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Go ahead, break my symmetry!

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Abstract

Work in this issue advances our understanding of how the small G protein Cdc42 functions to polarize budding yeast. Remarkably, Cdc42 can polarize in the absence of upstream cues or positive feedback from the cytoskeleton. Polarization requires the scaffold protein Bem1 and cycling of Cdc42 between its GTP- and GDP-bound states.

Polarity is a fundamental feature of almost all cells, and how cells break symmetry to achieve polarity is an important and topical question. The budding yeast *Saccharomyces cerevisiae* is a good experimental system in this regard because bud formation represents a simple case of polarity. Indeed, much progress has been made. First, we know that the cell specifies the location of the bud formation site, and some genes/proteins that direct site selection have been defined. These bud-site selection proteins appear at the place on the membrane where the bud will form, directed by polarized structures from the previous cell division. Second, a signalling network centred around the Rho-type GTPase Cdc42 polarizes to this site. Finally, the cytoskeleton (including actin and septins) assembles at the site. We know that Cdc42 directs polarized assembly of the cytoskeleton and that Bud proteins direct the positioning of Cdc42 (refs 1,2). Now, work from Lew and colleagues on page 1062 further our understanding of how polarization of Cdc42 occurs³.

One interesting question concerns to what extent positive feedback loops or cooperative protein interactions contribute to the polarization of Cdc42; for example, does the polarized actin cytoskeleton provide positive feedback to reinforce polarization of Cdc42? In particular, actin cables deliver secretory vesicles to the site of bud formation and growth, causing polarized secretion and growth. Perhaps Cdc42 is delivered to the site by these vesicles, a hypothesis supported by the fact that Cdc42 is directly associated with membranes through a lipid modification.

In terms of cooperativity, can Cdc42 alone self-assemble into a structure at the bud site? Indeed, yeast cells lacking Bud proteins still form a bud. Furthermore, only one bud forms, and that bud is both associated with and dependent on polarization of Cdc42 at that location. So, one might imagine that Cdc42 has a strong intrinsic tendency to self-assemble and that Bud proteins merely need to nudge the process at the desired location.

Self-assembly is well understood and accepted for the cytoskeleton, where protein subunits such as actin spontaneously form filaments that possess polarity. Actin-binding proteins associate with the filaments and with each other to create larger polarized structures. In addition, spontaneous fluctuations of position can be sufficient to break symmetry in a model system containing actin filaments⁴.

In signalling, assembly is based largely on the concept of scaffolding, where one protein binds to several other proteins. If the scaffold or any of its binding proteins can interact with itself or with other proteins then one can readily envision the formation of a polymer of multicomponent protein complexes. Polymerization may be cooperative and self-propagating, so that only one polymer forms at a given space and time. So, does such a phenomenon exist

for Cdc42 in yeast? The answer seems to be yes. Lew and colleagues asked whether Cdc42 could polarize to a single location at the plasma membrane in the absence of upstream cues (Bud genes/proteins) and in the absence of potential positive feedback from the actin cytoskeleton³. First, they deleted two genes involved in bud-site selection, producing a cell with no evidence of an upstream cue. Next, they treated the cells with an actin toxin under conditions that remove all evidence of structures based on filamentous actin (notably, the cables that mediate polarized secretion) and then asked if Cdc42 was polarized to one site on the membrane: it was.

At this point, one might imagine that Cdc42 polarizes entirely on its own through cooperative self-assembly. That seems to be a stretch: the scaffold notion is more likely. Indeed, Cdc42 is known to interact with many other proteins. On the other hand, almost all of the Cdc42 interactors are considered to be either upstream cues, such as Bud proteins, or downstream effectors that induce actin and septin assembly. Lew and colleagues found that the Bem1 protein seems to function as a scaffold: first, Bem1 was found to be necessary for self-polarization of Cdc42; second, mutations changing single amino acids in any one of several domains of Bem1 caused loss of Cdc42 polarization, similarly to the complete absence of Bem1.

G proteins seem to function in two ways —switching or cycling. In the switch model, the protein has some activity when in a particular nucleotide state (usually when bound to GTP). The strength of activity is proportional to the amount of GTP-containing protein; oncogenic Ras seems to function in this way. In the cycling model, activity is produced only through transitioning between states. The G protein EF-Tu regulates translation elongation by a cycling mechanism. Cycling is also used by cytoskeletal motors, which hydrolyze ATP to produce a conformational change that is converted into molecular motion. In mammalian cells, cycling of Cdc42 has been implicated in oncogenic transformation⁵.

Lew and colleagues asked whether the switch or the cycling model accounts for the observed polarization of Cdc42 by expressing a GTP-locked Cdc42 mutant. In addition, they used different promoters to control expression levels. When expressed at levels comparable with that of the endogenous protein, polarization of Cdc42 was not observed. High expression levels did result in polarization. This is in agreement with another recent study showing that polarization of Cdc42 is dependent on the actin cytoskeleton⁶, suggesting that positive feedback through the actin cytoskeleton and secretion may promote Cdc42 polarization along the lines discussed above. Bi and colleagues recently proposed such a model to account for poor polarization of Cdc42 in mutants defective for secretion⁷. Together, these studies question the extent to which Cdc42 polarization is caused by Bud proteins, filamentous actin, polarized secretion and scaffolding proteins such as Bem1 in wild-type cells. Elucidating this complex issue will most probably require quantitative methods to follow Cdc42 polarization in cells in response to small changes in the activity of each proposed cause of polarization.

The results from Lew and colleagues support a cycling, rather than switch, mode of action for Cdc42 during self-polarization. Other recent studies also indicate that Cdc42 cycles between the two nucleotide-bound states to induce bud formation⁸ and septin assembly^{9,10}. How might this be explained? Perhaps Cdc42 uses the energy of GTP hydrolysis to execute a conformational change in itself and associated proteins, allowing new interactions that are necessary for polymerization of the Cdc42 structure (the 'Cdc42-some'). Lew and colleagues suggest another interesting possibility. Cells contain more kinds of guanine nucleotide-exchange factors (GEFs) than they do G proteins. A common explanation for this difference is that individual GEFs are localized or activated by different upstream signals, thus placing the G protein into its active GTP state at different times and places. Instead, one might now imagine that a specific GEF mediates the assembly of Cdc42-GDP with other proteins into a

macromolecular complex. In this model, GTP exchanges for GDP and the complex cooperatively self-assembles into a polymer (Fig. 1).

In summary, the data of Lew and colleagues, in combination with other recent studies, makes important advances in our understanding of how small G proteins function in budding yeast. Novel ideas for the molecular action of Cdc42 are provided by the findings that it can polarize without positional cues or actin filaments, that scaffold proteins are involved, and that GTPase cycling is required. G proteins are essential for mediating signals that direct cell polarization and macromolecular assembly in all eukaryotes, so the lessons learned in yeast may help illuminate the path of research in other systems.

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Figure 1.

Symmetry breaking by Cdc42. In the absence of upstream polarity cues, Cdc42 associates with assorted proteins, including a scaffold, a GEF, a GTPase-activating protein (GAP) and an effector, to form a multisubunit complex. Next, the complex assembles into a polymer, a process driven by GTP/GDP cycling of Cdc42. One polymer forms, and its location establishes the new bud site.