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Corresponding author email id: C.W.Gourlay@kent.ac.uk

Actin – a biosensor that determines cell fate in yeasts

Daniel G.J. Smethurst¹, Ian W. Dawes² and Campbell W. Gourlay^{1*}

¹Kent fungal Group
School of Biosciences
University of Kent
Canterbury
Kent, CT2 7NJ
England, UK.

²School of Biotechnology and Biomolecular Sciences
University of NSW
Kensington
Sydney
NSW 2052
Australia

*Corresponding Author

Abstract

The decision to proliferate, to activate stress response mechanisms, or to initiate cell death lies at the heart of the maintenance of a healthy cell population. Within multi-cellular and colony forming single celled organisms, such as yeasts, the functionality of cellular compartments that connect signalling to cell fate must be maintained to maximise adaptability and survival. The actin cytoskeleton is involved in processes such as the regulation of membrane micro-compartments, receptor internalisation and the control of

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master regulatory GTPases which govern cell decision making. This affords the actin cytoskeleton a central position within cell response networks. In this sense a functional actin cytoskeleton is essential to efficiently connect information input to response at the level of the cell. Recent research from fungal, plant and mammalian cells systems have highlighted that actin can trigger apoptotic death in cells that become incompetent to respond to environmental cues. It may also be the case that this property has been appropriated by microorganisms competing for niche environments within a human host. Here we discuss the research that has been carried out in yeast that links actin to signalling processes and cell fate that supports its role as a biosensor.

1. The Yeast Actin Cytoskeleton

Actin is an ancient molecule whose property of dynamic filament assembly has been appropriated into a host of cellular processes within pro- and eukaryotes. The yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have been widely used as model eukaryotes in which to study actin related processes, enabled by the conserved fundamental principles of actin dynamics and associated regulatory proteins. The best studied examples of yeast actin function lie within processes that engage the movement and stabilisation of membrane compartments such as cell polarisation, endocytosis and vesicle trafficking, contractility and cytokinesis (for recent reviews see (Mooren et al. 2012a), (Kovar et al. 2011) . Yeast cells contain monomeric (G) and filamentous (F) actin that can be assembled into three primary higher order networks, cables, patches and the cytokinetic ring. The assembly and dynamic nature of actin filaments requires the binding and hydrolysis of ATP. Actin monomer addition to a fast growing filament end, subsequent hydrolysis of ATP, release of ADP bound actin monomers, ADP/ATP exchange and re-addition of the re-charged ATP bound monomer to a new fast growing end is termed treadmilling. This repetitive process allows force generation in the direction of filament growth and is aided by a number of conserved actin regulatory proteins that control the process such as the actin filament severing protein cofilin and the actin monomer binding and ADP/ATP exchange protein profilin (Moseley & Goode 2006). Actin patches are

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assembled by the activity of the branched filament promoting, Arp2/3 complex whose activity is regulated by the Nucleation Promoting Factor, Las17p (homologous to mammalian WASp). The assembly of actin into cortical patches has been well studied and aids the internalisation of endocytic vesicles (Mooren et al. 2012b). The process of endocytosis serves to regulate receptor internalisation and re-cycling and maintains the plasma membrane environment. It therefore serves as a crucial interface between environmental sensing and cell response. Actin cable assembly is facilitated by the formin proteins, Bni1p and Bnr1p, which nucleate unbranched actin filaments at bud tip and bud neck respectively (Moseley & Goode 2006). Actin cables, which are held together by bundling proteins such as Sac6p (homologue of human fimbrin) and stabilised by tropomyosin, are crucial for cell polarisation and transport of cargo to the growing bud which occurs in a type V myosin dependent fashion. In addition actin cables are the means by which organelles such as ER, mitochondria and peroxisomes are transported within yeast cells. Actin cables can also facilitate the movement of damaged proteins and mitochondria to facilitate homeostasis control (see below). The completion of cell division relies upon the combined action of actin and myosin within the contractile ring. Constriction of the actomyosin ring drives the formation of the septum which provides a physical diffusion barrier for both mother and daughter cell until membrane and cell wall synthesis is complete. Actin structures are therefore crucial to yeast cell survival and adaptability.

