COMMENTARY

Deconstructing formin-dependent actin cable assembly

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Eukaryotic cells are organized in three dimensions to allow for both cell division and for them to perform their physiological functions. This organization is dependent upon the arrangement of the cytoskeleton, which can either generate force by subunit polymerization or serve as tracks for molecular motors to deliver protein complexes, RNAs, or membrane-bound compartments. In general, long-range transport is mediated by microtubules, whereas microfilaments often contribute more local structural and motile roles, being especially prominent in association with the plasma membrane. Microtubules and microfilaments can be quite dynamic, and this requires the appropriate spatial regulation of polymer assembly, then association with factors to generate functional structures, and also control of polymer disassembly. For microfilaments, two major nucleators of actin assembly are the actin-related protein 2/3 (Arp2/3) complex, which generates branched filaments and formins that generate unbranched filaments (1). Formins are present in all eukaryotes—humans have 15 different ones—so understanding their biology is of significant importance. Miao et al. (2) have now established a formin-dependent actin cable assembly reaction using yeast cell extracts that will permit a dissection of how formin activity is regulated in vivo.

Budding yeast is an ideal system to study actin assembly: microtubules orient the nucleus and build the mitotic spindle, whereas a single essential gene encodes actin that is used to build actin-based patches and cables (Fig. 1*A*) (3). Patches are sites of endocytosis (4, 5), and actin cables, which extend from the bud into the mother, are tracks for the myosin-Vs that transport secretory vesicles for polarized growth, organelles for their segregation, and mRNAs for fate determination (6, 7). The branched actin filaments in patches are nucleated by the Arp2/3 complex largely through activation by Las17 (yeast WASP) and, together with a plethora of additional proteins, this drives the invagination of endocytic vesicles against the internal turgor pressure (5). The genetically redundant formins, Bni1 and Bnr1, nucleate the assembly and catalyze the elongation of actin cables (8, 9). Bni1 is localized by its N-terminal regulatory domain to sites of cell growth, and the C-terminal catalytic half containing the FH1 domain is able to recruit profilin-actin that is then added to the growing filament end by the adjacent FH2 domain (Fig. 1*B*) (10, 11). It is known that actin cables are stabilized by tropomyosin (12, 13) and destabilized by the combined action of cofilin and Aip1 (14), yet no system until now has attempted to reconstitute formin-mediated actin cable assembly in a physiological context.

The study of Arp2/3 actin dynamics has benefitted enormously from a combination of genetics and in vitro assembly reactions. The Drubin laboratory pioneered the use of beads with immobilized Las17 to drive Arp2/3-dependent actin assembly in extracts from either wild-type cells or from cells bearing mutations in actin regulatory proteins, allowing them to dissect the molecular mechanisms involving actin during endocytosis (5, 15). Miao et al. (2) now establish an analogous system for actin cable assembly by formins.

The FH1-COOH region of Bni1 both nucleates actin assembly and elongates the nascent filament; therefore, Miao et al. (2) attached this region to beads and added yeast extracts in the expectation of driving formin-dependent actin assembly (Fig. 1*C*). To visualize assembled filaments, the extract was made from yeast harboring GFP-tagged ABP140, an actin-binding protein. Surprisingly, no assembly occurred when extracts from unsynchronized cells were used. However, when extracts were made from cells arrested in mitosis, actin filaments were formed and bundled into cables. We return to cell-cycle regulation later, after first discussing the physiological relevance of this reaction.

Actin cables are stabilized by tropomyosin and, reassuringly, no cables were found when extracts lacking the major tropomyosin were used. Formins compete with capping protein for the growing end of a filament—capping protein terminates assembly, whereas formins catalyze it (16)—and more cables resulted when extracts lacking capping protein were used. Actin cable disassembly is enhanced by cofilin, yet reactions made using extracts containing mutant cofilin assembled cables normally. This finding is not surprising, as cofilin affects disassembly, not assembly. Consistent with this result, cables made from extracts with mutant cofilin

