blunt-ended substrates. Perhaps, in the case of longer DEAD-box proteins, the flanking amino- and carboxy-terminal extensions direct the protein to a single-stranded overhang and increase its local concentration. This hypothesis cannot be tested in the light of the Vasa structure, as the fragment of Vasa that was crystallized lacked most of the residues outside of the core domain. However, it is in accordance with data from the bacterial DEAD-box proteins CsdA and SrmB, which need a minimum length of the single-stranded tail of the substrate for efficient activity (Bizebard et al., 2004). The absence of the flanking sequences from the structure also makes it impossible to fully model the binding sites for Vasa-specific partner proteins such as eIF5B and Gustavus (Styhler et al., 2002).

Another key issue that will require further experimentation is how substrate specificity is achieved. The particular role for Vasa in germline development suggests that it regulates only certain substrate RNAs, but the basis for this specificity is not clear from its structure, which seems to rule out sequence specificity in RNA binding. Conceivably, the flanking regions, protein cofactors, or posttranslational modifications are involved in conferring specificity to particular target RNAs. Therefore, defining the target dsRNA or RNA-protein complexes for Vasa binding remains a crucial point for further understanding of its function. Another crucial question is the regulation of DEAD-box protein activity. It is known from other DEAD-box proteins, such as yeast Ded1, which is closely related to Vasa (Cordin et al., 2004), that binding to RNA is largely stimulated by ATP binding, but binding to RNA stimulates ATP hydrolysis. Thus, to function, the DEAD-box protein needs to be directed in a timely fashion to its target RNA or kept inactive and then activated in a timely manner. How this occurs is another challenge for future experiments in the field.

REFERENCES

Benz, J., Trachsel, H., and Baumann, U. (1999). Structure *15*, 671–679.

Bizebard, T., Ferlenghi, I., Iost, I., and Dreyfus, M. (2004). Biochemistry *43*, 7857–7866.

Cordin, O., Tanner, N.K., Doere, M., Linder, P., and Banroques, J. (2004). EMBO J. *23*, 2478–2487.

Johnstone, O., and Lasko, P. (2004). Development *131*, 4167–4178.

Linder, P., Lasko, P.F., Ashburner, M., Leroy, P., Nielsen, P.J., Nishi, K., Schnier, J., and Slonimski, P. (1989). Nature *337*, 121–122.

Raz, E. (2000). Genome Biol. *1*, REVIEWS1017. 10.1186/gb-2000-1-3-reviews1017.

Sengoku, T., Nureki, O., Nakamura, A., Kobayashi, S., and Yokoyama, S. (2006). Cell *125*, this issue.

Story, R.M., Li, H., and Abelson, J.N. (2001). Proc. Natl. Acad. Sci. USA *98*, 1465–1470.

Styhler, S., Nakamura, A., and Lasko, P. (2002). Dev. Cell *3*, 865–876.

Tanner, N.K., and Linder, P. (2001). Mol. Cell *8*, 251–262.

Nck Links Nephrin to Actin in Kidney Podocytes

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Two papers, one in *Nature* (Jones et al., 2006) and the other in the *Journal of Clinical Investigation* (Verma et al., 2006) show that Nck adaptor proteins connect phosphorylated nephrin with actin polymerization in podocyte foot processes, structures important for slit-diaphragm formation in the kidney. Their results further our understanding of podocyte development and repair in glomerular disease.

