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Yeast studies reveal moonlighting functions of the ancient actin cytoskeleton.

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Abstract

Classic functions of the actin cytoskeleton include control of cell size and shape and the internal organisation of cells. These functions are manifest in cellular processes of fundamental importance throughout biology such as the generation of cell polarity, cell migration, cell adhesion and cell division. However, studies in the unicellular model eukaryote *Saccharomyces cerevisiae* (Baker's yeast) are giving insights into other functions in which the actin cytoskeleton plays a critical role. These include endocytosis, control of protein translation and determination of protein 3-dimensional shape (especially conversion of normal cellular proteins into prions). Here we present a concise overview of these new "moonlighting" roles for the actin cytoskeleton and how some of these roles might lie at the heart of important molecular switches. This is an exciting time for researchers interested in the actin cytoskeleton. We show here how studies of actin are leading us into many new and exciting realms at the interface of genetics, biochemistry and cell biology. While many of the pioneering studies have been conducted using yeast, the conservation of the actin cytoskeleton and its component proteins throughout eukaryotes suggests that these new roles for the actin cytoskeleton may not be restricted to yeast cells but rather may reflect new roles for the actin cytoskeleton of all eukaryotes.

Conservation of the actin cytoskeleton from yeast to humans

Baker's yeast (*Saccharomyces cerevisiae*) was first used for genetic studies in the 1930s (1), however it was only in 1984 that *S. cerevisiae* was first shown to possess a cytoskeleton comprising actin and tubulin (2). It is now evident that *S. cerevisiae* possesses a set of cytoskeleton components that is analogous to, albeit less extensive than, that found in human cells (Table 1). Yeast cells do not physically resemble human cells which are not ellipsoid, lack a cell wall, and do not grow by budding. Nevertheless, many regulatory pathways that control the cytoskeleton have been conserved throughout the evolution of eukaryotes. There are numerous regulatory pathways that control the cytoskeleton and most key pathways are conserved between *S. cerevisiae* and humans: G-protein-coupled receptors, Ras GTPases, Rho GTPases, MAP kinase cascades, cyclin-dependent kinases, protein kinases A and C, phosphatidylinositol 4,5-bisphosphate (PIP₂), etc (for a comprehensive database see the Saccharomyces Genome Database (SGD), www.yeastgenome.org, (3)).

Advantages of yeast as an experimental tool for studying the cytoskeleton

There are six major advantages of *S. cerevisiae* as an experimental tool for exploring fundamental aspects of cytoskeleton function: 1) its short doubling time (90 min) (4); 2) its ability to be maintained as either a stable haploid or stable diploid (4); 3) the ability of haploids to be mated and put through genetic crosses and that one can collect and analyse all four meiotic products (4); 4) that it has only one (rarely two) member of each family of cytoskeleton component (www.yeastgenome.org); 5) gene knockout is easy and fast (3 days) (4) and a genome-wide collection of clean gene knockouts and conditional gene knockouts is widely available (e.g. (5,6)); and 6) regulation of the cytoskeleton is not already compromised by pre-existing genetic changes prior to experimentation (which can be an issue with cancer cell lines and immortalised primary animal cell lines). In fact, yeast is being frequently used as model organism for biomedical research (e.g. (7)).

Yeast studies contribute to a better understanding of the human actin cytoskeleton

One of the many major contributions of *S. cerevisiae* and other yeasts (e.g. *Schizosaccharomyces pombe*) to the cytoskeleton field was the discovery in genetic screens of actin cytoskeleton components, most of which were subsequently found to have human homologues, e.g. Arp2p and Arp3p (8) (human Arp2 and Arp3 (8)) (Table 1). Arp2 and Arp3 are components of a conserved multisubunit complex known as Arp2/3 that initiates the assembly of branched actin filaments (8). These branched actin filaments form extensive dendritic arrays (9). Among the many other actin cytoskeleton components discovered in yeast but with mammalian homologs are: Rvs167p, Rvs161p (human amphiphysins/endophilins), Abp1p (mammalian Abp1), Sla2p (mammalian Hip1R), Sla1p (human CIN85), and Vrp1p/verprolin (human WIP) (10,11) (Table 1).

