Life Without Nephrin: It’s for the Birds

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It is difficult to conceive how podocytes establish slit diaphragms without nephrin, but a recent paper\(^1\) suggests that an entire class of vertebrates have podocytes that are able to do just that. Why did this discovery take so long, and why should it be surprising or exciting? The answer to the first question may simply be that no one had thought to look; some thoughts about the second question follow.

Our understanding of glomerular cell biology and physiology, as well as the direction of glomerular disease research, was changed forever by the discovery of Karl Tryggvason and colleagues in 1998 that \(NPHS1\)/nephrin is mutated in congenital nephrotic syndrome of the Finnish type.\(^2\) Nephrin is a transmembrane protein with eight extracellular immunoglobulin-like domains, a fibronectin type 3 domain, a single pass transmembrane domain, and a cytoplasmic tail. After its discovery, nephrin became a major focus of research efforts around the world and was recently referred to as the signature molecule of the glomerular podocyte.\(^3\)

It has been reported that nephrin is a required structural component of the glomerular slit diaphragm; forms a pore structure capable of restricting the passage of albumin-sized molecules; binds numerous other proteins that are both present at the slit diaphragm and required for a proper filtration barrier; and is a lipid raft-associated signaling molecule at the slit diaphragm that undergoes tyrosine phosphorylation and is linked to the actin cytoskeleton within podocyte foot processes.\(^4–6\) Although many questions still remain, these results provide important mechanistic insights into the potential roles of nephrin and the slit diaphragm in maintaining podocyte health and in establishing and maintaining a size-selective barrier to albumin.

Despite its weak homology (<50% similarity) to some other immunoglobulin superfamily molecules, such as the Nep, hemicentin, and roundabout proteins, mammalian nephrin comprises a family of one; there are no closely homologous proteins, either in sequence or structure, that would be capable of fully compensating for its absence. In contrast, there are three members of the mammalian Neph family (paralogs), each with significant homology to one another, as well as to the irregular-archiasm-C (irreC)/roughest and kin of irreC (KIRRE) proteins of \(Drosophila\) and the SYG-1 protein of \(Caenorhabditis\) elegans. Similarly, nephrin exhibits clear homology of sequence and structure to the \(Drosophila\) sticks and stones (SNS) and hibris proteins and to nematode SYG-2.

Studies in these two invertebrates have shown that the nephrin and Neph-like proteins interact with each other in a heterophilic fashion to carry out their developmental roles. For example, in \(Drosophila\), an interaction between KIRRE (Neph-like) and SNS (nephrin-like) leads to formation of a slit diaphragm-like structure in the nephrocyte, which has a filtration-related function.\(^7,8\) Moreover, all three mouse Neph proteins, when expressed in the appropriate cell, can partially substitute for nematode SYG-1 (Neph-like) in synaptogenesis by interacting with SYG-2 (nephrin-like) in an adjacent cell.\(^9\) This finding demonstrates a high degree of functional conservation even after hundreds of millions of years of evolutionary divergence. Furthermore, given that mouse nephrin and Neph1 have been shown to physically interact,\(^10,11\) and mutation of either one causes nephrotic syndrome,\(^12–15\) a functional interaction between nephrin and Neph family members in podocytes, and perhaps in other cell types, would logically be a requirement across diverse species.

In a very recent paper in \(Histochemistry and Cell Biology,\) Kispert and colleagues\(^1\) investigate the expression of Neph proteins in mouse and chick embryos by \textit{in situ} hybridization. Their goal was to determine in detail the expression patterns of Neph1, Neph2, and Neph3 to gain insights into their potential functions, with a focus on the nervous system. Unlike nephrin, which is expressed in only a few nonrenal cell types,\(^12\) Neph proteins are expressed widely.\(^1,13,16\) This suggests that heterophilic interactions between nephrin and Neph proteins can only be required for a limited number of specific cellular processes, such as, for example, podocyte slit diaphragm formation and/or maintenance.

