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Uncoordinated (UNC)119: Coordinating the trafficking of myristoylated proteins

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ABSTRACT

The mechanism by which myristoylated proteins are targeted to specific subcellular membrane compartments is poorly understood. Two novel acyl-binding proteins, UNC119A and UNC119B, have been shown recently to function as chaperones/co-factors in the transport of myristoylated G protein α-subunits and src-type tyrosine kinases. UNC119 polypeptides feature an immunoglobulin-like β-sandwich fold that forms a hydrophobic pocket capable of binding lauroyl (C12) and myristoyl (C14) side chains. UNC119A in rod photoreceptors facilitates the transfer of transducin α subunits (Tα) from inner segment to outer segment membranes by forming an intermediate diffusible UNC119–Tα complex. Similar complexes are formed in other sensory neurons, as the G proteins ODR-3 and GPA-13 in *Caenorhabditis elegans unc-119* mutants traffic inappropriately. UNC119B knockdown in IMCD3 cells prevents trafficking of myristoylated nephrocystin-3 (NPHP3), a protein associated with nephronophthisis, to cilia. Further, UNC119A was shown to transport myristoylated src-type tyrosine kinases to cell membranes and to affect T-cell receptor (TCR) and interleukin-5 receptor (IL-5R) activities. These interactions establish UNC119 polypeptides as novel lipid-binding chaperones with specificity for a diverse subset of myristoylated proteins.

1. Introduction

Gα subunits carrying the myristoylation consensus sequence (Farazi, Waksman, & Gordon, 2001) are presumed to be acylated cotranslationally, and combine with Gβγ subunits following the prenylation of Gγ. While there is general agreement that Gαβγ heterotrimer formation is essential for targeting (Marrari et al., 2007), it remains unclear how the complex traffics to the plasma membrane in non-polarized cells, or to cilia of polarized sensory neurons. Trafficking of Gα subunits to cilia mediated by the acyl-binding proteins, UNC119A and UNC119B, has been proposed recently for mouse rod photoreceptors and *Caenorhabditis elegans* olfactory neurons. Here we discuss the structure and function of the two lipid-binding proteins and the effects of their deletion or knockdown on trafficking/localization of the respective binding partners.

2. UNC119 supergene family

UNC119 polypeptides derive from a supergene family encoding proteins whose function has been maintained through metazoan evolution. Members of this family include prenyl-binding proteins encoded by the PDE6D gene, and two UNC119 paralogs generated by duplication of an ancestral gene (Fig. 1). Polypeptides of this supergene family share an immunoglobulin-like β-sandwich structure for lipid-binding and participate in trafficking of lipidated proteins.

Mammalian *UNC119* encodes a widely-expressed 27 kDa polypeptide (Higashide et al., 1996). UNC119 homologs have been identified in plants (POC7 in *Chlamydomonas reinhardtii*, (Keller et al., 2009)) and all animals, including unicellular protozoa such as *Paramecium* and *Tetrahymena*. Subcellularly, UNC119 homologs were identified in the basal body proteome of *C. reinhardtii* (Keller et al., 2009), the flagellar rootlet of *Naegleria* (Chung et al., 2007), neurons of *C. elegans* (Maduro & Pilgrim, 1995) and in the mouse photoreceptor sensory cilium complex (Liu et al., 2007).

3. The multifunctional UNC119A/RG4 protein

The *C. elegans unc-119* gene was first discovered due to a spontaneous mutation resulting in defects in chemosensation, locomotion and feeding behavior (Maduro & Pilgrim, 1995). Predominant expression of an *unc-119/LacZ* fusion construct in *C. elegans* neurons suggested that the *unc-119* mutant phenotype was caused by a nervous system defect. A human UNC119A ortholog termed HRG4 (Human Retina-specific Gene 4) was identified shortly thereafter by subtractive cloning. Retina specificity referred to abundant

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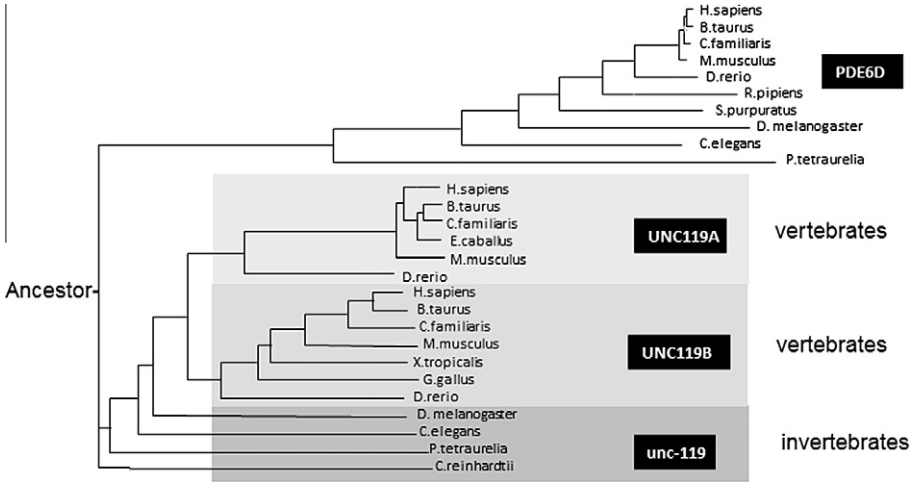

