and glomerulosclerosis.[6] Furthermore, clustering of ectopically expressed nephrin or nephrin cytoplasmic domain leads to Nck recruitment and localized actin polymerization[6,8] and, in some cases, global changes in cell morphology.[7] Using antibodies raised against a specific phosphotyrosine epitope of nephrin, Verma *et al.*[8] further demonstrated that nephrin is tyrosine-phosphorylated at Y1191 during mouse development, and again during protamine-induced foot process effacement, but not during normal adult life. This is in contrast to findings reported from rat glomeruli, where nephrin is constitutively recognized by an anti-phosphotyrosine antibody, but phosphorylation decreases during puromycin aminonucleoside-induced podocyte injury.[7] Whether this discrepancy represents a species difference, a result of different specificities of the antibodies, or other technical issues, remains to be resolved. In any case, these studies do demonstrate a pathway through which nephrin could recruit the machinery necessary to initiate actin polymerization.

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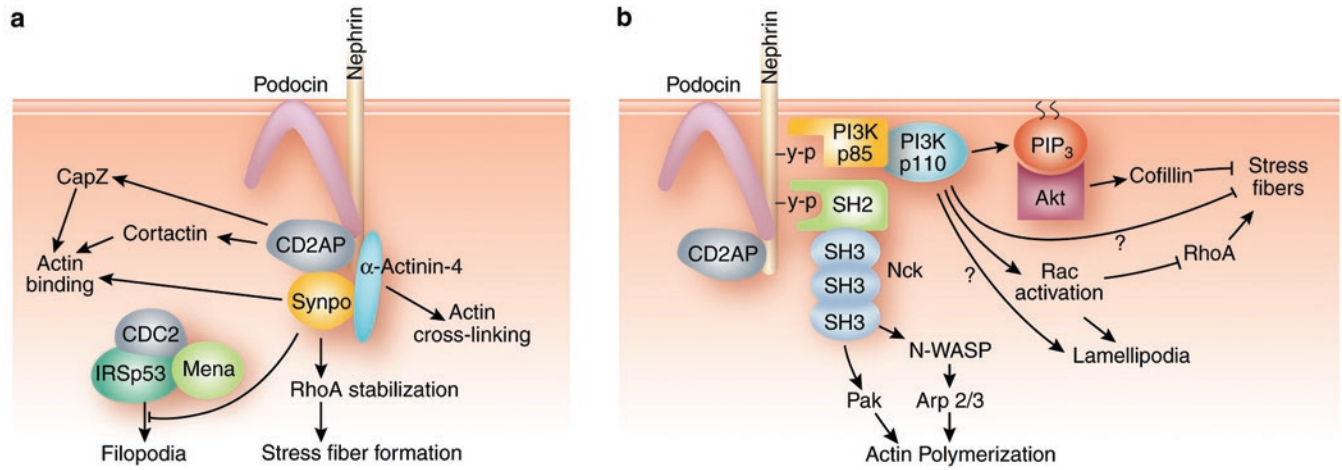

Figure 1 | The nephrin–actin network. (**a**) A subset of interactions through which nephrin is proposed to be linked to the actin cytoskeleton. Nephrin is shown in the unphosphorylated state. However, the effect of phosphorylation on most of the interactions depicted here has not been reported. (**b**) Nephrin interactions in response to tyrosine phosphorylation, such as can be mediated by Fyn. The *in vivo* signals regulating nephrin phosphorylation remain obscure. Phosphoinositide 3-kinase (PI3K) binding depends on Y1152 of rat nephrin, whereas Y1204 and Y1228 (and Y1191 in mice) lie within Nck SH2 binding consensus sequences. PI3K activation leads to an increase in PIP_3 at the inner leaflet, recruiting various PH domain-containing proteins, including Akt and Rac guanine-nucleotide exchange factors. In the case of downstream effects of Akt and Rac activation, arrows are not meant to imply a direct effect. Please see Faul *et al.*[4] for additional network connections between the actin cytoskeleton and slit diaphragm proteins other than nephrin. Synpo, synaptopodin.

Zhu *et al.*[9] (this issue) examine the role of phosphoinositide 3-kinase (PI3K) in mediating nephrin's effect on the actin cytoskeleton. The 85-kilodalton regulatory domain of PI3K has previously been shown to bind to both nephrin and its binding partner, CD2AP, in a phosphorylation-dependent manner.[10] Furthermore, overexpression of nephrin in murine podocyte cell lines was reported to stimulate the PI3K–AKT pathway, thereby enhancing AKT-mediated pro-survival signals. Zhu *et al.*[9] confirm the phosphorylation-dependence of the nephrin–PI3K interaction, further demonstrating that overexpression of Fyn is sufficient to mediate this interaction. By mutation analysis, nephrin Y1152 is shown to be necessary for the interaction. This is of particular interest, as Y1152 does not represent a Nck binding site. The authors then proceed to examine the effect of overexpressing nephrin and podocin in a rat podocyte cell line. They find that overexpression of these two proteins leads to activation of two downstream targets of PI3K, Akt and Rac, a small Rho-GTPase (Figure 1b). The activation of both of these effectors is abolished by a Src-kinase inhibitor, a PI3K inhibitor, or the Y1152F mutation in nephrin. In addition, nephrin overexpression leads to decreased RhoA activity and decreased cofilin phosphorylation (and thus presumed increased cofilin activation).

