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COMMENTARY

Are prenyl groups on proteins sticky fingers or greasy handles?

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This Commentary discusses the work of Dietrich et al. in this issue of the *Biochemical Journal*, which sheds new light on the biological roles of protein-bound prenyl groups by providing evidence that the α-subunit of the heterotrimeric G-protein transducin has a binding site for the geranylgeranyl group of the γ-subunit.

Key words: farnesyl, geranylgeranyl, membranes, prenylation, proteins.

Prenylation of proteins has been recognized as a major post-translational modification for nearly 15 years, and its biochemistry is well understood. Two types of prenyl groups, C_{15} farnesyl and C_{20} geranylgeranyl, are known to be attached to cytoplasmic proteins at C-terminal cysteine-rich signal sequences of the CaaX (C, Cys; a, aliphatic; X, any residue except Pro) type or alternatively of the double-cysteine type [1]. The linkage is a highly stable thioether and is followed, in the case of the CaaX sequences, by proteolysis of the last three amino acids and carboxylmethylation of the C-terminus (which also occurs for the CXC termini). Prenylation uses diphosphate derivatives of isoprenoids as donors and is catalysed by one of three types of protein:prenyl transferases, namely protein farnesyl transferase (FT), protein (CaaX) geranylgeranyl transferase (GGT type-I) or Rab geranylgeranyl:protein transferase (RGGT; also known as GGT type-II). For FT and GGT type-I, a single prenyl group is transferred to a CaaX sequence. The majority of RGGT substrates, restricted to members of the Rab GTPase family, undergo double-geranylgeranylation on adjacent cysteine residues at or near the C-terminus in motifs including CXC, XCC, CCXX, CCXXX, CCX and CXXX [2].

In every known case, prenylation confers on the modified protein the ability to interact with cellular membranes, although many prenylated proteins have a substantial soluble pool, especially the doubly geranylgeranylated Rab proteins. It has usually been assumed that the long-chain hydrophobic prenyl groups act like the acyl chains of lipids and intercalate into the lipid bilayer of biological membranes, and most reviews on the topic contain cartoons depicting this type of membrane interaction, once dubbed the 'sticky finger' [3]. Calculations of the hydrophobicity of prenyl groups indicate that they are at least as hydrophobic as fatty acids [4], and *in vitro* studies have shown that prenylated peptides can interact with artificial bilayers [5–10]. However, it is difficult to demonstrate unequivocally that prenyl groups intercalate into membrane bilayers *in vivo*, because of the complexity of cellular membranes, nor has actual intercalation *in vitro* been directly demonstrated.

There are reasons for believing that this simple model may not apply *in vivo*, at least not in all cases. Prenyl groups are unlike acyl chains in being relatively rigid and containing a double bond and bulky methyl side chain for every four carbon atoms along the main chain. These 'bumpy' lipid groups may not pack well with the most abundant biological phospholipid acyl chains, which tend to be longer and mono- or di-unsaturated. This is particularly relevant within plasma membrane 'lipid raft' domains, which are enriched in saturated sphingolipids and cholesterol and are believed to exist in a liquid-ordered state where the acyl chains are highly extended and tightly packed [11]. Nevertheless, many prenylated proteins, such as Ras proteins and heterotrimeric G-proteins, have been reported to be enriched in lipid rafts or the related structures caveolae [12–14].

