Cdc42 – the centre of polarity

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Summary

All cell types polarize, at least transiently, during division or to generate specialized shapes and functions. This capacity extends from yeast to mammals, and it is now clear that many features of the molecular mechanisms controlling polarization are conserved in all eukaryotic cells. At the centre of the action is Cdc42, a small GTPase of the Rho family. Its activity is precisely controlled both temporally and spatially, and this can be achieved by a wide variety of extracellular cues in multicellular organisms. Moreover, although the functional characteristics of cell polarity are extremely variable (depending on the cell type and the biological context), Cdc42 has an amazing capacity to co-ordinate the control of multiple signal transduction pathways.

Key words: Polarisation, Rho GTPases, GEF, PAR proteins

Introduction

Polarity touches on essentially every aspect of cell and developmental biology. Cell polarity is of fundamental importance for proliferation, differentiation and morphogenesis; yet, despite these diverse contexts, its broad features, such as the ability of cells to interpret an extracellular cue and transduce a signal to generate intracellular asymmetry, are quite general. Evidence for a conserved molecular mechanism is emerging from studies in many different organisms and cell types. In particular, Cdc42, a small GTPase of the Rho family, stands out as playing a central role in establishing cell polarity in all eukaryotic cells, irrespective of the biological context. I review here the current understanding of the involvement of Cdc42 in cell polarity, beginning with yeast. Yeast has proven to be an excellent model, in which Cdc42 was first discovered and in which many of its activities have subsequently been revealed. Two fundamental questions have been highlighted by this work: how can a diverse set of polarization signals regulate Cdc42 and how can this small molecule control the multiple cellular activities required for cell polarization? In the latter part of this Commentary, I discuss some recent results obtained in multicellular organisms, where similarities and significant differences compared with yeast have emerged.

Controlling cell polarity: lessons from yeast

Budding yeast is a single-celled organism in which cell polarity directly underlies morphogenesis and cell division. Because of its simple, tractable genetics, it provides an excellent model system for studies of polarity.

Cell polarization requires Cdc42 activity

Budding yeast cells grow isotropically until they reach a critical size and form a bud (Fig. 1). The normal direction of budding is specified by the position of the bud scar formed by the previous division. Evidence pointing to Cdc42p (yeast Cdc42) as a major component of the polarity machinery first appeared in this context, and, in the absence of Cdc42p, cell growth is no longer polarized but instead gives rise to large, round, unbudded cells (Adams et al., 1990).

Like all Rho GTPases, Cdc42 cycles between a GTP-bound and a GDP-bound state and signals to its effectors when it is in the active GTP-bound state (Etienne-Manneville and Hall, 2002). Cdc42-GTP levels can be regulated either positively by guanine nucleotide exchange factors (GEFs) or negatively by GTPase-activating proteins (GAPs). Cdc42 can also be maintained in an inactive state by binding to guanine nucleotide dissociation inhibitors (GDIs) that retain it in the cytosol.

In *Saccharomyces cerevisiae*, Cdc24p is the major GEF controlling Cdc42p activity and cell polarization (Chang and Peter, 2003). Overexpression of a constitutively active form of Cdc42p or a mutant that is slow to hydrolyse GTP can bypass the requirement for the GEF; however, this does not fully restore polarity but instead generates multiple, randomly localized buds (Caviston et al., 2002). By contrast, more physiologically relevant levels of expression of constitutively active Cdc42p cannot establish a polarization site (Irazoqui et al., 2003). These observations led to three important conclusions: (1) Cdc42p activation is both necessary and sufficient to promote spontaneous cell polarization; (2) Cdc42p activation must be restricted temporally and spatially; (3) in physiological conditions, Cdc42p function requires its ability to hydrolyse GTP.

Spatial and temporal control of Cdc42p activity

A first key step during polarization is the local activation of Cdc42p at the presumptive growth site. It is thought that this is driven by the recruitment of the GEF Cdc24p, which is sequestered in the cell nucleus in a complex with Far1p during G1 phase of the cell cycle (Nern and Arkowitz, 2000). In late G1 phase, activation of the cyclin-dependent kinase (CDK)-cyclin complex Cdc28-Cln2 triggers degradation of Far1p, resulting in the nuclear exit of Cdc24p. Cdc24p is then recruited to the polarization site, where it binds to the bud-site