Studies using *S. cerevisiae* have also assigned functions to human cytoskeleton proteins. For example, the proteins known as formins were first discovered in vertebrates as proteins with critical roles in limb development (12). However, that formins function to initiate assembly of linear (i.e. non-branched) actin filaments emerged from studies on the equivalent formins Bni1p and Bnr1p in *S. cerevisiae* (13) with subsequent confirmation that mammalian formins also function in initiation of linear actin filament assembly (14). Another example is the role of the actin cytoskeleton in endocytosis, the process by which cells internalise cell surface receptors, other membrane material, and extracellular fluids and particles (**Fig. 1**). Studies with a variety of mammalian cells in culture gave conflicting results as to whether a functional actin cytoskeleton is essential for endocytosis (15-17). The first decisive evidence for a generalised role for the actin cytoskeleton in endocytosis came from genetic screens in yeast that identified numerous actin cytoskeleton components as proteins essential for uptake of both cell surface receptors and extracellular fluids (18,19). It is now clear that although there are many different pathways of endocytosis in mammalian cells, most, if not all of these, are dependent on a functional actin cytoskeleton (20).

Structures that comprise the yeast actin cytoskeleton

S. cerevisiae cells possess several recognisable types of actin filament (F-actin)-containing structures (Fig. 1). Underlying the plasma membrane are numerous small spots of F-actin. These are cortical actin patches and comprise branched actin filaments assembled by the Arp2/3 complex. The cortical actin patches have a distribution during the cell cycle that is polarised towards the site of polarised growth and have been proposed to be sites of endocytosis (2,18,21) (Fig. 1). Another type of F-actin-containing structure are cytoplasmic actin cables. These are fibres that extend the length of the cell and comprise thousands of linear actin filaments assembled by formins. Actin cables align along the mother cell-bud axis with their tips near the clustered cortical actin patches. Actin cables have been proposed to serve as tracks for movement of vesicles and organelles to sites of polarised growth (2,18,21). Finally, during mitosis a continuous ring of F-actin forms precisely at the bud neck. This ring comprises linear actin filaments assembled by formins and a conventional non-muscle myosin (myosin II). This is the contractile actomyosin ring and is equivalent to the actomyosin contractile ring found in mammalian cells. The actomyosin ring does not alter its subcellular distribution, however, it contracts to a small dot as cells divide (22).

A new role for actin in protein synthesis/translation

Spatial and temporal regulation of protein synthesis is a central theme throughout biology, e.g. for development, cellular migration and differentiation, adaptation, and long term memory formation. An intriguing idea, now supported by an increasing number of studies, is that the actin cytoskeleton is a key contributor to spatial and temporal regulation of translation. In several organisms it was found that a significant proportion of mRNAs, ribosomes, aminoacyl-tRNA synthetases, and some translation factors are anchored to the actin cytoskeleton (23), suggesting that the actin cytoskeleton acts as a scaffold for the organisation of the translation machinery components. In fact, perturbation of the actin cytoskeleton is associated with a dramatic reduction in the rate of global protein synthesis in yeast and mammalian cells (23). Direct evidence for

efficient translation requiring an intact actin cytoskeleton was finally provided via genetic studies in yeast. For example, deletion of *TPM1* or *MDM20* leading to fewer actin cables led to a drastic reduction in global translation, and/or in the step of translation initiation (23,24). As both actin and translation factors are conserved among eukaryotes, roles for actin in translation are also likely conserved.

