THREE-DIMENSIONAL STRUCTURE OF THE GLOMERULAR SLIT DIAPHRAGM AS REVEALED BY ELECTRON TOMOGRAPHY

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The renal glomerular filter constitutes three layers: a fenestrated endothelium, glomerular basement membrane (GBM), and the slit diaphragm located between the interdigitating foot processes of the podocytes that cover the capillary surface. While the unique morphology of the glomerular filtration barrier has been well known for decades, the molecular nature of the filtration barrier, thought to be a size- and charge-selective filter, has been poorly understood. However, in recent years, the significant role of the slit diaphragm has become increasingly appreciated (1, 2).

The slit diaphragm has for long been observed in electron microscopy as a fine line between the podocyte foot processes. In the 1970s Rodewald and Karnovsky (3) proposed, based on their electron microscopic findings, that the slit diaphragm is an about 40 nm wide, isoporous zipper-like structure where staggered cross-bridges extend from adjacent podocytes to a longitudinal central filament and form rectangular pores in the slit diaphragm. This model was questioned following results with freeze-etching replicas of unfixed rat kidney, which suggested a sheet-like, rather than a zipper-like substructure (4). The molecular nature of this vital ultrafilter remained obscure until in the late 1990s.

Through positional cloning of the gene mutated in congenital nephrotic syndrome of the Finnish type (CNF), nephrin, a unique component of the slit diaphragm was identified (5). Nephrin that is specifically located in podocyte foot processes (6), is a transmembrane protein with a short intracellular domain short intracellular domain rich in serine and tyrosine residues, a transmembrane domain and an extracellular domain containing eight distal IgG-like motifs and a proximal fibronectin type III repeat. Based on the primary structure, it could be predicted that each nephrin molecule is about 30 nm long and it was hypothesized that nephrin molecules from adjacent foot processes interact in the center of the slit to form a zipper like formation (7). This model would allow for holes on each side of a central density, essentially as predicted by Karnovsky. Support for such a structure came from studies showing homologous interactions of nephrin molecules in solution and in transfected cells, interactions that could be disrupted with antibodies directed against the extracellular domain. Three novel membrane proteins, Neph1, Neph2 and a large protocadherin FAT1 have also been localized to the slit diaphragm proper and shown to be essential for its function (see (1, 2, 8). In addition to those, several intracellular proteins such as, podocin, CD2AP, ZO-1 have been shown to be an important part of a slit diaphragm protein complex (for review, see (1, 2)). Ablation of the genes for most of those proteins causes proteinuria, demonstrating the importance of the proteins for filtration.

In order to elucidate the three dimensional-structure of the slit diaphragm, we have used a novel electron tomography (ET) technique. ET has opened up for new possibilities for molecular analysis of cellular constituents (9, 10). It is the only 3D reconstruction method not based on averaging. Therefore, it makes imaging (11, 12) and immunoidentification (13, 14) of individual macromolecular structures possible in their cellular context, usually at some 5 to 10-nm resolution. Combined with high-resolution structural approaches (e.g. X-ray crystallography, nuclear magnetic resonance, and single-particle analysis by cryo-electron microscopy), ET also provides the possibility to perform multi-resolution docking of structural details of component molecules into macromolecular complexes in cellular organelles and substructures (15).This has opened up wider prospects for mapping territorial distribution of macromolecules and analysis of molecular interactions *in situ*, both in sections and in intact frozen cells.

In our analysis of thin resin and cryo-sections of fixed human, rat, and mouse kidneys, the SD was shown to be as a porous network of molecular strands with globular substructure (16). The results were confirmed in fixed and unfixed mouse kidneys using high-pressure-freezing and freeze-substitution. Immunoelectron microscopy and immuno-ET revealed the presence and orientation of nephrin molecules in the SD network. Glomeruli from CNF patients and nephrin knockout mice lacked the SD network and its group of characteristic strands, and the slit had collapsed to only about 10-15 nm in width. Analysis of individual nephrin molecules in transfected cells and in solution showed that nephrin molecules, identified by anti-nephrin antibodies, appear as convoluted strands similar to those observed in the normal slit diaphragm *in situ*. Based on these findings, we propose a nephrin-containing network-model for the structure of the slit diaphragm. It takes into consideration the EM localization of nephrin, the structural characteristics of the molecule, and the novel ET findings. The present findings result in a partly molecular-level version of the Rodewald and Karnovsky zipper-model (3) where nephrin strands from the two opposing slit sides cross the slit diaphragm and form filtration channels, too small for albumin-sized molecules to pass. Molecular interactions may exist between the nephrin strands, most notably at the slit center. Other molecular types, at present not identified on the ET level, may well contribute to the network. The model also allows for a proposed dynamic nature of the slit diaphragm Changes in the slit diaphragm width could take place by stretching of the coiled and bent cross-strands and explain, in part, the variations seen by different methods in slit diaphragm length and appearance.

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