Opinion

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Ras moves to stay in place

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Ras is a major intracellular signaling hub. This elevated position comes at a precarious cost: a single point mutation can cause aberrant signaling. The capacity of Ras for signaling is inextricably linked to its enrichment at the plasma membrane (PM). This PM localization is dynamically maintained by three essential elements: alteration of membrane affinities via lipidation and membrane-interaction motifs; trapping on specific membranes coupled with unidirectional vesicular transport to the PM; and regulation of diffusion via interaction with a solubilization factor. This system constitutes a cycle that primarily corrects for the entropic equilibration of Ras to all membranes that dilutes its signaling capacity. We illuminate how this reaction–diffusion system maintains an out-of-equilibrium localization of Ras GTPases and thereby confers signaling functionality to the PM.

The cell expends energy to maintain Ras at the PM

The PM is an adaptive barrier that allows selective passage of information from the extracellular to the intracellular environment [1]. Transmembrane receptors allow this communication by triggering a cascade of protein reactions in the cytoplasm. An integral role in this transduction relies on the presence of peripheral plasma membrane proteins of the rat sarcoma (Ras) family, which relay receptor activity into the cytoplasm. One of the current views regarding why Ras traffics is to ensure the propagation of signals inside the cell. Ras has been shown to traffic to and signal from endomembrane compartments such as endosomes and the Golgi.

However, recent biophysical analyses of Ras trafficking are beginning to reveal another role for Ras trafficking that may extend beyond the transmission of signals to endomembranes. If all of the energy-consuming processes that maintain a cell were halted, transmembrane receptors would stay in the PM, whereas peripheral membrane proteins (such as Ras) – loosely attached by a lipid anchor – would continue to move around inside the cell by Brownian motion and thereby distribute to all available membranes. Such thermodynamic equilibration would favor endomembranes due to their large surface area. However, to be able to transduce signals from receptors in the PM, a cell must counter this entropic equilibration by expending energy. Breaking such energy-consuming systems that maintain PM enrichment automatically causes relaxation to a distributed endomembrane localization of Ras that is incapable of transducing signals from activated receptors at the PM. From this perspective, signaling benefits from the trafficking mechanisms that maintain Ras localization, but it does not give rise to such localization. We illustrate that – by tinkering with vesicular trafficking – the cell has evolved a dynamic localization cycle that maintains Ras in place. Although other peripheral membrane proteins that are central signaling hubs may utilize similar strategies to maintain PM localization, we concentrate on Ras proteins as one example of the constant uphill battle against entropic equilibration across all membranes.

The proto-oncogene product Ras

Wild type Ras cycles between a GDP-bound, inactive and GTP-bound, active state by the action of guanine nucleotide exchange factors (GEFs) that induce activation by nucleotide exchange to GTP and GTPase-activating proteins (GAPs) that accelerate the low intrinsic hydrolysis rate [2]. Ras proteins are mutated in approximately 20–30% of human cancers [3]. Oncogenic mutations of Ras – most commonly affecting the amino acids at positions 12, 13, and 61 – maintain Ras in a constitutively active GTP-bound state [3–7] and thereby falsely portray the presence of growth factors to the cytoplasm. There are three major Ras isoforms, H/N/KRas, of which the latter has two splice variants (KRas4A and 4B) and is most commonly affected in cancer, with activating mutations occurring in, for example, around 90% of pancreatic and 40% of colorectal tumors [3,8]. Active, GTP-loaded Ras recruits effector proteins like Raf kinase, the catalytic p110 domain of phosphoinositide 3-kinase, or the Ral guanine dissociation stimulator to the PM, thereby initiating signaling cascades that result in proliferation and cell survival [9].

The recruitment of these cytosolic effectors results in a dimensionality reduction in their diffusional space [10–12] that potentiates their reaction in the subsequent step of the signaling cascade. For example, the Ras-mediated concentration of Raf on the PM facilitates its dimerization, which induces Raf activation [13,14]. Therefore, the amount of Ras that resides on the PM is crucial for growth factor signaling output. Several post-translational modifications on the C-terminal hypervariable region (HVR) of Ras proteins confer an affinity for membranes.

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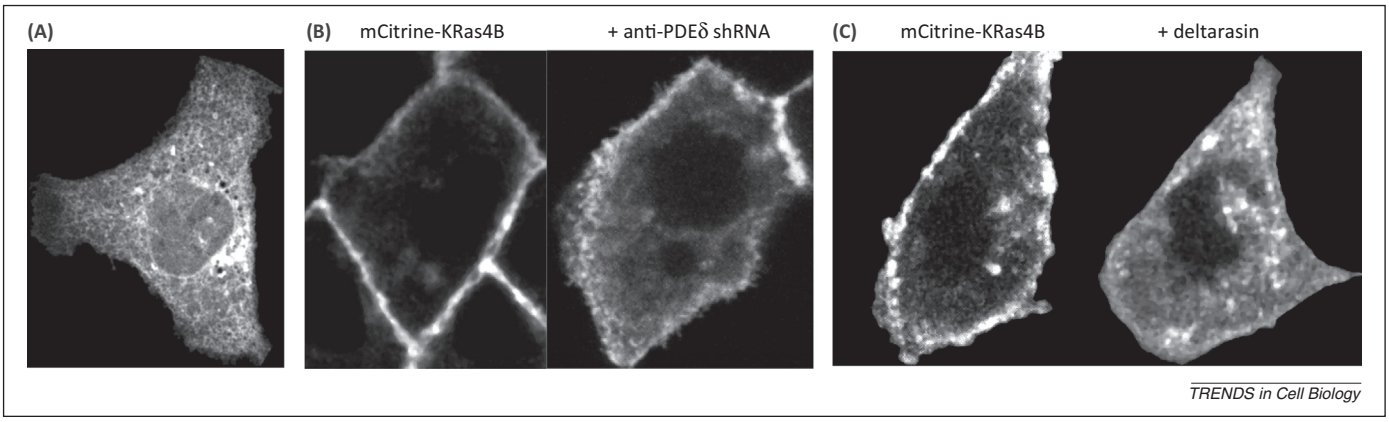

