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A Systems-Biology Approach to Yeast Actin Cables

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Abstract

We focus on actin cables in yeast as a model system for understanding cytoskeletal organization and the workings of actin itself. In particular, we highlight quantitative approaches on the kinetics of actin cable assembly and methods of measuring their morphology by image analysis. Actin cables described by these studies can span greater lengths than a thousand end-to-end actin monomers. Because of this difference in length scales, control of the actin-cable system constitutes a junction between short-range interactions—among actin monomers and nucleating, polymerization-facilitating, side-binding, severing, and cross-linking proteins—and the emergence of cell-scale physical form as embodied by the actin cables themselves.

1 Introduction

Many basic cell functions such as cell motility, endocytosis, cytokinesis, and establishment of cell polarity depend on actin filaments [1]. Actin filament nucleation, polymerization, and controlled disassembly keep actin subunits in a state of constant turnover between the monomer and filament states. Groups of regulating proteins marshal this adaptable actin cytoskeleton for diverse tasks. A huge body of work considers the actin system both in controlled *in-vitro* situations and in eukaryotic and bacteria cells that use actin or its homologs [1, 2]. These studies raise questions about how cells employ the actin system to move, polarize, divide, transport material, resist stress, contract, and signal. Here, we discuss how budding and fission yeast can serve as model systems for universal molecular mechanisms of the actin cytoskeleton.

During growth, yeast cells build two actin structures: patches and cables [1, 3, 4, 5]. The actin patches, dense dendritic networks of actin filaments nucleated by the Arp2/3 complex, assist endocytosis. The actin cables, which we focus on here, are bundles of actin filaments that run across the cell and guide the transport of secretory vesicles and organelles (see Fig. 1). Formin proteins at the cell cortex generate these bundles by promoting nucleation of new filaments out of monomers and by processive polymerization [6, 7, 8]. As actin filaments polymerize away from the cell tip, they become bundles held together by cross-linking proteins, often as long as the cell. Budding and fission yeast differ in shape and, accordingly, in where they direct formins to sow cables at specific sites. Two formins, Bni1p and Bnr1p, activate cable growth in budding yeast [3]. A single formin, For3p, initiates actin-cable growth in fission yeast [4]. The cell breaks down and disassembles long cables through the

coordinated action of a set of proteins. As a whole, the actin-cable system manages an interface between actin biochemistry and cell geometry.

As a system to study how cells respond to information about location, actin cables offer many advantages. Yeast serves well for genetic manipulation, allowing researchers to exploit homologous recombination and deletion libraries. Regulating proteins and the cables themselves can be monitored by fluorescence microscopy. Methods have been developed to measure protein concentrations in yeast [9, 10]. Also, the cables may be one of the simpler actin structures: in fission yeast, they appear to require far fewer assisting proteins than actin patches or contractile rings for division [4]. But although actin cables may be a relatively simple system, they certainly behave in ways that would be hard to predict by knowing only the interactions among components. Understanding the complexity of regulating these dynamic structures by timing and location seems to require the approach of systems biology: simultaneous measurements of multiple components and rigorous statistical analyses combined with mathematical models [11]. Here we highlight recent quantitative studies of yeast actin cables and discuss our view of the direction of this field.

2 Quantifying the Polymerization Kinetics of Actin Cable Assembly

Actin cables are very dynamic structures with lifetimes of order one minute. The constituent filaments grow by adding monomers from the cytoplasm, at their barbed ends, and losing monomers to the cytoplasm by severing and depolymerization. Many actin-binding proteins modulate these kinetics. For instance, formins seed cables and increase the rate of actin-monomer addition at the barbed end. Reaction rates and protein concentrations, and their regulation, can affect qualitative behavior and so understanding the actin-cable system depends on measuring their values.

Recent studies suggested a detailed molecular mechanism for formin-mediated actin-cable assembly [13, 14]. Formin proteins promote actin filament nucleation and elongation by processive association with the polymerizing end of actin filaments [6, 7, 8]. In fission yeast, formin For3p localizes in cortical foci at the growing tips of the cell (see Fig. 1B). Budding-yeast actin cables are nucleated by formins Bni1p and Bnr1p. Bnr1p localizes at the bud neck (see Fig. 1D). Bni1p localizes as foci at the tip of the growing bud and subsequently joins Bnr1p at the bud neck (see Fig. 1D). Both Bni1p and For3p associate with large cortical macromolecular structures where they nucleate actin filaments for cables. These filaments are bundled by actin cross-linking proteins such as fimbrin [3], and undergo retrograde flow away from the bud (or away from the cell tips in fission yeast) at speeds of order $0.3 \mu\text{m/s}$ and larger [15, 16]. Long cables disassemble through the coordinated action of tropomyosin, cofilin, actin interacting protein Aip1, coronin, and twinfilin [17, 3].