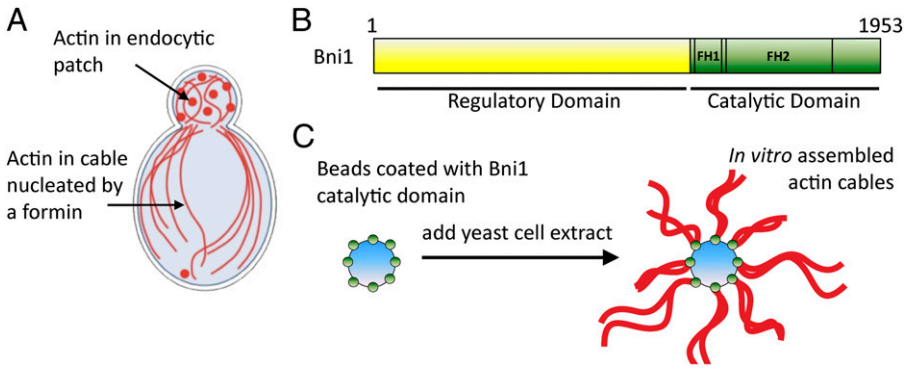

Fig. 1. (*A*) Diagram of actin-contain patches and cables in a growing yeast cell. (*B*) Overall domain organization of the formin Bni1. (*C*) Diagram of the assay used in the study by Miao et al. (2).

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were more stable to the subsequent addition of Latrunculin A, a drug that sequesters monomeric actin from the polymerization reaction. Thus, the in vitro system largely recapitulates what is known about cable dynamics in vivo.

Having established the system, Miao et al. (2) then used proteomics to generate a list of over 100 proteins enriched with the assembled cables. Satisfyingly, the authors find enrichment for known cable components (tropomyosin and the actin-bundling protein fimbrin), but also recover actin binding proteins thought to exist primarily in patches. For example, the patch protein Abp1 was recovered, but repeating the formin-driven actin assembly assay in extracts lacking Abp1 did not affect the outcome. This result illustrates the power of the approach: researchers can now do assembly reactions from extracts of cells lacking each of the components detected by proteomics to see which might affect the reaction and be relevant.

We now return to the finding of Miao et al. (2), that only extracts from mitotically arrested cells can drive cable formation from Bni1 FH1-COOH–coated beads. Supporting this finding, inhibition of the master cell-cycle regulator Cdk1 completely abolished the ability of mitotic extracts to assemble actin cables. These results are surprising, because cables are present throughout the cell cycle. Nevertheless, detailed quantitative imaging revealed that metaphase cells have a modestly higher level of actin cables than G1 cells, where Cdk1 activity is low. In cells lacking Bnr1, where the only formin is Bni1, inhibition of Cdk1 activity in mitotic cells reduced actin cables 40%, in strong support of the in vitro results. Thus, the assembly reaction has revealed that Bni1-dependent actin cables are regulated by Cdk1.

The study by Miao et al. represents the first step in dissecting the formin-dependent actin assembly reaction in a complex protein mixture. As with any initial study, it has limitations that will no doubt be resolved by further experimentation. For example, why are cables only assembled from mitotic extracts? In vivo Bni1 directs actin cable formation at other stages of the cell cycle; therefore, do the unsynchronized cell extracts unleash a negative regulator? It would be interesting to test if cables are assembled in vitro when mitotic and unsynchronized extracts are mixed. The protein Bud6 binds the FH1-COOH region of Bni1 as a positive regulator of cable formation (17), explaining why *bud6*Δ cells have very few cables (18). However, extracts from *bud6*Δ cells yielded robust cable assembly. Little Bud6 is recovered in extracts of wild-type cells, so it would be very interesting to supplement the extracts with additional Bud6 protein and maybe thereby drive assembly in nonmitotic extracts. Another limitation is that no cable assembly was seen when the FH1-COOH region of Bnr1 was used, although this region can nucleate and elongate filaments, and cells expressing just Bnr1 have actin cables (19). To address these issues, it is likely that the method used for preparing the extract may need to be adjusted, perhaps increasing its concentration, or adding an ATP regenerating system, or "spiking" it with additional purified yeast actin. Of course, this system now employs just the catalytic domain of Bni1, so when the whole protein is ultimately used it should uncover new mechanisms of formin regulation. Despite these current limitations, the development of this assembly assay is a major first step.

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Finally, Miao et al. (2) show that their results may be generally applicable: beads coated with the FH1-COOH region of mouse formin mDia2 can drive cable assembly in extracts made from *Xenopus* cells arrested in mitosis, but not when made from interphase cells. Thus, the door to a detailed biochemical analysis of factors regulating formin-dependent actin assembly has been thrown open, and we can look forward to how this approach, together with in vivo analyses, will shed light on the factors that work with these important actin nucleators.

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