Rho signalling at a glance

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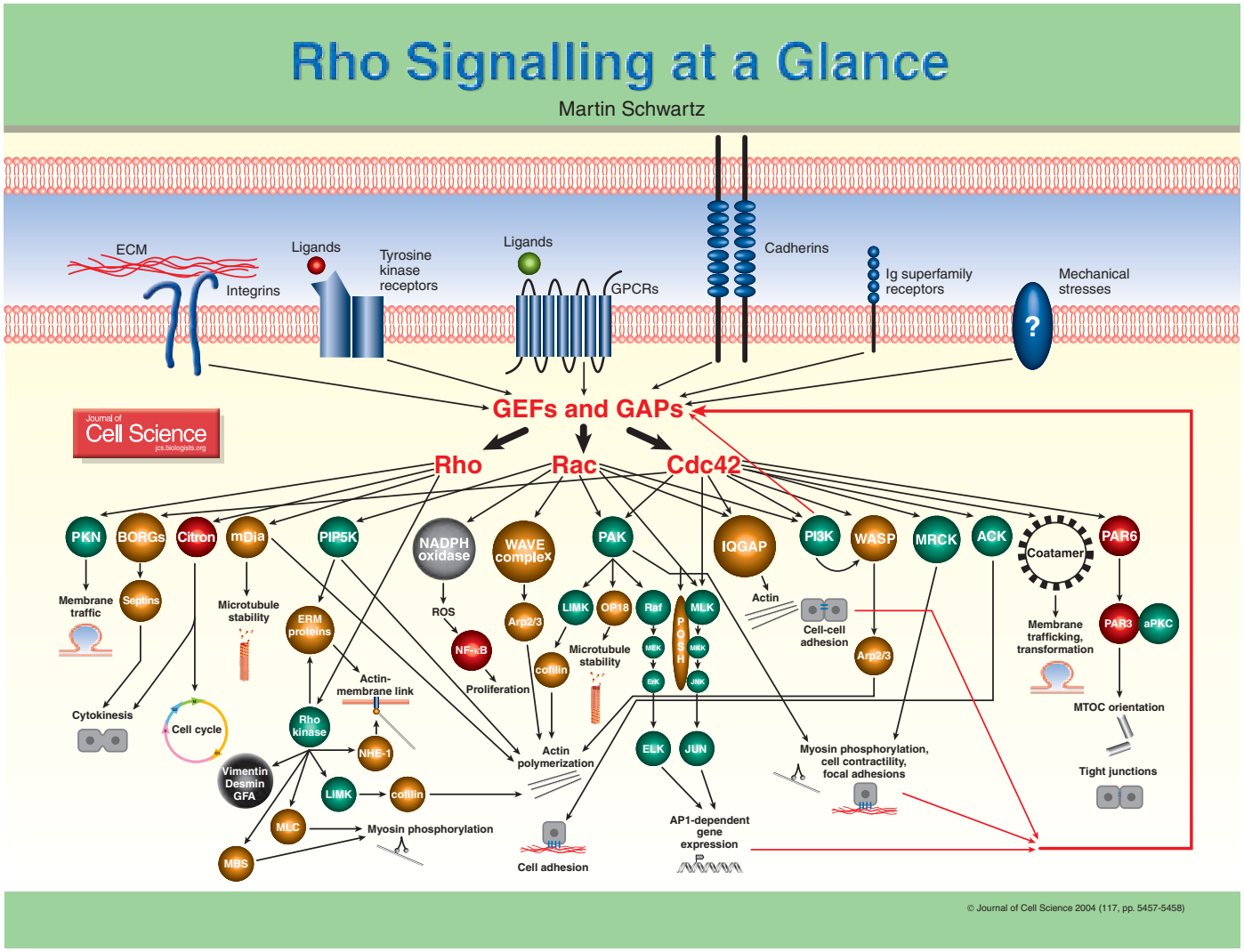
Cells receive extracellular stimuli in the form of soluble molecules (growth factors, cytokines and hormones) that bind to cell surface receptors, adhesive interactions (extracellular matrix and cell-cell adhesion) or mechanical stresses (tension, compression and fluid shear stress). These stimuli act upon guanine-nucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs) to control the activation state of the small GTPases Rho, Rac and Cdc42. Once activated, the GTPases bind to a spectrum of effectors to stimulate downstream signaling pathways. The pathways shown in the poster represent the major effector pathways for these Rho family GTPases. The total number of effectors is too large to be shown here, and I apologize to authors whose work could not be included. [Excellent reviews for these effector pathways are available; for detailed information, please see (Bishop and Hall, 2000; Symons and Settleman, 2000; Bokoch, 2003; Ridley, 2001; Yoshimi et al., 2001; Riento and Ridley, 2003).] Other Rho family proteins are not included but readers are referred to a recent review (Wennerberg and Der, 2004).