The molecular mechanisms by which actin regulates translation are, in general, still not well understood. The best studied example so far is the regulation of eEF1A, the translation factor that in its GTP-bound form delivers aminoacyl-tRNAs to the ribosome (24). Binding of filamentous (F-) actin to eEF1A promotes GTP hydrolysis and prevents eEF1A from binding GTP, suggesting that actin drives eEF1A into its translation-inactive form (21). Another example has come from recent findings suggesting that the actin monomer (G-actin) binding protein Yih1p links translational regulation with actin filament assembly (25). The general amino acid control (GAAC) regulatory network controls the response of yeast cells to amino acid starvation. Upon recognition of amino acid starvation, a protein kinase component of the GAAC network (Gcn2p) phosphorylates the alpha subunit of translation initiation factor 2 (eIF2), resulting in reduced global translation but concomitant increased specific translation of a transcriptional activator necessary to respond and adapt to starvation (26). To sense starvation, Gcn2p must directly bind to an effector protein (Gcn1p) (27). Overexpression studies suggest that Yih1p acts to bind and sequester Gcn1p (thereby diminishing Gcn1p-Gcn2p interaction and consequently Gcn2p activity). Furthermore, genetic studies suggest that Yih1p can only do this after being released from the complex with G-actin (25,26) (**Fig. 2**). The finding that the GAAC response is not enhanced in *yih1Δ* strains suggests that Yih1p only inhibits Gcn2p under specific physiological conditions, e.g. when Gcn2p activity is deleterious to the cell. Alternatively, Yih1p may inhibit Gcn2p only at specific intracellular sites and this inhibition escapes detection when using conventional experimental procedures (that involve cell breakage and mixing of the cell content) (26). Yih1p orthologues are found in mammals (called IMPACT). When overexpressed in yeast, IMPACT inhibits Gcn2p

function and co-precipitates actin. Furthermore, overexpression of IMPACT in mammalian cells impairs GCN2 function as found in yeast, suggesting that this regulatory mechanism is conserved (26). Interestingly, local regulation of GCN2 activity is very likely a contributing factor for long term memory formation in neurons, and in neuron-like N2a cells the GCN2-IMPACT module is involved in modulating neurite outgrowth (26). Recently, it was discovered that in yeast eEF1A binds and inhibits Gcn2p under nutrient-replete conditions (28). In addition, considering that actin regulates eEF1A activity (see below) in yeast and mammals, this raises the possibility that actin also utilises eEF1A to modulate Gcn2p/ GCN2 activity.

Conversely, translation factors are required for proper actin cytoskeleton function. For example, eEF1A binds and bundles actin filaments in all eukaryotes (23,24). Taking into account that eEF1A is the second most abundant cellular protein after actin, and according to work in the slime mold *Dictyostelium*, eEF1A has high affinity for actin and more than 70% of eEF1A is actin bound (29), one may suggest that eEF1A is a major contributor in regulating the actin cytoskeleton. Supporting this idea, specific eEF1A mutations, or eEF1A overexpression, disrupt the actin cytoskeleton in *S. cerevisiae* (24). Furthermore, eEF1A overexpression leads to synthetic growth defects when combined with mutations in actin. In mammals, eEF1A has been implicated in tumour metastasis and it has been shown that eEF1A from metastatic cells has reduced F-actin affinity (30).

The relationship between translation factors and actin is dynamic and regulated. A good example is the interaction between eEF1A and actin. Studies have shown that eEF1A binds exclusively to either F-actin or aminoacyl tRNA, and to either F-actin or its activation factor eEF1B α (23,24). While bound to F-actin, eEF1A does not participate in translation, possibly due to its inability to bind aminoacyl-tRNAs. *In vitro* and genetic studies in yeast suggest that the alpha (catalytic) subunit of the guanine nucleotide exchange factor complex (eEF1B) promotes dissociation of eEF1A from F-actin, thereby switching eEF1A function from F-actin bundling to translation (23,24). Together, this suggests that the sub-compartmental stoichiometric balance between aminoacyl-tRNA, eEF1A, eEF1B α and F-actin is crucial for determining the rate of

translation elongation as well as actin bundling, illustrating a complex relationship between the eEF1A function in translation and actin organisation (**Fig. 2**).