The association of Bni1p and For3p with the cortex is transient: within seconds, these formins dissociate from the cortex and passively follow actin cable retrograde flow and disassembly, thus following a turnover cycle similar to actin. Based on these observations, Buttery and Pellman [13] and Martin and Chang [14] proposed the mechanism shown in Fig. 2A. The movement of the formins away from the cortex (process 4 in the Fig. 2A) was found to be dependent on actin polymerization, indicating the existence of coupled control mechanisms between actin and formins. This feedback mechanism indicates the possibility for rich dynamical behavior by the cable system. Unlike Bni1p, Bnr1p appeared to remain associated with the neck [13]. The cartoon of Fig. 2A appears to describe a summary of what is seen in experiments. But, without the support of a quantitative model, it is unclear if the model is even a consistent representation of an actin cable assembly mechanism.

To explore the quantitative implications of the proposed model of Fig. 2A, Wang and Vavylonis added rate constants, protein concentrations, and diffusion coefficients in an

analytical and computational model [18]. They considered fission yeast due to its simpler geometry as compared to budding yeast, and the fact that actin cables are nucleated by only one formin, For3p. Fig. 2B shows the processes described by the rate equations of the model, with A_{cyto} and A_{cable} being the numbers of actin subunits in the cytoplasm and in actin cables, respectively, and F_{cyto} , F_{cable} , and F_{tip} , are the numbers of For3p in the cytoplasm, along the body of actin cables, and at cable tips, respectively. One rate constant depends on the processivity parameter, p , the average number of actin subunits polymerized per cortical For3p before its detachment into the cable. Whole-cell numerical simulations of actin and For3p reaction and diffusion were performed in 3D (Fig. 2C). The model considers a continuous field of cytoplasmic actin due to its abundance and individual For3p particles moving on a lattice due to their rarity.

The simulations validated the cartoon model. Using a combination of measured and fitted parameters, the model could explain experimental results, such as fluorescence recovery after photobleaching (FRAP) curves of For3p-3GFP and the response of the actin cables to treatments with the drug Latrunculin A (LatA), which promotes cable disassembly by sequestering available actin monomers.

In addition, the model suggests a description of the system in the form of “dynamical phase diagrams” (Fig. 2D, E) that describe how parameter values (concentrations, rate constants) affect physiological properties of actin cables: polymerization rate, thickness, and length. Do cells tweak these parameter values to manipulate form (as in Fig. 2D) and thus optimize function? The facility of genetic engineering in yeast may allow future tests of these results. For example, systematic For3p overexpression and/or reduction of For3p expression levels are possible. Similarly, changes in the polymerization rate constant and processivity parameter could be tested by targeted changes in the FH2 and FH1 domains of For3p that mediate polymerization and processive motion [19, 7, 20, 16]. The above could be combined with treatments with drugs such as LatA, which effectively reduces the actin-polymerization rate constant.

With a constant processivity parameter, this model admits a single steady state for the actin-cable system. A cooperative mechanism for For3p detachment, meaning that the detachment rate depends sensitively on the polymerization rate, would introduce nonlinearities that could lead to additional steady states of actin-cable organization. This suggests the intriguing possibility that the cell might gain fitness through the ability to signal a switch between these states, allowing a rapid reorganization of the actin-cable system.

