

## Rac1 gets fatter

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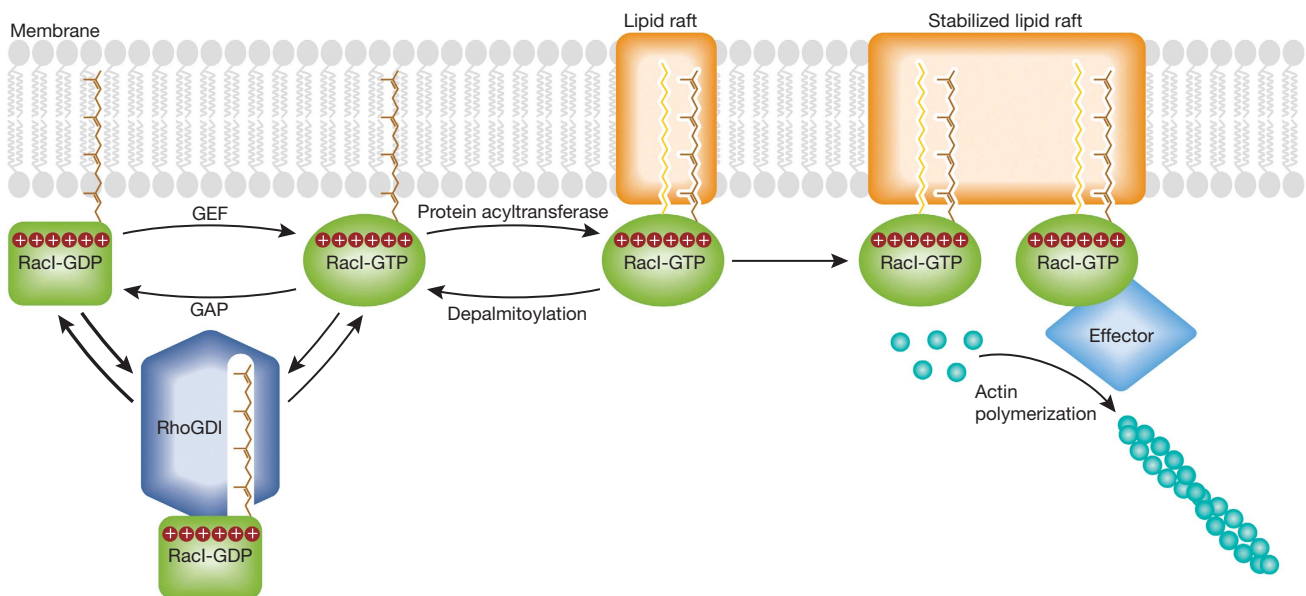
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Since its description more than two decades ago as a substrate for botulinum C3 exoenzyme (Didsbury *et al*, 1989), Rac1 has been one of the most studied small GTPases of the Ras superfamily. Interest in Rac1 exploded in 1992 when Ridley and Hall published their seminal work showing that Rac1 regulates the actin cytoskeleton to promote lamellipodia formation (Ridley *et al*, 1992). Since that report, >4350 studies have been published on Rac1 and seemingly every detail of its regulation and biological function has been dissected, including its post-translational modifications. It therefore comes as somewhat of a surprise when del Pozo and colleagues (Navarro-Lérida *et al*, 2012) report for the first time in this issue of *The EMBO Journal* that Rac1 can be palmitoylated and that acylation directs its location in plasma membrane microdomains and modulates its signalling output.

Palmitoylation is a common and reversible form of post-translational modification that has received much attention recently because a long sought family of protein acyltransferases (PATs) have been described (Lobo *et al*, 2002) and

because global proteomic analysis has revealed a great many substrates for PATs (Roth *et al*, 2006). No consensus amino-acid sequence for palmitoylation has been discerned such that genomic data cannot predict protein acylation. GTPases are no strangers to palmitoylation. Indeed, H-Ras is among the best studied of all palmitoylated proteins and N-Ras, K-Ras4A, RhoB, and TC10 are other examples of palmitoylated small GTPases.

Small GTPases are directed to the plasma membrane as a consequence of a three-step post-translational modification of a C-terminal CAAX sequence that adds a farnesyl or geranylgeranyl lipid to the CAAX cysteine. Also required is a so-called second signal immediately upstream of the CAAX sequence that consists either of a polybasic region or one or more cysteines that serve as sites for palmitoylation (Choy *et al*, 1999). The prototypical polybasic region is that of K-Ras4B, which possesses a net charge of +8. Rac1 has a similar polybasic region with a net charge of +6 and this has been considered to be its functional second signal for plasma membrane targeting.