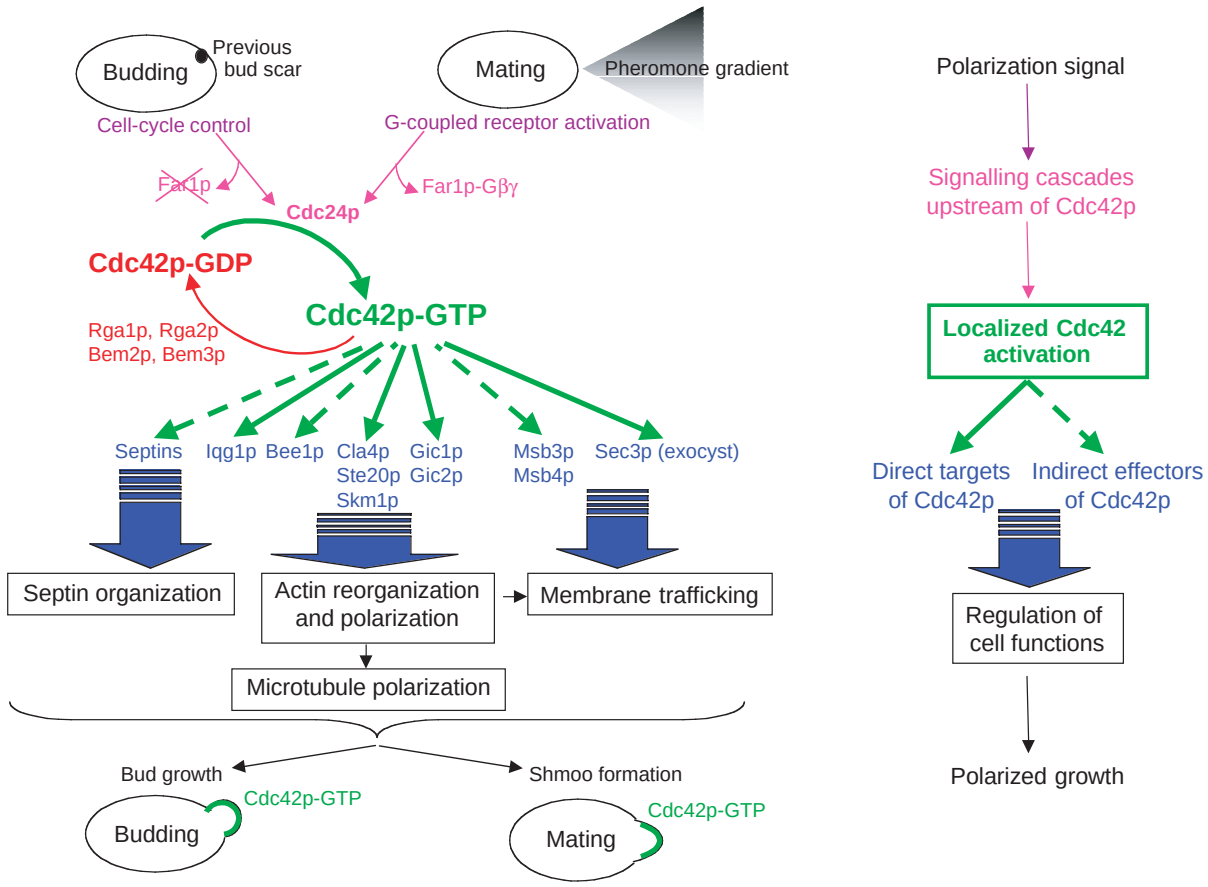

Fig. 1. Cdc42 during budding yeast polarization. Budding yeast can polarize in response to two main stimuli: they undergo polarized growth, which leads to budding, and they respond to pheromone gradient during mating by forming a shmoo. These stimuli both lead to Cdc42 recruitment and activation at the site of polarized growth through distinct signalling cascades (pink). Far1p associates with the Cdc42p-GEF Cdc24p and plays a key role in Cdc42p activation. During budding, Far1p degradation allows Cdc24p exit form the nucleus and, during mating, it binds Gβγ and thereby recruits Cdc24p to the site of polarization. The active, GTP-bound form of Cdc42p (green) regulates multiple direct (solid line) or indirect (dashed line) effectors (blue), which control several cell functions. The co-ordinate polarization of septins, actin and microtubule structures, and of membrane trafficking, allows a polarized growth that leads during budding to the formation of a bud and during mating to the formation of a shmoo.

selection protein Rsr1p/Bud1p and membrane adaptors such as Bem1p (Shimada et al., 2000). During mating, polarized growth (shmoo formation) occurs in response to a gradient of pheromone (Fig. 1), which acts through G-protein-coupled transmembrane receptors. Far1p then associates with the Gβγ subunits, leading to the accumulation of Cdc24p at the site of receptor stimulation (Nern and Arkowitz, 1999). Subsequently, active Cdc42p and its target proteins accumulate at the shmoo tip.

The spatial control of Cdc42p activity is not only achieved through GEF recruitment but also by recruitment of Cdc42p itself to the site of polarized growth. Indeed, localization of endogenous Cdc42p or a GFP-Cdc42p fusion protein shows that, during division, Cdc42p clusters at the bud site prior to bud emergence (Richman et al., 2002; Ziman et al., 1993). The mechanisms controlling Cdc42p clustering are not understood. In particular, it is not clear whether the GEF plays a role, since, in G1-arrested cells, in which Cdc24p is thought to be inactive, a constitutively active form of Cdc42p still concentrates at the plasma membrane, but randomly, which points to a GEF-independent clustering mechanism (Caviston et al., 2002). Mathematical simulations suggest that the local concentration of Cdc42p is generated by a stochastic increase of activated Cdc42p on plasma membrane sites and is amplified by a positive feedback loop, which may involve Cdc42-driven actin rearrangements (Wedlich-Soldner et al., 2003). However, incubation with the actin-depolymerizing drug latrunculin A does not disrupt the polarized localization of Cdc42p (Ayscough et al., 1997). By contrast, the actin cytoskeleton is involved in clustering and signalling of pheromone receptors and might also consolidate Cdc42p signalling by concentrating Cdc42 targets to one region of the cell cortex (Ayscough and Drubin, 1998). Other downstream effectors of Cdc42, such as Gic1p and Gic2p, favour Cdc42 signalling by recruiting additional Cdc42p effectors such as Cla4p and Bni1p to the site of polarized growth (Kawasaki et al., 2003). Finally, the scaffold protein Bem1p, which can bring together Cdc24p, Cdc42p and downstream effectors of Cdc42p, provides an actin-independent feedback mechanism to amplify stochastic fluctuations in local Cdc42p concentration (Bose et al., 2001; Irazoqui et al., 2003). Feedback loops are also involved in the downregulation of Cdc42p. For instance, the Cdc42p effector

Cla4p can phosphorylate and perhaps inactivate Cdc24p in a cell-cycle-dependent manner, triggered by Cdc28p (Gulli et al., 2000).