2. Actin and quality control

The formation of protein aggregates is observed in a variety of ageing related and neurodegenerative disorders. The accumulation of mis-folded proteins and their assembly into small oligomers (often thought to represent the toxic species) and larger aggregations, for example those caused by expanded polyglutamine rich (Poly-Q) tracts, is generally thought to reflect cell dysfunction that may contribute to disease (Todd & Lim 2013). Actin receives comparatively little attention in this respect despite the fact that aggregations containing cross-linked or damaged actin are often reported within the literature. The

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appearance of actin aggregations may represent, as may be the case with many protein aggregations, a benign structure in which cells store useless filaments that are difficult to clear. However, good evidence exists to suggest that actin damage and aggregation can have a profound effect on cellular function, stress management and the control of apoptosis. Actin and actin-binding protein rich aggregations such as Hirano bodies and ADF/cofilin rods can be observed to form in response to a variety of stresses, in ageing cells and in neuronal populations from patients suffering from neurodegenerative diseases such as Huntington's and Alzheimer's (Barbara W Bernstein & James R Bamburg 2010). The formation of actin structures such as ADF/cofilin rods can occur as part of a normal stress response mechanism (Barbara W Bernstein et al. 2006) but may also persist and participate in cellular damage (J R Bamburg et al. 2010). The persistence of actin rich aggregations can also be promoted under conditions of oxidative stress. This can be attributed, in part, to the fact that actin proteins possess surface exposed cysteine residues whose oxidation leads to a dramatic reduction in the dynamic capabilities of F-actin filaments (Farah et al. 2011). Another twist in the actin tale comes from evidence, generated in a number of systems including budding yeast, which cite actin dynamics and actin associated processes as a catalyst for protein aggregation that is directly linked to human disease. The expression of an expanded human Huntingtin (103-Q) repeat protein readily aggregates in yeast (Sokolov et al. 2006). This aggregation event is toxic and is accompanied by an increase in cell death that exhibits a number of phenotypes associated with apoptosis (Sokolov et al. 2006). The actin patch and endocytosis regulator Sla1p has been shown to interact with Poly-Q tracts and the deletion of genes involved in the regulation of the actin cytoskeleton and endocytosis were found to exhibit an increased sensitivity to the presence of Poly-Q aggregates (Meriin et al. 2007). Indeed it has been suggested that the loss of endocytic function may underlie the cellular toxicity associated with PolyQ aggregation (Meriin et al. 2007). It should be noted at this stage that actin regulation can also promote amyloid protein aggregation in a manner that does not confer toxicity as is the case in the formation of the PSI⁺ prion (the prion form of the yeast translation termination factor eRF3 encoded by the *SUP35* gene) in yeast (Chernova et al. 2011).

In addition to a role in the formation of aggregations, the actin cytoskeleton has been identified as important in the clearance of damaged proteins and organelles from cells. Recent work has identified that yeast cells can partition damaged proteins and mitochondria into the mother cell to protect the newly forming bud from accumulating age associated damage (Aguilaniu et al. 2003(Klinger et al. 2010). The movement of protein aggregations from the daughter to mother has been shown to require the efficient production of folded actin by the CCT chaperonin complex (Liu et al. 2010). In addition, the movement of mitochondria, which are key players in the regulation of homeostasis and apoptosis, also rely on actin for their distribution in yeast. Actin dysfunction leads to aggregation of mitochondria (Lazzarino et al. 1994) and an inability of mitochondria to bind to actin leads to a reduction in the stability of mtDNA (Lazzarino et al. 1994). Damaged mitochondria and aggregated material can be removed from cells by the autophagic process. Interestingly, autophagic proteins that are involved in the selective degradation of mitochondria, Atg9p and Atg11p, have been shown to require a functional actin cytoskeleton for their correct

localisation. Atg9p has been shown to localise to the mitochondria and where it interacts with Atg11p which in turn appears to target Atg9p bound organelles, in an actin dependent manner, to the pre-autophagosomal structure which then fuses with the vacuole (Lazzarino et al. 1994). The *act1-159* mutant, which exhibits reduced nucleotide exchange rates and a less dynamic cytoskeleton, has been shown to display an aberrant localisation of Atg11p. This mutant also displays defects in Atg9p cycling and the cytosol to vacuole targeting (Cvt) pathway (Lazzarino et al. 1994). Actin therefore seems to play an important role in the maintenance of a healthy mitochondrial population at a number of levels.

3. Actin links environmental sensing to cell fate

The yeast actin cytoskeleton is acutely responsive to stress. A variety of insults ranging from centrifugal force, osmotic and heat shock, nutritional deprivation and oxidative stress result in the MAPK dependent re-organisation of actin into a static de-polarised ensemble that facilitates the cessation of growth (Guo et al. 2009). Removal of such stresses results in the rapid re-establishment of actin dynamics which is necessary for cell growth and division to continue. Clear links have been established between the actin cytoskeleton and a number of signalling pathways that connect environment sensing to cell response. These include the mitogen activated protein kinase (MAPK) cascades, TORC1 and TORC2 signalling and Ras/cAMP/PKA pathways. Yeast cells are now known to initiate programmes of apoptosis and necrosis in response to a range of insults that range from nutritional stress, antifungal treatment, DNA strand breaks and oxidative stress (Carmona-Gutierrez et al. 2010). Regulation of the actin cytoskeleton has been shown to influence the decision to commit to apoptosis (C W Gourlay & K R Ayscough 2006) and to offer protection in the face of stress (Farah et al. 2011). This raises the question, to what extent is actin embedded within environmental sensing pathways as a modulator of cell fate?

