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Ras Hitchhikes on PDE6δ

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Abstract

Ras GTPases are tethered to cellular membranes by a farnesyl lipid that modifies a C-terminal cysteine. Among the ways Ras traffics between membranes is via fluid phase diffusion, suggesting that a cytosolic chaperone might be needed to shield the farnesyl lipid during transport. PDE6δ is now revealed to be a farnesyl-binding Ras chaperone that facilitates its trafficking and signaling.

Ras family GTPases are targeted to membranes by virtue of post-translational modification with either a farnesyl (C15) or geranylgeranyl (C20) polyisoprene lipid. Ras proteins themselves are farnesylated and some are further lipidated with the acyl chains of one or two palmitic acids. Since Ras was shown to be a lipoprotein more than two decades ago, an unresolved question has been whether there exists a cytosolic chaperone capable of delivering farnesylated Ras to membranes, extracting it from membranes and solubilizing it in the cytosol. The characterization of RhoGDI as just such a chaperone for geranylgeranylated Rho family GTPases intensified the interest in this question. Recent observations demonstrating fluid-phase transport of farnesylated Ras between membrane compartments^{1, 2} have lent urgency to the quest for Ras chaperones as a way to better understand Ras trafficking. A study by Bastiaens and colleagues on page XXX of this issue³ now establishes the δ subunit of cGMP phosphodiesterase type 6 (PDE6δ) as a cytosolic chaperone for Ras that contributes to its subcellular trafficking and thereby to its function as an oncogene.

Several farnesyl-peptide binding proteins have been described including PRA1⁴, PDE6δ⁵, galectin-1⁶ and smgGDS⁷. Among these, PDE6δ is particularly interesting because it has structural similarity to RhoGDI^{5, 8}. PDE6δ is a protein abundantly expressed in the retina that was originally described as a regulatory subunit of the cGMP phosphodiesterase that mediates signaling in photoreceptors. PDE6δ was reported to bind to both farnesylated PDE6α and geranylgeranylated PDE6β⁹ as well as to other prenylated components of the visual signal transduction pathway including farnesylated GRK1 and geranylgeranylated GRK7¹⁰. The high level of expression of PDE6δ in photoreceptors is believed to be required for the huge flux through the connecting cilium of prenylated proteins trafficking between the inner and the outer segments⁹. Unlike the catalytic subunits of PDE6 that are restricted to the retina, PDE6δ is ubiquitously expressed. In 2002 Nancy et al. reported that PDE6δ bound to H-Ras and several related proteins, including geranylgeranylated Rap1, and that

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overexpressed PDE6 δ could solubilize GFP-tagged H-Ras and Rap1 but not Ral from cell membranes. Interestingly, Arl2/3, small GTPases that are not prenylated, also bind to PDE6 δ ⁸. In a recent, elegant structural study to which Bastiaens' group contributed, the authors showed that the prenyl-binding pocket of PDE6 δ is allosterically regulated by GTP-bound Arl2/3, which act as releasing factors that cause discharge of farnesylated Rheb from PDE6 δ ¹¹. Importantly, the co-crystal revealed that PDE6 δ bound the farnesyl group of Rheb without making contacts with the Rheb protein, explaining the observed lack of specificity of PDE6 δ for GTPases or for their guanine nucleotide binding states. Thus, PDE6 δ is a cytosolic chaperone for a wide range of prenylated proteins and transfer of its cargo onto membranes is regulated by Arl2/3.

Farnesylation is insufficient to target Ras proteins to the plasma membrane. Also required is a so-called "second signal" that consists either of palmitoylated cysteines in proximity to the farnesylated C-terminus or, in the case of K-Ras4B, a polybasic region that forms an electrostatic interaction with the negatively charged headgroups of the phospholipids in the inner leaflet of the plasma membrane. Recent studies have shown that each of these second signals can be reversed: the palmitate modification can be removed by a thioesterase^{1, 2, 12, 13} and the polybasic region can be partially neutralized by phosphorylation¹⁴. Bastiaens and colleagues have produced a compelling series of studies establishing an acylation/deacylation cycle for N-Ras and H-Ras that regulates trafficking of these Ras proteins between the plasma membrane and Golgi². According to Bastiaens' model, the restriction of palmitoylation to the Golgi drives the affinity trapping of Ras on this compartment and the vectorial delivery of Ras proteins to the plasma membrane via secretory vesicles that counteract entropy that would otherwise distribute Ras proteins evenly on all membranes¹³.

In the study published in this issue of *Nature Cell Biology*, Bastiaens and colleagues extend our understanding of PDE6 δ by showing that this chaperone is required for proper trafficking and signaling of Ras³. Tracking localization of Ras and related proteins in live cells and confirming their findings with fluorescence resonance energy transfer (FRET), Chandra et al. show that co-expression of PDE6 δ can extract some but not all Ras family proteins from cellular membranes. With regard to palmitoylated Ras proteins the degree of palmitoylation controls PDE6 δ binding: doubly palmitoylated H-Ras bound poorly while monopalmitylated N-Ras, Rap2a and Rap2c bound relatively well. These results suggest that PDE6 δ is involved in the retrograde traffic of Ras from the plasma membrane to the Golgi and explains the observation that the kinetics of this pathway were most consistent with fluid phase diffusion^{1, 2}. Indeed, fluorescence loss after photoactivation of cytosolic non-palmitylated H-Ras revealed that PDE6 δ enhanced the effective diffusion of the Ras protein.