Adding to the complexity, several proteins have been found to modulate eEF1A-actin interaction directly or indirectly. For example, the F-actin binding and bundling activity of eEF1A is inhibited by association with the formin Bni1p (21). Bni1p regulates the actin cytoskeleton through its ability to nucleate the assembly of linear actin filaments, interacts with the actin-monomer-binding protein profilin, and is also a downstream target of Rho1p, a protein belonging to the Rho family of small GTPase proteins involved in important signalling processes such as regulation of the actin cytoskeleton. In *Dictyostelium*, it was found that the eEF1A-F-actin interaction is pH-dependent. Chemoattractants such as cAMP lead to an increased intracellular pH thereby dissociating eEF1A from actin, while pH changes do not affect eEF1A-aminoacyl-tRNA interaction (31,32), suggesting that pH alterations can stimulate protein synthesis. Interestingly, in mammals intracellular alkalinisation has been associated with the growth and metastasis of tumor cells (33). It was found that intracellular alkalinisation correlated with increased dissociation of eEF1A from actin, and that siRNA-mediated knockdown of eEF1A reverted the effects of alkalinisation-induced cell growth (34). Furthermore, eEF1A is subject to regulation by key signalling molecules or enzymes. For example, the actin bundling activity of *Tetrahymena* eEF1A is inhibited by the Ca^{2+} /calmodulin complex (35).

Actin-mediated mRNA transport to specific intracellular sites is another powerful and fast mechanism to spatially and temporally control translation of specific proteins. Interestingly, eEF1A is involved in anchoring beta-actin mRNA to F-actin in the protrusions of motile cells (36), thereby allowing local synthesis and efficient polymerisation of actin at the site of demand. Studies suggest that actin-dependent mRNA transport and its local translation are also important for yeast pseudohyphal growth and the filamentation and virulence of *Candida albicans* (37).

Another way to regulate protein synthesis is to translationally silence mRNAs in RNA granules. RNA granules are macromolecular aggregates found in all eukaryotes that in addition to

mRNA may contain components of the translation machinery. RNA granules may require microfilaments for their dynamics (38). Of the RNA granules one type referred to as processing bodies exist constitutively and take up or release mRNAs in response to particular stimuli. Processing bodies are also involved in RNA degradation. In contrast, the RNA granules known as stress granules occur only under certain stress conditions.

Actin-mediated spatial organisation of translation machinery components would increase the local concentration of these components and thus enhance the efficiency of protein synthesis. The actin-translation linkage may also allow the cytoskeleton to convey internal/external cues (e.g. environmental stress) to the translation machinery for optimal and quick adaptation. Conversely, translation factors, or their regulators, may convey information to and thereby regulate the actin cytoskeleton. Taken together, it becomes increasingly evident that the actin cytoskeleton and protein synthesis machinery reciprocally regulate and require each other, which in turn allows an optimal cellular adaptation to any given condition. More work is necessary to gain a full understanding of this complex interdependency and reciprocal regulation.

A new role for actin in protein folding

There is emerging evidence that the actin cytoskeleton also plays a critical role in the folding of certain proteins, in particular proteins that can form cross-beta fibrous aggregates known as amyloids. Self-perpetuating amyloids termed prions transmit heritable traits in yeast (39) and neurodegenerative diseases in mammals (including humans), e.g. Creutzfeldt-Jakob Disease (CJD), scrapie, and bovine spongiform encephalopathy (BSE) (40). Many other amyloid diseases, including Alzheimer's, Parkinson's and Huntington diseases, possess at least some prion properties. Similar to mammalian prions, yeast prions propagate by immobilizing and converting other polypeptides of the same amino acid sequence into an amyloid form and generate aberrant protein aggregates. However, mammalian prions are transmitted via extracellular infection, whereas yeast prions are inherited via the cytoplasm in cell divisions, mating or cytoplasm exchange (cytoduction)

(39,41). Some yeast prions are pathogenic to the yeast host (reviewed in (39)). However the recent discovery of traits controlled in a prion-like fashion in at least about 1/3 of the natural and industrial isolates of *Saccharomyces cerevisiae* confirm that prions are widespread and suggest that some of them are adaptive (42). Traits controlled by prions are usually associated with the decrease or loss of protein function, although gain of function has also been reported. Prion formation by regulatory proteins may result in altering a range of cellular processes, including metabolism and gene expression pathways (39). Yeast prions provide a robust, but dynamic system for epigenetic regulation of phenotype controlled by the cellular and environmental factors (41).