**Figure 1** The Rac1 acylation cycle. Rac1 localizes to the plasma membrane by virtue of its geranylgeranyl modification (shown in brown) and its +6 polybasic region (shown in red) once it has been released from its cytosolic chaperone, RhoGDI. At the plasma membrane, Rac1 is activated by a guanine nucleotide exchange factor (GEF) and also undergoes palmitoylation (palmitate is shown in yellow) by an as yet uncharacterized PAT. Palmitoylated Rac1 segregates to cholesterol-rich, liquid-ordered domains (lipid rafts) in the plasma membrane and stabilizes these microdomains in a process associated with actin polymerization.

Whereas both K-Ras4B with its polybasic region and H-Ras with its palmitoyl modifications localize to membrane microdomains, only H-Ras localizes to cholesterol-rich, liquid-ordered domains sometimes known as lipid rafts (Prior *et al*, 2003). Indeed, other palmitoylated proteins such as linker of activated T cells are enriched in lipid rafts and palmitoylation is believed to be the principal determinant of protein partition into these domains. Thus, it presented a conundrum when 7 years ago del Pozo *et al* (2004) showed that Rac1 with its geranylgeranyl modification and polybasic region was enriched in lipid rafts.

Navarro-Lérida and del Pozo have now resolved this paradox by describing the palmitoylation of Rac1 and showing how this modification affords another level of spatio-temporal regulation of its activity. Using metabolic labelling with tritiated palmitic acid, they show that Rac1 incorporates palmitate at its C-terminus in a manner dependent on both its prior CAAX processing and its polybasic second signal. Furthermore, they demonstrate that inhibition of palmitoylation, by either treatment with 2-bromopalmitate or mutation of the palmitoylated cysteine, affects both the plasma membrane distribution and GTP loading of Rac1, and consequently, its ability to activate effectors. These results suggest that Rac1 undergoes an acylation cycle that modulates its signalling activity (Figure 1), as has been described for H-Ras and other similarly modified proteins (Chiu *et al*, 2002; Rocks *et al*, 2005).

For this class of protein, the cycle of palmitoylation/depalmitoylation serves to distribute and limit protein signalling at specific cellular compartments (Rocks *et al*, 2010), and Rac1 proves no different in this regard. As one might predict from what is known about the function of Rac1, loss of palmitoylation resulted in defects in cell spreading and migration. In contrast, the observations that Navarro-Lérida *et al* made using total internal reflection fluorescence microscopy were quite unexpected. They found that GFP-Rac1 was enriched in 'worm-like' regions of plasma membrane that

were also marked by GM1 staining, were cholesterol dependent, and were associated with regions of polymerized actin. Not only was the incorporation of GFP-Rac1 into these membrane domains dependent on Rac1 palmitoylation, but so too was the formation of these microdomains. Among the remarkable features of this observation is the scale of the domains. Recent work has shown that the lipid rafts that are enriched for H-Ras are highly dynamic and exist on a nanoscale that can accommodate fewer than ten Ras molecules (Prior *et al*, 2003). The membrane domains induced by palmitoylated Rac1 are at least one order of magnitude larger.

By characterizing Rac1 palmitoylation, Navarro-Lérida and del Pozo simultaneously answer a long-standing question of Rac1 membrane distribution and define a new and fertile area for future investigation. How and where is Rac1 palmitoylation controlled? Is there a Rac1-specific thioesterase that depalmitoylates Rac1? A recent report describes a role for prolyl isomerization in regulating H-Ras palmitoylation (Ahearn *et al*, 2011), and Rac1 contains conserved prolines in proximity to its palmitoylation site. Is Rac1 palmitoylation also regulated by peptidyl-prolyl isomerization? Palmitoylation blocks the binding of RhoB to RhoGDI (Michaelson *et al*, 2001) but has no effect on Rac1 binding to the chaperone. How is the palmitate accommodated in the complex in a way that allows solubilization of Rac1? Given the large number of palmitoylated proteins at the plasma membrane, what are the special characteristics of palmitoylated Rac1 that drive stabilization of the worm-like microdomains? Whatever are the answers to these questions, the identification of palmitoylation as a mechanism by which Rac1 function is modulated proves that, even after 20 years of scrutiny, there is still much to be learned about this remarkable molecule.

## Conflict of interest

The authors declare that they have no conflict of interest.

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