Cilia and Nuclear Pore Proteins: Pore No More?

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Nuclear pore proteins at the base of cilia were thought to regulate transport into cilia. In this issue of Developmental Cell, Del Viso et al. (2016) challenge this view, showing instead that pore proteins localize to ciliary basal bodies and that their perturbation leads to congenital heart disease.

Cilia are microtubule-based structures found protruding from almost every cell in vertebrates. In both their motile and immotile forms, cilia play a critical role in the formation of left-right (LR) asymmetric patterning of the body ensuring the appropriate placement of organelles and associated vascular networks, as reviewed in Koefoed et al. (2014). The heart has a clear LR asymmetry, attributed to the distinct functions between the left and right sides of the heart. During early embryonic development, LR asymmetry begins with signaling in a region known as the LR organizer (LRO) (reviewed in Nakamura and Hamada, 2012). Motile cilia in LRO beat in one direction, generating a one-sided flow sensed by immotile cilia, leading to a cascade of asymmetric gene expression that sets up LR asymmetry development (Nakamura and Hamada, 2012). In a previous study, Fakhro et al. (2011) genotyped 262 patients suffering from Heterotaxy (Htx), a disorder of left-right patterning, and found five genes whose morpholino knockdown led to a severe disruption of LR development. Strikingly, one of these proteins was the nucleoporin Nup188, found in the NPC’s inner ring (Figure 1). Following up on this, Del Viso et al. (2016) now explore in this issue of Developmental Cell the role and localization of Nup188 in the context of Htx and ciliary function.

Nucleoporins (Nups) are components of the nuclear pore complex (NPC), a large multiprotein assembly (100 MDa in vertebrates) embedded within the nuclear envelope that mediates all transport between the nucleus and the cytoplasm (Simon and Rout, 2014). The NPC consists of two inner rings and two outer rings that anchor, in the central channel, a cloud of disordered Nups rich in phenylalanine dipeptide repeats (FG-Nups) (Figure 1). FG-Nups are responsible for the NPC’s permeability barrier. Many diseases are associated with defective Nups, including cancer (Simon and Rout, 2014). Recently, Nups have been shown to have localizations and functions outside of the NPC, including at the kinetochores, centrosome, and even chromatin (Mossaid and Fahrenkrog, 2015; Verhey and Yang, 2016). A key question arising from the association of Nup188 with Htx is whether Nup188’s disease phenotype is associated with its functions at the NPC or with a secondary function of Nup188 elsewhere in the cell.

To test these alternative possibilities, Del Viso et al. (2016) used Xenopus embryos, a tractable system with a heart structure similar to humans. They injected morpholino oligos or mRNA into one cell of a two-cell embryo and identified embryos in which only the left or right side was targeted. Notably, embryos showed stronger cardiac defects when Nup188 was depleted from the embryo’s left side, compared with the right side, and specificity was confirmed by rescue of these defects with co-injection of human NUP188 mRNA. The authors then tested a series of Nups that belong to different NPC subcomplexes, including FG Nups. Only one other Nup gave similar cardiac defects: Nup93, another member of the inner ring complex of the NPC that directly binds Nup188. The authors also noted that tampering with levels of either Nup93 or Nup188 led to an absence of the developmental transcription factor pitx2 from the left flank of the embryo, which was preceded by loss of asymmetry of cocom expression, an extracellular nodal antagonist that is initially expressed symmetrically across the LR axis, becoming asymmetric as the LR axis is specified. The authors speculated that the above effects of the Nup93 and Nup188 knockdown embryos were suggestive of a disruption of cilia motility or signaling.

Looking by immunofluorescence for acetylated tubulin, the authors observed a specific reduction of cilia density in LRO cells targeted for Nup188 depletion, explaining the disease phenotype. In fact, Nup93 or Nup188 knockdown also specifically depleted cilia in other types of ciliated Xenopus and mammalian cells. Interestingly, overexpression of Nup188 also recapitulated the loss of cilia in the LRO, corroborating the observation that a duplication of the Nup188 locus was likely the underlying cause of Htx and CHD in one patient (Fakhro et al., 2011), although it remains unclear why increases in Nup188 copy number mimics the same phenotype as a decrease in Nup188 copy number. Remarkably, neither Nup93 nor Nup188 depletion seemed to affect NPC number, distribution, or functionality. Given the lack of an NPC phenotype, together with the recent reports of Nup localization at the base of cilia (Verhey and Yang, 2016), the authors wondered whether these observed phenotypes were a result of the perturbation of some sub-population of Nup93 and Nup93 moonlighting at cilia, rather than their “normal” localization at the NPC.

Although cilia are not completely encapsulated by a physical membrane like other cell organelles, they nonetheless concentrate signaling molecules while simultaneously excluding cytoplasmic proteins that would otherwise diffuse into the cilia. There has been a great interest in piecing together the molecular mechanism underlying the basis of this so-called ciliary selective diffusion barrier. The potential existence of an NPC-type transport barrier at the base of cilia has been suggested based on the observation that several scaffold and

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FG-Nups localized to the cilium via a specific ciliary localization signal and that nuclear transport factors appear to contribute to the localization of some proteins to the cilium (Verhey and Yang, 2016). FG-Nups bind to transport factors and mediate NPC transport, so they were prime candidates for forming proposed “ciliary pore complexes” (CPCs), analogous to NPCs (Verhey and Yang, 2016). However, detecting Nups at the base of cilia has proven challenging, with different groups disagreeing on which (or even whether) Nups are actually localized there. For example, one study (Breslow et al., 2013) confirmed the presence of a diffusion barrier at cilia but raised doubts about the role of Nups in establishing this barrier. Likewise, a comprehensive proteomic study of the transition zone, where the diffusion barrier is located (Figure 1), in the ciliated unicellular algae Chlamydomonas found a host of proteins there but no Nups (Diener et al., 2015).

To address this issue, Del Viso et al. (2016) turned to a new, high-sensitivity, super-resolution microscopy approach (Huang et al., 2016). The method showed that Nup93 and Nup188 do indeed localize near the base of the cilium, as well as to their more usual NPC abode. In the NPC, the ring-like structure of the inner ring could be discerned, confirming the extreme resolution achieved. However, strikingly, no ring-like “CPCs” were seen at the base of cilia. Rather, the nucleoporins appeared as numerous diffuse blobs surrounding the centrioles, in the same region as the pericentriolar material and thus somewhat distal from the entrance to the cilia, where the diffusion barrier is known to reside (Figure 1). Importantly, the authors did not detect FG-Nups (which are required to set up an NPC-type permeability barrier) anywhere at the cilia, suggesting a structural role for ciliary Nups as opposed to involvement in transport. This study therefore now raises even more questions about the role of Nups at the base of cilia.

Links between the centrosome and NPC components are well known (Mossaid and Fahrenkrog, 2015). Additionally, ciliary transport complexes (such as the intraflagellar transport complex) and vesicle-coating complexes (such as COPI, COPII, clathrin, and tethering complexes) share architectural features suggestive of a common evolutionary origin for NPCs, coated vesicles, and some ciliary components (Field et al., 2011). Based on the work from the Verhey group and the Khokha and Lusk groups leading the Del Viso et al. (2016) study, it appears that there are Nups at cilia; however, whether they form an analogous selective diffusion barrier and mediate transporter with FG-Nups remains unclear. Regardless, their presence there is important, with a crucial but still obscure role in the earliest stages of embryonic development. If Nups are not forming a ciliary pore, the challenge now is to find out just what is their molecular function at the base of the cilium.

REFERENCES