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Author

Munn, Alan, Sattlegger, Evelyn

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Yeast Studies Reveal New Roles for an Ancient Skeleton

Alan Munn[1]* and Evelyn Sattlegger[2]

[1]*School of Medical Science, Griffith University Gold Coast, Southport, QLD 9726*
[2]*Institute of Natural Sciences, Massey University, Albany, Auckland 0632, New Zealand*
**Corresponding author: a.munn@griffith.edu.au*

Conservation of the Actin Cytoskeleton

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. 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 (although clearly not all) 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 (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:

- its short doubling time (90 minutes) (4)
- its ability to be maintained as either a stable haploid or stable diploid (4)
- the ability of haploids to be mated and put through genetic crosses and that one can collect and analyse all four meiotic products (4)
- it has only one (rarely two) member of each family of cytoskeleton component (5-7)
- 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 (8,9)
- 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).

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 (10) and Arp3p (11) [human Arp2 and Arp3 (12)] (**Table 1**). Arp2 and Arp3 are components of a conserved multisubunit complex known as Arp2/3 that initiates the assembly of branched actin filaments (12). These branched actin filaments form extensive dendritic arrays (13). Among the many other actin cytoskeleton components discovered in yeast but with mammalian homologs are: Rvs167p (5-7,14) [human amphiphysins/endophilins (5-7,14,15)], Abp1p (5-7) [mammalian Abp1 (16)], Sla2p (5-7) [mammalian Hip1R (17)], Sla1p (5-7) [human CIN85 (15)], and Vrp1p/verprolin (5-7,14) [human WIP (18)] (**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 (19). 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* (20) with subsequent confirmation that mammalian formins also function in initiation of linear actin filament assembly (21,22). 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 (23-25). 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 (5,6,14). It is now clear that although there are many different pathways of endocytosis in mammalian cells, all are dependent on a functional actin cytoskeleton (6,26).

Structures that Comprise the Yeast Actin Cytoskeleton

S. cerevisiae cells possess several recognisable types of actin filament (F-actin)-containing structure (**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 (2,5-7) (**Fig. 1**). Another type of F-actin-containing structure is the cytoplasmic actin cable. 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 (2,6,7). 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 (27).

Table 1. Examples of functionally equivalent actin cytoskeleton proteins in yeast and humans.

Human protein(s)	Yeast protein(s)	Function
Actin	Act1p (End7p)	Filament component/polarity signalling/endocytosis/cytokinesis
Arp2/3 complex	Arp2p, Arp3p, Arc40p, Arc35p (End9p), Arc19p, Arc18p, Arc15p	Filament nucleation/filament binding/polarity signalling/ endocytosis
Type I myosin (role in filament nucleation is likely yeast-specific)	Myo3p, Myo5p	Filament nucleation/filament binding/motor/ polarity signalling/ endocytosis/cytokinesis
Type II myosin	Myo1p	Filament binding/motor/polarity signalling/cytokinesis
Type V myosin	Myo2p, Myo4p	Filament binding/motor/polarity signalling
Wiskott-Aldrich Syndrome Protein (WASP)	Las17p (Bee1p)	Filament nucleation/binds actin monomers/polarity signalling/ endocytosis/cytokinesis
WASP-Interacting Protein (WIP) (role in cytokinesis remains to be shown)	Vrp1p (End5p)	Filament nucleation/binds actin monomers/polarity signalling/ endocytosis/cytokinesis
Tropomyosin	Tpm1p, Tpm2p	Filament binding/filament stabilisation/polarity signalling
Profilin	Pfy1p	Filament nucleation/binds actin monomers/polarity signalling
Capping protein (CP α/β) (role in endocytosis remains to be shown)	Cap1p (α), Cap2p (β)	Filament binding/filament end capping/polarity signalling/ endocytosis
Cofilin	Cof1p	Filament binding/filament severing/binds actin monomers/ polarity signalling/endocytosis
Fimbrin/plastin (role in endocytosis remains to be shown)	Sac6p	Filament binding/filament bundling/polarity signalling/ endocytosis
Twinfilin	Twf1p	Filament binding/filament severing/binds actin monomers
Coronin	Crn1p	Filament nucleation/filament binding/filament bundling
Formin	Bni1p, Bnr1p	Filament nucleation/filament binding/polarity signaling/ cytokinesis
Calmodulin (conflicting reports of a role in endocytic uptake)	Cmd1p	Polarity signalling/endocytosis
Cdc42	Cdc42p, Rho5p	Filament nucleation/polarity signalling
RhoA	Rho1p, Rho2p, Rho3p, Rho4p	Polarity signalling
Eps15 (role in filament nucleation remains to be shown)	Pan1p	Filament nucleation/polarity signalling/endocytosis
CIN85	Sla1p	Polarity signalling/endocytosis
Amphiphysin/ endophilin/Bin1/Bin2	Rvs167p	Polarity signalling/endocytosis
Bin3 (role in endocytosis remains to be shown)	Rvs161p (End6p)	Polarity signalling/endocytosis
mAbp1 (role in filament nucleation remains to be shown)	Abp1p	Filament binding/filament nucleation/polarity signalling/ endocytosis
Hip1/Hip1R	Sla2p (End4p)	Polarity signalling/endocytosis
Eps15	End3p	Polarity signalling/endocytosis
Cyclase associated protein (CAP)	Srv2p (End14p)	Binds actin monomers/polarity signalling/endocytosis
eEF1A	Tef1p, Tef2p	Filament binding/filament bundling/translation elongation factor
IMPACT (actin association remains to be shown)	Yih1p	Regulation of the key translational regulator Gcn2p, binds actin monomers

