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HOW DO SMALL GTPase REGULATE MICROFILAMENT ASSEMBLY

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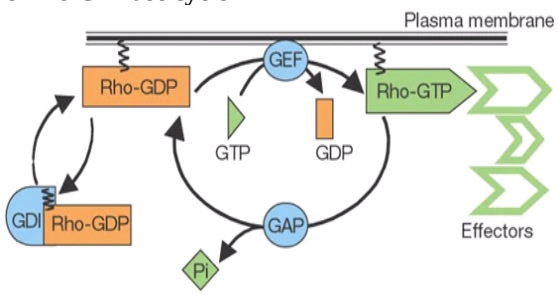
GTPase
Small GTPase is a kind of GTP-binding protein commonly found in eukaryotic cells. It plays an important role in cytoskeletal reorganisation, cell polarity, cell cycle progression, gene expression and many other significant events in cells, such as the interaction with foreign particles. The most prominent member of the small GTPase family is the Ras GTPase, thus the family is also called the Ras superfamily. The analysis of the small GTPase protein crystal structure indicates that the GTP binding domain of this type of protein can be subdivided into five relatively conserved motifs G1–G5. G1 motif (I) is a purine nucleotide binding signal; G2 motif (E) is in one of two segments that redirects with GDP or GTP binding function and provides major component of the effector binding surface; G3 motif (II) is related to the binding of nucleotide-related Mg2+; G4 motif (III) brings the hydrogen bond in contact with the guanine ring; G5 motif (IV) makes indirect associations with the guanine nucleotide. Taken together, these elements constitute the conserved ~20 kDa domain and shared by all Ras superfamily proteins.

Action of GTPase
While participating in physiological activity, the molecular structure of small GTPases present two forms which support mutual transformation, GTP-binding activated state and GDP-binding non-activated state, which can also be called as "ON" state and "OFF" state, respectively. In the active state, they interact with one of over 60 target proteins (effectors). The cycle is highly regulated by three classes of protein: in mammalian cells, around 60 guanine nucleotide exchange factors (GEFs) catalyse nucleotide exchange and mediate activation; more than 70 GTPase-activating proteins (GAPs) stimulate GTP hydrolysis, leading to inactivation; and four guanine nucleotide exchange inhibitors (GDIs) extract the inactive GTPase from membranes. All Rho GTPases are prenylated at their C terminus, and this is required for function.

Rho GTPases
Rac induces actin polymerization and integrin adhesion complex assembly at the cell periphery, leading to membrane protrusion, and it is essential for the migration of cells. The biochemical mechanisms by which Rac catalyses actin polymerization are a focus of great interest. Four Rac targets (IRSp53, phosphatidylinositol-4-phosphate 5-kinase, p65Pak and LIM kinase) have been implicated, as has the Arp2/3 complex, which catalyses de novo nucleation of actin polymerization and the formation of new filament branches.

The Rho GTPase cycle



Rho, Rac and Cdc42 have each been shown to regulate a signal transduction pathway in Swiss 3T3 cells that links extracellular signals to the formation of stress fibres, lamellipodia, and filopodia, respectively. These different compartments of the cellular cytoskeleton are composed of filamentous actin associated with integrin adhesion complexes, and their dynamic assembly and disassembly provide the driving force for cellular movement.

Discovery
The Rho gene was identified in 1985, but it was observations reported in 1992 that provided the first insights into the cellular function of Rho GTPases. Constitutively activated (GTPase deficient) mutants of Rho and Rac were found to induce the assembly of contractile actin and myosin filaments (stress fibres) and actin-rich surface protrusions (lamellipodia), respectively, when introduced into fibroblasts. Later, Cdc42 was shown to promote the formation of actin-rich,