In light of the known ability of PI3K and its effectors to induce actin cytoskeleton rearrangement,[11] Zhu *et al.*[9] examine the effect of overexpressing nephrin and podocin on the actin cytoskeleton of rat podocytes. Nephrin overexpression led to a loss of stress fibers and cortical actin, with a concomitant increase in lamellipodia formation. Using a series of constitutively active and dominant-negative forms of Rac and Akt, the authors demonstrate that Rac is probably at least partially responsible for the increase in lamellipodia seen upon nephrin expression, and that Akt1 is able to reduce stress fibers in podocytes but is not necessary for nephrin-mediated inhibition of stress fibers. Interestingly, overexpression of Y1152F mutant nephrin decreased lamellipodia formation and increased stress fiber formation in relation to wild-type glomerular epithelial cells, suggesting either that this mutant inhibits subtle effects mediated by endogenous nephrin, or that other pathways antagonistic to PI3K effectors (such as those mediated by synaptopodin) may also be activated by nephrin.

Finally, as would be predicted from the reported decrease in nephrin phosphorylation in rats treated with PAN to induce transient proteinuria,[7] Zhu *et al.*[9] report a decrease in the nephrin–PI3K interaction and a decrease in Akt phosphorylation *in vivo* after PAN treatment.

Two important caveats must be kept in mind in contemplating the results presented by Zhu *et al.*:[9] (1) The signaling events and actin rearrangements described all depended on the overexpression of nephrin. It remains very much a matter of speculation what might regulate nephrin phosphorylation and PI3K activation at the slit diaphragm *in vivo*. This is of particular interest given the discrepancy in the data described above regarding nephrin phosphorylation *in vivo*. (2) A large canyon lies between the cytoskeletal arrangement of podocyte cell lines and their *in vivo* counterparts, making any conjecture about how loss of stress fibers or increased lamellipodia in tissue culture relates to foot process effacement *in vivo* highly speculative.

In summary, Zhu and colleagues[9] have added substantially to the list of proteins through which nephrin appears to modulate the podocyte cytoskeleton.

Two points stand out as being of particular interest: (1) Nck binding and PI3K binding appear to depend on distinct tyrosine motifs, though binding of both appears to be dependent on Fyn. (2) Nephrin-mediated PI3K activation leads to a loss of stress fibers, whereas another slit diaphragm-associated protein, synaptopodin, has been reported to enhance stress fiber formation.[12]

Several interesting questions will now need to be addressed: What are the *in vivo* stimuli that lead to nephrin phosphorylation? Are Nck and PI3K recruited to and activated by nephrin independently, simultaneously? Once activated, are the signaling pathways they activate complementary, synergistic, or antagonistic? Do synaptopodin and PI3K play antagonistic roles at the slit diaphragm? Perhaps most importantly, what role do these different pathways play *in vivo* in the creation of foot processes during development and during foot process effacement and recovery in adult life? Although much remains to be discovered, it appears clear that the web connecting the slit diaphragm and the actin cytoskeleton will continue to grow in complexity and in importance for proteinuric kidney disease.

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Animal models of renal disease

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Mice have become a favored species to model disease. Many mouse strains have proven relatively resistant to some manipulations that have generated renal disease in other species. Kirchhoff *et al.* describe a means of producing hypertension, proteinuria, and glomerular sclerosis in a mouse strain.

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Clinical work is not enough. The all-important thing is to start the derivation of first approximation answers to clinical questions through experimental work on animals.

—Thomas Addis[1]

Animal models have been an essential component of medical research for centuries. Fulfilling Koch's postulates usually requires an animal model as one step. Many mechanistic questions can be answered only through invasive procedures or extreme exposures possible only in animals. Development of therapies and detection of toxicities almost always involve animal testing. To be sure, models can sometimes be uninformative or even misleading. Rats resist diphtheria toxin, but guinea pigs (and humans) succumb to it. Attempts to induce lung cancer with cigarette smoke in animals failed. Fleming's initial studies of penicillin in rabbits may have retarded its trial in humans, as it was rapidly cleared in the animal.

Mice have become a favored species for study because their gene expression can be altered with relative ease. Indeed, this year's Nobel Prize in Physiology or Medicine went to three scientists instrumental in making gene targeting possible. Models that test the alteration of a gene suspected of having a key role have in some cases supported that suspicion.[2] In other cases, the lack of phenotype in a knockout mouse may be perplexing.[3] Overexpression has also been useful in testing a pathway of injury, for example, transforming growth factor-β.[4] For medical researchers, the weights of both the positive and the negative results depend, of course, on whether the gene of interest functions and is regulated similarly in mice and humans. This may not always be the case.[5]

Mouse models using single gene knockout or overexpression have in many cases produced palpable renal disease and have in some cases produced diseases very faithful to the human condition. However, mice have generally not been tractable for producing more complex

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