Actin cytoskeleton: Thinking globally, actin' locally

Actin cytoskeleton: **Thinking globally, actin' locally**

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A class of proteins dubbed pipmodulins bind to and sequester the phospholipid PIP_2 in the plasma membrane. Local release of PIP_2 controls actin dynamics in specific subcellular regions and plays a critical role in regulating actin-based cell motility and morphogenesis.

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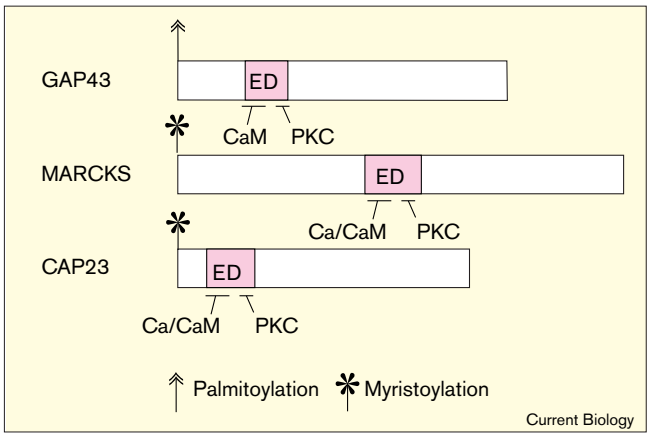
Phosphatidylinositol 4,5 bis-phosphate (PIP_2), is a relatively abundant acidic phospholipid found on the inner leaflet of the plasma membrane of a eukaryotic cell. PIP_2 binds to, and can regulate, the localization and function of a diverse spectrum of proteins, including molecules involved in phospholipid metabolism, second messenger signaling, and cell adhesion and motility [1]. Given the relative abundance of PIP_2 — it makes up 0.1–5% of inner bilayer lipids [2] — and its apparent global distribution throughout the plasma membrane, the question arises as to how PIP_2-dependent functions could be regulated locally and specifically. Recent work from the Caroni laboratory [3,4] suggests that the 'GMC' proteins GAP43, MARCKS and CAP23 function as quasi interchangeable modulators of PIP_2 availability. The authors show that the GMC proteins bind PIP_2 and sequester it in discrete microdomains. They propose that, in response to local signals, GMC proteins dissociate from PIP_2, creating local pools of free PIP_2. The net effect of this release will then depend on the relative local concentration of the many PIP_2 ligands. Based on this function, the authors have christened the GMC proteins as 'pipmodulins'.

Although the GMC proteins have almost no sequence homology to one another, they share a number of characteristic properties (Figure 1). First, they associate with the membrane by palmitoylation or myristoylation. Second, GMCs contain an effector domain which is rich in basic amino acids and binds PIP_2, calmodulin or actin filaments in a mutually exclusive manner. Third, phosphorylation of sites within the effector domain by protein kinase C (PKC) disrupts GMC interaction with PIP_2. Finally, GMC proteins have been shown to play important roles in actin-dependent processes of cell motility and morphogenesis. Despite these similarities, it was not until recently that the extent of the functional relatedness of the various GMC proteins was fully appreciated.

In an impressive series of experiments with transgenic mice, Frey *et al.* [3] have recently demonstrated that CAP23 plays critical roles in regulating nerve sprouting and that, despite their lack of sequence homology, GAP43 expression can almost completely rescue the defects in CAP23 mutants. This observation also provides an explanation for the relatively restricted defects in axonal growth and guidance that have been reported for GAP43 mutants, and suggests that elimination of all GMC proteins may be necessary to fully understand their role in nervous system development and plasticity. Indeed, in a companion paper Laux *et al.* [4] report that transgenic animals expressing a dominant-negative form of GAP43 in postnatal neurons exhibit dramatic defects in axonal outgrowth and nerve sprouting, phenotypes reminiscent of CAP23 mutants.

Analysis of cultured DRG neurons has revealed that, in addition to their shared functions, CAP23 and GAP43 have distinct expression patterns and unique functions in neurite outgrowth. These unique functions may be conferred by differences in the regulatory properties of their effector domains. The effector domain of CAP23 displays high-affinity, calcium-dependent calmodulin binding and appears to be only partially phosphorylated by PKC, whereas the effector domain of GAP43 displays low affinity, calcium-independent calmodulin binding and is phosphorylated efficiently by PKC. Furthermore, CAP23 association with the plasma membrane requires both myristolyation and the presence of its effector domain,

Figure 1



The GMC proteins: GAP43, MARCKS and CAP23 have amino-terminal sites for palmitoylation or myristoylation that are important for membrane association. The basic effector domain (ED) binds PIP_2, F-actin, calmodulin (CaM) or PKC in a mutually exclusive manner.

whereas membrane association of GAP43 is determined solely by palmitoylation.