Cdc42p activates multiple polarity pathways

Polarized cell growth is a complex process that requires the co-ordination of multiple cellular activities, including polarization of the actin and microtubule cytoskeletons and directed vesicle trafficking to deliver cell wall and plasma membrane components to the bud site. How does Cdc42 co-ordinately regulate these diverse cellular functions?

The localized activation of Cdc42p first characterizes the site of bud emergence. Cdc42p directly promotes the self-organization of septins, leading to the formation of a ring that demarcates the mother and the bud (Caviston et al., 2003; Gladfelter et al., 2002; Kinoshita and Noda, 2001). Actin structures accumulate at the bud site and, in budding yeast, these are responsible for most of the subsequent events associated with cell polarization. Several pathways downstream of Cdc42p orchestrate actin rearrangements (Fig. 1). A Cdc42p effector, Iqg1p, a yeast homologue of the mammalian IQGAPs (reviewed by Briggs and Sacks, 2003), is subsequently recruited. Ipg1p serves as a scaffold protein and participates in the regulatory effects of Cdc42p on the actin cytoskeleton (Osman and Cerione, 1998). It allows the proper localization of the spatial landmark protein Bud4p and might therefore facilitate the accumulation of septins (Osman et al., 2002). Members of the p21-activated kinase (PAK) family, Cla4p and Ste20p, regulate the type I myosins. In addition, Ste20p controls the phosphorylation of Bni1p and thereby might contribute to its activation (Goehring et al., 2003). Gic1p and Gic2p interact and colocalize with Cdc42p at the bud tips (Brown et al., 1997). They function as adaptors that might link activated Cdc42p to components involved in actin organization and polarized growth, including Bni1p, Spa2p and Bud6p (Jaquenoud and Peter, 2000; Ozaki-Kuroda et al., 2001). Formin homologues (Bni1p and Bnr1p) contribute to the formation of actin cables and the assembly of the cytokinetic actin ring (Evangelista et al., 2002; Evangelista et al., 2003; Sagot et al., 2002). Although Cdc42p is not required for the formin-dependent assembly of actin cables, it might play a role in properly recruiting the active formins to the nascent bud site (Dong et al., 2003). Through the regulation of formin homologues and PAKs, Cdc42p recruits and activates an Arp2/3-activating complex formed by Bee1p/Las17p (the orthologue of mammalian WASp) and Vrp1p, which allows the local assembly of actin filaments (Lecher et al., 2001).

In *S. cerevisiae*, actin filaments serve as tracks for the delivery of secretory vesicles to growth sites. Cdc42p not only regulates actin organization, but also directly regulates exocytosis (Adamo et al., 2001). Msb3p and Msb4p genetically interact with Cdc42p and are recruited to the presumptive bud site, the bud tip and the mother-bud neck (Bi et al., 2000). They control vesicular trafficking by regulating the Rab-type GTPase Sec4p (Adamo et al., 2001; Gao et al., 2003; Zhang et al., 2001). Cdc42p might also direct exocytosis by interacting directly with Sec3p, a component of the exocyst complex, which is thought to contribute to polarized exocytosis (Zhang et al., 2001).

Polarization of the actin cytoskeleton also contributes to transport of cytoplasmic microtubules to the bud, where they interact with the cell cortex and lead to orientation of the spindle along the polarity axis (Bloom, 2000; Lee et al., 2000; Miller et al., 2000). Kar9p acts as a link between the microtubule-plus-end-binding molecule Bim1p and actin. The myosin motor Myo2p, which binds and moves along actin filaments, interacts with Kar9 and transports microtubules along polarized actin cables (Hwang et al., 2003).

Studies in yeast highlight some general principles underlying the establishment of cell polarity: (1) the localized activation of Cdc42, induced through various intrinsic or extrinsic signals; (2) the activation of multiple Cdc42 target proteins, leading to the co-ordinated regulation of cell functions; and (3) the use of feedback loops involving Cdc42 effectors that tighten the spatial and temporal regulation of the polarization machinery. An unresolved question is how Cdc42 regulates a precise subset of effectors. Is Cdc42 directly responsible for their recruitment at the polarized growth site? How is the succession of effectors temporally set up? One possibility is that the pathways regulating Cdc42 are also involved in the selection and recruitment of downstream targets. For instance, Bem1p, which is a Cdc42 target, binds directly to the GEF Cdc24p and to another Cdc42 target, Cla4p, and might therefore help to concentrate proteins involved in polarity establishment at a discrete site (Bose et al., 2001).

Cdc42 and polarity establishment in multicellular organisms

General mechanisms of cell polarization appear to be conserved throughout evolution. However, the complexity encountered in multicellular organisms has led to an even more diverse range of Cdc42 regulators and downstream effectors.

In multicellular organisms, cell polarity is determined primarily by external stimuli. Contact receptors such as integrins and cadherins, as well as receptors for soluble ligands such as chemokines, allow individual cells to sense their environment and organize polarity accordingly. This is controlled by Cdc42 and, as in yeast, the localized recruitment and activation of Cdc42 is likely to be a key event leading to cell polarization (Etienne-Manneville and Hall, 2002).

