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# Actin microfilaments in fungi

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## ABSTRACT

The cytoskeletal protein actin is among the most abundant proteins in nature. It is almost ubiquitous, occurring in all eukaryotes and in an ancestral form in prokaryotes. Actin monomers can polymerise to form microfilaments, structures that play a critical role in a number of fundamental cell processes in fungi such as morphogenesis, cytokinesis and the movement of organelles. Microfilaments are extremely dynamic structures and can be rapidly modified through their interactions with a number of actin binding proteins (ABPs). The purpose of the following review is to introduce actin and microfilaments in fungi to a general mycological audience and to provide a basic framework from which further study is possible.

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## 1. Introduction

The fungal cytoskeleton comprises two main structural components: microfilaments, which are polymers of the protein actin, and microtubules, which are polymers of the protein tubulin. These pervade the cytoplasm in what can be quite often intricate 3-dimensional arrangements, yet they are not simply frameworks around which cells are built or organised. They are in fact very dynamic and play a multitude of roles in fungal biology. With respect to actin, its importance can perhaps best be illustrated with the variety of phenotypic changes that can be observed when the gene that encodes actin (*act1*) is mutated, as listed in the yeast genomic database (<http://db.yeastgenome.org/cgi-bin/locus.pl?locus=actin>). These include altered sensitivities to temperature, osmotic and ion concentrations, delocalised chitin deposition, abnormal nuclear segregation and/or cytokinesis, defective bud site selection, alteration of the organisation and distribution of intracellular organelles and finally, a variety of secretion/endocytosis and gross morphological defects.

In view of the importance of actin in fungal biology and an extensive and rapidly expanding body of literature, this review is intended to introduce actin to a general mycological

audience. Where relevant, details of more comprehensive/in depth reviews have been included should the reader wish to investigate particular areas further. Because of the advantages pertaining to their use as experimental organisms, much of what is known about F-actin and its behaviour has arisen from studies on yeast. These studies provide a useful framework for work on filamentous species but as pointed out by Harris and Momany (2004), care is required when direct extrapolations are made from yeast to hyphal species. Because of this, where relevant the organism(s) on which particular studies have been carried out will be indicated. Initially the structure of actin will be considered, followed by a review of microfilament dynamics and finally the roles that these play in fungi. A forthcoming review will consider the other major component of the cytoskeleton, microtubules.

## 2. Actin, ABPs and the dynamics of microfilaments

Actin is a protein with a molecular weight of around 42 kDa, which can exist as a monomer (referred to as G-actin) or as a linear polymer of repeating monomer subunits. When two

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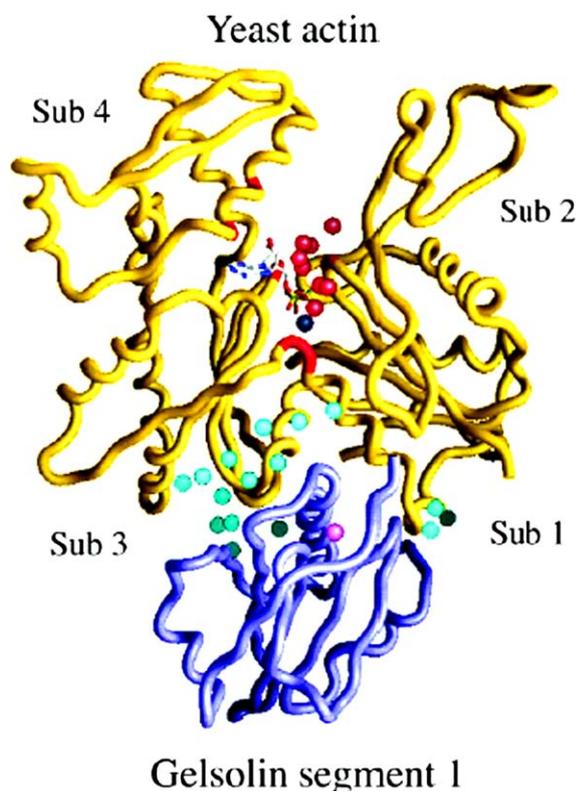
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polymers helically interweave, a microfilament (or F-actin) is formed with a diameter of  $\sim 7$  nm that can join with other microfilaments to form linear cables and/or intricate 3-dimensional networks. A key characteristic of these structures is that they are able to undergo rapid and extensive modification in response to cellular requirements. This is made possible through the interaction of actin with a number of actin binding proteins (ABPs).

