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A GDI/GDF like system for sorting and shuttling ciliary proteins

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Abstract

Post/co-translational modifications by the addition of lipids take place in a vast number of proteins. Rab and Rho are small G proteins which are prenylated and targeted to membranes in complex with solubilising factors called guanosine dissociation inhibitors (GDIs). The release of Rab and Rho at the correct destination from their cognate GDI has been proposed to be mediated through GDI displacement factors. However this mechanism is yet to be established and it has been shown that loading of Rab proteins with GTP at the destination can be sufficient for their correct targeting. PDE6D shares structural homology with Rho GDI and solubilises several prenylated proteins and mediate their targeting to different destinations including cilia. In a paper published by Fansa et al, the authors propose that sorting of cargo is dependent on the differential release by bona fide GDFs, Arl2 and Arl3, and the localisation of the active Arl3GTP in cilia.

Significant number of proteins is post/co-translationally modified by the addition of a lipid moiety. By changing the physicochemical properties of proteins, lipid modification can mediate protein-protein interactions, influence protein stability and mediate interaction with membranes (*1*). Rab and Rho proteins are small GTPases that are post translationally modified by prenylation which in turn mediates their localisation at their target membrane. The addition of a prenyl group results in increased hydrophobicity and in order for Rab and Rho proteins to stay in solution ready to be shuttled to their destination they interact with a class of proteins called guanosine dissociation inhibitors GDI. GDIs sequester the hydrophobic prenyl group and hence solubilise Rab and Rho in a GDP dependent mode (*2, 3*).

There are more than 80 different Rab and Rho proteins and only two isoforms of Rab GDIs, GDI-α and GDI-β, and three Rho GDIs, RhoGDI1, 2 and 3. Furthermore, Rho and Rab interact with their cognate GDIs with high binding affinities forming kinetically stable complexes. This begs the question of how different Rabs and Rhos are targeted to different destinations. A class of proteins called GDI displacement factors, GDFs, have been proposed to catalyse the dissociation of the Rho/Rab-GDI complexes at the destination for proper delivery at the target membrane (*4, 5*). Nevertheless the molecular mechanism of these GDFs has been elusive. Furthermore it has been reported that targeting of Rabs to their destination is rather dependent on the localisation of their cognate guanine exchange factors (GEF)(*6*). In favour of this model, where targeting is dependent on GEFs and not GDFs, the *legionella pneumophila* protein DrrA ability to displace Rab1 from the Rab1-GDI complex has been shown to be due to its GEF activity and not due to a GDF activity as previously reported (*7, 8*). Nevertheless, GEFs do not accelerate the disruption of Rab-GDI complexes but ensure entrapment of on the correct membrane.

PDE6D, UNC119a and UNC119b are homologous proteins that share structural homology with RhoGDI. PDE6D binds to prenylated proteins whereas UNC119a and UNC119b bind to myristoylated proteins (*9, 10*). Similar to GDIs, UNC119a/b and PDE6D solubilise lipid modified proteins and hence play part in their shuttling; because of this functional and structural similarity we name them GDI like solubilising factors (GSFs). Using a different interface and in a lipidation independent manner, GSFs interact with the small G proteins Arl2 and Arl3 in a GTP dependent mode (*11, 12*). It has been shown that Al2 and Arl3 function as bona fide GDFs for PDE6D and UNC119a/b (*13, 14*). In case of PDE6D the allosteric interaction with Arl2 or Arl3 results in closure of the hydrophobic pocket and the release of the prenylated cargo. For UNC119 interaction with Arl3 results in widening of the hydrophobic pocket and loss of crucial hydrophobic interactions with the lipid group and hence release of the cargo. Arl3 is activated by another G protein, Arl13b, which acts as a GEF whereas XRP2 acts as an Arl3 GTPase activating protein (GAP) (*15, 16*). The tubulin cofactor TBCC and ELMOD proteins function as GAPs for Arl2 but there are no known Arl2 GEFs nevertheless due to the relatively fast dissociation of nucleotides from Arl2, the need for a GEF can be questionable (*17, 18*).

PDE6D is involved in shuttling INPP5E into cilia and GRK1 and PDE6 catalytic subunits to the photoreceptors outer segment, a specialised form of cilia (*10, 19*). Furthermore PDE6D plays a role in localisation of non-ciliary proteins as knocking down of PDE6D resulted in mislocalisation of Ras proteins (*20*). This raises the question, similar to that pertaining Rab/Rho-GDI complex targeting, how cargoes are targeted to different destination yet shuttled by the same chaperone. In a paper by Fansa et al , the authors put forward a sorting model that depends on the affinity of cargo to PDE6D and the presence of the active Arl3 exclusively in cilia (Fig2) (*21*).