The glomerular podocytes contribute to the kidney filter in a unique manner. They extend primary processes to the capillary surface where they form fine secondary foot processes that interdigitate with foot processes of a neighboring podocyte (Figure 1). This interdigitation results in a 40 nm wide slit between foot processes which contains a porous ultrafilter called the slit diaphragm. The foot processes have coiled actin microfilaments along their axis. The slit diaphragm proper is formed by the extracellular domains of specific transmembrane proteins, such as nephrin, the Neph proteins, and two large cadherins FAT1 and FAT2. Little is known about the extracellular interactions between the different SD proteins, but nephrin mol-

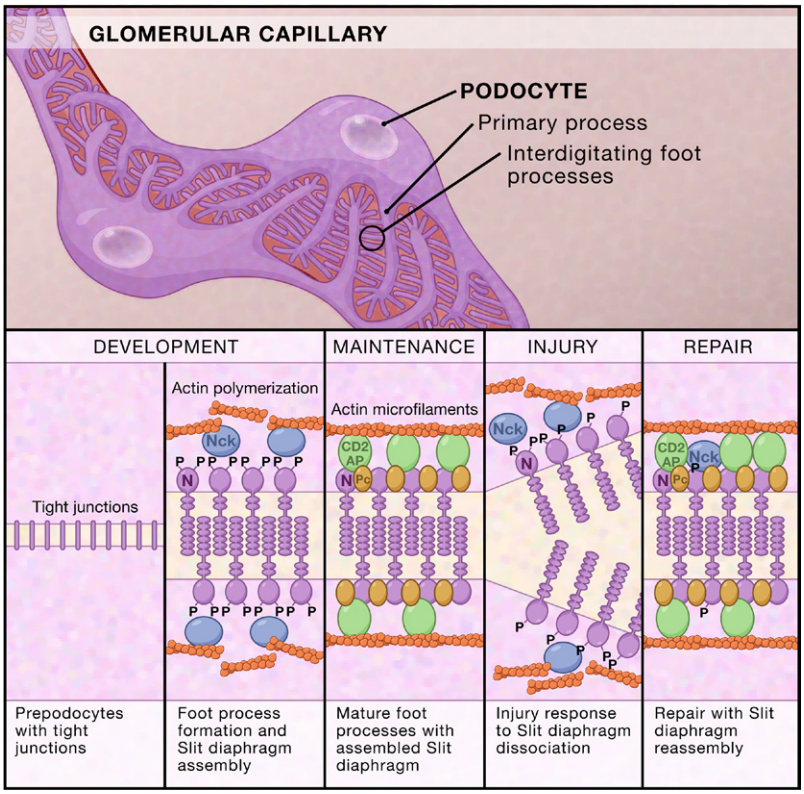

Figure 1. Glomerular Podocytes and the Slit Diaphragm

(Top panel) Podocytes cover the outside wall of the glomerular capillaries and extend primary processes that are attached to the surface of the glomerular basement membrane. Secondary foot processes extend from the primary processes to interdigitate with foot processes of a neighboring podocyte. The slit between the foot processes contains a thin porous filter called the slit diaphragm. (Bottom panel) Prior to formation of the foot processes and the slit diaphragm, prepodocytes covering the glomerular capillaries are connected by tight junctions. During formation of the foot processes, nephrin (N) becomes phosphorylated (P), possibly upon encountering its extracellular ligand, which results in recruitment of Nck adaptor proteins and induction of actin polymerization. Once the slit diaphragm is assembled, the nephrin molecules are dephosphorylated and nephrin is connected to actin through CD2AP, podocin (Pc), and possibly some other proteins. Following injury of the slit diaphragm leading to foot-process effacement and proteinuria, nephrin molecules become clustered, which induces their phosphorylation, Nck association, and actin polymerization. Finally, upon injury repair, the nephrin molecules are dephosphorylated and the slit diaphragm-actin filament complex is restored as in normal mature foot processes.

ecules of adjacent foot processes are believed to interact in the center of the slit. Furthermore, they have been shown to interact with Neph1. Intracellularly, proteins important for podocyte structure, ZO-1, podocin, and CD2AP, are located in the slit-diaphragm region and they are thought to play a role in the connection of the slit diaphragm to the actin microfilaments (for review, see Tryggvason et al. [2006]).

