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Focus on Molecules: Rootletin

Jun Yang, Tiansen Li*

The Berman-Gund Laboratory for the Study of Retinal Degenerations, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA 02114, USA

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1. Structure

Rootletin (accession number NP_742120) is a large coiled coil protein initially identified as a structural component of the ciliary rootlet (Yang et al., 2002). Its formal designation (ciliary rootlet coiled-coil, rootletin), as listed in most databases, reflects these two known aspects of the protein. The murine rootletin comprises 2009 amino acid residues and has a calculated molecular weight of about 220 kDa. The C-terminal three quarters of rootletin sequence are predicted to form an extended rod domain consisting entirely of coiled-coils. The N-terminal one-quarter is thought to assume a globular structure. The rod domain alone is sufficient to polymerize into elongated fibrils, whereas the globular domain is dispensable. Rootletin is conserved among mammalian species, with 82% identity and 88% similarity in the amino acid sequence between mouse and human (accession number NP_055490). C-Nap1, a centrosomal protein involved in centriolar cohesion, is the closest known homologue of rootletin in the mammalian genome, with 29% identity and 48% similarity between the human C-Nap1 and rootletin sequences.

2. Function

As its name implies, the primary function of rootletin is to serve as a constituent of the rootlet. Over-expression of recombinant rootletin in cell culture leads to the formation of rootlets that are morphologically similar to those of native tissues. No co-polymers of rootletin have been found by immunoprecipitation studies. Targeted disruption of rootletin gene in mice abolished the formation of ciliary rootlets (Yang et al., 2005). These data indicate that rootletin is the major, and likely only, structural constituent of the rootlets. A working model has been proposed in which rootletin forms parallel and in-register homodimers. These homodimers are further polymerized into 10-nm thin protofilaments through lateral interactions between the coiled-coil segments. The resulting protofilaments are bundled together to form the ciliary rootlet (Fig. 1; Yang et al., 2002).

While the conclusion that rootletin polymerizes to form the rootlet is well established, a more interesting and biologically relevant question inevitably follows. What, then, is the in vivo function of the ciliary rootlet? Familiar to cell biologists for decades, the rootlet is seen by electron microscopy as a large, striated cytoskeleton-like structure. The rootlet originates from the basal body and extends proximally toward the cell nucleus. Structurally related to centrioles, basal bodies nucleate the formation of cilia and rootlets at their distal and proximal ends, respectively (Fig. 1). The rootlets are particularly well developed in retinal photoreceptors. In surveys of tissue expression patterns, retinal photoreceptors are where rootletin expression level is by far the highest. In mice lacking ciliary rootlets, there is a striking fragility at the ciliary base of photoreceptors. When exposed to mechanic stress in vitro, photoreceptor outer segments break off easily just below the basal bodies. Photoreceptors in mice lacking ciliary rootlets slowly degenerate. These findings demonstrate that the rootlet provides structural support to ciliated cells. And this support is essential for the long-term viability of photoreceptors (Yang et al., 2005). All ciliated cells presumably benefit from the structural support provided by the rootlets. Because photoreceptors elaborate exceptionally enlarged distal cilia, they are especially sensitive

* Corresponding author.
E-mail address: tli@meei.harvard.edu (T. Li).

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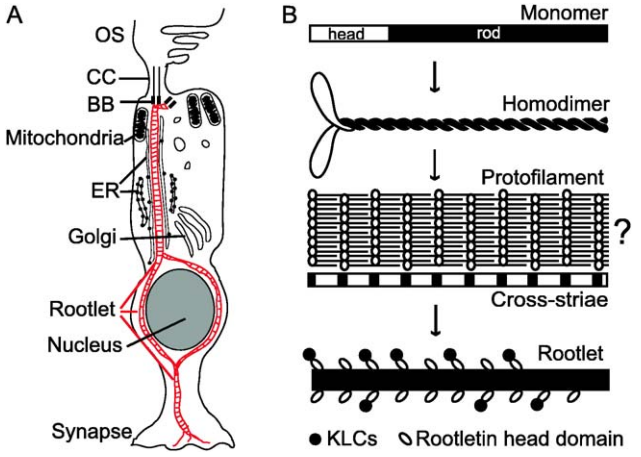