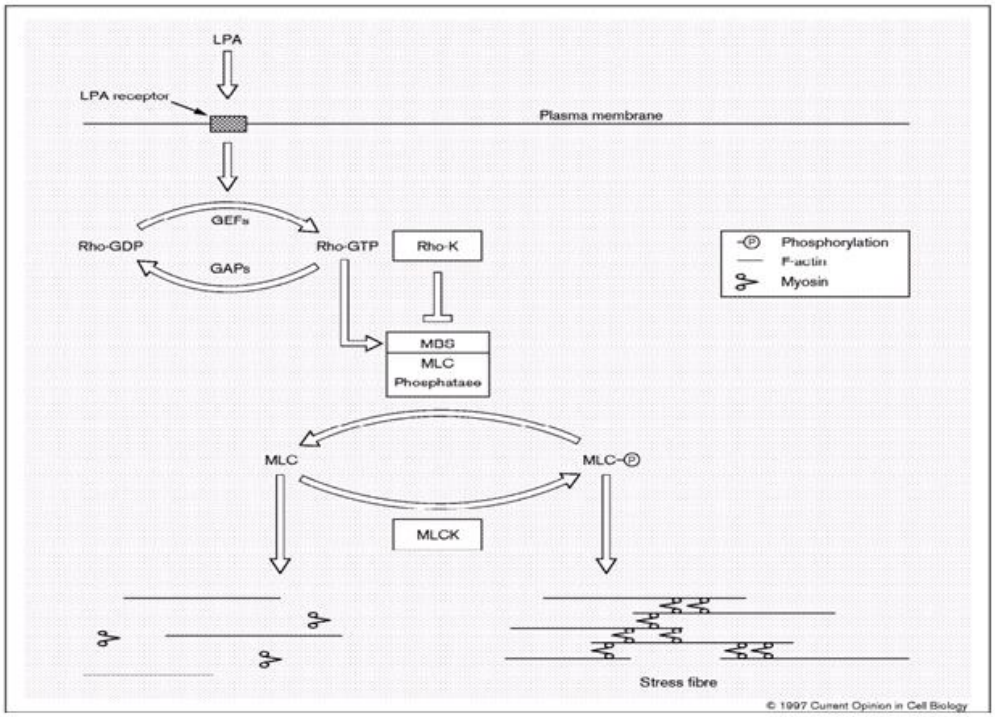
finger-like membrane extensions (filopodia). The conclusion that Rho, Rac and Cdc42 regulate three separate signal transduction pathways linking plasma membrane receptors to the assembly of distinct filamentous actin structures has since been confirmed in a wide variety of mammalian cell types as well as in yeast, flies and worms.

Rho

Two groups identified protein kinase N (PKN; also known as PRKl/Z) as a Rho -interacting protein. This 128 kDa leucine-zipper-bearing protein has a serine/threonine-kinase domain that is highly related to that of protein kinase C (PKC). PKN interacts specifically with GTP-bound Rho (not with Rat or Cdc42) and this interaction can be blocked by pretreatment of the GTPase with C3 transferase, a bacterial enzyme known to inactivate Rho through ADP ribosylation. The interaction of Rho with PKN leads to increased kinase activity both in vitro and in viva. Another serine threonine kinase, ~164 Rho-associated kinase (Rho-K; also known as ROKa), and its close relative, p 160 Rho-associated coiled-coil-containing protein kinase (ROCK), also interacts with Rho in a GTP-dependent manner.

An in viva target for Rho-K has been identified, though in a rather unexpected way. Using an affinity chromatography technique to isolate Rho-interacting proteins, Kaibuchi and colleagues identified the myosin-binding subunit (MBS) of myosin light chain (MLC) phosphatase. They subsequently went on to show that MBS is a substrate for ~164 Rho-K in vitro and that phosphorylation of MBS leads to a decrease in MLC-phosphatase activity.

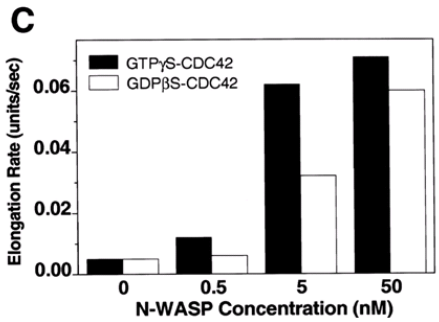
As the level of phosphorylation on MLC is regulated positively by MLC kinase and negatively by MLC phosphatase, it was predicted that Rho activation should lead to increased phosphorylation of MLC. Increased MLC phosphorylation could be a major contributor to the effects of Rho on actin organisation and perhaps even focal adhesion assembly. Rho activation results in an elevated level of MLC phosphorylation (through decreased phosphatase or increased kinase activity), thus leading to the bundling of dispersed actin filaments.



A potential mechanism for Rho-induced stress-fibre formation through the regulation of myosin phosphorylation.