In yeast, as in other eukaryotes, the actin monomer consists of a single polypeptide that contains a large and a small domain (Vorobiev *et al.* 2003) (Fig. 1). Within these are four subdomains (denoted Sub1-4 in Fig. 1). Two structural features are of particular importance with respect to the dynamics of microfilaments. Firstly, a central cleft contains a high-affinity binding site for a nucleotide (ATP or ADP) and a cation (usually  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ ); hydrolysis of the nucleotide in this cleft influences F-actin dynamics. Secondly, the monomer is longitudinally asymmetrical (owing to the nature of the functional groups exposed), thus when they associate they form a filament with an intrinsic polarity. Monomers can both join and leave a filament, but because of the biochemical differences between the two ends, they typically do so at different rates. Association (or assembly) predominates at one end (the

“plus” or “barbed end”) and dissociation (or disassembly) occurs more readily at the other (the “minus” or “pointed end”). Dissociation of the monomers from the pointed end of the filament and their subsequent incorporation into the barbed end is referred to as treadmilling.

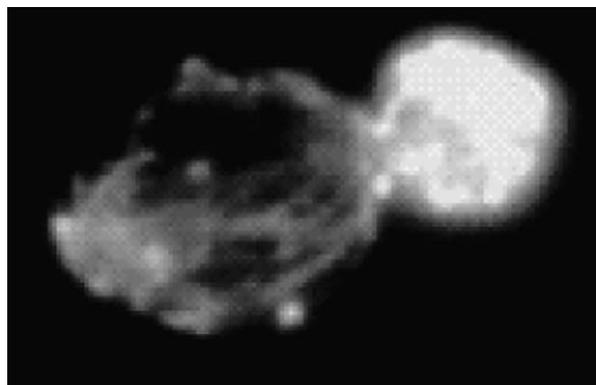
In yeast and hyphae, F-actin dynamics are influenced by cellular conditions, actin monomer concentration and, as mentioned above, the action of ABPs. The ABPs have been the focus of many studies and it appears that a number of these proteins share a conserved actin-binding domain that binds actin in the hydrophobic cleft between subdomains 1 and 3 (Dominguez 2004). This may in part explain why actin is thought to participate in more protein/protein interactions than any other known protein. ABPs that have been described in yeast include; cofilin/ADF, which severs and depolymerises microfilaments (Moon *et al.* 1993); profilin, which regulates microfilament formation (Haarer *et al.* 1990); capping protein, which regulates microfilament length (Amatruda & Cooper 1992); fimbrin/SAC6, which promotes the bundling of microfilaments (Adams *et al.* 1991); formin which initiates cable formation (Sagot *et al.* 2002); and myosin, which is involved in organelle movement (Watts *et al.* 1985). For a comprehensive review of actin binding proteins see Dos Remedios *et al.* (2002).



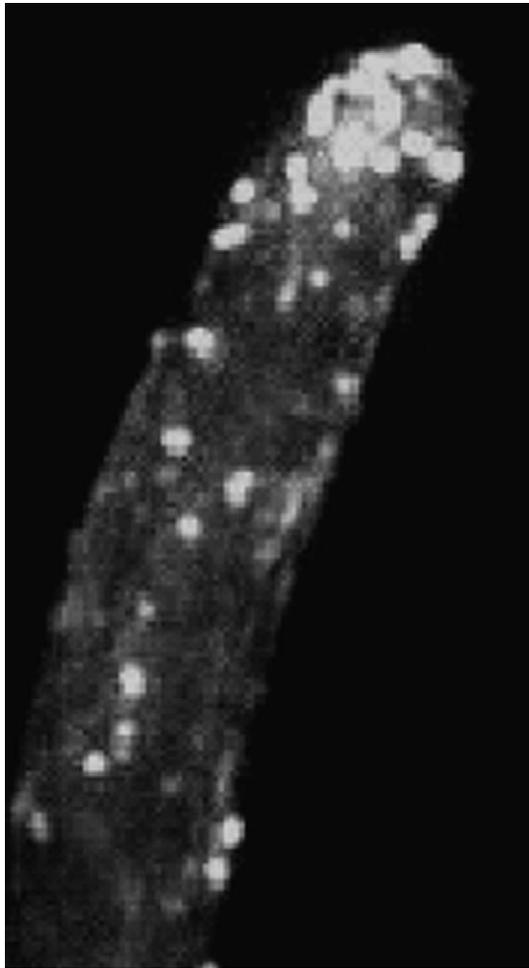
**Fig. 1** – The structure of yeast actin (yellow) complexed with gelsolin segment-1 (blue). G-Actin is composed of four subdomains (Sub 1, 2, 3 and 4), which form a nucleotide binding cleft (red backbone). An adenine nucleotide is shown in the cleft along with a divalent cation (black) and associated water molecules (aqua). Reproduced from Proceedings of the National Academy of Sciences of the United States of America, 100, 5760-5765. © 2003 The National Academy of Sciences of the United States of America, all rights reserved.