Fig. 1. Phylogram of PDE6D (PrBP/δ) and UNC119 paralogs, generated by ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Four main branches are PDE6D, UNC119A, UNC119B and the invertebrate Unc-119. Only sequences from selected species are shown (from hundreds of genbank hits). PDE6D and UNC119 homologs are present throughout the animal kingdom (*unc119* even in plants, *Chlamydomonas reinhardtii*).

expression in the retina as observed by northern blotting. A closely related paralog, UNC119B, was identified later in zebrafish by library screening with degenerate primers (Manning et al., 2004). Ubiquitously-expressed human UNC119A and UNC119B are comprised of 240 and 251 amino acids, respectively. *UNC119A* mRNA exhibits strong expression in retina with the protein localized to photoreceptor synapses and inner segments (Higashide, McLaren, & Inana, 1998). The human *UNC119* and *UNC119B* genes (Fig. 2) have an identical gene structure consisting of 5 exons (Higashide & Inana, 1999). *UNC119A* produces a splice variant in which the 3′ most intron is retained, yielding a distinct C-terminal end (Swanson et al., 1998) lacking the β-sandwich structure. Both paralogs exhibit significant sequence similarity with PrBP/δ, particularly at the PrBP/δ C-terminal region, which forms the β-sandwich fold into which lipid side chains may insert.

A heterozygous stop codon in the *UNC119* gene (K57ter) was linked to cone dystrophy in a single patient (Kobayashi et al., 2000) and this phenotype was replicated in a transgenic mouse model expressing the murine *Unc119* gene with an identical stop codon (Kobayashi et al., 2000). Further, a UNC119(G22V) dominant negative mutation has been suggested to cause idiopathic CD4 lymphopenia (ICL), an immunodeficiency disorder associated with reduced T-cell stimulation (Gorska & Alam, 2011).

4. UNC119A-interacting partners

Yeast two-hybrid screening and *in vitro* pulldown assays reveal that UNC119A interacts with a large number of both related and unrelated polypeptides, including acylated Gα-subunits, receptor-associated src-type tyrosine kinases, non-receptor protein kinases,

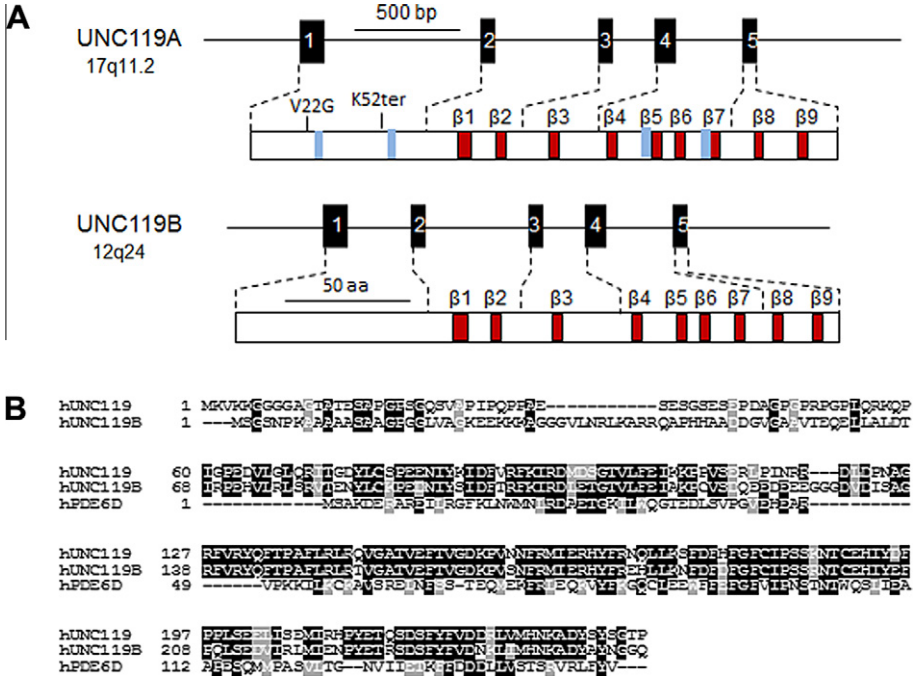

Fig. 2. *Unc119* genes and proteins. (A) Structure of *Unc119* genes and gene products. Mutations associated with disease are noted in the UNC119A protein. Blue bars denote approximate positions of SH2- and SH3-interacting domains. (B) Sequence alignment of UNC119 paralogs and PrBP/δ. Conserved residues are shown white-on-black background, conservative substitutions white-on-grey background.