Yeast heritable element [*PSI*⁺] is a prion isoform of the yeast translation termination factor (eRF3/Sup35p) (39). Conformational change of soluble Sup35p into the amyloid-like aggregated form leads to reduced efficiency in termination of the polypeptide synthesis at stop codons that may potentially result in production of elongated polypeptide chains (39). Several lines of evidence link the actin cytoskeleton to the yeast prion [*PSI*⁺]. First, the prion-forming domain of Sup35p physically interacts with a number of cortical actin patch proteins (43,44). Furthermore, during the initial transition from normal protein to [*PSI*⁺] prion (induced by Sup35p over-expression) Sup35p forms filamentous aggregates that exhibit subcellular colocalization with several cortical actin patch components (43). These aggregates appear to be an intermediate found only in cells converting from [*psi*⁻] to [*PSI*⁺] and colocalization with cortical actin patch components appears to specifically occur in this conversion phase. Mutations that affect the actin cytoskeleton reduce the ability of Sup35p to form visible intracellular aggregates required for conversion to the [*PSI*⁺] prion and increase the toxicity of Sup35p when over-expressed in cells that harbour [*PSI*⁺], suggesting that the assembly of protein polymers into the large visible aggregates may counteract cytotoxicity (43). Interestingly, actin assembly proteins also interact with the polyglutamines, expressed in yeast in the yeast model of Huntington's disease. These interactions might be mediated by prion form of another yeast protein, Rnq1, that promotes polyglutamine aggregation in yeast, and result in the

inhibition of endocytosis (apparently due to sequestration of the actin assembly proteins by polyglutamines) and respectively, in cytotoxicity (reviewed in (39)).

The assembly of amyloid fibres in some ways resembles the assembly of actin filaments in that both require a rate-limiting nucleation event followed by elongation and final breaking up of existing polymers in order to nucleate new polymers (43). This, together with the finding that the conversion of Sup35p to $[PSI^+]$ appears to occur at sites that also contain cortical actin patch components and is perturbed by mutation of cortical actin patch components, has led to the proposal that amyloid fibre assembly may be regulated by the same machinery that functions in actin filament assembly and actin-dependent endocytosis (43). Indeed, depletion of actin associated Las17p (WASP) binding protein Lsb2 results in the destabilization of $[PSI^+]$ prion aggregates during and after thermal stress (45). At the same time, high levels of Lsb2p trigger conversion of Sup35p into the prion form (45). Levels of Lsb2p protein are dramatically increased by heat shock, while its prion inducing ability is strictly dependent on its association with cortical actin patches (45). These findings directly implicate actin cytoskeleton in regulating prions during the environmental changes. Interactions of actin cytoskeleton with the prion aggregates somewhat parallel the proposed role for the actin cytoskeleton in asymmetric segregation of aggregated oxidatively damaged protein, resulting in generation of the aggregate-free daughter cells (buds) in the cell divisions following stress (46).

The actin cytoskeleton itself may contain amyloidogenic proteins. Lsb2p as well as some other actin assembly proteins interacting with Sup35p, e. g. Sla2p (homolog of human HIP1R), possess QN-rich domains similar to prion domains of yeast prion proteins (reviewed in (39)). In addition, many actin cytoskeleton components in yeast (including Lsb2p) and mammals possess Src Homology 3 (SH3) domains - a type of small (~60 amino acids long) protein-protein interaction module first found in the non-receptor tyrosine kinase Src (47). Evidence suggests that SH3 domains can be converted to fibrous amyloids in specific conditions *in vitro* (48). The amyloid form