So far there have been no detailed quantitative models of actin cable dynamics in budding yeast. However, some experimental studies treat the actin cables from a systems point of view [21]. For example, upon Bni1p overexpression the actin cables become shorter and more dense within the bud [22, 23]. In these overexpression studies, the actin cables within the mother cell (presumably nucleated by Bnr1p) become short and thin [22], though some mother cells become unusually large and contain multiple cable-like fragments [23]. This change in the actin cables in the mother cell could be due to the Bni1p-induced depletion of the actin monomer pool available to Bnr1p. Because of uncertainties in the mechanisms of Bnr1p cortical dissociation and association, the effects of Bnr1p overexpression [21] are harder to interpret. Full length Bnr1p overexpression has small effects [21], though overexpression of unregulated Bnr1p leads to serious defects that can be rescued by an increase in the concentrations of proteins that bind to actin monomers or with treatment with LatA, possibly by reducing Bnr1p-mediated nucleation of actin filaments in the cytoplasm [21]. A more recent study showed that the two formins, Bni1p and Bnr1p, assemble kinetically separable cable populations [16]. A future quantitative modeling approach may help provide insight into the importance of these experimental findings.

3 Analyzing the Morphology of Actin Cables

The model of Fig. 2A described the kinetics of nucleation, assembly, disassembly, and severing in 3D but treated each cable as a 1D object. Further cable traits arise however in 3D: cables bend, twist, and buckle; organelles deflect the cables and cell geometry confines them; and cables cross or become bundled with one another. Actin-binding proteins regulate this cable morphology. Cross-linking proteins, such as fimbrin [3, 4], can bind to multiple filaments and stiffen a single cable or help to bundle multiple cables. Together with side-binding proteins, such as tropomyosin [24] they mediate bending and twisting and may make filaments more or less susceptible to severing [25]. Actin cables also appear to associate with actin patches [26]; further, association with myosin V [16] may attach cables to organelles. Mutations in these proteins can disrupt normal actin cable morphology, indicating that these proteins regulate the spatial distribution of actin cables.

Measuring actin-cable morphology requires clear images of actin cables. This is possible since yeast actin cables are dilute as compared to actin structures in other cells. Several fluorescent markers can illuminate cables in live cells. For example, a fusion of the calponin homology domain from the IQGAP Rng2p to GFP (GFP-CHD) [14], the seventeen-amino-acid peptide Lifeact [30], and actin binding protein 140 tagged with GFP [15] all mark filamentous actin. Confocal microscopy allows for the reconstruction of the cable position in three dimensions. Sub-diffraction microscopy could enhance the precision with which cables can be located.

Extracting the numbers that describe morphology from images and movies of the cables presents another challenge, but recent work shows that this can be done. Smith and others provide an open-source tool, JFilament, which fits this task [28]. JFilament uses stretching open active contours [27] to find flexible filaments in a noisy image. The algorithm starts with a proposed filament skeleton and modifies it to minimize an energy, which includes internal terms that penalize bending and stretching and external terms that account for crossing a gradient in the image (see Fig. 3A). With adjustments to the relative contribution of these terms and some manual interaction, JFilament allows efficient capture of many actin-cable statistics. Using the program, Smith and others analyzed cables with a clear trajectory across the cell (Figs. 3B and 3C). They found two length scales that described the cables, one less than the persistence length of single actin filaments and one closer to the persistence length of microtubules. The smaller length scale could correspond to short-scale deformations from pulling and motor buckling [31, 32], interaction of cables with patches [33], or fixed fluctuations during actin-cable assembly [18, 34]. The longer length scale could reflect the stiffness of the bundles and the fact that the actin cables are confined to the cell interior, which behaves as a rigid tube [35]. In any case, these analyses suggest that the equilibrium semiflexible-polymer description needs a few additions to capture the behavior of actin cables.

Looking forward, work towards complete, reliable automation of the data extraction should allow for the leveraging of the statistics of thousands of cells' worth of actin cables, possibly to reveal subtle changes in the regulation of morphology across the breadth and cycle of the cell. Reported automated two-dimensional methods that distill stacks of images into filament locations and network topologies [29] (Fig. 3D) could be extended to three dimensions. Also, automated separation of actin patches and cables remains challenging.

From this data, modeling studies will attempt to answer open questions: Do actin cables interact with or attach to the membrane? If so, can they grow while attached? Is cable position tightly regulated, or do cells allow random processes to determine their precise location? Are multiple nucleators required in budding yeast because it has a more complex

shape than fission yeast? What minimal set of regulating processes can capture the salient aspects of actin-cable morphology? Theoretical work addresses the behavior of semi-flexible polymer bundles under confinement [35, 36, 37], but this has yet to be applied to actin cables in yeast. A model may show that only a few simple assumptions are necessary to reproduce most characteristics of measurable behavior, and this could become a framework for understanding how cells regulate the morphology of actin cables. These mathematical models will help us understand how proteins guide this measured morphology through collective behavior.