### 3. F-actin staining patterns

The arrangement of F-actin seen in fungal cells varies dependant on the species, on the stage of the cell cycle and on the methodology that has been used to preserve and/or visualise F-actin (for a good critical appraisal of F-actin staining patterns and methodologies see Heath (2000)). Despite this, there are several recurring arrangements. The most common of these are peripheral patches or plaques (Figs 2 and 3). These are dynamic structures that are able to rapidly assemble, disassemble and reassemble. Patches concentrate at the sites of bud formation in budding yeast (Fig. 2), at both growing ends



**Fig. 2** – The arrangement of the actin cytoskeleton in a budding yeast that has been stained with rhodamine phalloidin. A dense accumulation of plaques are present in the daughter cell. Distinct linear cables extend from the daughter cell along the mother/daughter cell axis. Image kindly supplied by Prof. John Cooper, Washington University, St Louis.



**Fig. 3 – The arrangement of the actin cytoskeleton in a hypha of the fungus *Ashbya gossypii*. Plaques are concentrated at the tip of the hypha and cables are orientated along its longitudinal axis. Reproduced from *Mol. Biol. Cell* 2004 15: 4622–4632 with the permission of The American Society for Cell Biology.**

of fission yeast and in areas of growth at or close to the tips of hyphae (Fig. 3). In budding yeast, actin patches have been partially purified and found to contain branching networks of short filaments (Young *et al.* 2004). Each patch typically contains 85 filaments, which have an average length of 50 nm (or 20 actin subunits). Associated with the filaments is the ARP2/3 complex, which consists of several proteins including the actin-related proteins Arp 2 and Arp 3. These act as a template for branched filament formation (Winter *et al.* 1997) and appear to be important for patch assembly and motility. Patches also contain the ABP fimbrin/sac6, which appears to be important for the stability of the structure. Patches are thought to be actin coats that cover tubular invaginations of the plasma membrane, which suggests that they function in endocytosis (Engqvist-Goldstein & Drubin 2003). There is also data supporting a role in cell wall deposition (Utsugi *et al.* 2002).

Another common arrangement of F-actin are cables (Fig. 2). These vary in length and form when numerous microfilaments

bundle together due to the activity of fimbrin/sac6. In budding yeasts, cables can extend in a polarised manner from the bud along the mother cell/bud axis (Fig. 2). Likewise, in filamentous species, cables typically align parallel to the longitudinal axis of the hypha (Heath 1990). In both yeast and hyphae, these may form pathways along which organelles are transported (Adams & Pringle 1984; Howard 1981).

Two other F-actin patterns are observed in fungal cells; diffuse networks of microfilaments that pervade the cytoplasm and contractile actin rings that are present in dividing cells, which are thought to function in pulling the plasma membrane inwards at the end of mitosis, thus facilitating septum deposition (which is considered in more detail below).

It is of interest to note that the actin cytoskeleton of the other group of hyphal organisms, the oomycetes differs somewhat from that observed in the fungi. The oomycetes typically contain a cap of peripheral microfilaments at the tip of the cell. This cap may be complete or may contain an actin-depleted zone at the apex of the cell (Yu *et al.* 2004). In subapical regions, this cap gives way to a more diffuse arrangement of fibrils and plaques. Thus it appears that the convergent evolution of fungal and oomycete hyphae may have occurred with different underlying arrangements of F-actin.