of SH3 domains is highly cytotoxic when taken up by cultured mammalian cells (49), however it is not known whether SH3 domains naturally switch to amyloid *in vivo*. Some specific yeast SH3 domains become highly cytotoxic *in vivo* following either mutation or loss of their binding site in a partner protein (a cytotoxicity that is effectively cured by deletion of the offending SH3 domain) (50-52). Although the molecular basis for this SH3-domain-dependent cytotoxicity is not understood, one possibility is that association with a binding partner prevents the SH3 domain from undergoing conversion to an amyloid. An ability to form amyloid may reflect some as yet overlooked physiological signalling function of SH3 domains. Much more work is needed to test these hypotheses. The ability of the actin cytoskeleton to affect stable and heritable changes in protein conformation and activity may play important roles in long-term adaptation of cells to changes in the environment with similar effects to (but not necessarily with the permanency of) mutation of DNA.

Taken together, strong evidence is accumulating to support the possibility that the cellular roles of actin go far beyond its well-studied skeletal functions and may range from control of vital cellular processes like protein translation to promotion of disease development and in particular those diseases attributable to protein aggregation. More studies are necessary to fully understand the crosstalk between the actin cytoskeleton and specific cellular processes, which simultaneously will provide insight into how certain diseases may develop and how they may be treated or prevented in the future. Clearly, several studies have demonstrated that the amenable yeast eukaryotic model is a prime tool for contributing to the further decipherment of the many new roles actin plays in the cell.

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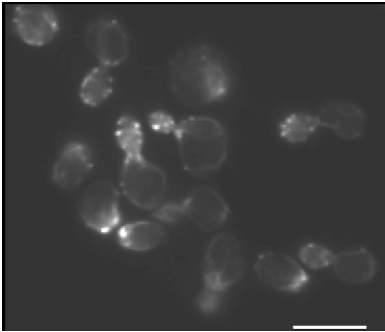
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Figure legends

Figure 1 The yeast actin cytoskeleton and its importance for endocytosis

A. Visualisation of actin cytoskeleton via fluorescence microscopy of *S. cerevisiae* cells, fixed and stained with fluorophore-conjugated phalloidin (F-actin specific reagent). Scale bar, 5 μm .



B. Internalisation and vacuolar accumulation of the fluorescent endocytic dye Lucifer Yellow by endocytosis in *S. cerevisiae* cells. Scale bar, 5 μm .

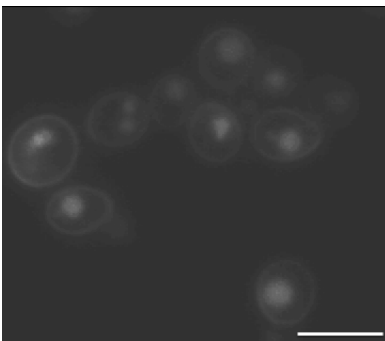


Figure 2 Actin-Translation connections found in yeasts

A. eEF1A bound to F-actin and Yih1p bound to G-actin do not participate in translation and in controlling Gcn2p-mediated translational regulation, respectively. **B.** eEF1A can be released from F-actin by either Bni1p or the α subunit of the guanine nucleotide exchange factor eEF1B, and may then participate in protein synthesis. Studies support the idea that Yih1p released from G-actin sequesters Gcn1p thereby inhibiting Gcn2p function. For more detailed explanation please see text.

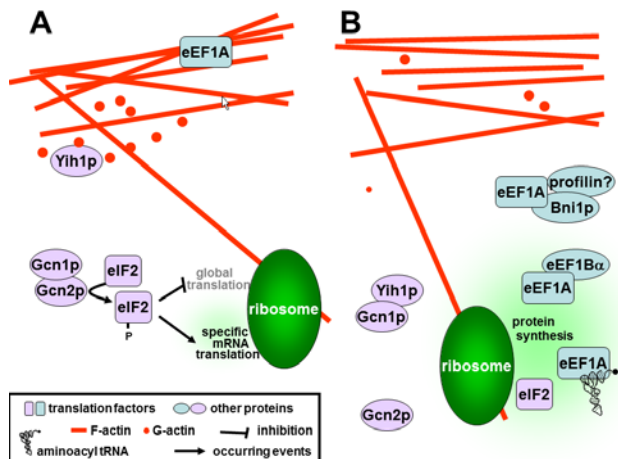


Table 1:

Examples of functionally related actin cytoskeleton proteins in the yeast *Saccharomyces cerevisiae* and humans (assembled from the *Saccharomyces* Genome Database (SGD), www.yeastgenome.org, (3))

<i>Human protein(s)</i>	<i>S. cerevisiae protein(s)</i>	<i>Function</i>
actin component/ polarity signalling/ endocytosis/	Act1p (End7p)	Filament cytokinesis/ regulation of protein translation/ prion amyloid formation
Arp2, Arp3, p40, p35, p19, p18, p14	Arp2p, Arp3p, Arc40p, nucleation/ filament binding/ Arc35p (End9p), Arc19p, signalling/ endocytosis Arc18p, Arc15p	Filament polarity
Type I myosin (role in filament nucleation/ filament binding/motor/ nucleation is likely fungal-specific) signalling/ endocytosis/cytokinesis	Myo3p, Myo5p	Filament polarity
Type II myosin binding/motor/ polarity signalling/	Myo1p	Filament cytokinesis
Type V myosin binding/motor/ polarity signaling	Myo2p, Myo4p	Filament
Wiskott-Aldrich Syndrome Protein nucleation/ binds actin monomers/ (WASP and N-WASP) signalling/ endocytosis/cytokinesis	Las17p (Bee1p)	Filament polarity
WASP-Interacting Protein nucleation/ binds actin monomers/ (WIP) (role in cytokinesis signalling/ endocytosis/cytokinesis remains to be shown)	Vrp1p (End5p)	Filament polarity
tropomyosin binding/ filament stabilisation/ polarity signaling	Tpm1p, Tpm2p	Filament
profilin	Pfy1p	Filament nucleation/ binds actin monomers/ polarity signalling

capping protein (CP α/β) (role in binding/ filament end capping/ polarity signalling/ endocytosis remains to be shown)	Cap1p (α), Cap2p (β)	Filament endocytosis
cofilin	Cof1p	Filament binding/ filament severing/ binds actin monomers/ polarity signalling/ endocytosis
fimbrin/plastin (role in endocytosis binding/ filament bundling/ polarity signalling/ remains to be shown)	Sac6p	Filament endocytosis
twinfilin	Twf1p	Filament binding/ filament severing/ binds actin monomers
coronin	Crn1p	Filament nucleation/ filament binding/ filament bundling
formin nucleation/ filament binding/ polarity signaling/	Bni1p, Bnr1p	Filament cytokinesis
calmodulin signalling/ endocytosis	Cmd1p	polarity
Cdc42 nucleation/ polarity signalling	Cdc42p, Rho5p	filament
RhoA signalling	Rho1p, Rho2p, Rho3p, Rho4p	Polarity
Eps15 (role in filament nucleation nucleation/ polarity signalling/ endocytosis remains to be shown)	Pan1p	Filament
CIN85 signalling/ endocytosis/ prions in yeast	Sla1p	Polarity interactions with

amphiphysin/endophilin/Bin1/Bin2 signalling/ endocytosis	Rvs167p	Polarity
Bin3 (role in endocytosis signalling/ endocytosis remains to be shown)	Rvs161p (End6p)	Polarity
mAbp1 (role in filament nucleation binding/ filament nucleation/ remains to be shown) signalling/ endocytosis	Abp1p	Filament polarity
Hip1/Hip1R signalling/ endocytosis/ prions and polyglutamines	Sla2p (End4p)	Polarity interactions with
Eps15 signalling/ endocytosis/ prions in yeast	End3p	Polarity interactions with
cyclase associated protein (CAP) monomers/ polarity signalling/	Srv2p (End14p)	Binds actin endocytosis
eEF1A binding/ filament bundling/ elongation factor	eEF1A (Tef1p/ Tef2p)	Filament translation
IMPACT (direct actin association remains to be shown)	Yih1p	Regulation of the key translational regulator Gcn2p, binds actin monomers