From a developmental standpoint, how the foot process and the slit diaphragm form are central questions. The slit diaphragm originates from apically located tight junctions of prepodocytes. During formation of the glomerulus, these apical-junction complexes migrate toward the basal surface, and prior to reaching it, nephrin appears in the complex. This appearance of nephrin is associated with the development of foot processes and appearance of CD2AP and other slit-diaphragm proteins. The outgrowth of foot processes from the primary process requires polymerization of actin microfilaments and their connection to nephrin and other slit-diaphragm proteins. The proteins that are crucial for this process have not been identified. Also unknown are the molecular events that occur in slit-diaphragm injury when plasma proteins leak into the urine (proteinuria), a condition frequently accompanied by retraction of the foot processes (a phenomenon referred to as "effacement"). In proteinuria disorders, the effacement can be irreversible, leading to progressive proteinuria and development of end-stage renal disease, or it is reversible with restoration of the foot processes and slit diaphragm.

In addition to its role as a structural component of the slit diaphragm, nephrin has been implicated in podocyte intracellular signaling. Nephrin contains several tyrosine residues in the cytoplasmic domain that are conserved from humans to zebrafish. Some of these reside within motifs which, if phosphorylated, could provide docking sites for SH2 domain-containing kinases or adaptor proteins. Indeed, several groups have reported that nephrin is phosphorylated on tyrosine residues in vivo by the Src family kinase Fyn (Lahdenpera et al., 2003; Li et al., 2004; Simons et al., 2001; Verma et al., 2003). Fyn was found to associate with nephrin and become rapidly but transiently activated upon clustering of nephrin (induced by antibodies). Nephrin also interacts with PI 3-kinase in a phosphotyrosine-dependent manner. Additionally, nephrin tyrosine phosphorylation appears to modulate podocyte gene expression as well as the association of nephrin with podocin (Huber et al., 2001; Li et al., 2004). Whether or not nephrin tyrosine phosphorylation influences the development of foot processes or glomerular injury and repair has remained unknown. So far the only hint of a possible connection has been the observation that Fyn kinase knockout mice develop proteinuria and foot-process effacement.

Two new reports, recently published in *Nature* (Jones et al., 2006) and the *Journal of Clinical Investigation* (Verma et al., 2006), provide

compelling evidence that nephrin tyrosine phosphorylation controls podocyte cell morphology through the Nck adaptor proteins (Figure 1). The Nck subfamily of adaptor proteins, which has been implicated in the regulation of actin dynamics, contains two members, Nck1 and Nck2. Both proteins contain one SH2 and three SH3 domains. The interaction between phosphotyrosines and the SH2 domain brings Nck to the site of action, where actin polymerization is mediated by the Nck SH3 domain, which recruits various proteins involved in the regulation of actin cytoskeleton including N-WASp, and protein kinases such as Pak and Abl. Jones et al. (2006) identified the major Src family kinase phosphorylation sites on the human nephrin cytoplasmic domain and showed that each of these sites, Y^{1176}DEV, Y^{1193}DEV, and Y^{1217}DQV, binds Nck. In fact, a triple mutant with Tyr to Phe substitutions at these positions completely abolished the interaction of nephrin with Nck. Verma et al. (2006) showed that Fyn phosphorylates at least five tyrosines on the mouse nephrin cytoplasmic domain and that the Y^{1176} and Y^{1193} are crucial for the interaction with Nck. Previous studies showed that mice deficient for either of the two Nck proteins are viable with no obvious kidney defects. The double knockouts, on the other hand, are early embryonic lethal. Both groups showed that both Nck1 and Nck2 are expressed in podocytes, and Jones et al. (2006) created a podocyte-specific ablation of *Nck2* in Nck1-deficient mice. These mice developed proteinuria associated with pathological changes in the glomeruli. Newborn mice and 16.5-day-old embryos lacked foot processes, whereas wild-type embryos of the same age have foot processes and slit diaphragms. They concluded that *Nck* ablation in podocytes leads to severe defects in foot-process formation.