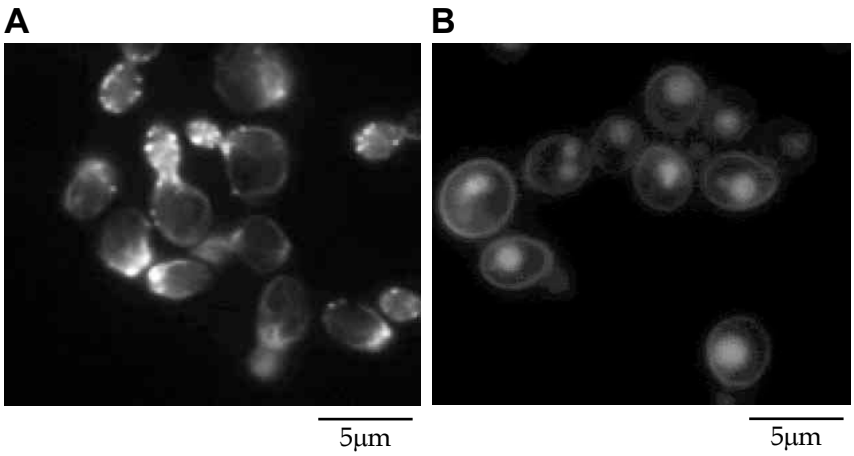

Fig. 1. The yeast actin cytoskeleton and its importance for endocytosis.

A. Fluorescence microscopy images of *S. cerevisiae* cells that have been fixed and stained with fluorophore-conjugated phalloidin (F-actin specific reagent) to visualise the actin cytoskeleton.

B. Fluorescence microscopy images of *S. cerevisiae* cells that have internalised the fluorescent endocytic dye Lucifer Yellow showing dye accumulation in the vacuole.

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 cellular differentiation and adaptation to external/internal cues. An intriguing idea, now supported by an increasing number of studies, is that the actin cytoskeleton provides (at least in part) this spatial and temporal regulation. Ribosomes synthesise proteins in three steps: initiation, elongation, and termination. To accomplish each step, ribosomes associate with specific translation factors, the so-called eukaryotic initiation, elongation, and release factors (eIFs, eEFs, eRFs). In several organisms, a significant proportion of mRNAs, ribosomes, and some translation factors are anchored to the actin cytoskeleton (28). Perturbation of the actin cytoskeleton affects translational fidelity and initiation in yeast, and protein synthesis in mammals (29-31). This suggests that the actin cytoskeleton plays crucial roles in protein synthesis, e.g. as a scaffold for translation machinery components and/or translation regulator. 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, not well understood. However, new insights into such a mechanism has come from recent findings suggesting that the actin monomer (G-actin) binding protein Yih1p links translational regulation with actin filament assembly (32). 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 translation initiation factor eIF2α, resulting in reduced global translation but concomitant increased specific translation of a transcriptional activator necessary to respond and adapt to starvation (33). To sense starvation, Gcn2p must directly bind to an effector protein (Gcn1p) (34). Yih1p acts to bind and sequester Gcn1p (thereby diminishing Gcn1p-Gcn2p interaction and consequently Gcn2p activity), but studies suggest that it can only do this after releasing its bound G-actin (32) (**Fig. 2**). Yih1p appears to only inhibit Gcn2p under specific physiological conditions or at specific intracellular sites (32,34). Yih1p orthologues are found in mammals, suggesting that this regulatory mechanism is conserved (35).