Laux *et al.* [4] have reported compelling evidence that the primary function of GMC proteins may be to modulate the availability of PIP_2. In a series of cell-culture experiments, they have shown that GMC proteins accumulate in plasmalemmal microdomains, where they co-localize with PIP_2. GMC–PIP_2 microdomains are triton-insoluble, enriched in some GPI-linked proteins, and are dispersed by the cholesterol sequestering drug cyclodextrin, indicating that these microdomains are associated with lipid rafts [5]. The fact that GMC-enriched rafts do not contain Src or caveolin [6] suggests they represent a distinct subtype of lipid raft.

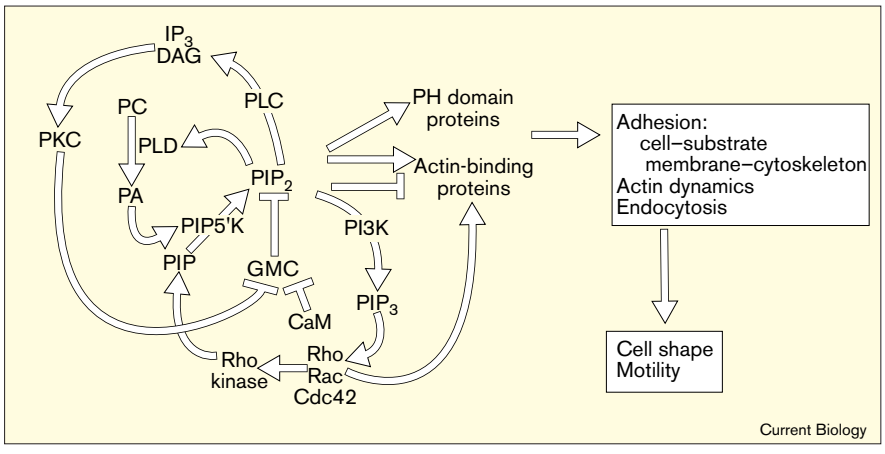
Overexpression of GMC proteins was found to increase the size of GMC–PIP_2 clusters and to partially protect PIP_2 from dispersion by cyclodextrin. In contrast, overexpression of GMC mutants lacking the effector domain (GMCΔED forms) had a dominant-negative effect on PIP_2 accumulation in microdomains, most likely because the mutant proteins compete with endogenous GMC proteins for localization in the rafts. Bulk levels of PIP_2 and its metabolites were found to be largely unaffected by manipulation of GMC–PIP_2 microdomain size and number, by overexpression of either GAP43 or GMCΔED mutants.

On the basis of these observations, Laux *et al.* [4] conclude that accumulation of PIP_2 within microdomains does not have a global effect on PIP2 metabolism; they suggest that the GMC proteins function to sequester PIP_2 within microdomains. Interestingly, the GMCΔED mutants were found to co-localize with endogenous GMC proteins, indicating that unidentified protein interactions outside the effector domain must be involved in GMC targeting. GMC–PIP_2 microdomain assembly also appears to be independent of the actin cytoskeleton, as treatments that disrupt filamentous actin (F-actin) were found to have little effect on the size or distribution of the GMC–PIP_2 microdomains.

Studies of neurite outgrowth in PC12B cells, induced by nerve growth factor (NGF), showed that GMC–PIP_2 microdomains play an important role in regulating actin dynamics [4]. Neurite outgrowth requires the asymmetric induction of actin polymerization in certain regions of the lamellipodia, and repression of actin dynamics in other regions [7]. That GMC proteins play a role in regulating actin assembly during NGF-induced neurite outgrowth is supported indirectly by experiments showing that treatment of dorsal root ganglia cultures with cytochalasin D, a drug that interferes with actin polymerization, largely phenocopies the neurite-outgrowth defects observed in *CAP23*$^{-/-}$ cultures. In PC12 cells, overexpression of wild-type GMC proteins was found to enhance the accumulation of actin-rich filopodia at the periphery of cells and to potentiate NGF-induced neurite outgrowth, whereas expression of GMCΔED mutants suppressed both NGF-induced translocation of actin to the cell periphery and neurite outgrowth.

