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Cortical actin dynamics

Generating randomness by formin(g) and moving

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The actin cytoskeleton plays essential roles in cell polarization and cell morphogenesis of the budding yeast *Saccharomyces cerevisiae*. Yeast cells utilize formin-generated actin cables as tracks for polarized transport, which forms the basis for a positive feedback loop driving Cdc42-dependent cell polarization. Previous studies on cable organization mostly focused on polarized actin cables in budded cells and their role as relatively static tracks for myosin-dependent organelle transport. Using quantitative live cell imaging, we have recently characterized the dynamics of cortical actin cables throughout the yeast cell cycle. Surprisingly, randomly oriented actin cables in G_1 cells exhibited the highest level of dynamics, while cable dynamics was markedly slowed down upon cell polarization. We further demonstrated that the rapid dynamics of randomly oriented cables were driven by the formin Bni1 and Myosin V. Our data suggested a precise spatio-temporal regulation of the two yeast formins, as well as an unexpected mechanism of actin cable rearrangement through myosins. Here we discuss the immediate significance of these findings, which illustrates the importance of generating randomness for cellular organization.

Key words: actin, formin, myosin, polarity, self organization

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Introduction

The budding yeast *Saccharomyces cerevisiae* has been extensively used as model to study the molecular mechanisms of actin regulation.[1] Filamentous actin (F-actin) in *S. cerevisiae* assembles into three types of structures, namely actin patches, actomyosin rings and actin cables. Actin patches are bouquets of branched filaments nucleated by the Arp2/3 complexes. Together with Myosin I this branched actin is thought to generate the forces required for the first steps of endocytosis.[2] Actomyosin rings assemble transiently at the bud neck after mitotic exit. In these rings, bundles of actin filaments together with Myosin II provide the constricting force for cytokinesis.[2]

Actin cables are thought to be linear bundles of F-actin generated by the two yeast formins Bni1 and Bnr1.[3,4] Formins promote both nucleation and elongation of F-actin via processive stepping on the barbed ends.[5] In unpolarized *S. cerevisiae* cells actin cables are randomly oriented.[6] In contrast, in cells with small or medium-sized buds, Bni1 becomes mostly localized to the bud tip, whereas Bnr1 is restricted to the bud neck.[4,7-9] These polarized localization patterns of formins enable cells to build distinct arrays of oriented actin filaments in mother and daughter cells, that drive polarized transport of organelles and molecules into the bud.[1,10-12] In later stages of the cell cycle, Bni1 relocates to the bud neck and actin cables reorient accordingly, supporting cytokinesis and possibly abscission.[7] It has been suggested over a decade ago that actin cables are highly dynamic structures that undergo constant turnover throughout the cell cycle.[6,13] However, in vivo data on cable dynamics had been scarce due to the difficulty of visualizing cables in living cells. Several studies focused on the behavior of polarized cables.[14,15] These studies utilized Abp140-GFP as a reliable in vivo marker for actin cables and showed that actin

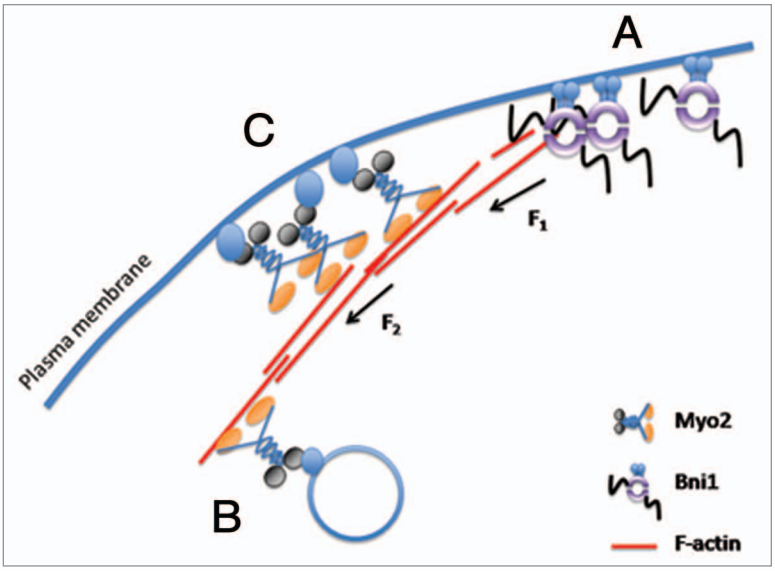

Figure 1. Proposed mechanism of actin cable dynamics driven by formin and Myosin V. (A) Actin cables are generated by cortical Bni1 molecules. (B) Actin cables can serve as tracks for Myo2 to drive cargo transport. (C) Cortically anchored Myo2 can function as a motor that moves actin cables. Cortically anchored Bni1 and Myo2 generate forces (F_1 and F_2) toward the pointed ends, driving the fast extension/motility of actin cables.

cables can move with speeds of roughly 0.4 μm/s. However, the molecular mechanism behind this movement remained unclear.