In their paper, the authors show that all three Neph paralogs are expressed in the developing CNS of the mouse embryo, and they reasonably conclude these proteins could be involved in axon pathfinding.\(^1\) The fact that other studies have found nephrin to be expressed in radial glia in the cerebellum\(^12\) is consistent with the notion that heterophilic interactions between nephrin and Neph proteins could be involved in some aspects of neural development. However, interactions between Neph family members (either homophilic or heterophilic) are also likely to occur, given the very limited domain of...
nephrin expression in the CNS and the fact that children with severe nephrin gene mutations do not usually exhibit neurologic defects.17

Of the Neph proteins, Neph3 exhibits the most restricted pattern of expression during mouse development, being found in pancreas bud, otic vesicle, metanephros, and some CNS segments. In contrast, Neph1 and Neph2 are expressed more widely, with at least one of the two expressed in limb bud, lung, heart, branchial arches, pituitary, and other sites, in addition to the nervous system.1

When the authors turned to performing similar analyses in the developing chick, they found that not only does the chicken genome lack Neph3, which is not too surprising given the evolutionary distance between mammals and birds, but it also lacks nephrin.1 However, despite the absence of nephrin, chickens have glomeruli with the very familiar three-layered capillary wall structure: a fenestrated endothelium, a glomerular basement membrane, and podocytes with foot processes connected by what appear to be typical slit diaphragms.18,19

This unexpected finding leads to important questions about the nature of the glomerular filtration barrier in birds, as well as in mammals, and it should challenge our perception of the slit diaphragm, which has been viewed and treated as a requisitely nephrin-based structure for over a decade.

A basic question to consider. How can chickens establish a slit diaphragm, clearly visible by electron microscopy,18,19 without nephrin? One obvious explanation is that Neph1 and/or Neph2 may be participating in interactions between adjacent foot processes. However, nephrin and Neph3 have dissimilar extracellular domain sizes (~115 and ~55 kD, respectively), and Neph3 lacks a fibronectin type 3 domain. Thus, the physical and structural characteristics of what might be a Neph-based chicken slit diaphragm should be very different from those of a nephrin-based slit diaphragm. On the other hand, slit diaphragms certainly contain other types of transmembrane junctional proteins, including placental (P)-cadherin20,21 and the giant atypical cadherin Fat122; mutation of the latter causes a lack of both slit diaphragms and podocyte foot processes,23 demonstrating its importance. These proteins may be playing cooperative roles in maintaining the integrity of the chicken slit diaphragm and could contribute to its ultrastructural characteristics and perhaps also to those of the mammalian slit diaphragm.

A second question—how do chickens retain serum albumin, which is ~66 kD and homologous to mammalian serum albumin, in the blood? If one subscribes to the view that the glomerular basement membrane plays the major role as an albumin barrier,24–26 that the endothelial glyocalyx is paramount,27,28 or that electrical forces matter most,29 the lack of nephrin in avian podocytes is not so difficult to reconcile with an efficient albumin barrier.

However, what still needs explanation is why does the lack of nephrin in mice, humans, and zebrafish30 cause heavy proteinuria? Perhaps the bird is telling us that it is the lack of slit diaphragms—rather than the lack of nephrin per se—that causes albuminuria. Birds have apparently evolved different mechanisms to establish and maintain slit diaphragms, which may have more important roles in regulating flow across the capillary wall than in conferring permselectivity.24 Such mechanisms might involve signaling by Neph proteins rather than nephrin; both nephrin and Neph proteins have substantial cytoplasmic tails capable of being tyrosine phosphorylated and interacting with various signaling, scaffolding, and polarity proteins, including podocin and Par3.31–33

In any event, comparative studies of the composition and function of mammalian, fish, and bird slit diaphragms may provide important clues as to how defective slit diaphragms associated with human glomerular disease—even in cases with nephrin defects—might be remedied.

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DISCLOSURES

None.

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Induction of IgA Deposits and Glomerulonephritis by IgA Rheumatoid Factor

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In this issue of *JASN*, Otani et al.1 describe an interesting murine model for studies of IgA-associated GN. The authors explore the nephritogenic potential of two different monoclonal IgA rheumatoid factors (designated as 6-19 IgA and 46-42 IgA) specific for murine IgG2a in relation to potential differences in glycosylation of these IgA antibodies. Previous research by this group showed that 6-19 IgG3 anti-IgG2a rheumatoid factor monoclonal antibody, derived from lupus-prone MRL-Fas+ mice, induces glomerular lesions.2 These lesions are characterized by IgG subendothelial deposits of the IgG3 cryoglobulin. To assess the role of the IgG3 heavy chain...