In cultured cells, both groups provided ample evidence that the observed phenotypes are at least partly due to the absence of the nephrin-Nck interaction. For example, Jones et al. (2006) showed that in Nck1/Nck2-knockout fibroblasts with ectopic Nck2 expression, clustering of a chimeric nephrin receptor that had an intact cytosolic domain induced Src family kinase-dependent tyrosine phosphorylation of this domain and association of Nck2 and its effector molecule N-WASp. These downstream signaling events did not occur with the triple Tyr to Phe nephrin mutant. Strikingly, the clustering of the intact cytosolic domain of nephrin resulted in actin polymerization that colocalized with ectopic Nck2. Similar actin polymerization was not seen in the Nck1/Nck2 knockout cells without restored Nck2 expression or in the Nck2-restored cells expressing the chimeric nephrin triple mutant.

These findings strongly linked nephrin tyrosine phosphorylation to the rearrangement of the actin cytoskeleton in foot processes and indicate that the Nck adaptor proteins function as mediators of this connection. Interestingly, the nephrin cytoplasmic domain contains multiple Nck binding sites. Are all these sites biologically significant? In this regard, it is interesting to note that a high local concentration of the Nck SH3 domains on the plasma membrane was found to be sufficient to induce cytoskeletal rearrangements (Rivera et al., 2004).

Although actin filaments are dynamic structures, it is possible that in a steady-state situation, the rate of actin turnover in the podocyte foot processes is low, and there is no need for a high number of nephrin-Nck interactions. However, the situation is likely to be different during the recovery process after foot-process effacement, when rapid actin polymerization and cytoskeletal reorganization is needed. Indeed, transient induction of nephrin tyrosine phosphorylation has been detected in many animal models with foot-process effacement. To support this idea, Verma et al. (2006) found nephrin to be phosphorylated transiently in a proteinuria model with foot-process effacement using an antibody specific for nephrin phosphorylated at the Nck binding sites. The same antibodies also recognized epitopes in developing glomeruli, further demonstrating the association of nephrin phosphorylation with foot-process and slit-diaphragm formation. One could envision that when the cytoskeleton has been rearranged and nephrin redistributed to its correct position, its level of tyrosine phosphorylation decreases, which results in a low number of nephrin-Nck interactions. Thus, it is very likely that Nck does not mediate the association of nephrin to the cytoskeleton at all times. Once the actin filaments have been assembled and stabilized, other interactions, such as those mediated by CD2AP (Shih et al., 1999), may be important. The concept that nephrin has Nck-independent interactions with the cytoskeleton is supported by the fact that nephrin knockout mice die within the 24 hr after birth (Tryggvason et al., 2006), whereas the podocyte Nck-knockout mice survive the neonatal period.

To substantiate the results of these two studies, it would be important to generate nephrin knockin mice carrying point mutations at its major tyrosine phosphorylation sites. The existence of such mice could reveal the role of the nephrin-Nck interaction in vivo. Do these mice develop proteinuria? If not, how do they respond in an experimental proteinuria model? Another question is how the first foot processes develop during the embryonic development. How does nephrin become activated in that situation? Does transient tyrosine phosphorylation occur when nephrin encounters its extracellular ligand? What about mice in which Nck function is ablated by repressing its expression from an inducible promoter in podocytes? Shutting off Nck expression in adult mice will reveal the function of the nephrin-Nck interaction in maintaining foot processes. Although normal-like foot processes are not completely absent from the kidneys of patients with mutations in NPHS1 (the gene that encodes nephrin) or the nephrin-knockout mice, it is very likely that the nephrin-Nck

interaction has an important function both in the formation and maintenance of the foot processes. These and other questions await answers; however, the two papers by Jones et al. (2006) and Verma et al. (2006) have provided new central pieces to help solve the puzzle of identifying the molecular mechanisms of podocyte foot-process development, injury, and repair.

