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Mum, this bud's for you: where do you want it? Roles for Cdc42 in controlling bud site selection in *Saccharomyces cerevisiae*

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Summary

The generation of asymmetric cell shapes is a recurring theme in biology. In budding yeast, one form of cell asymmetry occurs for division and is generated by anisotropic growth of the mother cell to form a daughter cell bud. Previous genetic studies uncovered key roles for the small GTPase Cdc42 in organizing the actin cytoskeleton and vesicle delivery to the site of bud growth,^(1,2) but a recent paper has also raised questions about how control of Cdc42 activity is integrated into a proposed hierarchical regulatory pathway that specifies a unique site of bud formation.⁽³⁾

Cell asymmetry in budding yeast

Cells have a wide variety of shapes; for example, the shape of an epithelial cell and a neuron are very different. Even the same cell can often change its shape rapidly, for example during cell migration or cell division.⁽⁴⁾ How does this asymmetry in cell shape arise? What defines the point of asymmetry, and how are cytoskeleton and membrane dynamics altered to drive changes in cell shape? The budding yeast, *Saccharomyces cerevisiae*, is an extremely useful organism to define how asymmetric cell shape is generated.^(5,6) During vegetative growth, the cell (termed the mother cell) initiates new membrane growth at one site on the plasma membrane, termed a bud. The bud continues to grow during the cell cycle and eventually becomes the new daughter cell upon completion of the next round of karyokinesis and cytokinesis. Formation of the daughter cell bud is dependent on delivery of vesicles from the mother cell, which requires that the actin cytoskeleton and vesicle delivery are oriented along the mother–daughter cell axis. During this time, the mother cell does not increase in size.

A molecular framework for regulating bud formation

The site for membrane growth and formation of the daughter cell bud is specified by “landmarks” retained on the plasma membrane close to (axial and bipolar patterns), or opposite (bipolar pattern) the site of the preceding cytokinesis, which is marked by a bud scar. “Landmark” genes for axial (BUD3, BUD4 and BUD10/AXL2) and bipolar budding (BUD8 and BUD9) are different, and a third class is required for both budding patterns (BUD1/RSR1, BUD2, BUD5).^(7–9) Some of the “landmark” proteins (Bud8p, Bud9p) are transmembrane proteins that could mark the site for membrane growth, whereas others are part of a protein module for a Raslike small GTPase, Bud1p/Rsr1p and its GTPase exchange factor (GEF), Bud5p, and GTPase activating protein (GAP), Bud2p.^(5,6) Recent studies indicate that Bud5p regulates the distribution and, therefore, location of Bud1p/Rsr1p activity.⁽¹⁰⁾ Although a biochemical link between the Bud1 GTPase module and the transmembrane “landmark” proteins is not known, Bud5p is mis-localized when *BUD3*,

BUD10/AXL2 or *BUD9* are disrupted, which results in a loss of localized activation of Bud1p/Rsr1p GTPase.^(11–13)

Cdc42: a central regulator of bud formation

Deletion of BUD genes still result in formation of a daughter bud, albeit randomly on the surface of the mother cell.^(5,6) In contrast, deletion of *CDC42* results in isotropic growth of the mother cell and no daughter bud formation or growth, placing Cdc42p as a central regulator of bud formation.⁽¹⁴⁾ Cdc42p is a well-characterized Rho family small GTPase that regulates actin cytoskeleton assembly and organization, and vesicle dynamics, both of which are required for bud formation and growth. Moreover, Cdc42p becomes concentrated, relative to the rest of the plasma membrane, at the incipient bud site several minutes prior to bud emergence^(3,15) indicating that its localization is part of the cellular machinery that prescribes the site of bud emergence (see also Ref. 16). Concentration of active Cdc42p at the bud site leads to global changes in the polymerization and orientation of the actin cytoskeleton, and consequently exocytosis, in the mother–bud axis.^(5,6)

How is Cdc42p localized to the site of bud formation? One idea is that the GEF Cdc24p is specifically localized to the membrane at the right time and place during the cell cycle. For example, in vitro binding studies showed that active GTP-bound Bud1p/Rsr1p binds Cdc24p, which in the cell would localize the activity of Cdc42p to the bud site (reviewed in Refs. 5 and 6). Other studies⁽¹⁷⁾ point to a role for the nuclear protein Far1p which binds Cdc24p^(18,19) and sequesters Cdc24p in the nucleus until late G1 when Far1p is degraded and Cdc24p is released into the cytoplasm.⁽¹⁷⁾ These results support an essential role for Cdc24p in activating Cdc42p and, through cell-cycle-dependent availability, to bind the Bud1p/Rsr1p module, thereby localizing Cdc42p activity at the right time to a specific site on the plasma membrane defined by the “landmark” proteins.

