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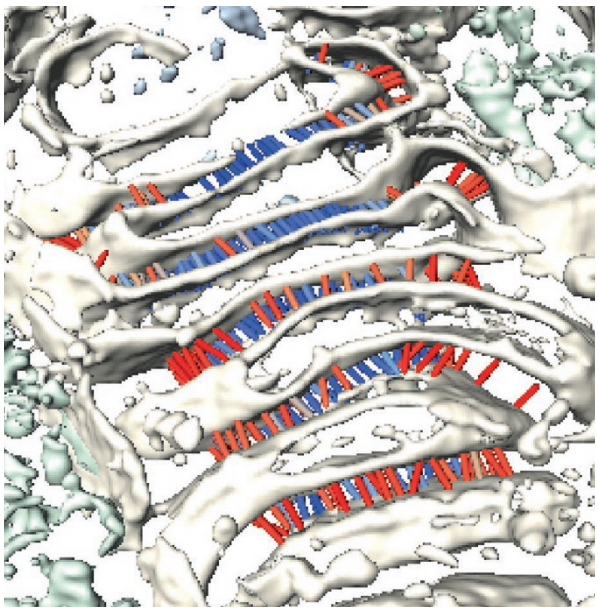
NEPHROTIC SYNDROME

Insights into protein scaffolds of the slit diaphragm

> Our structural findings… indicate that the slit diaphragm is likely to represent a highly dynamic cell–cell contact

The slit diaphragm is a specialized type of intercellular junction that connects neighbouring podocyte foot processes in the glomerulus; mutations in components of the slit diaphragm, such as NEPHRIN, have been linked to inherited proteinuric diseases. Although imaging and mutational studies have improved understanding of the molecular composition of the slit diaphragm, our understanding of the slit diaphragm structure is incomplete. Using a combination of genetic and imaging approaches, researchers now show that the slit diaphragm is composed of NEPHRIN and NEPH1 molecules arranged in a layered, bipartite composition that is thought to create a flexible, dynamic filtration barrier. "We came up with a novel and completely unexpected concept of a flexible, dynamic and multi-layered architecture of the slit diaphragm," explain the researchers Florian Grahammer, Achilleas Frangakis and Tobias Huber. "Our structural findings, in combination with the flexibility inherent to the repetitive immunoglobulin folds of NEPHRIN and NEPH1, indicate that the slit diaphragm is likely to represent a highly dynamic cell–cell contact that forms an adjustable, non-clogging barrier within the renal filtration apparatus."



Cryo-electron tomography reveals that NEPHRIN (red) and NEPH1 (blue) molecules are arranged in the slit diaphragm in a layered, bipartite pattern. Image courtesy of A. Frangakis. University of Frankfurt. Germany.

To examine the function of the slit diaphragm components, Grahammer and colleagues generated a set of mice in which *Nphs1* (which encodes Nephrin) and *Neph1–3*, were constitutively deleted. Mice lacking *Nphs1* or *Neph1* were born with nephrotic-range proteinuria and died perinatally, and exhibited evidence of podocyte and slit diaphragm abnormalities. In particular, a proportion of slit diaphragms in *Nphs1*-knockout mice were shorter than normal, a finding that was corroborated in samples from human patients with congenital nephrotic syndrome caused by mutations in *NPHS1*. By contrast, mice lacking *Neph2* or *Neph3* appeared normal, suggesting that NEPHRIN and NEPH1 molecules contribute to podocyte intercellular junction formation whereas NEPH2 and NEPH3 do not.

The researchers then used cryo-electron tomography of high-pressure frozen mouse renal cortex to examine the native ultrastructure of NEPHRIN and NEPH1. They identified a quasiperiodic arrangement of molecular strands that differed between basal and apical layers but were of similar thickness and distance to neighbouring molecules, suggesting that the two molecules do not form homomeric or heteromeric complexes. The researchers also identified differences in the localization of the molecules and in the size of the junctions formed. "Single NEPH1 molecules seem to form the lower part of the junction close to the glomerular basement membrane with a width of ~23 nm whereas single NEPHRIN molecules form an adjacent junction more apically with a width of ~45 nm," says Frangakis, the cryo-electron tomography expert of the group. "We think that the molecular spring properties of NEPH1 and NEPHRIN might have extensive implications for the filtration properties of podocytes, and participate in the formation of an adjustable barrier to proteins," say the researchers.

Huber and colleagues now plan to use techniques that have been established to study molecular springs, such as muscle fibres, to further examine the molecular characteristics of NEPHRIN and NEPH1 to fully understand their role within the glomerular barrier. "Once we know how these molecules work under physiological conditions we will draw our attention to known disease states of the slit diaphragm and will try to decipher how these molecules are, for example, affected by diabetic conditions or inflammation," he says.

Susan J. Allison

ORIGINAL ARTICLE Grahammer, F. *et al.* A flexible, multilayered protein scaffold maintains the slit in between glomerular podocytes. *JCI Insight* 1, e86177 (2016).