Polarization induced by cell-cell contact

Morphogenesis is a key feature of all multicellular organisms because they organize their cells to form distinct sub-populations and tissues. One well-studied example of this is the formation of epithelial sheets, during which cells polarize to generate distinct apical and baso-lateral surfaces upon cell-cell contact (Rojas et al., 2001). This is initiated by adhesion proteins such as nectin and E-cadherin (Takai and Nakanishi, 2003), whose engagement induces Cdc42 activation (Arthur et al., 2002; Honda et al., 2003; Kawakatsu et al., 2002). Although the pathway leading to GTPase activation is not clear, p120catenin, which binds to the cytoplasmic domain of cadherins, and Vav2, a GEF for Cdc42 and Rac (Noren et al., 2000) (Fig. 2), might play a role. Morphogenesis in neurons also involves molecules of the cadherin family. In particular, N-cadherins control synaptic structure and function, and again cell-cell contact leads to stimulation of Cdc42 activation,

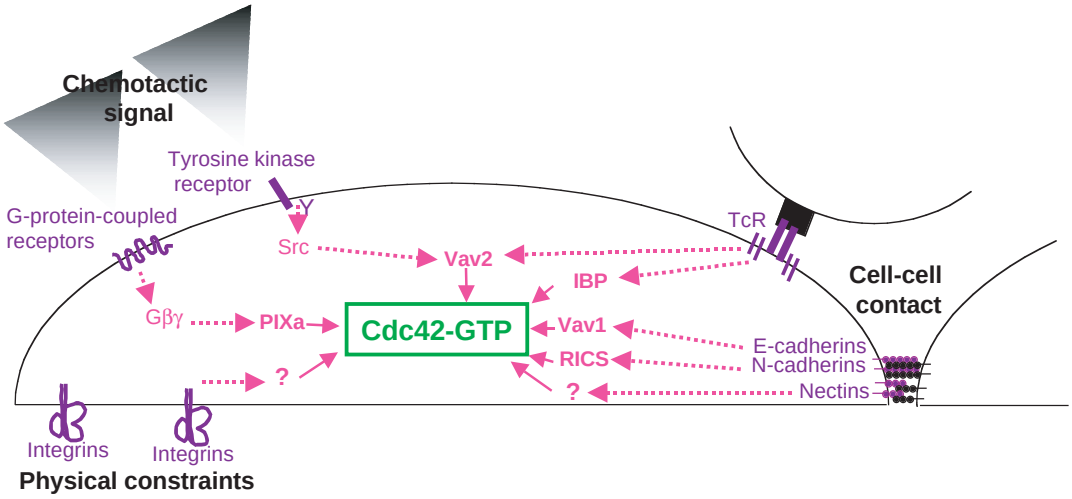

Fig. 2. Multiple pathways controlling Cdc42 activation. In multicellular organisms, cells respond to a wide range of external signals that promote cell polarization (chemotactic signals, physical stress, cell-cell contacts). These signals are transduced by different families of receptors (purple) that can all regulate Cdc42. The signalling pathways involve several intermediates (pink) that eventually recruit and activate a Cdc42-GEF. Although some of the GEFs have been identified, the precise mechanisms involved are still to be determined.

although in this case β-catenin and the Cdc42/Rac GEF RICS are thought to be involved (Okabe et al., 2003) (Fig. 2).

Cell-cell contact also initiates a polarized functional response in other cells. For example, during interactions between T cells and antigen-presenting cells, the actin cytoskeleton is actively remodelled to form a long-lived adhesive structure, referred to as the immunological synapse. Moreover, the centrosome and the Golgi apparatus become oriented in the direction of this cell-cell contact in a Cdc42-dependent manner, and this is required for the efficient delivery of secretory vesicles to the site of attachment. Engagement of the T-cell receptor (TCR) induces the recruitment of Cdc42, WASp and other components of the actin polymerization machinery (Cannon and Burkhardt, 2002). The Cdc42 GEF Vav1 is required for the reorientation of the microtubule-organizing centre, and its activation is dependent on the kinase Itk, which functions downstream of the TCR (Labno et al., 2003). However, another GEF, IBP, is recruited to the immunological synapse and might also be involved in Cdc42 activation (Gupta et al., 2003).

Polarization induced by soluble factors

During development, as well as in the adult, cells undergo chemotaxis in response to a variety of extracellular signals. For example, chemotactic leukocytes migrate towards the highest concentration of a variety of soluble chemoattractants, and this requires polarization of the actin cytoskeleton, which allows cells to protrude at the front and retract at the back. In macrophages, Cdc42 is required for directionality but does not appear to be required for movement per se (Allen et al., 1998). Dominant-negative Cdc42 mutants introduced into neutrophils exposed to a chemoattractant gradient induce formation of multiple, unstable pseudopods, whereas constitutively active Cdc42 completely blocks any change in morphology (Srinivasan et al., 2003).

So far, two pathways leading to Cdc42 activation and recruitment at the leading edge of polarized cells have been described (Merlot and Firtel, 2003). The phosphoinositide 3-kinase (PI 3-K) pathway, which is required for cell polarization during chemotaxis, activates Rac and Cdc42 GTPases at the leading edge of migrating cells (Benard et al., 1999; Wang et al., 2002). However, PI 3-K promotes pseudopod formation but is not involved in direction sensing. Rac induces a positive-feedback loop responsible for phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] accumulation and organizes the leading edge (Srinivasan et al., 2003; Weiner et al., 2002). By contrast, Cdc42 is not involved in this feedback loop but constrains the leading edge where PtdIns(3,4,5)P_3 accumulates, thereby maintaining a persistent direction of migration (Srinivasan et al., 2003).