#### 4. Roles of actin

As detailed above there are a large number of different phenotypes that arise upon mutation of the actin gene in yeast suggesting that F-actin plays a number of roles. These include morphogenesis, mitosis, meiosis, cytokinesis and septation, and the movement and positioning of organelles. Due to space limitations, each of these can only be considered briefly although a number of key references are provided should further reading be required.

##### *Morphogenesis*

Morphogenesis in both hyphae and yeasts is dependant upon the localised yielding at, and the directed transport of vesicles to, sites of growth. At such sites, F-actin, in concert with the cell wall, is thought to resist turgor pressure. Thus, changes to the arrangement of F-actin could in part regulate yielding. Microfilaments could also directly drive growth. In yeast the polymerisation of a single microfilament in association with the ABP formin, has been shown to generate a force of at least 1 pN (Kovar & Pollard 2004). Thus, when turgor is low or absent microfilaments could provide a protrusive force in a manner similar to that which is responsible for the extension of lamellipodia in animal cells (Heath & Steinberg 1999).

Cables and filaments, in association with the ABP myosin, are likely to direct the delivery of vesicles to sites of growth. This is suggested by the subapical ballooning of hyphae upon treatment with latrunculin, which inhibits the formation of F-actin. This is presumed to lead to delocalised vesicle delivery. Furthermore, actin and myosin mutants contain accumulations of vesicles and display a loss of polarised growth. There may be variations in the mode of vesicle delivery as the tips of some hyphal species contain aggregations of F-actin and vesicles, which together form a structure called the Spitzenkörper. The precise location of this correlates

with the direction of growth, which has led to the suggestion that it may act as a vesicle supply centre (Bartnicki-Garcia *et al.* 1989). The exact role for F-actin in this structure is unclear although it is possible that it could enable movement of the Spitzenkörper to allow directional changes in growth.

The positioning of F-actin at sites of growth is crucial to morphogenesis and this is controlled by a complicated series of events. In yeast, cortical landmark proteins such as Bud3p first establish sites of polarity, to which a number of proteins are recruited, including the Rho GTPase Cdc42p. These in turn recruit two protein complexes, the Arp2/3 complex and the polarisome, which contains the ABP formin. From these branched networks, microfilaments and cables form respectively. A polarisome that has been fused with green fluorescent protein is shown in Fig. 4 which demonstrates a close association between the polarisome and sites of growth (Bauer *et al.* 2004). Many of the proteins responsible for the series of events described are conserved between yeast and filamentous species (Harris & Momany 2004; Wendland 2003), so models of budding and hyphal growth share a number of similarities.

### Mitosis and meiosis

While the major components of the mitotic spindle, microtubules, appear more important with respect to mitosis and meiosis, F-actin has been hypothesised to play a number of roles in these processes. In a number of fungi, F-actin is present in the nucleoplasm close to where the nuclear envelope associates with the spindle pole bodies. This has led to suggestions that it may play a role in the migration of the spindle pole bodies during prophase and/or provide a mechanism that enables the separation of chromatids and their movement towards the spindle poles during anaphase A. Other potential roles of actin in mitosis and meiosis include the alignment of the spindle in the correct orientation and acting as a support that enables astral microtubules to generate force and thereby separate daughter nuclei during telophase. Each of these roles is discussed in more depth by Heath (1995). Whatever the exact role of F-actin, it is clear that any role in nuclear division would necessitate co-operation and/or interaction between microfilaments and microtubules, for which there is evidence with respect to a number of processes in fungi (Yarm *et al.* 2001).

The increasing use of supplementary videos published with the electronic versions of some journal articles means that it is now often possible to view the dynamics of the

