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Review

Coordinating the uncoordinated: UNC119 trafficking in cilia

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ABSTRACT

Constructing the distinct subcellular environment of the cilium relies in a large part upon intraflagellar transport (IFT) proteins, which traffic cargo both to and within the cilium. However, evidence from the last 10 years suggests that IFT alone is not sufficient to generate the ciliary environment. One essential factor is UNC119, which interacts with known IFT molecular switches to transport ciliary cargos. Despite its apparent importance in ciliary trafficking though, human *UNC119* mutations have only rarely been associated with diseases commonly linked with ciliopathies. This review will outline the trafficking pathways required for constructing the cilium by highlighting UNC119's role and the complexities involved in ciliary trafficking. Finally, despite important roles for UNC119 in cilia, UNC119 proteins also interact with non-ciliary proteins to affect other cellular processes.

1. Introduction

Cilia are microtubule-based organelles that project from the cell body and are covered by a ciliary membrane that is continuous with, but compositionally distinct from, the plasma membrane. Within the membrane, microtubules form the axoneme, the core cytoskeletal structure of all cilia. The axoneme protrudes from the cell body and is anchored to the cell by the basal bodies, which are formed from centrioles (Dawe et al., 2007). The basal body is where axonemal formation begins and is the site where ciliary proteins are docked for trafficking via intraflagellar transport (IFT), which is an evolutionarily conserved process essential for ciliogenesis and to maintain the distinct composition of the ciliary membrane as a signaling center (Ishikawa and Marshall, 2011; Pazour et al., 2005, 2000).

Although cell biologists have been studying cilia for several decades, it has been only in the last 20 years that cilia have been implicated in apparently disparate human diseases (Pazour et al., 2000). These diseases are characterized by a pleiotropic set of clinical features that occur in varying combinations, including renal cysts, vision defects, respiratory problems, sterility, obesity, polydactyly, and cerebral malformations that can result in developmental delays and cognitive impairment. However, despite their clear importance, much is still unknown regarding establishing the proper ciliary environment. For instance, the roles of many of the several hundred proteins associated with the ciliary proteome have yet to be uncovered (Hsiao et al., 2012). Since these proteins are synthesized within the cell body and must be trafficked to the cilium, this illustrates that complex trafficking systems must be in place in order to facilitate proper cilia function.

Ciliary trafficking largely involves two biochemically distinct IFT complexes (A and B), molecular motors, and accessory proteins, such as the Bardet-Biedl syndrome (BBS) proteins and small GTPases (eg. ARLs (ADP-ribosylation factor-like proteins) and RABs) (Blacque et al., 2004; Cantagrel et al., 2008; Knodler et al., 2010; Kozminski et al., 1995, 1993; Nachury et al., 2007; Pazour et al., 1998; Schrick et al., 2006). The IFT complexes travel together along the ciliary axoneme although they have complementary functions. IFT complex B is composed of about 16 proteins and is responsible for anterograde transport whereas complex A contains about 6 proteins and conducts retrograde transport (Behal et al., 2012; Katoh et al., 2016). The two complexes travel either to the tip of the cilium via kinesin-2 or back to the cell body via cytoplasmic dynein 2. The BBSome binds to the IFT complexes and is thought to function as an adaptor for both cargo import and export (Domire et al., 2011; Lechtreck et al., 2013; Nager et al., 2017). Many small GTPases function as molecular switches and have roles that include maintaining ciliary length, docking the BBSome at the basal body, stabilizing the interaction between the two IFT complexes, delivering vesicles to the ciliary base, and acting as release factors for ciliary targeted cargos (Cevik et al., 2010; Li et al., 2010; Lu et al., 2015; Mourao et al., 2014; Nachury, 2008; Nachury et al., 2007; Wang et al., 2012; Wiens et al., 2010; Wright et al., 2011). Altogether, these proteins facilitate the delivery of key proteins for both ciliogenesis and ciliary function. However, although IFT is considered the classical mechanism of ciliary trafficking, it is not sufficient to maintain the ciliary proteome and thus additional trafficking methods are essential.

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For example, in recent years, it has been shown that one IFT protein, IFT20, also functions outside of the cilium to promote the trafficking of vesicles between the Golgi and the cilium and for docking of these vesicles at the ciliary base (Follit et al., 2006).

UNC119 is a key protein in these additional trafficking mechanisms, although previously, this protein has been described in several contexts that are apparently distinct from cilia. Nonetheless, UNC119 proteins function in combination with known IFT effectors and are required to transport essential ciliary cargos. This review will begin with the protein description of the UNC119 family and will subsequently highlight their molecular activities and how these relate to cilia function. Finally, we will describe other molecular roles for UNC119 proteins and how these might have arisen over evolutionary history in combination with cellular trafficking.

2. UNC119 introduction

UNC119 family members are among the most highly conserved metazoan proteins, described in protozoa to mammals (Chung et al., 2007; Higashide et al., 1996; Keller et al., 2009; Maduro and Pilgrim, 1995; Manning et al., 2004; Swanson et al., 1998). Despite its high structural conservation, loss-of function mutations in *unc119* genes in different metazoans produce distinct phenotypes. For example, while UNC119 proteins are required for ciliary function in cell culture and protozoa, they are also required for initiating the immune response in mammals, patterning the nervous system in zebrafish and *C. elegans*, and promoting proper cell division (Chung et al., 2007; Gorska and Alam, 2012; Keller et al., 2009; Lee et al., 2013; Manning et al., 2004; Ohshima et al., 2010; Wright et al., 2011). These phenotypes have complicated how the molecular function has been interpreted and have also raised the question of whether UNC119 proteins have been functionally re-appropriated in each system in order to facilitate such diverse cellular functions. This seems unlikely as either *Drosophila unc-119* or human *UNC119* transgenes can rescue a *C. elegans unc-119* mutant, demonstrating that the UNC119 proteins are functionally conserved, in addition to being structurally conserved, across metazoans (Maduro et al., 2000). Therefore, despite performing such diverse roles in different organisms, this suggests that UNC119 proteins must somehow be involved in a common molecular mechanism that underlies all of these cellular functions.