Cortical Actin Dynamics Driven by Formins and Myosin V

Using total internal reflection microscopy (TIRFM), we recently performed an extensive analysis of actin cable dynamics in *S. cerevisiae*.[16] We found that cables were reorganized throughout the cell cycle via a combination of growth, shrinkage, bending, bundling and translational motility.[16,17] In contrast to previously published results,[14,15] cable extension and movement occurred at a wide range of speeds between 0.5 and 5 μm/s. To our surprise, cable velocity and turnover were highest in unpolarized G_1 cells, and reduced upon cell polarization. To determine the molecular basis for cable dynamics we analyzed cable dynamics in various mutants of actin regulators and polarisome components. We found that the formin Bni1 was associated with cable extension at 1–2 μm/s, while the other formin Bnr1 mediated slower cable extension at rates below 1 μm/s. In addition, we could show that the type V myosin Myo2 drove rapid translational movement of cables along the cell periphery at speeds above 2 μm/s.

The contribution of formins and Myo2 were differentially regulated through the cell cycle. In unpolarized G_1 cells, cable dynamics was dominated by Bni1 and Myo2. Strikingly, both molecules assembled into distinct cortical patches associated with the plasma membrane. Double color TIRFM experiments suggested that Bni1 patches were sites of actin cable assembly, whereas Myo2 patches generated translational motility by sliding actin cables along the inner plasma membrane surface. Upon cell polarization, cortical Bni1 and Myo2 patches in mother cells became destabilized and both proteins now instead became concentrated at polarization sites and bud tips. At the same time Bnr1 was activated and localized to the bud neck. As a consequence of these changes, Myo2 no longer participated in cable motility. Bnr1, with an actin binding affinity tenfold higher than Bni1,[18] dominated cable dynamics in mother cells, resulting in the observed slow-down of cable reorganization in polarized cells.

Our findings can be summarized in a model of dynamic actin reorganization through spatio-temporal interplay of three motor molecules with different modes of action and kinetics (reviewed in ref. 16, **Fig. 1**). It was shown previously that the two formins Bni1 and Bnr1 have distinct biochemical properties and localize to different cellular locations.[9,18] We now demonstrated that in cells these differences are actually used to generate kinetically distinct actin cables. Combined with the stable association of Bnr1 with septins at the bud neck[19] and the dynamic localization of Bni1 on the mother cell cortex,[9] this enables cells to switch between two different modes of actin organization by simple activation/inactivation of the formin Bnr1.

Cortical Localization of Bni1 and Myo2

Bni1 and Myo2 were previously shown to mainly localize to bud tips and bud necks.[1,7] This view was first challenged when triple-GFP labeled Bni1 were reported to form cytoplasmic foci in mother cells.[9] In our study the improved signal to noise ratio of TIRFM allowed us to investigate the cortical localization of single-GFP labeled proteins. We found that both Bni1 and Myo2, but not Bnr1, assemble into randomly distributed cortical patches. These patches are most abundant and long lived in unbudded G_1 cells, and become destabilized as cells commit to polarized growth. We also showed that cortical Bni1 and Myo2 patches assemble independently of F-actin. Interestingly, Bni1 patches were partially dependent on Bud6, whereas cortical association of Myo2 required its C-terminal cargo-binding domain.[17] Our data suggests that Bni1 and Myo2 anchor to the plasma membrane indirectly through adaptor proteins. We propose that the observed Myo2 and Bni1 patches allow cells to maintain an isotropic cortex organization by constantly producing randomly oriented, rapidly moving actin cables.