Conversely, translation factors are required for proper actin cytoskeleton function. For example, the elongation factor eEF1A binds and bundles actin filaments (36). In *S. cerevisiae* eEF1A over-expression affects actin distribution, cell morphology, and budding, and reduced eEF1A-actin interaction affects the actin cytoskeleton and cell morphology (37,38). In mammals, eEF1A has been implicated in tumour metastasis (e.g. see ref 39).

The relationship between translation factors and actin is dynamic and regulated. A good example is the interaction of eEF1A and actin (40). eEF1A binds exclusively to either F-actin, aminoacyl tRNA, or its activation factor eEF1Bα (41,42). While bound to F-actin, eEF1A does not participate in translation, possibly due to loss of interaction with eEF1Bα. This suggests that eEF1Bα binding may switch eEF1A function from F-actin bundling to translation (40,42) (**Fig. 2**). The F-actin binding and bundling activity of eEF1A is also inhibited by association with the formin Bni1p (43). Bni1p regulates the actin cytoskeleton through its ability to nucleate the assembly of linear actin filaments (20), interacts with the actin-monomer-binding protein profilin, and is also a downstream target of the Rho GTPase Rho1p (44). In other eukaryotes, several additional cues were found to modulate interactions between eEF1A and F-actin (e.g. see refs 45-47), and another elongation factor (eIF4E) has been reported to also undergo regulated interactions with actin (48,49).

Actin-mediated mRNA transport to specific intracellular sites is another powerful and fast mechanism to spatially and temporally control translation of specific proteins (50,51). Well-studied examples are the polarised localisation of the *ASH1* mRNA (which encodes a daughter-cell-specific transcriptional repressor) to the yeast bud (50) and spatiotemporal regulation of actin mRNA translation in mammalian cells in cell motility and neuronal development (51).

Actin-mediated spatial organisation of translation machinery components would increase the local concentration of the translation machinery 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.

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 regular fibrous aggregates known as amyloid. A major recent discovery was heritable traits in yeast and other fungi that exhibit cytoplasmic (rather than Mendelian) inheritance and are associated with aberrant protein folding rather than mutations in DNA – a phenomenon known as 'protein-only inheritance' (52-54). In some cases, the trait is simply loss of protein function (52); however, in other cases the trait can be a gain of function (54). One of the first examples of this is the yeast translation termination factor (eRF3/Sup35p). After Sup35p undergoes conformational change to the aberrant form (known as [*PSI*$^+$]), it is no longer able to efficiently terminate the synthesis of polypeptides when the ribosome encounters an mRNA stop codon, resulting in synthesis of elongated polypeptide chains. The aberrantly folded [*PSI*$^+$] form of Sup35p causes all newly synthesised Sup35p polypeptides to adopt the [*PSI*$^+$] conformation (52-54).

The altered proteins responsible for protein-only inheritance in yeast are reminiscent of infectious prions that cause neurological disease in humans and other animals, e.g. Creutzfeldt-Jakob Disease, scrapie and bovine spongiform encephalopathy (55-57). Both involve amyloidogenic proteins, heritable conformational changes, propagation by conversion of other polypeptides with the same amino acid sequence to the altered form, and aberrant protein aggregate formation. One difference between yeast prions and the prions of animals is that the latter can spread from cell to cell (55-57), whereas yeast prions seem unable to spread from cell to cell (except by cell division or mating) (58).

Several lines of evidence link the actin cytoskeleton to yeast prions. Firstly, the prion-forming domain of Sup35p physically interacts with a number of cortical actin patch components (59). Furthermore, during the initial transition from normal protein to [*PSI*$^+$] prion (induced by Sup35p over-expression), Sup35p forms ring-like and worm-like aggregates that exhibit subcellular colocalisation with several cortical actin patch components (59). The ring-like and worm-like aggregates appear to be an intermediate found only in cells converting from [*psi*$^-$] to [*PSI*$^+$] and colocalisation 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 large visible aggregates are less toxic (59).