An alternative pathway leading to Cdc42 activation is induced by heterotrimeric G protein signalling during neutrophil responses to the chemoattractant C5a, which acts through serpentine receptors (Li et al., 2003) (Fig. 2). Free Gβγ binds to the Cdc42 effector p65PAK1, which in turn interacts with the Cdc42 GEF PIXα. This binding stimulates Cdc42 and leads to the activation of p65PAK1. This pathway, which activates Cdc42 but not Rac, is essential for direction sensing and persistent polarized migration. The PI 3-K pathway is also required here to localize the p65PAK1-PIXα-Cdc42 complex. Note that, in this case, an effector of Cdc42 participates as a scaffold protein in the initial phase of Cdc42 activation.

Molecules such as epidermal growth factor (EGF) or platelet-derived growth factor (PDGF) that induce polarization by binding to receptor tyrosine kinase also activate Cdc42 and this is required for polarized migration (Chou et al., 2003; Liu and Burridge, 2000). Inhibition of Cdc42 in EGF-stimulated fibroblasts leads to the formation of multiple transient protrusions without effective cell migration. Interestingly, the tyrosine kinase Src is involved during Cdc42 activation by EGF. Src induces Cdc42 activation by the Vav2 GEF and also promotes phosphorylation of Cdc42, which stimulates the binding of RhoGDI, perhaps to direct the movement of Cdc42 to a specific cellular site (Tu et al., 2003).

Polarization induced by release of physical constraints

Polarized cell migration can also be induced by the release of physical constraints. For example, in vitro models of scratch-

induced migration have shown that cells polarize in a direction perpendicular to the wound; the actin machinery faces the front, the microtubule system is aligned along the direction of migration, and the centrosome and Golgi are reoriented in front of the nucleus. Cdc42 is involved in all of these polarization events, and its activity is induced by integrin-matrix interactions at the front of the cell as a consequence of wounding (Etienne-Manneville and Hall, 2001; Nobes and Hall, 1999; Palazzo et al., 2001). Dominant-negative Cdc42 alters the polarized cell morphology and abrogates the reorientation of the Golgi apparatus and centrosome. As in yeast, expression of a constitutively active form of the GTPase or of a deregulated Cdc42 GEF prevents the correct orientation of the cell. Endothelial cells exposed to shear stress similarly polarize and in this case migrate in the direction of flow. The actin and microtubule cytoskeletons align along the polarity axis, and Cdc42 is required for the reorientation of the centrosome towards the direction of movement (Tzima et al., 2003). Antibodies to fibronectin that block integrin engagement prevent Cdc42 activation and cell polarization. Although integrin stimulation can lead to Cdc42 activation (Arthur et al., 2002; Price et al., 1998), the exact mechanisms and, in particular, the GEF involved in this pathway are still to be determined (Fig. 2).

Cdc42 is essential for different aspects of cell polarization. It is important to note that, in case of migrating cells, Cdc42 is implicated in the orientation and maintenance of the polarized morphology rather than in the formation of a polarized shape. The formation of a polarized morphology in migrating cells generally involves another Rho GTPase, Rac, which, with PI 3-K, regulates protrusion at the leading edge. It seems that the effect of Cdc42 on protrusion formation becomes evident only when the cells move slowly (e.g. astrocytes, fibroblasts) and therefore requires a sustained localized protrusive activity. By contrast, absence of Cdc42 in fast-moving cells, such as neutrophils, does not block protrusion formation but rather affects its maintenance and therefore the establishment of a single, stable leading edge.

Multiple Cdc42 targets mediate polarization

Cdc42 regulates the actin cytoskeleton

One of the best-characterized targets of Cdc42 and Rac is the p65PAK family of serine/threonine kinases (reviewed by Bokoch, 2003). p65PAK associates with F-actin in membrane ruffles and lamellipodia at the leading edge of polarized migrating cells, such as leukocytes (Dharmawardhane et al., 1999). It also localizes to cell-cell contacts in epithelial cells and in phagocytic cups in neutrophils and macrophages. It apparently plays an important role in actin rearrangements by regulating LIM kinase, which in turn phosphorylates and inactivates the actin-severing protein cofilin (Edwards et al., 1999).

In its GTP-bound form, Cdc42 binds to and activates WASp, the product of the gene mutated in Wiskott-Aldrich syndrome. WASp in turn recruits and activates the Arp2/3 complex (Kim et al., 2000; Machesky and Insall, 1999). This pathway leads to actin polymerization and filopodia formation (Pollard and Borisy, 2003). The exact role of filopodia is not clear. They seem to be closely coupled to polarity establishment, but then this is perhaps not so surprising since Cdc42 plays such a prominent role in both processes. In migrating cells, filopodia are not required for polarization per se, but they might help cells sense the local environment and transmit this information to the polarization machinery. In epithelial cells, they seem to play a more direct role in establishing intimate contact between neighbouring cells to drive cell-cell junction assembly, which in turn leads to adoption of a polarized morphology. One other interesting activity of Cdc42 is its ability to activate Rac (Nobes and Hall, 1995). The target proteins mediating this cross-talk are not known, but they could provide a mechanism to couple the polarization and protrusion machineries during directed cell migration.

Cdc42 regulates the microtubule cytoskeleton

Cell polarization is often characterized by microtubule reorganization. The microtubule cytoskeleton is required for polarization of the *Caenorhabditis elegans* embryo, in which the positioning of the asymmetric spindle is dependent on microtubule-mediated forces (Grill et al., 2001). These are regulated by the PAR proteins (PAR-1 to PAR-6), which are asymmetrically localized. The scaffold protein PAR-6 interacts with another scaffold, PAR-3, and the atypical protein kinase C PKC-3 at the anterior pole of the embryo (reviewed by Etienne-Manneville and Hall, 2003a). This PAR complex is highly conserved throughout eukaryotes (although not in yeast) and plays a key role in cell polarization in all higher eukaryotes examined so far (Etienne-Manneville and Hall, 2003a; Henrique and Schweisguth, 2003). In its active form, Cdc42 interacts with a semi-CRIB motif and the adjacent PDZ domain of PAR-6 (Garrard et al., 2003). By binding to the complex, Cdc42 induces a conformational change in PAR-6 that activates the aPKC (Garrard et al., 2003). Inhibition of Cdc42 function by RNA-mediated interference prevents the polarized localization of PAR proteins and leads to the disruption of polarity in the *C. elegans* embryo (Gotta et al., 2001; Kay and Hunter, 2001).

