Formin' actin filament bundles

Daniel J. Lew

The formin family of proteins have been implicated in regulation of cell polarity and cytoskeletal function in fungal and animal cells, but the manner in which they affect these processes has been mysterious. Two new studies report that formins in budding yeast are specifically required for the assembly of bundles of parallel actin filaments known as cables.

Cell shape and cell motility are governed by the dynamic behaviour of the actin cytoskeleton. Decades of intensive research into the factors controlling actin organization culminated recently in the successful reconstitution, using purified components, of branching-filament networks that bear a striking resemblance to those observed in the leading edges of migrating cells or in the actin 'comet tails' formed by intracellular parasites[1]. A major factor in this triumph was the identification of the Arp2/3 complex, which can bind to the side of an actin filament and nucleate polymerization of a new actin filament at a 70° angle, helping to explain the branched filament organization in these structures[2]. Another very common actin structure is the bundle of parallel filaments, which underlies the formation of filopodia, microvilli, fly bristles, and yeast actin cables, among many other structures[3]. The very properties that make the Arp2/3 complex ideally suited for generating branched actin networks make it difficult to understand how the same complex might generate parallel actin bundles, which has led to the proposal that some as-yet-unknown nucleator must underlie bundle formation. A significant step towards the identification of this mysterious nucleator was reported in two papers published in the last month's issue of *Nature Cell Biology*[4,5]; these papers demonstrate that formation of yeast actin cables depends on a family of proteins called formins.

Formins are a family of homologous proteins that share a common domain organization[6]. An amino-terminal domain can bind to members of the Rho protein family, and three 'formin homology' (FH) domains are thought to bind other proteins including profilin (which interacts with the proline-rich FH1 domain). Numerous studies have suggested that formins are important in controlling both the actin and microtubule cytoskeletons in a variety of organisms. In the budding yeast *Saccharomyces cerevisiae*, deleting the *BNI1* formin gene perturbs both the microtubule-dependent positioning of the mitotic spindle[7,8] and the actin-dependent process of cytokinesis[9] in vegetative cells, and impairs polarization of the cytoskeleton in mating cells[10]. Conversely, overexpression of 'activated' forms of Bni1p causes cell death associated with gross cytoskeletal aberrations[10]. This type of result, combined with the frequent localization of formins to cellular sites of polarization or to cytoskeletal elements, and the binding partners mentioned above, has led to general acceptance of the idea that formins act somehow as 'Rho effectors' for cytoskeletal reorganization. But the diversity of cytoskeletal derangements that result from perturbation of formin function has provided scant clues as to their specific role(s).

Genetic analysis of formin function has been hampered by the fact that most cells express more than one formin, and that deletion of the full complement of formin genes is usually lethal for the cell. To circumvent these problems, the new studies have generated conditional (temperature-sensitive) *bni1* mutants, and analysed their effects in yeast cells lacking other formins[4,5]. The results are crystal clear: formins are specifically required for the assembly of actin cables. Similar conclusions were recently reported for actin cables in fission yeast[11]. The new studies are particularly incisive because the kinetics of mutant inactivation are extremely rapid: actin cables disappear within two minutes of formin inactivation, and reappear equally rapidly after formin reactivation. This dramatic result strongly supports the idea that formins are directly involved in cable assembly or integrity. Previous studies that used similarly rapid conditional mutants of tropomyosin and type V myosin have been equally incisive in dissecting how the actin cables themselves function in yeast[12,13]. Indeed, despite the justified excitement associated with the latest genomic and proteomic approaches to understanding gene function, these studies illustrate how this classical genetic approach — looking carefully at the phenotypes of fast conditional mutants — remains perhaps the most powerful way of pinning down the precise function of an individual protein.

Yeast actin filaments are organized into two distinct structures, cables and patches, during bud growth (Fig. 1). (A third structure, the cytokinetic ring, forms briefly and

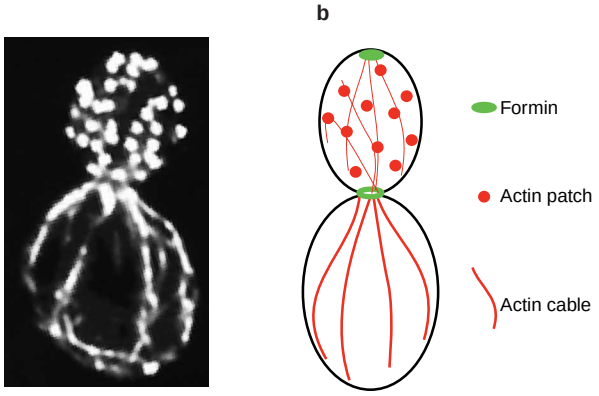

Figure 1 **Actin in yeast.** a, **Yeast cell stained with fluorescent phalloidin, which binds to filamentous actin (adapted with permission from David Amberg), and** b, **a diagrammatic representation . Actin cables are bundles of parallel actin filaments, and patches are motile actin-rich discs that migrate in association with the plasma membrane. Both structures are polarized during bud growth, with patches largely constrained to the bud and cables coursing towards the bud through the cortical cytoplasm of the mother cell. Finer cables continue into the bud and are thought to terminate at the tip of the bud. Formins are concentrated at the bud tip and the bud neck. The new studies suggest that formins control the assembly of actin cables at those locations.**