Can Cdc42 functions be separated from upstream regulators?

The proposed hierarchical pathway, “landmark” proteins → the Bud1p/Rsr1p GTPase module → Cdc24p that properly localizes Cdc42p activity for bud growth is challenged, at least conceptually, by experiments in which constitutively active mutants of *cdc42p* are expressed in cells.^(1–3) In these cells, *cdc42p* activity is mostly independent of the normal regulatory components of the Cdc42p module (GAPs and GEF). However, bud formation still occurs and, in some cases, multiple buds are formed in the same cell cycle. Significantly, the location of these buds is random on the cell surface, i.e., the site of bud formation is independent of the location of “landmarks”. These results have led to a model that Cdc42p (activity) becomes concentrated at a site on the plasma membrane through a stochastic process, rather than a deterministic process involving “landmarks”.⁽²⁰⁾

Richman and Johnson⁽¹⁾ isolated temperature-sensitive mutations in the 25 amino acid effector binding domain of Cdc42p, and tested their effect on bud formation. One of the mutants, *cdc42^(D38E)p* partially complemented *cdc42* loss-of-function mutants and initiated bud emergence (polarized actin and vesicle organization) next to a “landmark”, but bud enlargement ceased prematurely. Subsequently, another bud emerged, this time randomly on the mother cell surface (i.e., not adjacent to a “landmark”), and this second bud either ceased growth, or grew normally or elongated to form a budded daughter cell. Formation of multibudded cells appeared to be the result of sequential initiation of new buds during the same cell cycle. These results indicate that normal Cdc42p function is required for bud emergence, maintenance of bud growth, and prevention of more than one bud formation per cell cycle.⁽¹⁾ *cdc42^(D38E)p* had altered interactions with the downstream effectors Cla4p and the GAP, Bem3p, indicating that proper regulation of these downstream effectors is important in sustained growth of a single bud.⁽¹⁾

Caviston et al.⁽²⁾ took a slightly different approach to examine functions of Cdc42p. Instead of specific mutations in the effector domain, or in the GTP-binding domain as discussed later, they performed random mutagenesis of *CDC42* and isolated temperature-sensitive mutants. Four of these mutants formed multiple buds (45% had >2) on each mother cell; note that ~75% of cells with >2 buds grew twice as slowly as wild-type cells and were non-viable. Caviston et al.⁽²⁾ also observed that each bud contained F-actin and new membrane growth, that multiple buds occurred in the same cell cycle and, hence, were not linked to cell cycle progression, and that budding occurred at sites on the membrane that were independent of the location of “landmark” proteins.

Caviston et al.⁽²⁾ showed that these four *cdc42* mutants had a single amino acid substitution at residue 60 (G60A, G60D, G60C), as well as changes in other residues. Mutations in residue 60 resulted in the expression of two to five times more protein compared to wild-type cells. However, it is not the amount of protein expressed, per se, that gives rise to the multibudded phenotype, because Caviston et al.⁽²⁾ found that expression of wild-type Cdc42p at these high levels still produced only one bud during the cell cycle. Thus, it is the nature of the mutation in *cdc42*, possibly increased GTPase activity and/or changes in normal GTP → GDP cycling, that cause the multibudded phenotype. Mutations in residue 60 are in the Cdc42p GTP-binding and hydrolysis domain, and result in a hyperactive GTPase. However, these *cdc42* mutants retain some GAP-sensitive GTPase activity since overexpression of the Cdc42p GAP, Rga1p, reduced the number of multibudded *cdc42* cells. Analysis of the GEF Cdc24p in *cdc42* mutants revealed a two- to three-fold increase in protein level, but this again did not account for the increase in GTPase activity, since a *cdc4Δ* strain expressing *cdc42* mutants in residue 60 also had a multibudded phenotype. Mutant *cdc42* required normal downstream effectors of Cdc42p to produce the multibudded phenotype since *cdc42* mutants were synthetically lethal with *cla4Δ*, *bni1Δ*, *gic1Δ*, and *gic2Δ*, presumably due to the decrease in amounts of effector proteins required to organize the actin cytoskeleton for multibud formation and growth.⁽²⁾