Mammalian Par6 and the atypical PKC PKCζ are involved in microtubule-mediated-centrosome reorientation during wound-induced astrocyte migration and during shear-stress-induced endothelial cell polarization (Etienne-Manneville and Hall, 2001; Tzima et al., 2003). Several downstream targets of the Cdc42-Par6-PKCζ complex have been identified recently. Glycogen synthase kinase 3β (GSK3β) activity is spatially inhibited by PKCζ-induced phosphorylation and this leads to the association of the adenomatous polyposis coli protein (APC) with microtubule plus-ends (Fig. 4). This is required for centrosome reorientation, perhaps regulating microtubule dynamics or microtubule plus-end capture, or both, at the leading edge (Etienne-Manneville and Hall, 2003b). The Par6-aPKC complex also interacts with the tumour suppressor Lethal giant larvae (Lgl), with aPKC phosphorylating it at highly conserved residues (Betschinger et al., 2003). Lgl localizes to the leading edge of migrating fibroblasts, and a non-phosphorylatable Lgl mutant inhibits aspects of polarization induced by wounding (Plant et al., 2003), but its role in microtubule dynamics has still to be investigated (Fig. 4).

Cdc42 can also contribute to the polarization of the microtubule system by promoting microtubule capture. Cdc42 binds to and recruits IQGAP1, which interacts with the