Dual Function of Myosin

Type V myosins are well known for their role in polarized organelle transport.[1,10-12] We now found that plasma membrane-bound Myosin V also directly mediate motility of actin cables. Such a behavior has been repeatedly observed from in vitro gliding assays but has not been

demonstrated in vivo, although various myosins have been proposed to move actin filaments within cells.[20,21] The dual function of Myo2 in generating forces for polarized transport as well as actin reorganization is perfectly suited to cell cycle specific requirements of yeast. In unpolarized G_1 cells the cell periphery is covered with randomly oriented cables generated by cortical Bni1. The faster elongation rate and low affinity of Bni1 to filament ends very likely leads to frequent detachment of shorter cables. These can then immediately be captured and moved by cortical Myo2 (**Fig. 1**). An intriguing possibility that still remains to be tested is that Myo2 may actively pull on Bni1 attached actin filaments and thereby either enhance or terminate the filament elongation. The combination of rapid polymerization and fast motility generates a highly dynamic and randomized network of actin cables, thereby maintaining the unpolarized state of G_1 cells and supporting isotropic growth. In polarized cells most cables in mother cells are nucleated by the bud neck-associated formin Bnr1.[16,22] While Bnr1 has a slower elongation rate than Bni1, it has much higher affinity to filament barbed ends and therefore remains tightly associated with cable ends.[18] A cortical Myo2 motor that encounters such a stably anchored cable will very likely not be able to detach it from the bud neck. Instead, the anchorage of Myo2 to the cortex could be dislodged and myosin motors become free to support polarized organelle transport toward the bud neck.

Several recent studies in fission yeast demonstrated that tropomyosins differentially regulate motor activities of Myosins I, II and V. Binding of tropomyosins to actin filaments can alter ATPase activity of myosin heads, as well as their binding affinities to actin.[19,23] Interestingly, it was previously suggested that *S. cerevisiae* tropomyosin Tpm2 impedes retrograde actin cable flow in polarized mother cells by inhibition of myosin-actin interaction.[15] Therefore, *S. cerevisiae* tropomyosins may also be involved in the regulation of rapid cable dynamics we observed. Whether tropomyosin isoforms (Tpm1 and Tpm2) associate with specific formins, and thus regulate distinct subpopulations of cables, or they regulate cable sliding by cortical Myosin V, are intriguing options for further investigations.

The Importance of Generating Randomness

Cell polarization requires most actin cables to be oriented with their barbed ends pointing toward the polarization site. Paradoxically, cortically anchored Bni1 and Myosin V should both generate forces toward the pointed ends of actin filaments. Therefore, Bni1 and Myo2, both required for proper cell polarization, also seem to antagonize it by randomization of F-actin. Why then, do cells expend so much energy to generate randomness, although they all eventually commit to polarized growth? One reason could be that in *S. cerevisiae* the decision for a polarization site must be made within an extremely narrow time window with high accuracy.[24-26] This requires the cell to balance the need for rapid decision making with the need for error detection and correction. A highly dynamic network of actin cables serves both ends. On the one hand, it allows the cell to rapidly respond to a polarization cue, since the reorganization of entire cable network only takes several seconds in G_1 cells.[16,17] On the other hand, randomization of cables by Bni1 and Myo2 prevents the cell from falling into kinetically trapped states, such as the formation of multiple polarization foci. Indeed, when cable dynamics were forced to slow down in cells already defective in Rho GTPase recycling, a significant proportion of them formed double polarization caps.[16] This result suggests that cells evolved a coupled mechanism of GTPase recycling and actin cable dynamics to ensure the fidelity of cell polarization.

An alternative function for Bni1-Myo2 dependent actin movement could be found in the cellular stress responses. In a recent work, Nyström and colleagues reported a mother-specific damage retention mechanism of protein damage. After heat stress in *S. cerevisiae*, the daughter cells were cleared of protein damage by actin-dependent retrograde flow of Hsp104-labelled misfolded protein aggregates.[27] Interestingly, their genetic analyses also pinpointed the importance of Bni1 and Myo2 in this process, which is consistent with their roles as motors generating pointed end-directed actin movement. Nonetheless this remains to be examined further, since the organization and function of formins, actin and myosins may be altered in cells under high stress.[28]

Concluding Remarks

In summary, our work provides a prototypical example for a self-organized biological system, in which motors and their polymeric tracks differentially interact according to cellular needs. We showed that, the same cytoskeletal components required for promoting cell polarization might also function to prevent it from happening in an erroneous manner. Such mechanisms illustrate fundamental distinctions between the logic of biological decisions and that of engineered mechanical processes. The former is characterized by adaptability and reversibility, while the latter is usually inflexible and irreversible.

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