Wedlich-Soldner et al.⁽³⁾ also examined effects of expressing mutant *cdc42* on budding using a constitutively active mutant of *CDC42*, *cdc42^{Q61L}* that is GTPase deficient and cannot undergo normal GTP → GDP cycling. GAL4-induced expression of *cdc42^{Q61L}* resulted in levels of protein that were five- to six-fold more than that of wild-type Cdc42p. Overexpression of *cdc42^{Q61L}* resulted in the appearance of multibudded cells, and there was a positive correlation between the level of *cdc42^{Q61L}* expression and the number of buds formed. Time-lapse imaging of GFP-tagged *cdc42^{Q61L}* showed initially that mutant protein was localized randomly at the plasma membrane, but gradually accumulated in dot-like, motile structures at the plasma membrane that were the precursors of bud formation. These sites coincided with accumulation of F-actin and Sec4p. Wedlich-Soldner et al.⁽³⁾ showed that *cdc42^{Q61L}*-induced bud formation was independent of pre-existing bud scars (“landmarks”); in a *bud1Δ* background, *cdc42^{Q61L}* still resulted in multiple budding although the frequency decreased from 71% to 55%.

Early studies by Li et al.,⁽²¹⁾ using permeabilized cells to reconstitute actin assembly during budding, showed that active Cdc42p (wild-type Cdc42p + GTP, or an activated *cdc42p* mutant) is essential for F-actin assembly at the bud. Wedlich-Soldner et al.⁽³⁾ examined whether *cdc42^{Q61L}*-induced bud formation was linked to F-actin organization. Treatment with the F-actin depolymerizing drug Latrunculin-A (LatA) during or after *cdc42^{Q61L}*-induced bud initiation resulted in disruption of bud formation showing that F-actin assembly was required for both the formation and maintenance of bud growth. However, it should be noted that Ayscough et al.⁽²²⁾ reported previously that LatA did not effect correct localization of wild-type Cdc42p to the insipient bud site, and observed multiple buds often formed on the mother cell under these conditions; the reason for this difference in LatA

effects between the two studies is unclear, although one likely possibility is the difference in proper regulation of Cdc42p activity. To test whether the requirement for F-actin was due to its role in vesicle transport to the bud, Wedlich-Soldner et al.⁽³⁾ examined *cdc42^{Q61L}*-induced bud formation in strains lacking Myo2p or the exocyst protein Sec6p. In both cases, *cdc42^{Q61L}*-induced bud formation was reduced to <10% suggesting that *cdc42^{Q61L}* might be delivered to, and thereby concentrated and maintained at a localized site(s) on the plasma membrane by vesicle delivery.⁽³⁾ To test this idea, they fractionated cells by differential centrifugation and found that *cdc42^{Q61L}*p was enriched with the plasma membrane(P2) but not vesicles (P3). However, in a *sec4Δ* mutant background, in which transport vesicles are unable to fuse with the plasma membrane and accumulate in the cytoplasm, *cdc42^{Q61L}*p was enriched in the vesicle rather than the plasma membrane fraction.⁽³⁾

(Re-)integrating cdc42 functions into a hierarchical pathway

Overall, these studies (1,2,3) arrived at a similar conclusion: a constitutively active *cdc42p* mutant localizes randomly on the plasma membrane, but through a stochastic process becomes concentrated at one or more sites, whereupon downstream Cdc42p effectors orient F-actin and vesicle delivery to those sites to produce multiple buds in one cell cycle. That these *cdc42* mutants form random, multiple buds is evidence that they can bypass normal control points that specify cell-cycle-dependent growth and completion of only one bud per cell cycle.

Analysis of mutant *cdc42* defects indicates several control points for regulating Cdc42p activity (see also Ref. 23). One is the spatiotemporal control of recruitment of Cdc42p to the plasma membrane and insipient bud site by Cdc24p and the Bud1p GTPase (“landmark”) module. A second control point is normal GTP → GDP cycling through activity of the Cdc42pGAPs(e.g., Rga1p) and GEF(Cdc24p). A third control point is the proper regulation and availability of downstream Cdc42p effectors (Cla4p, Bni1p).

Does the fact that mutant *cdc42* can bypass these control points undermine conceptually the proposed hierarchical regulatory pathway that specifies normal bud growth during the cell cycle that involves “landmark” proteins → the Bud1p/Rsr1p module → the Cdc42p module → downstream effectors of Cdc42p? Although studies of mutant *cdc42* used nonphysiological manipulations of cellular pathways, they do point to a constitutive ability of Cdc42p to generate localized membrane growth that must be adapted and properly controlled by cells. In fact, studies of mutant *cdc42* underscore the importance of the hierarchical regulatory pathway in the spatiotemporal regulation of where active Cdc42p accumulates initially during the cell cycle, the importance of the Cdc42p module in regulating normal GTP → GDP cycling in modulating Cdc42p activity at different stages of bud growth, and proper activation of downstream effectors of Cdc42p in stabilizing the orientation of actin cytoskeleton growth, and thereby exocytosis, to only one site on the plasma membrane.

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