4 Outlook

Cells may have optimized the actin-cable parameter values to be robust [38], corresponding to a large parcel of parameter space for the physiological region in Fig. 2D. However, the actin cable system adapts for reorganization, as when the cables disassemble and actin filaments move to the division site for cytokinesis in fission yeast [39, 5]. The size of the physiological region may balance robust behavior for actin cables, which requires a large region, with the need for a malleable actin system that may be adapted to many purposes, which may require a small region. Here we motivate a systematic experimental exploration of parameter space to test these issues. Such studies should also reveal quantitative details on the role of other components of actin cables, such as regulatory pathways and bundling kinetics.

The results in yeast may have implications on the general role of formins in cells beyond yeast, such as the actin cable network in plants [40, 41, 42]. Because changes to parameter values establish different distributions of actin and formins within yeast, many other eukaryotic cells may have also used this property to establish different patterns and structures. Future work will uncover the extent of universality in the mechanisms of formin function. Much remains to be established, for example, on the precise function of fission yeast formin Cdc12p in nucleating disperse actin meshworks and/or actin cables during the assembly of the cytokinetic contractile ring [43, 44, 45, 46]. Hopefully, the modular structure of biological systems will allow us to proceed to a hierarchical understanding of the cell biological function of formin-mediated actin structures, starting from general features at a mesoscopic level of description, down to the full details of regulatory pathways that may differ across organisms.

Finally, we acknowledge some significant challenges. Attempts to measure cable elongation rates encounter technical difficulties—the end can be hard to locate and the dynamic nature of the marker complicates FRAP experiments. Models of actin cables become more complex as they include more elements, obscuring their interpretation. Also, the actin cables are only approximately a modular system, and incomplete knowledge of the systems with which they interact may limit understanding of the actin cables. However, we are optimistic that an increasing toolbox of quantitative methods will eventually help overcome such obstacles.

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Fig. 1.

Actin cables in fission and budding yeast. (A) Image of interphase yeast cells expressing actin marker CHD-GFP showing actin patches (bright spots) and actin cables (linear elements) [5]. (B) Actin cables (blue) in fission yeast polymerize away from the cell tips where formin For3p (green) nucleates actin filament assembly. (C) Image of budding yeast showing actin cables and actin patches (stained with rhodamine-phalloidin) from [12]. (D) Budding yeast actin cables (blue) are nucleated by formins Bni1p (red) and Bnr1p (purple) that localize at the bud and bud neck.

Fig. 2.

Model of actin cables in fission yeast [18]. (A) Schematic showing the basic processes (1–6) of the model. (B) Ordinary differential equations model. (C) 3D computational lattice model accounting for the small number of For3p which are treated as discrete units. (D) Qualitative dynamical phase diagram describing the morphology of the actin cable system as a function of actin and For3p concentration. (E) The model predicts different distributions of actin and For3p depending on rate constants.

Fig. 3.

Segmentation and tracking of actin cables using active contours. (A) Example of segmentation in a total internal reflection microscopy image of a single actin filament [27]. Initialization of the active contour away from the central line of the filament and position of active contour after 20, 40 and 60 iterations of deformation. Scale: 1 pixel = $0.17 \mu\text{m}$. (B) Images showing a fission yeast *cdc25-22* cell expressing GFP-CHD that marks actin cables and actin patches [28]. Left: 3D volume view and active contour of a segmented actin cable. Right: Image of an active contour together with x , y and z cross-sections of the image. Cell diameter is $\sim 3.5 \mu\text{m}$. (C) The tangent correlation function of actin cables from images as in panel B. A fit to a double exponential (continuous line) leads to length scales $l_1 = 2 \mu\text{m}$ and $l_2 = 1 \text{ mm}$ [28]. (D) Automatic segmentation of 2D filament network using multiple active contours from [29]. A meshwork is generated by initialization of multiple active contours at ridge points followed by growth, merging and splitting of active contours. Grouping analysis is used to classify segments. Image shows application to a 2D radial projection of a 3D confocal microscopy volume of a dividing *cdc25-22* fission yeast cell expressing GFP-CHD. Vertical axis is arc length.