Interestingly, the ability of GMC proteins to release PIP_2 appears to be just as important as their ability to sequester it. Global sequestration of PIP_2 by treatment with neomycin, a drug that binds to and masks PIP_2, led to translocation of F-actin to the periphery of the cell and symmetric induction of actin filopodia, but inhibited NGF-induced neurite

Figure 2



A simplified model showing a few of the many functions of PIP_2 in cell motility and morphogenesis. Binding of PIP_2 by GMC proteins inhibits interaction of the phospholipid with other proteins. Phosphorylation of GMC proteins by PKC, or binding to CaM, releases PIP_2. At least three feedback loops could stimulate the production of additional PIP_2, thereby locally amplifying the effect of PIP_2 release. Free PIP_2 is available to interact with actin-binding proteins and with PH-domain-containing proteins. The net effect of PIP_2 release on adhesion, actin dynamics and endocytosis may be determined by the localized concentration of competing PIP_2-binding partners. Abbreviations: IP_3, inositol triphosphate; DAG, diacylglycerol; PLD, phospholipase D; PA, phosphatidic acid; PC, phosphatidylcholine; PIP5'K, PIP 5-kinase; PIP, phosphatidylinositol 4-phosphate; PI3K, PI 3-kinase; PIP_3, phosphatidylinositol 3,4, phosphate.

outgrowth. The effect of neomycin could be partially reversed by overexpression of wild-type GMC proteins, and was mimicked most closely by overexpression of a GAP43 mutant that cannot bind calmodulin or be phosphorylated by PKC (and so cannot release PIP_2). Together, these results suggest that, although general sequestration of PIP_2, causing low global [PIP_2], promotes actin dynamics such as filopodia formation, localized release of PIP_2, causing high local [PIP_2], is required for neurite induction.

Other recent work highlights the diverse and critical roles of PIP_2 in regulating actin-dependent membrane dynamics. Raucher and colleagues [2] found that manipulation of either the level or availability of PIP_2 modulates cell 'adhesion energy', a measure of the force required to pull the plasma membrane away from the underlying cortical cytoskeleton. Expression of a PIP_2-specific pleckstin homology (PH) domain acted to mask PIP_2 and caused a reduction in adhesion energy. Similar effects were observed when signaling pathways that hydrolyse plasma membrane PIP_2 were activated. The effect of PIP_2 on membrane adhesion likely results from direct or indirect effects of PIP_2 on actin assembly, as the F-actin levels are reduced in cells with depressed PIP_2 levels. Furthermore, treatments that blocked F-actin accumulation reduced membrane adhesion, whereas stabilizing F-actin had the opposite effect.

Evidence that PIP_2 plays a role in actin driven-vesicle movement was reported recently by Rozelle *et al.* [8]. They found that expression of phosphatidylinositol 5-kinase (PIPKI), an enzyme that synthesizes PIP_2, induced the assembly of actin comet tails on cytosolic membrane vesicles. Interestingly, treatment with cyclodextrin reduced comet tail formation, indicating that cholesterol containing lipid rafts also play a major role in this type of PIP_2-dependent actin assembly. The motile vesicles were often endocytic, which according to Laux *et al.* [4] would suggest they lack GMCs that could sequester PIP_2. Interestingly, treatment with growth factors or drugs that increase tyrosine phosphorylation levels enhanced PIP_2-induced comet tail formation, indicating, once again, that signaling pathways can act synergistically with PIP_2 to stimulate actin assembly.

So how does PIP_2 regulate actin dynamics? The answer to this question is likely to be extremely complex (Figure 2). In the case of endocytic vesicles, N-WASP-induced activation of actin nucleation by the Arp2/3 complex was shown to be required for comet tail formation [8]. N-WASP is activated by PIP_2 binding, and expression of a construct that blocks recruitment of Arp2/3 to N-WASP inhibited comet tail formation. Whether PIP_2 plays other roles in this type of vesicular actin assembly remains to be determined. PIP_2 can bind to, and modulate, the localization and function of many, if not most, actin-binding proteins (reviewed in [9]). The precise effect of PIP_2 levels on actin dynamics is therefore likely to be determined by the local concentration of the various types of actin-binding proteins. As other classes of PIP_2-binding proteins are likely to compete with actin-binding proteins for biding to PIP_2, the local concentrations of non-actin-binding proteins may also influence the effect of PIP_2 on actin dynamics.

Finally, the ability of PIP_2 to regulate multiple signaling pathways and to mediate the recruitment of PH module containing proteins suggests there could be many indirect mechanisms by which PIP_2 regulates cell morphology and motility. A major challenge for the future will be to elucidate the mechanisms by which the plethora of PIP_2 effector molecules mediate the various cellular responses to localized liberation of PIP_2 from their keepers, the pipmodulins.

Acknowledgements

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