contracts during cell division). Strikingly, formin inactivation had extremely selective effects on the cables, with no apparent effect on the patches[4,5]. The patches are enriched in Arp2/3 complexes and their regulators, and cells deleted for the *ARP3* gene lacked detectable patches but retained formin-dependent cables[4]. There seems, therefore, to be a separate actin nucleator for cables — one exciting possibility is that the formins themselves either nucleate actin polymerization or regulate other factors that can do so. Alternatively, formins may simply promote the assembly of actin filaments (nucleated by some other factor) into cables.

Overexpression of formins promoted the assembly of supernumerary actin structures[4,5]. Similar to actin cables, the formin-induced filaments were coated with tropomyosin. The association of tropomyosin with cables may explain why the actin filaments in cables are not branched, as it was recently shown that tropomyosin blocks the binding of Arp2/3 complexes to the sides of actin filaments[14]. Alternatively, it is possible that the localization of most of the Arp2/3 complexes (and their activators) to the patches leaves few complexes available to form branches on the filaments generated (or assembled) by formins, allowing tropomyosin to coat the Arp2/3-free filaments. How yeast cells generate such distinct actin structures so close to each other remains a fascinating question.

Studies on formins in yeast have postulated separate roles in cell polarity, spindle orientation, and cytokinesis. Can the discovery that formins mediate actin cable assembly now provide insight into those defects that accompany partial loss of formin function? Cell polarity in yeast depends on properly oriented actin cables[15], and spindle orientation depends on components delivered into the bud by myosin-mediated transport along cables[16,17]. In addition, the cytokinetic ring may be a specialized version of an actin cable, also requiring formin function (this issue will be interesting to clarify in the future). It is conceivable, therefore, that all of the known functions of formins in yeast, and perhaps also in other cells, may boil down to the proper assembly of parallel bundles of actin filaments, which then indirectly affect various other structures, including the microtubule cytoskeleton. But it is possible that life is not that simple. One recent study suggested that formins might be involved in the polarization of actin patches in yeast, independent of cables[18]. In addition, studies on cytokinesis in yeast have suggested that the function of formins may go beyond the function of the cytokinetic actin ring[9]. Regardless of whether formins do or do not have additional functions, the new studies represent a major step towards understanding how actin cables form. Given the evolutionary conservation of formins across species, the insights gained from yeast will undoubtedly impact studies of parallel actin bundles in animal cells as well. □

Daniel J. Lew is in the Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA
e-mail: daniel.lew@duke.edu

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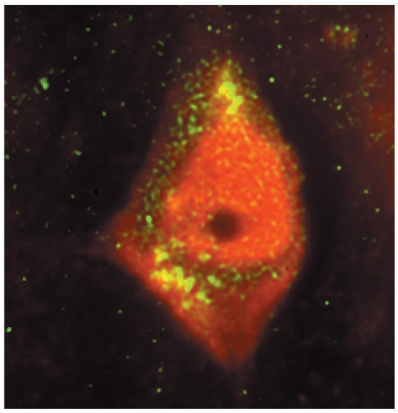
Hip, Hip, Hippi!

Huntington disease (HD) is caused by mutations in the *huntingtin* gene. Mutant Huntingtin protein (Htt) with expanded polyglutamine repeats forms aggregates in diseased brains. It has been suggested that the selective loss of neurons from the brain striatum of patients with HD is caused by apoptotic death. But how does mutant Htt activate the apoptotic pathway? Don Nicholson and colleagues now propose an attractive model on p.95 of this issue. The key is in the regulation of the interaction of Htt with its partner Hip-1. Nicholson and colleagues identify another partner for Hip-1, Hippi. The picture shows immunostaining of Hippi (yellow) in cortical pyramidal neurons (red) of the mouse brain. They show that the interaction between Hip-1 and Htt on the one hand, and between Hip-1 and Hippi on the other, are mutually exclusive, and that Hip-1/Hippi complexes can activate caspases, the effector enzymes of apoptosis.

In the normal brain, Hip-1 interacts with Htt. But the affinity of Hip-1 for mutant Htt is much lower than its affinity for wild-type Htt. So in diseased brains, levels of the Hip-1/Hippi complex are relatively higher than the levels of the Hip-1/Htt complex. In addition, the authors also demonstrate that the proenzyme procaspase-8 is recruited to Hip-1/Hippi complexes. Interestingly, previous studies have shown that active caspase-8 localizes to aggregates of mutant Htt, and that overexpression of mutant Htt induces apoptosis in a caspase-8-dependent manner.



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Nicholson and colleagues now show that Hip-1 and Hippi cooperate to induce apoptosis in a caspase-8-dependent manner when transfected into primary neurons. So the model suggests that Hip-1 and Hippi induce dimerization, and thus activation, of procaspase-8, thereby initiating the apoptotic cascade.

VALERIE FERRIER