Role of Cdc42 dynamics in the control of fission yeast cell polarization

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Abstract
Cell polarization is fundamental to many cellular processes, including cell differentiation, cell motility and cell fate determination. A key regulatory enzyme in the control of cell morphogenesis is the conserved Rho GTPase Cdc42, which breaks symmetry via self-amplifying positive-feedback mechanisms. Additional mechanisms of control, including competition between different sites of polarized cell growth and time-delayed negative feedback, define a cellular-level system that promotes Cdc42 oscillatory dynamics and modulates activated Cdc42 intracellular distribution.

Morphology control in fission yeast
The ability to grow in a polarized fashion is a property of fundamental importance for the emergence of cell form and for proper cell function and differentiation. Fission yeast is a genetically amenable organism that displays a regular rod shape and grows in a polarized fashion from the cell tips. Polarized cell growth is regulated during the cell cycle. Fission yeast cells divide by medial fission and, following cell separation, initially grow in a monopolar fashion, from the tip that existed in the previous cell cycle (the old end). Cells activate growth at the new end only once a minimal cell length has been achieved [NETO (new end take off)] [1], and then continue to grow in a bipolar fashion until the onset of the following mitosis. With its well-established genetics and its regular cell shape, fission yeast is thus an ideal system to study the cellular mechanisms of polarity control, and it has been extensively used in the past to identify novel gene functions that control different aspects of cell morphogenesis.

Role of Cdc42 GTPase in fission yeast cell polarization
The conserved GTPase Cdc42 plays a crucial role in the establishment of cell polarity by promoting cytoskeleton polymerization and vesicle exocytosis required for normal cell function, differentiation and motility [2,3]. Cdc42 is essential for viability in fission yeast, and loss-of-function cdc42 mutants display loss of polarity, resulting in a round cell shape [3,4]. Cdc42 is active when bound to GTP, and inactive when the GTP is hydrolysed to GDP [3]. Regulators of Cdc42 such as GEFs (guanine-nucleotide-exchange factors) promote GTP binding and activate Cdc42 [5]. In fission yeast, two Cdc42 GEFs have been characterized: Scd1 and Gef1 [6,7]. The phenotypes of scd1Δ and gef1Δ deletion mutants, which are viable, are different. scd1Δ cells display a wider diameter and a rounder shape, whereas gef1Δ mutants grow mainly in a monopolar fashion, delaying the onset of bipolar growth, suggesting that each GEF fulfills some specialized functions [6,7]. However, the double mutant scd1Δ gef1Δ is not viable, indicating that Scd1 and Gef1 perform an essential redundant role in promoting Cdc42 activation [7]. Scd1 and Gef1 proteins are localized mainly to the growing cell tips, consistent with their role in promoting local Cdc42 activation [8,9].

Another set of regulators, Cdc42 GAPs (GTPase-activating proteins), promote the GTPase activity of Cdc42, leading to GTP hydrolysis and Cdc42 inactivation. One Cdc42 GAP is currently known in fission yeast: Rga4 [10,11]. Consistent with a role in negatively regulating Cdc42 activity, Rga4 displays increased localization to the non-growing cell sides [10]. Rga4 also has a role in negatively controlling the dimensions of the growth zone, since rga4Δ mutant cells display a larger diameter, whereas cells overexpressing Rga4 display a smaller diameter [10]. Once inactivated, Cdc42 GTPase is released from the membranes by Cdc42 GDIs (guanine-nucleotide-dissociation inhibitors) [12]. One GDI, Rd1, is predicted by sequence homology to exist in fission yeast.

The active GTP-bound Cdc42 binds effector proteins most of which contain a specific domain, the CRIB (Cdc42/Rac-interactive binding) domain [13]. In fission yeast, GTP-bound Cdc42 localization can be studied using a bioprobe consisting of a GFP-tagged CRIB domain [11]. Whereas fluorescently tagged Cdc42 localizes to both the plasma and internal membranes, studies have shown that GTP-bound Cdc42, as visualized by the CRIB–GFP bioprobe, is localized mainly to the cell tips and the site of cell division, consistent with the established role of Cdc42 GTPase in the control of polarized cell growth [11] (Figures 1a and 1c).