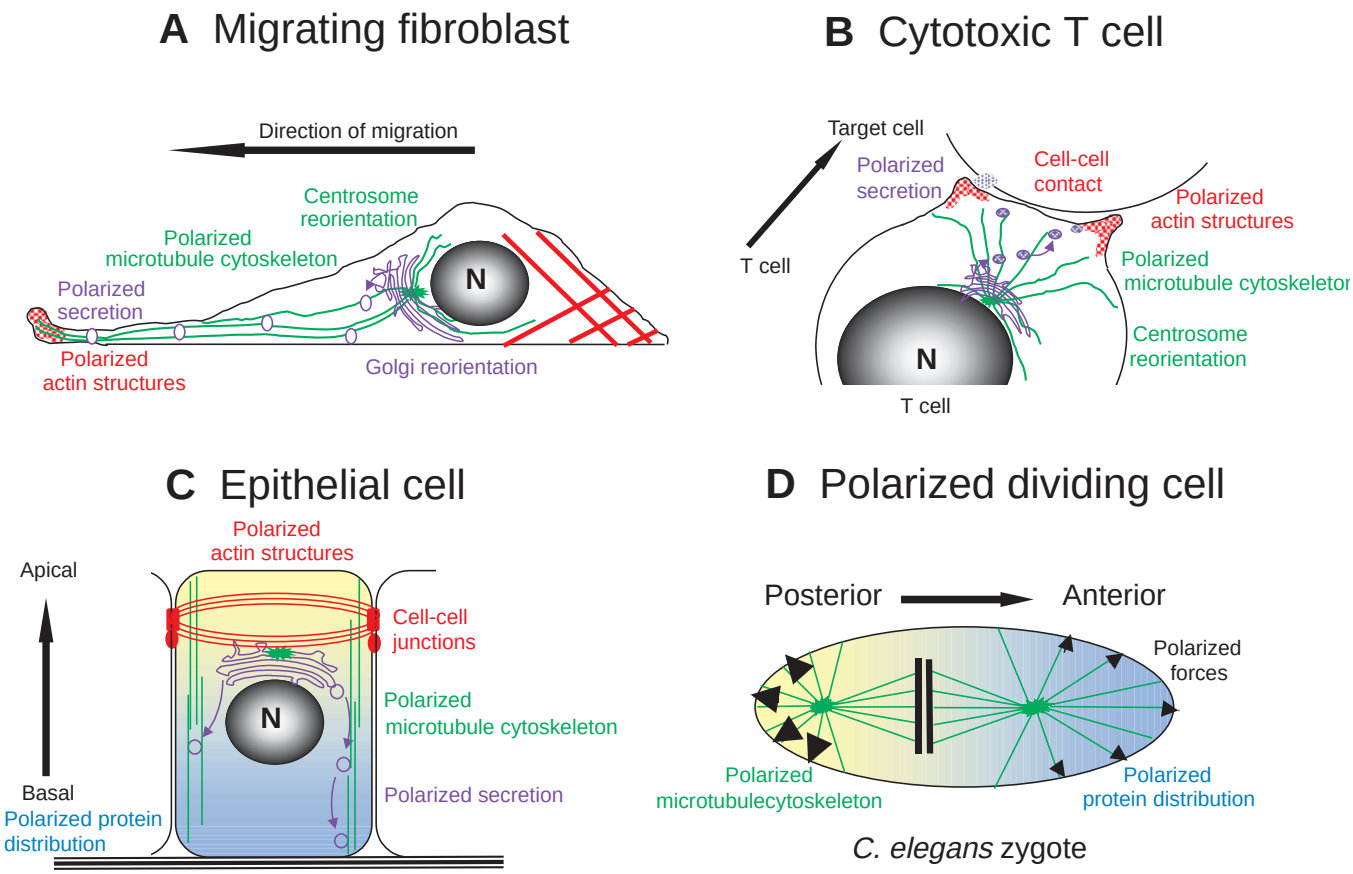

Fig. 3. Cellular functions regulated by Cdc42 in different polarized cell types. Most cell types can polarize under certain circumstances. This figure presents a few examples of polarized cells to highlight the multiplicity of aspects of cell polarity. Cell polarization results from the co-ordinate regulation of several cell functions. Most of these functions are under the control of Cdc42. (A) In migrating fibroblasts, the actin cytoskeleton promotes extension of the leading edge and retraction of the rear of the cell, the microtubule system associated with the centrosome aligns along the direction of migration and the Golgi apparatus faces the front of the cell and vesicular trafficking is oriented towards the leading edge. (B) During contact with a target cell, a cytotoxic T cell presents a polarized organization that allows the formation of a strong and stable cell-cell contact, and the orientation of the microtubule cytoskeleton and the secretory pathway in the direction of this contact. (C) In differentiated epithelial cells, the entire cell organization is polarized and allows the segregation of apical and baso-lateral proteins and membrane domains separated by tight junctions. The intracellular organization is also characterized by polarized cytoskeletal structures and polarized membrane traffic. (D) In the *C. elegans* zygote, the first division is asymmetric. The microtubule system is polarized, with asymmetric forces exerted on each pole of the embryo. This leads to an asymmetric positioning of the mitotic spindle. The protein distribution in the cytoplasm is also polarized, which will give rise to two non-equivalent daughter cells.

microtubule-plus-end-associated protein Clip-170. Interfering with IQGAP1 binding to Cdc42 or Clip-170 alters cell polarization and microtubule organization (Fukata et al., 2002; Fig. 4). The capture of microtubules at the plasma membrane might allow the generation of forces that reorient the microtubule cytoskeleton. Indeed, such forces are involved in the asymmetric positioning of the spindle during the first division of *C. elegans* (Grill et al., 2001; Wallenfang and Seydoux, 2000) (Fig. 3). The regulation of microtubule-associated motors such as dynein is likely to be involved in this process given that this is also required for centrosome reorientation during cell migration (Etienne-Manneville and Hall, 2001; Palazzo et al., 2001).

Cdc42 (and Rac) can regulate the stability of microtubules through another target protein, p65PAK (Bokoch, 2003). Activation of this kinase leads to the phosphorylation and inhibition of the microtubule-destabilizing protein stathmin (Daub et al., 2001). This mechanism could contribute to the protrusive activity during cell migration (Wittmann et al., 2003) (Fig. 4).

Cdc42 and the formation of intercellular junctions

The formation of epithelial junctions involves the co-ordinated activity of several protein complexes including Par6-aPKC (Petronczki and Knoblich, 2001; Yamanaka et al., 2001), and recent work in *Drosophila* has provided significant insights into the process (Bilder et al., 2003; Tanentzapf and Tepass, 2003). The Par complex initiates the formation of cadherin-containing adherens junctions and, thereby, an apical membrane region below which a complex containing Lgl and Scribble (Scrib) is recruited to define the lateral membrane. This initial baso-apical polarity is further enhanced by the recruitment of a third complex of proteins, containing Crumbs

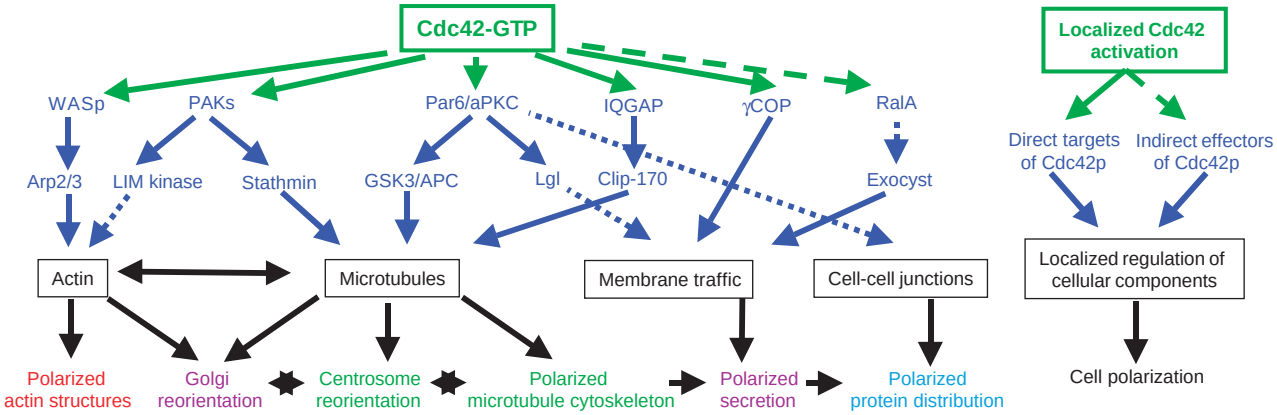

Fig. 4. Multiple signalling pathways controlled by Cdc42. Cell polarization requires the spatial and temporal regulation of several cell components. Orientation of the actin and microtubule cytoskeletons, regulation of cell contacts and organization of membrane traffic occur in concert. Multiple signalling pathways downstream of Cdc42 regulate these different cellular components (black box). These signals are transduced by different Cdc42 direct (solid line) or indirect (dotted line) effectors (blue) and involve several intermediates (blue). Cell polarization results from the localized activation of Cdc42, which leads to a localized regulation of cellular components and therefore to their asymmetric organization. The different cellular components cooperate and generate the general characteristics of cell polarization.

