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NEWS AND VIEWS

Importin-β2: a key to two gates?

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A key communication device of vertebrate cells is the primary cilium, a singular flagellum-like structure protruding from the cell surface. What do the primary cilium and the cell nucleus have in common? Very little, that is until a recent study from Kristen Verhey's laboratory of the University of Michigan Medical School at Ann Arbor, in which the authors showed that Importin-β2 and small GTPase Ran control the access of molecules into the primary cilium (Dishinger et al., 2010). Because the same importin-β2 and Ran are part of the cellular machinery guarding the entry into the nucleus through nuclear pore complexes (NPC), this observation raised a provocative possibility that a "pore-like opening" may stand in between the cilium and the rest of the cell body, a conjecture that has haunted the cilium research field for many years. If proven to be true, the existence of a "ciliary pore" will have profound implications.

Cilia are now widely found from protists such as the algae *Chlamydomonas* to humans. Inside a specialized ciliary membrane that is contiguous to cell's plasma membrane resides a core structure, called axoneme, which consists of nine sets of microtubule doublets (Rosenbaum and Witman, 2002). Ciliary defects in humans result in a variety of genetic and syndromic diseases such as infertility, polycystic kidney disease, obesity, mental illness and blindness, which are now collectively called the ciliopathies.

The cilium structure is built to execute motile and sensory functions, the latter of which relies on a special set of receptors and signaling molecules, such as components of the Wnt and Sonic Hedgehog pathways (Goetz and Anderson, 2010). Due to the lack of protein synthesis in cilia, all of hundreds of different ciliary proteins synthesized in the cell body need to be selectively loaded to the ciliary compartment and transported to the ciliary assembly site at the distal tip. Intraflagellar transport (IFT) is indispensable for the assembly, disassembly, and maintenance of nearly all cilia that have been studied. Originally discovered in *Chlamydomonas*, IFT moves trains of big particles containing IFT proteins, molecular motors, cargos, and associated factors continuously and bidirectionally along the axonemal microtubule tracts underneath the ciliary membrane. The anterograde IFT motor is kinesin-2, consisting of heterotrimeric KIF3A/KIF3B/KAP and homodimeric KIF17 in vertebrates. In addition to trafficking within the cilium, of the same IFT machinery also transports disassembled products out of the cilium, but how ciliary proteins enter cilia is unknown. The work of Verhey's laboratory now points to a new direction to study ciliary protein transport by comparing it to nuclear transport.

Similar to the cilium, the nucleus is not completely sealed and isolated from the cytoplasm. Nuclear pores on the nuclear envelope are tunnels that permit small molecules (below 40 kDa) to diffuse in while forcing large proteins to rely on active transport system to pass. The first step of this facilitative system is the recognition of nuclear localization sequence (NLS) on nuclear cargos by carrier proteins such as importin-β to form a transport complex (Stewart, 2007). The complex then interacts with FG-repeat proteins of the NPC and moves through the tunnel with assistance from accessory factors. By analyzing the amino acid sequence of KIF17, Verhey and her colleagues identified two potential NLSs in its C-terminal tail, one of them bearing a "KRKK" signature. Using fluorescent proteins fused to different truncated or mutant forms of KIF17, they found that if the KRKK signature is present, the fusion protein could enter either the cilium or the nucleus, but when this NLS was deleted or mutated, the fusion protein could enter neither. This indicates that KRKK is not only a NLS but also a ciliary localization sequence (CLS). These data suggest that nuclear import and ciliary import are parallel processes.

During nuclear import, importin-β localizes near the nuclear envelop and interacts with its cargo in a Ran-dependent manner. Ran, a small GTPase, is a key player in the nuclear transport system. The Ran-GDP form is predominantly in the cytoplasm where it enables the importin-cargo complex to form; a high level of Ran-GTP is maintained in the nucleus by a chromatin-bound guanine nucleotide-exchange factor for Ran (RanGEF) and facilitates cargo release. Verhey and her colleagues found that the transition zone and the cilium

proper contain the β2 isotype of importin β, and showed importin-β2 interacts with KIF17 specifically *in vitro* and *in vivo*. This interaction was dependent on the KRKK sequence on KIF17. Immunofluorescent staining with a conformation-specific antibody showed that Ran-GTP is enriched in the cilium, as it does in the nucleus. Furthermore, forced expression of a mutant Ran locked in the GTP-bound form in the cytoplasm abolished the ciliary localization of KIF17 and the interaction between KIF17 and importin-β2. These data support a model in which KIF17 and importin-β2 form a complex in the cytoplasm to move across the transition zone and enter cilia. The predominant Ran-GTP within cilia facilitates the disassociation of KIF17 from importin-β2, resulting in cargo release. This model was corroborated in biochemical studies of *Chlamydomonas*, in which key components of the nuclear transport machinery including importin-α, NTF2 (a nuclear import factor for Ran-GDP) and RBP1 (a GTPase regulator) were all found in the flagellum (Pazour et al., 2005). Therefore, a ciliary transport mechanism similar to nuclear transport may be conserved among ciliated organisms from *Chlamydomonas* to humans.

Like all exciting findings, the study by Verhey's laboratory raised more questions than it answered. First, where is exactly the ciliary pore? There are two possible locations: one is in the center of the cylinder of 9 doublet microtubules with its 9-fold symmetric fibers and meshwork inside at the transition zone, which is reminiscent to the 8-fold symmetric NPC structure. The diameter of the central space is about 50 nm, very similar to the diameter of NPC channels (~60 nm). The other possible location for the "pore" is the space between the ciliary membrane and microtubule doublets at the transition zone. Here, the space width is about 50 nm. However, homologs of the known NPC components such as FG-repeat proteins have not been found in any proteomic analysis of cilia and basal bodies. One possibility is that transition zone specific proteins such as nephrocystins substitute the classical NPC proteins for ciliary pore formation.

Second, do ciliary membrane proteins, soluble proteins in the matrix, and axonemal components all rely on the importin-Ran system to enter cilia? Taking into account of a recently discovered role of IFT in exocytosis, it has been proposed that not only ciliary membrane proteins but also axonemal proteins are transported to cilia by association with vesicles in the Golgi-derived exocytosis pathway (Baldari and Rosenbaum, 2010). The vesicles targeting to cilia are associated with IFT20, exocyst proteins, and Bardet-Biedl Syndrome proteins and fuse to the plasma membrane at the base of the cilium. Other IFT proteins and anterograde motors subsequently are assembled there and move the membrane protein patch across the transition zone.

It is obvious that the IFT-vesicles model is different from Verhey's implicated picture of ciliary pores using an analogy to nuclear transport. Since ciliary membrane proteins use divergent ciliary targeting sequences different from NLS for ciliary localization (Pazour and Bloodgood, 2008), it is likely that different categories of ciliary proteins utilize different machinery to enter cilia. To confirm this idea and to reconcile these two models, we need to identify other nuclear transport proteins and characterize their function in ciliary transport. For example, is there a RanGEF in cilia since it plays a critical role in keeping the Ran-GTP gradient? Is there a CLS similar to the NLS on axonemal proteins that also binds to importin-β? Since both ciliary transport and nuclear transport share the same targeting sequence and similar transport system, what factors control the protein entry to the cilium but not to the nucleus? Answers to these questions will certainly expand Verhey's exciting discovery as well as provide us with a clearer picture of protein trafficking among the cilium, the nucleus, and the rest of the cell.

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