Protein kinase N1 (PKN1, also known as PRK) and PKN2 are Rho effectors involved in endosomal trafficking. Citron is a ROCK-related kinase that is critical for cytokinesis and is also implicated in other aspects of cell cycle progression. Mammalian diaphanous protein 1 (mDia1, also known as dia-related formin or DRF), mDia2 and mDia3 mediate both actin polymerization through a profilin-dependent mechanism and stabilization of microtubule plus ends in cell migration.

Rho kinase 1 (also known as ROCK1) and ROCK2 are key Rho effectors that have multiple substrates. A partial list includes the myosin-binding subunit of the myosin phosphatase (MBS), which leads to inhibition of phosphatase activity, increased myosin light chain



(See poster insert)

phosphorylation and hence increased tension generation; LIM kinase (LIMK), which phosphorylates cofilin to release actin monomers and promote actin polymerization; and myosin regulatory light chain itself, which again promotes contractility. Rho kinase phosphorylates ezrin-radixin-moesin (ERM) family proteins to activate their function as linkers between actin and the plasma membrane. Rho kinases also phosphorylate the Na/H^+ antiporter NHE-1 to promote actin-membrane interactions and several intermediate filament proteins (desmin, vimentin and glial fibrillary acidic protein) to regulate intermediate filament structure.

Both Rac and Rho bind to phosphatidylinositol-4-phosphate 5-kinase (PIP5K); activation of PIP5K by Rho also requires Rho kinases. Production of phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] contributes to the activation of ERM proteins and to actin polymerization through WASP, profilin and multiple actin-capping proteins. Other reported substrates for Rho kinases that are not pictured include MARCKS, EF-1α, calponin, CPI-17 and collapsin-response mediator protein 2.

Rac has numerous effectors that mediate effects on the cytoskeleton and gene expression. Rac binds p67 PHOX to increase activation of the NADPH oxidase system and production of reactive oxygen species (ROS), which mediate activation of NF-κB-dependent gene expression, effects of Rac on cell cycle progression and inhibition of Rho activity. Rac binds the WAVE complex (also containing Abi and IRSp53/58), to release active WAVE, which promotes actin polymerization in lamellipodia through activation of the Arp2/3 complex. Both Rac and Cdc42 bind and activate the kinases PAK1, PAK2 and PAK3. PAKs have multiple substrates, including LIM kinase, which leads to actin polymerization; OP18/stathmin, which stabilizes microtubule plus ends; and Raf-1 and MEK1, whose phosphorylation by PAK enhances transmission of the signal to ERK. PAK activity also regulates myosin phosphorylation and cell contractility through several pathways, including myosin light chain kinase, myosin regulatory light chain, myosin heavy chain and caldesmon. Other pathways not listed on the diagram include filamin A, which cooperates with PAK to promote membrane ruffling; components of the paxillin-GIT/PKL-P1X complex, which regulates cell adhesion and motility; and the apoptotic regulator BAD, which promotes cell survival.

Rac and Cdc42 are important activators of Jun N-terminal kinase (JNK) and p38, which stimulate AP-1-dependent gene expression. Mixed lineage kinases (MLKs) appear to be the major Rac/Cdc42 effectors leading to JNK and p38 activity. Rac and Cdc42 also bind to the actin-binding protein IQGAP, which is implicated in regulation of cell-cell adhesion. Both Rac and Cdc42 also bind and stimulate PI 3-kinase. The resultant 3′-phosphorylated lipids bind to and stimulate Rac GEFs, creating a positive feedback loop that maintains cell migration.

WASP (and the more widely expressed N-WASP) are critical downstream effectors of Cdc42 that mediate formation of filopodia. These effectors also require PtdIns(4,5)P_2 and interact directly with Arp2/3 complex to promote actin polymerization. Cdc42 activates the serine/threonine kinase MRCK, which is related to Rho kinases and can promote myosin phosphorylation. Cdc42 also activates the tyrosine kinases ACK1 and ACK2; the latter regulates focal adhesion formation and organization of the actin cytoskeleton. Cdc42 promotes oncogenic transformation in part by binding to the α and γ coatamer proteins and regulating membrane trafficking. Finally, Cdc42 is the critical determinant of polarity in a wide variety of developmental systems; this involves binding of Cdc42 to PAR6. PAR6 is a component of a complex containing PAR3 and atypical protein kinase C (PKCζ or PKCη). This complex regulates positioning of the microtubule-organizing center (MTOC) in migrating cells and early embryos, and tight junction formation in epithelia. Borg proteins are Cdc42 effectors that connect to septins, structural proteins that can polymerize to form filaments involved in cytokinesis in yeast and mammalian cells, and that probably carry out additional structural roles in mammalian cells as well (Joberty et al., 2001).

Finally, the poster displays some of the major feedback loops among Rho GTPases and their effector pathways. Formation of integrin-dependent cell-ECM adhesion formation, cadherin-dependent cell-cell adhesion formation and PI 3-kinase activation influence the function of GEFs and GAPs for Rho family GTPases. These positive and negative regulatory loops appear to be important for cell motility and perhaps other processes.

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