Cdc42

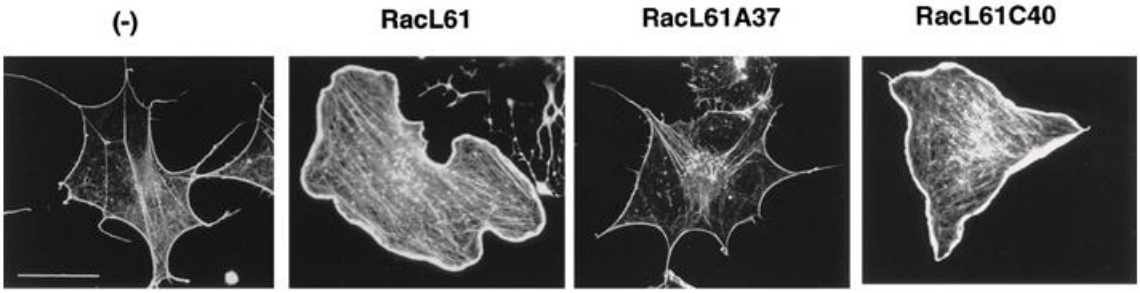
Detailed morphological analysis of T cells from Wiskott-Aldrich syndrome patients revealed unusual shapes and fewer microvilli than on normal cells, leading to the suggestion that WASP is important in organising the cytoskeleton of these cells. In vitro, the interaction of WASP with Rac is relatively weak, and WASP is most likely to be a target of Cdc42, with which it interacts strongly in a GTP-dependent manner. Containing adaptor molecule Nck and has some restricted sequence similarity to vasodilator-stimulated phosphoprotein (VASP), a protein known to interact with actin filaments. Overexpression of WASP in Jurkat cells induced the appearance of large cytoplasmic clusters of polymerized actin that was colocalized with WASP. Interestingly, this clustering effect was attenuated by coexpression of a dominant-negative Cdc42, suggesting that WASP is likely to be a cytoskeletal protein and that its interaction with actin may be controlled by Cdc42.



Effects of Cdc42 bound to different nucleotides on N-WASP.

Rac

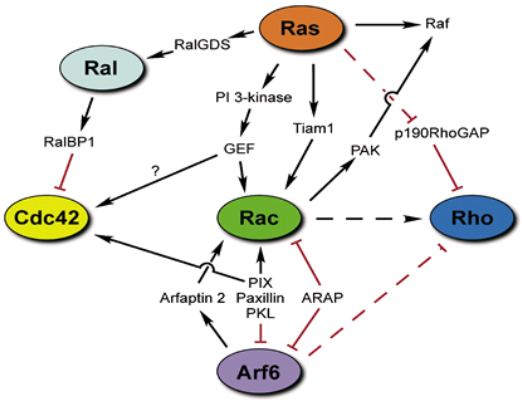
One of the major biological effects mediated by Rac is to control the polymerization of actin at the leading edge of the plasma membrane, leading to the extension of lamellipodia and the subsequent formation of membrane ruffles. Induction of actin polymerization at the cell margin by Rac is accomplished by the assembly of integrin-based focal complexes that can be visualised using anti-vinculin antibody. To explore the role of candidate Rac target proteins in this response, experiments were performed in which they microinjected eukaryotic vectors (pRK5) encoding myc-tagged Rac into serum-starved subconfluentSwiss 3T3 cells and examined the distribution of filamentous actin. 2 hr after microinjection of pRK5myc-RacL61 polymerized actin assembles at the leading edge of the plasma membrane to form lamellipodia; these are often seen folding back upon themselves to form ruffles.



Lamellipodia and Integrin Complex Formation Induced by Rac.

Cross-Talk between Cdc42, Rac, and Rho

A hierarchical relationship among the GTPases Cdc42, Rac, and Rho has been suggested in Swiss 3T3 cells, where Cdc42 activates Rac, which in turn activates Rho, resulting in coordinated actin reorganisation. However, the specific biochemical mechanisms and intermediate steps remain unclear. To investigate effector site mutations and their effect on this crosstalk, Rac or Cdc42 expression plasmids were microinjected into serum-starved Swiss 3T3 cells. Two hours post-injection, Rac injection led to peripheral actin formation; by four hours, stress fibres and focal adhesions appeared via Rho activation. The Y40C Rac mutant also activated Rho, unlike the F37A mutant. Similarly, Cdc42L61 injection induced Rac activation, while its F37A mutant resulted only in filopodia formation. This suggests that CRIB-containing proteins do not link Cdc42 to Rac or Rac to Rho, but that the F37A mutation disrupts crosstalk among Cdc42, Rac, and Rho.



Crosstalk between the Rho Family and Other Ras Superfamily GTPases (*Cell Journal*).

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