Turning their attention to K-Ras4B, the authors found that the strong polybasic region with a net positive charge of +8 prevented extraction from the plasma membrane by PDE6 δ . However, when they reduced the charge either artificially by mutating some of the lysines in the polybasic region to glutamic acids, or physiologically by stimulating phosphorylation of serine 181 in the polybasic region with the PKC agonist bryostatin-1¹⁴, K-Ras4B could be extracted from membranes and sequestered in the cytosol. Since the charge of the cytosolic

leaflet of endomembranes is much less than that of the plasma membrane, PDE6δ would be expected to extract K-Ras4B and other polybasic, farnesylated proteins from endomembranes and release them at the plasma membrane. Thus, whereas the cytosolic chaperone PDE6δ mediates retrograde trafficking of palmitoylated Ras proteins, it facilitates anterograde flow of K-Ras4B. In both cases, the action of PDE6δ is to counter entropy that would otherwise tend to randomly redistribute Ras proteins among all cellular membranes. PDE6δ thereby assures that each isoform of Ras is distributed properly on the subcellular compartments upon which it signals. To confirm the effect of PDE6δ on Ras signaling the authors used HepG2 hepatocarcinoma cells that express very little PDE6δ. All three Ras isoforms were mislocalized in these cells but ectopic expression of PDE6δ restored normal subcellular distribution and enhanced Ras/MAPK signaling. Thus, proper trafficking was required for efficient signaling. To establish a role for PDE6δ in Ras-transformed cells the authors silenced PDE6δ in human fibroblasts expressing oncogenic H-Ras and murine pancreatic adenocarcinoma cells expressing oncogenic K-Ras, and found diminished growth of each in a clonogenic assay that correlated with the degree of PDE6δ knockdown.

Several of the findings in Chandra et al. contradict previous reports. Whereas Nancy found by yeast-two hybrid and co-immunoprecipitation analysis that PDE6δ bound palmitoylated H-Ras and that overexpression of PDE6δ could extract both H-Ras and K-Ras4B from the plasma membrane, Chandra et al. found that both palmitoylation and the full positive charge of K-Ras4B inhibited binding. Chandra et al. also found that PDE6δ could not extract Ras proteins when the prenylation signal was changed from one that directs farnesylation to one that specifies geranylgeranylation. This is consistent with the co-crystal of PDE6δ and Rheb, which reveals a hydrophobic pocket that can best accommodate the fifteen carbon isoprene¹¹ in contrast to the deeper pocket of RhoGDI. This is also consistent with earlier work that showed that the dissociation constant for PDE6δ-bound farnesyl (0.7 μM) is twenty-fold lower than that of geranylgeranyl¹⁰. Yet numerous geranylgeranylated proteins, both in the photoreceptor¹⁰ and in the Ras superfamily⁵, have been reported to bind to PDE6δ and competition studies revealed that the binding of geranylgeranylated GRK7 to PDE6δ was stronger than that of farnesylated GRK1¹⁰. Further work will be required to resolve these inconsistencies.

Another remaining question is the relative importance of PDE6δ in Ras biology. PDE6δ null mice are small and have defects in GRK1 transport and visual signaling but are fertile and otherwise healthy¹⁵, suggesting that Ras proteins can function properly without PDE6δ. Does this mean that the trafficking efficiency afforded by PDE6δ is not required for Ras function, or do other cytosolic chaperones step in when PDE6δ is absent? Another open question is membrane specificity. If PDE6δ delivers Ras to the proper membrane compartment by diffusion and GTP-bound Arl2/3 is waiting there to stimulate release, what controls the spatial distribution of Arl2/3 and its regulators? No matter what prove to be the answers to these questions, it seems clear from the work of Bastiaens and colleagues, including Alfred Wittinghofer, that PDE6δ is a *bona fide* cytosolic chaperone for Ras and a particularly fascinating one since its function as a chaperone is regulated by Arl GTPases that are distantly related to Ras. The ability of Ras proteins to translocate from one membrane compartment to another without vesicular transport is well established. We now

know that among the ways Ras can accomplish this feat is by hitchhiking a ride with PDE6δ.

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Figure 1.

PDE6δ is a Ras chaperone that facilitates cytoplasmic trafficking. PDE6δ extracts Ras from a donor membrane and allows solubilization in the aqueous environment of the cytosol by sequestering the farnesyl chain in a hydrophobic pocket. Upon arrival by simple diffusion at the acceptor membrane, GTP-bound Arl2/3 binds to an allosteric site on PDE6δ causing it to release Ras onto the acceptor membrane where the farnesyl lipid can once again partition into phospholipid bilayer. In the case of H-Ras the donor membrane is the plasma membrane where H-Ras can be depalmitoylated and gain affinity for PDE6δ, and the acceptor membrane is the Golgi where H-Ras is re-acylated. In the case of K-Ras4B the donor membrane is endomembrane where the electrostatic interaction with its C-terminal polybasic region is relatively low and the acceptor membrane is the plasma membrane where that interaction is strong.