One possible explanation for these paradoxes is that protein-bound prenyl groups may not intercalate directly into biological membranes, but rather may interact with membrane-bound protein 'prenyl receptors', as previously proposed [15]. There is indeed much evidence that prenyl groups interact with proteins. Perhaps the best studied interactions involve GDP dissociation inhibitors (GDIs), cytosolic proteins that bind and prevent release of GDP from prenylated Ras-like GTPases, thereby stabilizing the inactive and soluble forms of the protein. These proteins regulate the activity of GTPases by controlling their membrane association/dissociation cycle. The molecular mechanisms underlying this activity remain unclear, but it certainly involves direct interaction with the prenyl group(s). There are two families of GDIs, namely RhoGDIs, which are specific for Rho family proteins, and Rab escort proteins (REPs)/RabGDIs, which are specific for Rab family proteins. RhoGDIs bind GDP-bound soluble Rho, Rac and Cdc42 proteins and may regulate their specific release (and consequent activation) to or from the membrane [16,17]. The crystal structure of the Cdc42–RhoGDI complex shows that the Cdc42 geranylgeranyl group binds to a hydrophobic surface of RhoGDI formed by the β-sheet-rich immunoglobulin-like domain [18]. The role of the RabGDI/REP family is more complex, as these proteins contribute at least two distinct activities. REP proteins bind RGGT, position Rabs for geranylgeranyl modification and deliver geranylgeranylated Rabs to membranes, while RabGDIs recycle geranylgeranylated Rabs on and off membranes [2,19,20]. The geranylgeranyl binding site(s) on REP and RabGDI remain to be determined, but certainly involve a distinct structure from RhoGDI, since REP/RabGDI proteins lack immunoglobulin-like domains. However, a distinct class of proteins typified by the rod phosphodiesterase δ-subunit (PDE-δ) form an immunoglobulin-like domain very similar to RhoGDI [21,22]. PDE-δ interacts with a variety of farnesylated and geranylgeranylated proteins, including PDE-α, PDE-β, RPGR (retinitis pigmentosa GTPase regulator), Ras, Rap and Rho; this broad specificity may be

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due to PDE-δ consisting of just a prenyl-binding domain, unlike RhoGDI, whose additional N-terminal domain confers specificity to interaction with a subset of prenylated proteins. Cytosolic PDE-δ may serve a general solubilization role to singly prenylated proteins and may regulate their interaction with the membrane and/or other proteins [21]. Another example of a protein that appears to bind prenyl groups is PRA1 (prenylated Rab acceptor) [23,24]. PRA1 binds Rab proteins and competes with RabGDI for Rab binding, suggesting that the balance between PRA1 and RabGDI activity controls the membrane/cytosol distribution of Rabs [24]. PRA1 belongs to a family of hydrophobic proteins called Ypt-interacting proteins (Yips) in *Saccharomyces cerevisiae*. Yip1 appears to bind selectively to doubly geranylgeranylated Rabs and could play a role in stabilizing them in the membrane [25]. This family of proteins may perform the long-sought-after role of prenyl receptor within the membrane bilayer.

The farnesyl group of Ras proteins is known to be crucial for their function. Kloog and collaborators [26–29] have shown that Ras proteins can be released from cellular membranes and their activity can be inhibited by long-chain prenylcysteine analogues. Some of these are also inhibitors of carboxylmethylation of Ras, but at much higher concentration than that required for the dislodgement of Ras. A major farnesylated Ras-binding protein is the cytoplasmic form of galectin-1, known to be associated with increased Ras activity and itself a transforming protein when overexpressed [30], although how the galectin-1–Ras interaction plays a role in activity is unclear.

In this issue of the *Biochemical Journal*, Dietrich et al. [31] report a similar approach, where prenylcysteine analogues have been shown to directly inhibit interaction between transducin α-subunit and the geranylgeranyl group of the γ-subunit. Both farnesyl and geranylgeranyl derivatives were effective, and inhibition was competitive and reversible. Importantly, the N-terminal S-acyl (palmitoyl) group of the α-subunit was not required for the interaction, showing that simple binding between the two lipid substituents is not responsible. This implies that the α-subunit has a specific binding site for the γ-subunit geranylgeranyl group and questions whether this group is involved in direct membrane binding.

In summary, despite the intuitive assumption that protein-bound prenyl groups intercalate directly into cellular membranes, there is little evidence to support this idea. Instead, most evidence suggests that prenyl groups are involved primarily in lipid–protein interactions. Perhaps the idea of the 'sticky finger' inserting into the lipid bilayer needs to be re-evaluated, at least in some cases, and a better analogy might be the 'greasy handle' that can be grabbed by proteins with appropriately hydrophobic binding sites.

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