Key words: Cdc42, cell polarity, fission yeast, morphogenesis, symmetry breaking.

Abbreviations used: CRIB, Cdc42/Rac-interactive binding; GAP, GTPase-activating protein; GDI, guanine-nucleotide-dissociation inhibitor; GEF, guanine-nucleotide-exchange factor; NETO, new end take off; Pak, p21-activated kinase.

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Activated Cdc42 recruits several effector proteins to the site of growth. Notable targets are the formin For3, which promotes actin cable polymerization [14], Pob1, which promotes localized protein secretion [15], and the signal transduction kinase Pak1 (p21-activated kinase 1) [16]. In fission yeast, known Pak1 targets are Tea1, Myo1 and Rga8, which regulate the function of the polarisome complex, endocytosis and Rho GTPase activity respectively [17–19].

**Oscillatory dynamics of Cdc42 GTPase**

One fundamental question concerns the mechanism whereby Cdc42 GTPase becomes activated in an asymmetrical manner to promote the polymerization of the actin cytoskeleton and local activation of exocytosis. The process that promotes Cdc42 asymmetrical activation is also called ‘symmetry breaking’. Previous studies in budding yeast have shown that Cdc42 breaks symmetry by clustering on a single membrane spot via a mechanism mediated by Cdc42 autocatalytic amplification [20,21]. This mechanism relies on a positive-feedback loop, which involves the recruitment of a Cdc42 GEF by a Cdc42 effector, the PAK kinase [20]. This mechanism results in a ‘winner-takes-all’ effect: through competition, a single spot of activated Cdc42 becomes dominant, leading to the emergence of asymmetry. This, however, cannot explain how multiple growing zones are maintained simultaneously in a cell, as is the case in a fission yeast cell growing in a bipolar fashion.

Trying to solve this apparent paradox led to the discovery of GTP-bound Cdc42 oscillations in fission yeast cells [22] (Figure 2a). Since one of the general requirements of biochemical oscillators is the presence of a delayed negative feedback, this finding shed light on additional mechanisms that govern Cdc42 activity. Negative feedback had not been...
Effect of changing Cdc42 GEF levels on the symmetry of anti-correlation of activated Cdc42 oscillations

Increasing Cdc42 GEF Gef1 levels leads to increased levels of active Cdc42, resulting in bipolar growth activation and decreased anti-correlation of activated Cdc42 oscillations at the cell tips. As mentioned above, on average, a more symmetrical not just of a specific regulatory event at one of the cell is the product of the general topology of the system, that the symmetry or asymmetry of Cdc42 distribution involve the whole reaction network, Cdc42 oscillations, since oscillators display system-level characteristics that Feedbacks regulate biochemical oscillations such as MinD oscillations at bacterial cell tips [23] as well as circadian rhythms and cell cycle progression in eukaryotes [24]. Thus Cdc42 behaviour can be modelled, including positive and delayed negative feedback as well as intracellular competition for Cdc42 or its effectors and regulators, on the basis of the observed GTP-Cdc42 anti-correlation between tips [22] (Figure 2b).

What limiting factors determine the extent of competition between the cell tips? We found that the anti-correlation of Cdc42 oscillations is sensitive to the levels of Cdc42 GEFs [22]. Thus it is likely that regulation of Cdc42 GEF availability plays a crucial role in the control of Cdc42 oscillatory dynamics. This finding might provide a key to deciphering the dependency of bipolar growth activation (or NETO) on the attainment of a minimal cell size. In the context of cell-wide Cdc42 dynamics, one cell tip will remain dominant until the system has accumulated sufficient available GEFs to fully support the competition by another tip (Figure 3). Visualization of activated Cdc42 GTPase by CRIB–GFP at cell tips during the transition to bipolar cell growth supports the idea that NETO is indeed a laboured noisy transition, where the new end displays increasing GTP-Cdc42 concentrations that eventually reach a minimal threshold to promote visible cell growth [22].