3. UNC119 history

unc-119 was first discovered in *C. elegans* as a strongly paralyzed mutant that arose from a cross between different wildtype strains (Maduro and Pilgrim, 1995). The phenotype arose from a transposable element insertion into a previously unidentified locus. The “uncoordinated” (“unc”) phenotype includes almost complete paralysis, defects in dauer formation, and constitutive pharyngeal pumping even in the absence of food. These phenotypes suggested a role of the protein in nervous system development and, consistent with this, reporter constructs show largely pan-neural expression beginning in the embryo (Maduro and Pilgrim, 1995, 1996). UNC-119 has been shown to act by stabilizing the structure of the nervous system after development; in it’s absence, neurons became excessively branched after the initial neural scaffold had formed properly (Knobel et al., 2001). In addition to the axon branching defect, *unc-119* mutant worms also display other neuronal phenotypes including defasciculated nerve fibers, chemosensation problems, and nerve elongation defects.

A cDNA sharing sequence identity with *unc-119* was subsequently reported in humans, mice, rats, rabbit, pig, calf, and monkey, suggesting strong mammalian conservation (Higashide et al., 1996; Swanson et al., 1998). Although these transcripts are predominantly expressed in retina, they are also detected in the brain (hippocampal cells and pineal gland), fibroblasts, liver and kidney (Bailey et al., 2009; Cen et al., 2003; May et al., 2014; Swanson et al., 1998). The human gene was termed human retinal gene 4 (*HRG4*; subsequently renamed *UNC 119*) and encodes a 1.4 kb (kilobase) transcript resulting in a 240 amino acid protein (Higashide et al., 1996).

4. Protein structure of UNC119

The UNC119 proteins are unusual in that there are very few (1–4) paralogues in any one metazoan tested, yet orthologues are extremely conserved. Although all previously identified UNC119 proteins form their own family, they are distantly related to PDEδ (renamed PrBP; prenyl binding protein), a protein responsible for trafficking prenylated proteins within the cilium, and even more distantly related to RhoGDI proteins, which may reveal some insight into the molecular roles of UNC119 (Hanzal-Bayer et al., 2002; Ismail et al., 2011; Maduro et al., 2000; Zhang et al., 2011, 2004). GDI (guanosine nucleotide dissociation inhibitor) proteins regulate small GTPase function and were initially named after their roles in preventing dissociation of guanine nucleotides (usually GDP) from GTPases, rendering the GTPase in the inactive state (Ueda et al., 1990). Since their discovery though, they have been found to fulfill two other important roles. First, GDIs are able to alternatively bind to GTP-bound GTPases and prevent GTPase activity thereby affecting subsequent interactions of the GTPase with effector targets (Chuang et al., 1993; Hart et al., 1992). Second, GDIs use an immunoglobulin-like β-sandwich fold which functions to solubilize GTPases from the membrane by shielding lipid modifications on the GTPase (Gosser et al., 1997; Hoffman et al., 2000; Keep et al., 1997). This is thought to allow their transport to different cellular compartments. While GDIs help to regulate small GTPase activity, GDIs are typically also regulated. For example, RhoGDI is phosphorylated by Src tyrosine receptors and other kinases; when RhoGDI is phosphorylated in vitro, it has a decreased ability to complex with Rho GTPases (DerMardirossian et al., 2006).

Both PrBP and UNC119 are GDI-like proteins due to their folded structure containing an immunoglobulin-like β-sandwich fold resulting in a narrow, hydrophobic cavity (Hanzal-Bayer et al., 2002; Ismail et al., 2011; Wright et al., 2011; Zhang et al., 2011, 2004). PrBP interacts with prenylated proteins whereas UNC119 interacts with myristoylated or lauroylated proteins, indicating that this lipid-binding capability is preserved (Zhang et al., 2011, 2004). Consistent with UNC119 acting as a GDI-like factor, UNC119 is able to solubilize myristoylated proteins from the membrane, although this is not restricted to small GTPases unlike Rho-GDIs, as UNC119 is also responsible for transporting other myristoylated proteins such as NPHP3 (Wright et al., 2011; Zhang et al., 2011). UNC119 proteins are able to bind to some small GTPases and prevent GTPase activity, thereby maintaining them in an active state, although this is also in contrast to RhoGDIs, which typically have lower binding affinity to GTP-bound GTPases (Gorska et al., 2009; Sasaki et al., 1993; Zhang et al., 2016). Again, this role is not restricted to small GTPases as UNC119 can also prevent GTP hydrolysis of Dynamin (Karim et al., 2010). The final similarity to RhoGDIs is that UNC119 interacts with Src tyrosine receptors; however, there has not been any evidence showing that Src phosphorylates UNC119 in order to regulate it, even though UNC119 proteins do contain potential phosphorylation sites (Cen et al., 2003). Regardless, this conserved interaction may represent a method by which UNC119 proteins are regulated.

5. UNC119 and cilia

The involvement of UNC119 proteins in functions associated with cilia has been studied in a variety of contexts, although a unified molecular mechanism is not yet clear. In protozoa, loss of UNC119 affects their motile cilium whereas only sensory cilia have demonstrated a requirement for UNC119 in metazoans (Chung et al., 2007; Keller et al., 2009; Ohshima et al., 2010; Ou et al., 2007; Zhang et al., 2011). Even within metazoans, UNC119 may affect ciliary trafficking in slightly