Actin and signalling from the RAS GTPase super-family

The Rho proteins are members of the Ras superfamily of small GTPases which are important in linking the actin cytoskeleton to the transduction of signals from external stimuli. These proteins act as switches where-by reduction of bound GTP to GDP leads to a conformational change that retards interaction with effector proteins (Perez & Rincón 2010). There are 6 yeast Rho GTPases (Boureaux et al. 2007) with one of the better studied examples, Cdc42p, being regulated in part by actin. Yeast Cdc42p localises to the plasma membrane at sites of growth and controls the formation of mating projections and buds. Recycling of Cdc42p to control morphogenesis is dependent in part on actin patch-mediated endocytosis (Slaughter et al. 2009). Cdc24p, the GEF for Cdc42p, is also required for polarized growth. Both actin and Cdc24p interact with Bem1p, an SH3-domain containing protein which also interacts with two components of the yeast pheromone pathway, Ste5p and Ste20p (Leeuw et al. 1995). The yeast pheromone response pathway is a canonical MAPK pathway which facilitates yeast mating by communication between haploid cells by pheromone and subsequent growth of mating projections and conjugation. The failure to

correctly execute the mating pathway in response to pheromone stimulation has been shown to trigger an apoptotic response in yeast cells (F F Severin & Hyman 2002, Nan-Nan Zhang et al. 2006). Functional actin structures are required to correctly position a number of proteins including the pheromone receptor and Cdc42p in response to extracellular pheromone (K R Ayscough & Drubin 1998). Actin therefore resides within a network that connects external stimuli to MAPK signalling, morphological change and the potential to initiate apoptosis.

Further links between actin and MAPK cascades are found in the control of polarisation and cell wall integrity (CWI). The CWI pathway begins with a signal from the sensory proteins Wsc1 and Mid2 within the cell wall. Here the pathway connects with the Rho1p GTPase switch via the GEF Rom2p. Activated Rho1p activates the kinase Pkc1p and this in turn activates the MAPK module of the pathway leading to Mpk1 activity (Fuchs & Mylonakis 2009). The CWI pathway also becomes activated by a number of environmental conditions including heat stress, osmotic shock, actin damage, and the presence of pheromone (Levin 2011). It has also been shown that directly depolarising the actin cytoskeleton by treating with latrunculin-B activates Mpk1 (Harrison et al. 2001). A strong link therefore exists between actin and the CWI. As cell wall stress has been shown to induce an apoptotic response in fungal systems (Levin 2011) it is tempting to suggest that a damaged actin cytoskeleton may promote the demise of cells exposed to stresses that challenge cell wall integrity.

TOR signalling

The Target of Rapamycin (TOR) is a highly conserved protein kinase that forms membrane-associated complexes that signal in response to environmental cues to regulate cell growth. The mammalian kinase is called mTOR, while in budding yeast two homologues exist, Tor1p and Tor2p. These TOR kinases are found in the structurally distinct complexes TORC1 and TORC2. Numerous evolutionarily conserved TORC2 functions have been reported including protein synthesis and actin organisation (Schonbrun et al. 2013; Wei Huang et al. 2013). TORC2 has been shown to regulate the cytoskeleton via downstream interactions with multiple Rho GTPases in yeast (Anja Schmidt et al. 1997) and is thought to interact similarly in mammals (H Zhou & S Huang 2011; Jacinto et al. 2004). In yeast there is crosstalk between TORC2 and the CWI pathway as they share components including Rho1 and Pkc1 and the downstream MAPK cascade, although Rho1 can polarise the actin cytoskeleton independently of TORC2 signalling (Helliwell et al. 1998; M Bickle et al. 1998). TORC1 also has actin regulation among its functions, as evidenced by the disrupted actin polarisation when TORC1 is inhibited by rapamycin, which does not affect TORC2 (Aronova & Wedaman 2007). TORC1 control of telomere length in response to starvation has been reported in yeast through interaction with the telomeric Ku complex (Ungar et al. 2011). Telomere length regulation is also linked to actin through the Arp2/3 complex, a highly conserved actin nucleation centre with roles in endocytosis, growth and polarity (Ungar et al. 2009). TOR pathway activity has been linked to apoptosis in yeast cells challenged with weak acid stress, whereby a functional TOR1 signalling system was shown to be required to induce a robust apoptotic response to acetic acid (Almeida et al.