(Crb), Discs-lost (Dlt) and Stardust (Sdt), to a site apical to the adherens junctions (reviewed by Nelson, 2003). This last complex limits the extension of the lateral membrane by antagonizing Lgl and Scrib. What is missing from the *Drosophila* work is an understanding of the biochemical relationship between these various protein complexes. The finding that Par6 can interact directly with members of the two other complexes, Lgl and Dlt (Betschinger et al., 2003; Nam and Choi, 2003), suggests that regulation of Par6-aPKC by Cdc42 could provide the initial signal leading to these multiple polarization events.

In mammalian cells, the Par6-aPKC complex contributes to the formation of tight junctions in a process that initiates epithelial polarization. It is believed that Par3 is recruited to sites of cell-cell contact by junctional adhesion molecule (JAM), a trans-membrane molecule found in tight junctions (Ebnet et al., 2001; Ebnet et al., 2004; Itoh et al., 2001). Feedback loops probably control this localization, because inhibition of aPKC disrupts Par3 localization (Suzuki et al., 2002). Lgl and Par6/aPKC are then recruited, whereupon Lgl becomes phosphorylated and relocalizes to the baso-lateral membrane (Yamada et al., 2003).

Many outstanding questions remain in this highly orchestrated process. It is tempting to speculate that Cdc42 initiates events by binding to Par6, causing phosphorylation of aPKC targets and the establishment of epithelial cell polarity. However, the requirement for Cdc42 activity during tight junction formation is still unclear (Gao et al., 2002).

Cdc42 regulates membrane traffic

In polarized cells, membrane traffic is oriented towards one particular membrane of the cell. In migrating cells, for instance, most of the exocytic machinery faces the front edge. In particular, the Golgi is localized in front of the nucleus in the direction of migration. This localization is dependent upon the integrity of the microtubule cytoskeleton. In migrating astrocytes, the Cdc42-Par6-aPKC pathway controls the orientation of the Golgi, probably indirectly by modifying microtubule organization. By contrast, in NIH3T3 cells, regulation of the actin cytoskeleton by Scar2 and the Arp2/3 complex has been implicated in Golgi reorientation (Magdalena et al., 2003). Moreover, active Cdc42 binds directly to the coatomer complex and regulates vesicular trafficking between the endoplasmic reticulum and Golgi (Wu et al., 2000). However, the biological significance of this is still unclear and whether it might directly participate in the reorientation of the Golgi apparatus has not been tested.

Polarized epithelial cells establish and maintain the distinct compositions of apical and baso-lateral membrane domains by regulating membrane trafficking (Fig. 3). A lack of functional Cdc42 in Madin-Darby canine kidney (MDCK) cells inhibits trafficking to the baso-lateral membrane in both the endocytic and secretory pathways, leading to a selective depolarization of baso-lateral membrane proteins (Kroschewski et al., 1999). The effects of Cdc42 on microtubules might contribute to this (Kreitzer et al., 2003). However, Cdc42 also controls the exit of baso-lateral proteins from the trans-Golgi network (Musch et al., 2001). Moreover, Cdc42 facilitates the targeting of exocytic vesicles. This might involve the establishment of polarized complexes on the plasma membrane, such as Par6-aPKC and Lgl-scribble. Lgl interacts with the t-SNARE syntaxin 4 and regulates baso-lateral exocytosis (Musch et al., 2002). Moreover, Crb activity is required for vesicle exocytosis at the apical surface (Myat and Andrew, 2002). Another complex found just below tight junctions contains Sec6-Sec8 and is orthologous to the yeast exocyst complex, which is found on vesicles targeted to the bud site. This complex is involved in the baso-lateral delivery of proteins in epithelial cells as well as in polarized vesicular traffic in neurons (Kreitzer et al., 2003; Murthy et al., 2003). Although a connection between Cdc42 and the mammalian Sec6-Sec8 complex has not been described, Cdc42 can activate another small GTPase, RalA, which interacts directly with Sec5, another component of the exocyst (Sugihara et al., 2001).

Conclusion and perspectives

In multicellular organisms, each polarized cell type has particular characteristics and yet, in many of these situations, Cdc42 plays a central role. Why is Cdc42 such a conserved element of the polarization machinery? It is tempting to speculate that the control of cell polarization requires a key molecule that integrates the variety of extra- and intracellular signals received by the cell in order to select a single appropriate polarity axis. This key molecule should also be able to control the polarization of several cellular activities. Cdc42 appears to fulfil all these requirements. First, it can be regulated by extracellular signals including soluble agonists, cell-matrix and cell-cell interactions and by intracellular signals generated by the cell-cycle machinery. Second, it functions as a molecular switch and, once activated, can potentially interact with more than ten distinct target proteins to control many essential cellular functions in a co-ordinated manner.

A major challenge now is to determine how a given stimulus applied to a given cell can direct Cdc42 to recruit and activate the appropriate effectors. In mammalian cells, more than 12 GEFs are potential activators of Cdc42, but to date almost nothing is known about the circumstances and molecular details underlying spatial regulation of this GTPase. In addition, very little is known about how the specificity in downstream signalling is achieved. However, there has been some significant progress in identifying individual targets and their associated pathways that mediate Cdc42-dependent polarization of the actin and microtubule cytoskeletons. There is much to suggest that positive-feedback loops play an important role in both the activation of Cdc42 and the selection of its downstream effectors and that, rather than separating GTPase-controlled pathways into upstream activation and downstream effects, one should view the two as intimately coupled. With cell polarity playing such a prominent role in most of cell biology, this is an area that deserves a great deal of attention.

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