Fig. 1. The ciliary rootlet in a photoreceptor cell and a model for rootlet assembly from rootletin monomers. (A) The ciliary rootlet (red) extends from the basal bodies to the synapse in a photoreceptor. OS, outer segment; CC, connecting cilium; BB, basal body. (B) The assembly of the ciliary rootlet from rootletin monomers proceeds through the intermediate steps of rootletin homodimers and the 10-nm thin protofilaments. KLCs interact with the head domain of rootletin.

to the loss of the rootlets. Other ciliated cells with smaller cilia may be less sensitive, hence the lack of a pronounced phenotype in the absence of a rootlet. The ciliary rootlet was found to integrate with actin filaments. Moreover, the recombinant rootletin fibers appeared very static with a turn over rate similar to that of intermediate filaments (Yang et al., 2005). These latter observations are consistent with a structural support role for the rootlet.

It is still not clear what additional roles rootlets may have in vivo other than the provision of mechanical support. By immunolabeling with rootletin antibodies, rootlets are found to have a much wider distribution than previously thought based on electron microscopic examinations. In general, large or motile cilia are associated with robust rootlets. Most cells, though, have at least a rudimentary rootlet. In fact, the proximal ends of all centrioles have small 'rootlets' attached to them. By protein interaction screens, rootletin binds kinesin light chains (KLCs), subunits of kinesin-1, which moves its cargos along the microtubules using the energy of ATP. In mouse rootletin interacts most strongly with KLC3, which is highly expressed in the retina. The interaction between rootletin and KLCs recruits some of the kinesin-1 in a cell to the rootlet. However, kinesin-1 does not appear to migrate along the ciliary rootlets as it does along the microtubules. In addition, a kinesin-1 vesicular cargo receptor (amyloid precursor protein) and a kinesin-1 vesicular cargo (presenilin 1) are recruited along the ciliary rootlet while other kinesin-1 cargo receptors (C-Jun N-terminal kinase-interacting proteins) are not. These data have led to the proposal that the rootletin-based ciliary rootlet is a docking site for a subset of kinesin-1 vesicular cargos and plays a role in intracellular trafficking (Yang and Li, 2005). However, conclusive evidence is still lacking. No apparent defects in intracellular trafficking in photoreceptors lacking rootletin have been found, suggesting that this putative function of rootletin is at least partially redundant in photoreceptors (Yang et al., 2005). Whether rootletin has any role in centriolar function is also of considerable current research interests.

3. Disease involvement

No human disease has been formally linked to rootletin at this time. Given the fact that rootletin is essential for photoreceptor maintenance in the mouse, it is a possibility that genetic defects in rootletin may be a cause of human retinal degeneration, in either simplex or syndromic forms. Based on all available data, the corresponding human retinal degeneration may be one of late onset, may present with systemic manifestations of a ciliary defect, and may be sensitive to environmental factors of a mechanical nature, such as physical stress or trauma.

4. Future studies

While our understanding of rootletin function has come a long way since its discovery three years ago, many questions remain. We do not yet know, first and foremost, whether rootletin is a causative gene for human retinal degeneration and/or other disease conditions. Second, it remains to be demonstrated if and to what extent rootletin is involved in intracellular trafficking. Third, whether rootletin has a role in centriolar function such as cohesion and replication needs to be explored. Future studies focused on these questions will help generate a complete picture of rootletin function in photoreceptors and perhaps in all cells.

References

Yang, J., Li, T., 2005. The ciliary rootlet interacts with kinesin light chains and may provide a scaffold for kinesin-1 vesicular cargos. Exp. Cell Res. 309, 379–389.

Yang, J., Liu, X., Yue, G., Adamian, M., Bulgakov, O., Li, T., 2002. Rootletin, a novel coiled-coil protein, is a structural component of the ciliary rootlet. J. Cell Biol. 159, 431–440.

Yang, J., Gao, J., Adamian, M., Wen, X.H., Pawlyk, B., Zhang, L., Sanderson, M.J., Zuo, J., Makino, C.L., Li, T., 2005. The ciliary rootlet maintains long-term stability of sensory cilia. Mol. Cell. Biol. 25, 4129–4137.