Rheb and INPP5E were used as examples for non-ciliary and ciliary cargo, respectively. Similar to previously reported data for non-ciliary cargo, Rheb binds to PDE6D with half a micromolar affinity whereas INPP5E bind to PDE6D with a nanomolar affinity. The crystal structure of a fully modified farnesylated INPP5E peptide in complex with PDE6D shows an isoleucine residue at position -1, relative to the farnesylated cysteine, favourably placed in a hydrophobic environment provided by several hydrophobic residues from PDE6D (Fig1). At position -3 a serine residue makes a hydrogen bond with a glutamic residue from PDE6D (Fig1). This serine is conserved among several prenylated ciliary proteins that are known to bind to PDE6D. Rheb has serine and lysine at positions -1 and -3 instead, and mutating residues -1 and -3 of Rheb to isoleucine and serine and that of INPP5E into serine (-1) and lysine (-3) increases the affinity of the Rheb peptide to PDE6D and decreases that of INPP5E. Finally a mutant INPP5E construct with serine (-1) and lysine (-3) failed to be exclusively localised in cilia indicating that the exclusive localisation of INPPE is at least in part dependent on the high affinity to PDE6D. Finally ciliary cargo, bound to PDE6D with high affinity, are released from PDE6D only by the more competent release factor Arl3 however the non-ciliary cargo, bound to PDE6D with low affinity are released by both Arl2 and Arl3.

Based on these results and on the localisation of the Arl3 GEF, Arl13b, in the cilia the following model has been proposed: Arl3 is activated by Arl13b in the cilia whereas Arl2 is active outside cilia. Prenylated cargos are solubilised by binding to PDE6D which accelerates their diffusion in cytosol. High affinity cargo-PDE6D complexes are delivered to cilia where they are released by active Arl3GTP. Low binding affinity complexes on the other hand will be released by Arl2GTP outside cilia.

In contrast to this this model, where targeting of the cargo is dependent on the localisation of an active GDF, the need for bona fide GDF in targeting Rab proteins is however disputable and a model where Rab targeting based on the spontaneous dissociation of Rab from the Rab-GDI has been proposed (*3, 6*)(Fig2). The need for GDF to accelerate the cargo unloading in ciliary targeting is however most likely to counter the diffusion of cargo out of the cilia. Indeed when Arl3 is knocked down, INPP5E still can be targeted to cilia but also can be seen outside of the cilia and the exclusive ciliary localisation is lost (*21*).

Figure 1 Residues responsible for the different affinities of INPP5E and Rheb to PDE6D. A schematic representation comparing the interaction of residues at positions -1 and -3 (in red) of INPP5E and Rheb to PDE6D. Polar interaction are shown as blue dotted lines and hydrophobic contacts are shown as violet dotted lines

Figure 2. GEF based Rab targeting model versus the ciliary INPP5E targeting model. RabGDP binds to GDI with high affinity (2.5nM) and spontaneously dissociates at the membrane where RabGEF accelerates nucleotide exchange to RabGTP. RabGTP has relatively low affinity to GDI (1700nM) and hence will stay on the membranes (*3*). INPP5E binds to PDE6D with high affinity (3.7nM) and dissociates on the membrane via the formation of a fast dissociating ternary complex with Arl3GTP (*14, 21*). The loading of Arl3 with GTP is accelerated by the Arl3GEF (Arl13b) (*15*)