The importance of history

Since oscillators display system-level characteristics that involve the whole reaction network, Cdc42 oscillations, which occur at both the old and the new end, indicate that the symmetry or asymmetry of Cdc42 distribution is the product of the general topology of the system, not just of a specific regulatory event at one of the cell tips. As mentioned above, on average, a more symmetrical state of Cdc42 distribution, promoting the transition to bipolar growth, occurs when the cell has achieved a minimal cell length, approximately 9 μm. However, mathematical modelling predicts that asymmetrical and symmetrical GTP-Cdc42 distributions can coexist for intermediate cell lengths [25]. Indeed, when CRIB–GFP tip fractions (amount of CRIB–GFP at one tip expressed as a portion of the total at both tips) are measured experimentally as a function of cell length, alternative CRIB–GFP distributions can be detected for cell lengths ranging from 8 to 12 μm, suggesting a coexistence region that allows both asymmetrical and symmetrical states [22]. Which of these two states occurs in each individual cell depends on initial conditions; possibly, the state of Cdc42 distribution when the new daughter cell is born, shortly after cell separation. Subtle differences or minor defects in the control of Cdc42 distribution at the time of cell division can give rise to different trajectories of the system during the newborn cell life cycle, and thus determine a pattern of cell growth that is more or less bipolar. The idea that Cdc42 dynamics and pattern of distribution follows trajectories influenced by the initial state of the system could be an explanation for the phenotype of deletion mutations in rga4A (encoding a Cdc42 GAP), for3Δ (Cdc42-dependent formin) and possibly pta2Δ (encoding a protein phosphatase type 2A regulator) genes [10,26,27]. In these cell populations, daughter cells of similar length display alternative patterns of growth, where one daughter cell grows in a bipolar fashion and the other continues to grow in a monopolar fashion.

A morphology control system to regulate cell diameter

Accumulation of Cdc42 GEFs at the membrane, either by loss of inhibition (a kinase-dead mutation in the Cdc42-dependent Pak1) or by increased expression of Gef1, stabilizes Cdc42 activity at one tip or both and dampens periodic falls in active Cdc42 levels [22]. Interestingly, all of these mutants display a greater diameter. This probably occurs because the increase of tip-bound Cdc42 results in Cdc42-dependent cell growth over a wider area. Indeed, Cdc42 GEF Gef1 levels positively correlate with increased GTP-Cdc42 cortical localization and with cell diameter [7,22]. These observations suggest that the Cdc42 oscillatory system plays an important role in regulating overall cellular dimensions. Being in a state of dynamic equilibrium, fostered by positive and negative feedback, this system keeps Cdc42 activity at the cell tips within a ‘normal’ range, thus maintaining a constant cell diameter. The system thus functions similarly to an automatic controller, which maintains the output (in this case, the levels of Cdc42 activity at the cell tips) within an acceptable range. Such a set-up may increase cell robustness by avoiding excessive excursions in Cdc42 activation and by limiting the variability of cell width. Preventing excessive fluctuations, for example in exocytosis, may allow for a better-constructed cell wall. Furthermore, although maintaining a constant cell diameter may not be particularly important under laboratory conditions, such a constraint may be advantageous in fungi during the extensile growth of a multicellular hypha, a
relevant possibility since fission yeast can grow as a dimorphic fungus.