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 (59). This, together with the finding that the

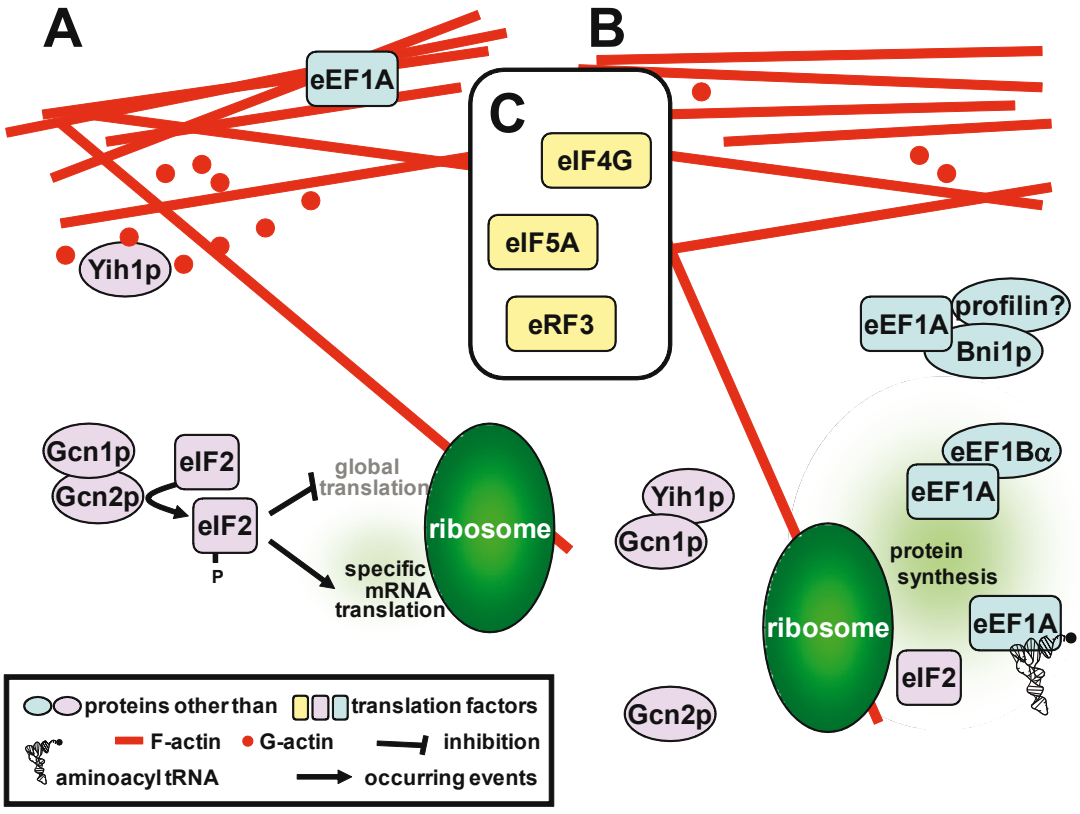

Fig. 2. Actin-translation connections found in yeasts.

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 eEF1Bα, and may then participate in protein synthesis. Yih1p released from G-actin sequesters Gcn1p thereby inhibiting Gcn2p function.

C. The indicated translation factors were found to affect the actin cytoskeleton or vice versa, however, the exact molecular mechanism is largely unknown (66-69). For more detail, please see text.

conversion of Sup35p to [*PSI*$^+$] appears to occur at cortical actin patches 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 (59).

The actin cytoskeleton itself contains amyloidogenic proteins. For example, many actin cytoskeleton components in yeast and mammals possess Src homology 3 (SH3) domains – a type of small (about 60 amino acids) protein–protein interaction module first found in the non-receptor tyrosine kinase Src (60). Some mammalian SH3 domains can be converted to amyloid under specific conditions *in vitro* (61). The amyloid form of these SH3 domains is highly cytotoxic when taken up by cultured mammalian cells (62), 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) (63-65). 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 inheritable 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 without the permanency of) mutation of DNA.

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