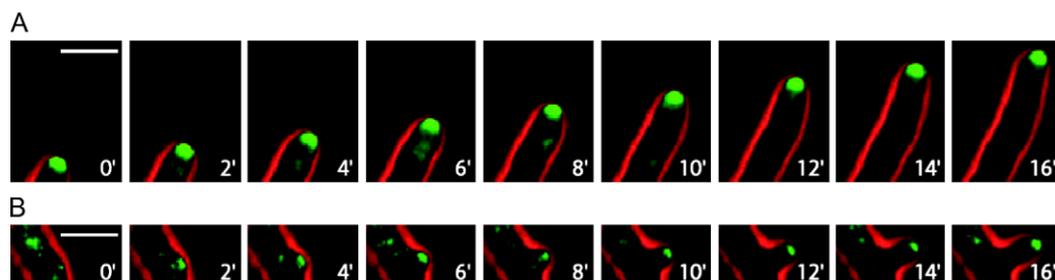
cytoskeleton or the effects of its disruption in complete experiments. Previously these could only be seen either during conference presentations or as incomplete records (showing various time intervals) in journal articles. In one very good example which is pertinent to mitosis it is possible to see how the actin inhibitor latrunculin affects the movement of centromeres in fission yeast (Videos 1 & 2 available with the electronic copy of Tournier *et al.* 2004). This particular experiment suggests that F-actin has a role in centromere movement to the spindle midzone prior to anaphase.

### Cytokinesis and septation

After nuclei have divided, the processes of cytokinesis and septation give rise to daughter cells in yeast and to partitions in hyphae. These processes are accomplished by a septal band that contracts inwards as septal wall material is deposited. The band contains F-actin and the ABPs formin (which is likely to be involved in the generation of the ring) and myosin (which is likely to give the ring its contractile property). Also present are septins, which are conserved eukaryotic proteins that form scaffolds at sites of cell division. It is thought that as the septal band contracts it pulls the plasma membrane inwards. As such, cytokinesis in fungi is similar to the process in animals and differs from that in plants where F-actin and microtubules form the phragmoplast. It is also possible that F-actin plays a role in the transport of vesicles to the newly forming cell wall.

There is clearly a need for close regulation of both the positioning, and the timing, of the formation of the septal band. The position of the band may be influenced by the mitotic spindle and in the case of budding yeast by the cortical landmark proteins mentioned previously. The first of these instances may provide another example of interaction between microfilaments and microtubules. The mechanisms controlling the timing of septal band formation is complicated and involves the mitosis exit network (MEN)/septation initiation network (SIN) signalling pathways (Walther & Wendland 2003). These may influence the recruitment of septins and formin, which then give rise to microfilament formation.

In addition to the above, F-actin also plays a crucial role in a number of aspects related to cytokinesis in sporangia. These include the proper alignment of cleavage apparatus and the distribution of organelles in zoospores. Furthermore, F-actin may be responsible for the amoeboid movement of some zoospores. These matters are considered further by Heath (1995).



**Fig. 4** – The polarisome protein AgSpa2p fused with green fluorescent protein localises to sites of hyphal growth (A) and branching (B) in the fungus *Ashbya gossypii*. This is thought to mark the spot from which actin cables will emanate. Reproduced from *Mol. Biol. Cell* 2004 15: 4622-4632 with the permission of The American Society for Cell Biology.

## Movement and positioning of organelles

As with vesicle transport, which was discussed above, there is evidence suggesting that actin is involved in the movement of certain organelles. Perhaps the most compelling evidence is for mitochondria, which in yeast co-localise with actin cables (Drubin *et al.* 1993) and the actin related protein Arp2 (Boldogh *et al.* 2001). Furthermore, destabilisation of actin cables affects directed mitochondrial movements (Fehrenbacher *et al.* 2004), mitochondrial morphology, and inheritance patterns (Simon *et al.* 1997). ABPs associated with the mitochondrial outer membrane are thought to mediate interaction between the mitochondria and actin cables (Boldogh *et al.* 1998). There have been suggestions that the force powering mitochondrial movement arises from Arp2/3 complex mediated actin nucleation and subsequent filament polymerization (Boldogh *et al.* 2001). The case is less clear for other organelles although nuclear movements can be affected by mutations in *act1* and some ABPs (Haarer *et al.* 1990; Watts *et al.* 1987). There is however ample evidence supporting the role of microtubules in nuclear movement potentially providing yet another instance where the two components of the cytoskeleton work in concert. For further details, regarding nuclear movements in filamentous species see the review by (Fischer 1999).

## 5. Summary

Microfilaments are dynamic structures that are composed of the protein actin. Much is known about the structure of actin and how, in collaboration with ABPs it can give rise to structures that can rapidly assemble and disassemble in response to cellular requirements. These structures play a critical role in many aspects of fungal biology. While the exact nature of these roles may, at present, be somewhat speculative, a rapidly expanding literature promises much in the future.

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