Figure 1. Endomembrane surface area significantly outweighs that of the plasma membrane. An unpalmitoylatable mutant of HRas that distributes to all endomembranes demonstrates the immensely larger endomembrane surface area compared with the plasma membrane **(A)**. Redistribution of KRas4B to endomembranes after knock down **(B)** or pharmacological inhibition **(C)** of the Ras-solubilizing factor phosphodiesterase 6δ (PDEδ).

De novo synthesized Ras family proteins are post-translationally modified in a series of three enzymatic steps at their C-terminal CAAX box (C, cysteine; A, aliphatic amino acid; X, any amino acid) [15–17]. If the X in the CAAX box is any amino acid other than leucine, which is the case for Ras isoforms, a 15-carbon farnesyl group is irreversibly attached to the CAAX box cysteine by the cytosolic enzyme farnesyl transferase [18–21]. Ras family proteins that feature a leucine in the X position will be geranylgeranylated. The subsequent two steps take place on the endoplasmic reticulum (ER): RAS-converting enzyme 1 (RCE1) proteolytically removes the three terminal amino acids, followed by methylation of the C-terminal prenylcysteine by isoprenylcysteine carboxylmethyltransferase (ICMT), which nullifies the negative charge of the C terminus [22–27]. The N-/HRas and KRas4A isoforms are additionally reversibly palmitoylated on one or two cysteines in the HVR. These post-translational modifications make Ras lipophilic and therefore enable its association with membranes. It is, however, not obvious how these modifications alone could result in a specific association of Ras with the PM, considering the overwhelming abundance of endomembranes compared with the PM (Figure 1).

Getting lost on endomembranes

A distinguishing feature of the inner leaflet of the PM is exposing negatively charged phospholipids to the cytoplasm. These offer binding sites for lysines, primarily those in the polybasic stretch of the HVR of KRas4B. Such an electrostatic interaction strengthens the weak, lipophilic PM association conferred by prenylation. Despite this tighter interaction, KRas4B is still lost from the PM by spontaneous dissociation and the constitutive internalization of the PM by endocytic vesiculation, resulting in a mean residence time of only 8 min [15,28]. Furthermore, internalized vesicles lose their charge asymmetry, which increases the dissociation of KRas4B from these vesicles. As a result, a small fraction of KRas4B is produced continuously that freely diffuses in the cytosol until encountering a membrane to associate with. By such rapid 'hopping' between endomembranes via its cytosolic fraction, KRas4B equilibrates over all membranes in the cell. Due to the excessive surface area of endomembranes relative to the PM (Figure 1A), KRas4B remains lost in the endomembrane system for a long time before it reencounters the PM. This prolonged residence of Ras on endomembranes compared with its shorter dwell time on the PM should therefore result in pronounced endomembrane localization. However, cells exhibit a strong enrichment of KRas4B on the PM, which means that such endomembrane partitioning of KRas4B is actively countered. How can KRas4B re-identify the PM in light of its surprisingly small relative surface area? A solution to this dilemma of relative membrane abundance versus KRas4B dissociation is an energy-consuming process that collects the KRas4B that is lost in endomembranes and then 'pumps' it continuously back to the PM [28]. To understand this process, consider Rab proteins, whose localization is determined by interaction with solubilizing factors.

Small GTPases of the Rab family as part of the Ras superfamily govern the organization of many membrane compartments. In their active, GTP-bound form, they regulate the collection and fusion of vesicles into larger organelles [29]. In their inactive, GDP-bound form Rab proteins are cytosolic, even though they are geranylgeranylated and should therefore have a generic membrane affinity. This stems from their interaction with specific solubilization factors that offer a binding pocket for the prenyl moiety of Rab proteins and thereby shield them from the hydrophilic cytoplasmic environment. The interaction of these solubilization factors, called guanine nucleotide dissociation inhibitors (GDIs), is much stronger for a GDP-loaded than a GTP-loaded Rab and locks Rabs in a GDP-bound state. The cytosolic complex of Rab-GDP with a GDI allows extensive and fast exploration of the cellular interior by diffusion. GDP-to-GTP exchange via GEF activity on membranes prevents rebinding of Rab to the GDI, resulting in its enrichment at the lipid bilayer harboring GEF activity [30]. Thus, GEF activity marks an endomembrane as the correct target membrane, causing highly specific localization. The concept of reversible prenyl binding to a solubilizing factor is also crucial to KRas4B localization.

Perinuclear release from phosphodiesterase 6δ (PDEδ) is essential for KRas4B localization at the PM

KRas4B that has been lost on endomembranes needs to explore the cell's interior by diffusion to reencounter the PM. To increase the fraction of KRas4B that can freely