REFERENCES

Huber, T.B., Kottgen, M., Schilling, B., Walz, G., and Benzing, T. (2001). J. Biol. Chem. *276*, 41543–41546.

Jones, N., Blasutig, I.M., Eremina, V., Ruston, J.M., Bladt, F., Li, H., Huang, H., Larose, L., Li, S.S., Takano, T., et al. (2006). Nature. Published online March 8, 2006. 10.1038/nature04662.

Lahdenpera, J., Kilpelainen, P., Liu, X.L., Pikkarainen, T., Reponen, P., Ruotsalainen, V., and Tryggvason, K. (2003). Kidney Int. *64*, 404–413.

Li, H., Lemay, S., Aoudjit, L., Kawachi, H., and Takano, T. (2004). J. Am. Soc. Nephrol. *15*, 3006–3015.

Rivera, G.M., Briceno, C.A., Takeshima, F., Snapper, S.B., and Mayer, B.J. (2004). Curr. Biol. *14*, 11–22.

Shih, N.Y., Li, J., Karpitskii, V., Nguyen, A., Dustin, M.L., Kanagawa, O., Miner, J.H., and Shaw, A.S. (1999). Science *286*, 312–315.

Simons, M., Schwarz, K., Kriz, W., Miettinen, A., Reiser, J., Mundel, P., and Holthofer, H. (2001). Am. J. Pathol. *159*, 1069–1077.

Tryggvason, K., Patrakka, J., and Wartiovaara, J. (2006). N. Engl. J. Med. *354*, 1387–1401.

Verma, R., Wharram, B., Kovari, I., Kunkel, R., Nihalani, D., Wary, K.K., Wiggins, R.C., Killen, P., and Holzman, L.B. (2003). J. Biol. Chem. *278*, 20716–20723.

Verma, R., Kovari, I., Soofi, A., Nihalani, D., Patrie, K., and Holzman, L.B. (2006). J. Clin. Invest. Published online March 16, 2006. 10.1172/JCI27414.

When a Motor Goes Bad: A Kinesin Regulates Neuronal Survival

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In this issue of *Cell*, Midorikawa et al. (2006) demonstrate that the kinesin superfamily member KIF4, a microtubule-based molecular motor, regulates the survival of electrically active neurons in the developing brain by modulating the function of poly(ADP-ribose) polymerase-1 in an unexpected way.

Naturally occurring neuronal cell death is a fundamental process whereby approximately half of the neurons produced in the mammalian nervous system die as part of a developmental mechanism to ensure the establishment of appropriate neural connections. Observations from Victor Hamburger and Rita Levi-Montalcini more than 50 years ago demonstrated that limiting amounts of trophic factors made by developing target tissues determined which neurons lived or died during this cell death period (Levi-Montalcini, 1987). Further studies made it apparent that trophic factors work in concert with neuronal activity—which regulates cell survival by increasing the concentration of cytosolic calcium—to precisely sculpt and refine the connections made in the developing nervous system. Identifying the intracellular mediators that regulate neuronal survival in response to these two environmental signals is thus important not only for our understanding of development but also for our efforts to devise approaches to maintain neuronal survival in the face of traumatic injury or neurodegenerative disorders.

Although great strides have been made toward identifying the key signals that allow trophic factors to regulate naturally occurring cell death, less progress has been made with neuronal activity. In this issue of *Cell*, Midorikawa et al. (2006) examine activity-dependent cell survival in developing central nervous system (CNS) neurons and come up with the surprising and unexpected finding that a kinesin superfamily protein (KIF4) is a crucial determinant of neuronal cell death. Members of the kinesin superfamily (KIFs) have long been known as neuronal molecular motors that move along microtubules, transporting cargo from the cell body to, and in some cases from, axons and dendrites (Hirokawa and Takemura, 2005). Forty-five members of the