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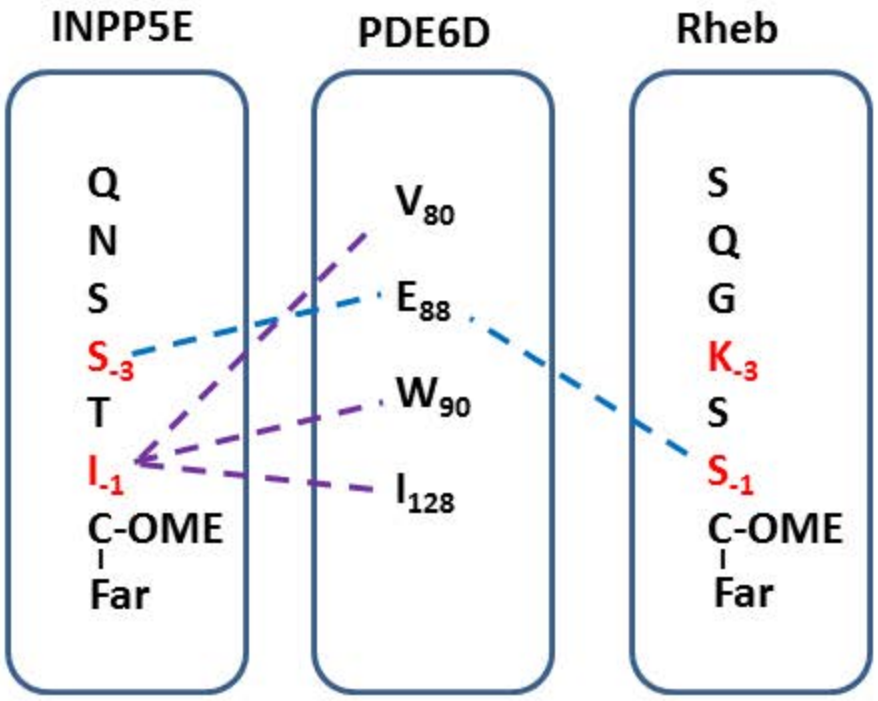
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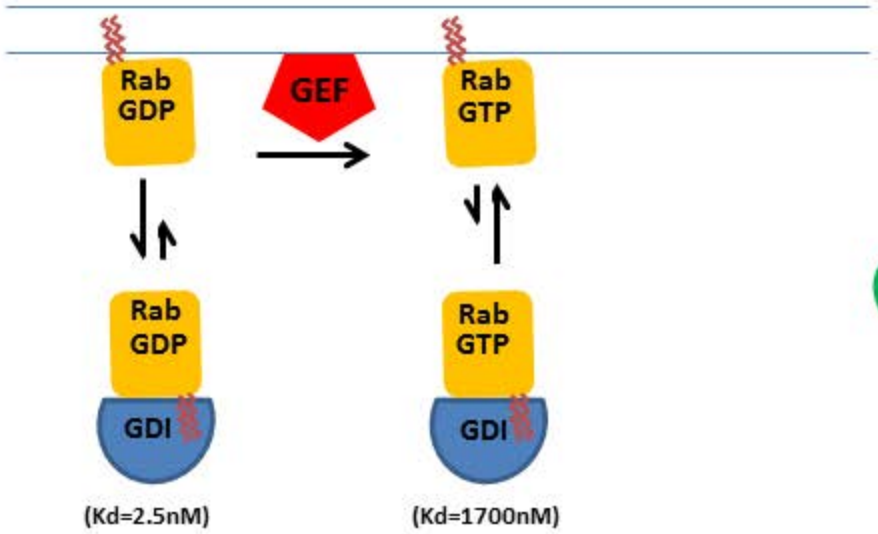
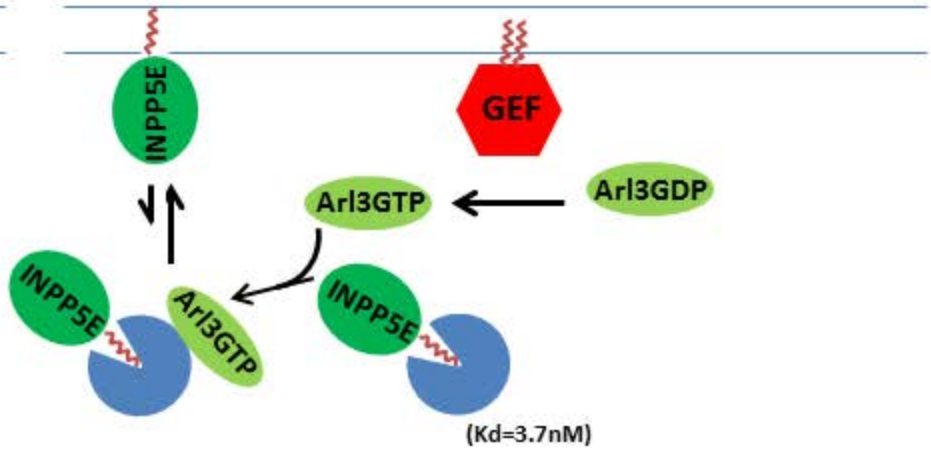
Rab Targeting



INPP5E ciliary Targeting