A way to explore space
Cdc42 oscillatory dynamics emerge from a process of self-organization, collective behaviour arising from molecular interactions and regulatory feedback loops. Why would the Cdc42 system maintain itself in an oscillatory state rather than a stationary state at any given cell length? Cdc42 oscillations may represent exploratory behaviour, a general strategy among self-organizing biological systems. Despite the cost associated with maintaining an exploratory process, biological systems benefit because they acquire the ability to quickly reach states that would otherwise be hard to access. For example, spindle assembly during mitosis is facilitated by the constant growth and shrinkage of microtubules at the expense of GTP hydrolysis [28,29]. Similarly, bacteria actively switch metabolic states to maintain optimal growth for a population in a changing environment [30]. The periodic, but brief, inactivation of Cdc42 at the cell cortex provides the opportunity for a new pattern of Cdc42 distribution to emerge and offers cells the flexibility to adapt the pattern of polarization in response to changing intracellular conditions, such as cell volume and length, cell stress or cytoskeletal organization. In an environment with changing external cues, such as nutrient availability or pheromone gradients, Cdc42 dynamics would allow cells to adapt and redirect their direction of growth. Recently, this latter idea has found experimental support in cells exposed to low levels of mating pheromone, which display short-lived GTP-Cdc42 clusters forming and dissipating throughout the whole cell cortex, a behaviour thought to enable the morphological response to an extracellular pheromone gradient [31].

Modulating the system
Observations of individual cell time courses suggest a significant role for randomness or variability. Sometimes cells may show one distinct transition, none or even temporarily revert to a more asymmetrical GTP-Cdc42 distribution. This suggests that, during growth, individual cell paths may vary stochastically from cell to cell, however, fitting a general description. Following cell division, cells display an asymmetrical state with most GTP-Cdc42 at the old end. Autocatalytic accumulation at the old end depletes the active Cdc42 available to the new end and prevents it from competing. Cell elongation and increased GTP-Cdc42 availability promotes a more asymmetrical Cdc42 distribution, whereas oscillations may boost the ability of cells to discover a favoured state among diverse possibilities.

What changes GTP-Cdc42 availability during cell elongation? The regulation of bipolar growth activation in fission yeast has been under intense scrutiny in the last few years. Many laboratories have contributed important findings, establishing the role of a diverse cohort of kinases and phosphatases in subtly modulating the pattern and direction of fission yeast cell growth. More recently, NETO has been linked to MPF (maturation-promotion factor) and Polo kinase activation [32]. Elegant studies have also linked NETO to the checkpoint kinase Cds1 and to calcineurin [33].

The microtubule cytoskeleton also has an important role in maintaining the direction of cell growth and in promoting bipolar growth activation. In the absence of microtubules, cells bend and curve, and grow in a predominantly monopolar fashion. The intrinsic dynamicity of the microtubule cytoskeleton, coupled with the rigidity of the microtubule lattice, promotes the natural alignment of microtubules along the main axis of the cell. Microtubules deliver the Tea1 protein complex to the cell tips, where it recruits important components of the polarity control machinery such as Tea1-complex protein Tea4, formin For3, protein phosphatase 1 Dis2 and DYRK (dual-specificity tyrosine-phosphorylated and -regulated kinase) family protein kinase Pom1 [34–37]. These observations indicate that the Tea1 protein complex functions to interpret the spatial information provided by microtubules, locally maintaining and reinforcing cell polarization at the cell tips. Our understanding of the mechanisms linking the microtubule cytoskeleton to Cdc42 activation is, however, currently very limited.

Conclusion
The Cdc42 oscillations suggest a general role for GTTPase dynamics in eukaryotic cell morphogenesis. Recent observations have shown Cdc42 oscillations in natural killer cell immunological synapse [38], activity fluctuations of Rho-GTPases within protruding and retracting lamellipodia in migrating mammalian cells [39], oscillating Rop1 GTTPase activity during pollen tube elongation in plants [40] and competition between Cdc42 clusters in artificially rewire budding yeast cells [41]. These findings indicate that GTP-Cdc42 oscillations in fission yeast are one example of a general strategy to control cell polarization and morphogenesis in eukaryotes. This flexible mechanism may facilitate the search for the optimal state of polarization to modulate growth asymmetry at the cellular level.

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