2009). Although speculative it is tempting to suggest that cells possessing a damaged actin cytoskeleton may be unable to correctly manage TOR signalling and so the activation of apoptosis in response to acetic acid.

Actin, cAMP/PKA signalling and apoptosis

The treatment of cells actin stabilising drugs such as Jasplakinolide induces apoptosis in yeast cells (Campbell W Gourlay et al. 2004), as is the case in mammalian cells (Franklin-Tong & Campbell W Gourlay 2008). Yeast cells expressing mutations in actin regulatory proteins that promote actin aggregation also trigger apoptosis (Campbell W Gourlay & Kathryn R Ayscough 2005). However it is interesting to note that mutations in actin regulatory proteins can also promote lifespan extension (Campbell W Gourlay et al. 2004) and induce a robust stress response (Vassilios N Kotiadis et al. 2012). These data suggest that in yeast, actin dynamics are linked to processes that can couple cell death control to stress response regulation. Evidence to support this comes from our findings that actin dynamics are involved in the control of the master regulatory small GTP-ase RAS, and its control of mitochondrial function via cAMP/PKA signalling. RAS/cAMP/PKA signalling co-ordinates cell growth and proliferation, stress response and mitochondrial activity with glucose availability (Thevelein & de Winder 1999). Activation of RAS signalling in response to glucose leads to proliferation and growth and suppression of stress response mechanisms. Therefore the control of Ras/cAMP/PKA is essential for effective cell cycle exit and initiation of the cellular stress response during starvation. Ras/cAMP/PKA signalling is also linked to mitochondrial health as cells expressing the constitutively active *ras*^{ala12val19} allele exhibit elevated ROS levels (Heeren et al. 2004). Actin aggregation triggers hyperactivity of the Ras/cAMP/PKA signalling pathway, which in turn is responsible for mitochondrial dysfunction, ROS accumulation and the triggering of apoptotic cell death (C W Gourlay & K R Ayscough 2006). In this pathway actin is coupled to RAS/cMPA/PKA signalling by the protein Srv2p/CAP which possesses an actin and adenylate cyclase (Cyr1p) binding activity (C W Gourlay & K R Ayscough 2006). ROS accumulation and subsequent death in actin aggregating cells requires the activity of the PKA subunit, Tpk3p, which is known to play an important role in the control of mitochondrial function. The control of Tpk3p activity levels appear to be critical for the maintenance of a healthy mitochondrial network. Cells lacking Tpk3p display mitochondrial defects and increased ROS levels (Chevtzoff et al. 2010). However elevation Tpk3p levels also lead to the production of dysfunctional mitochondria that promote ROS production. In this case of hyper-Tpk3p activity mitochondrial dysfunction is caused by transcriptional means that involves several stress linked regulators including *SOK2*, *SKO1* and the mitochondrial biogenesis regulator *HAP4* (Leadsham & Campbell W Gourlay 2010). The PKA subunit Tpk3p is therefore crucial in linking environmental sensing to mitochondrial function and apoptosis in *S. cerevisiae*.

The actin cytoskeleton is therefore embedded within signal transduction mechanisms that respond to environmental change and can trigger apoptosis when damaged. These data offer compelling evidence that actin damage functions as a biosensor that helps to terminate cells

that will be unable to react appropriately to a wide range of environmental challenges (Figure 1).

4. Harnessing actin triggered apoptosis within microbial warfare

As actin damage can trigger a powerful cell death signal in yeast cells, it would seem likely that this may have been exploited by competing micro-organisms. Although research in this area is embryonic, evidence is emerging to suggest that this is the case. Species of the human fungal pathogen *Cryptococcus* have been observed to undergo apoptosis in response to a range of stresses including peroxide, antifungal exposure and sodium citrate (Ikeda & Sawamura 2008; Semighini et al. 2011; Y-S Wang & Z-Y Wang 2012). Interestingly co-incubation of *C. neoformans* with the bacterium *Staphylococcus aureus*, which is commonly found in the human respiratory tract or on the skin, triggered aggregation of the actin cytoskeleton and an apoptotic cell death (Ikeda & Sawamura 2008). Treatment of cells with an inhibitor of the actin regulator ROCK1 prevented the aggregation of actin and the initiation of apoptosis (Ikeda 2011). Although more work is needed to confirm that actin aggregation triggers apoptosis when *S. aureus* contacts *C. neoformans* the involvement of the cytoskeleton in cell killing would seem likely. Whether this bacterial/fungal interaction represents a mechanism by which *S. aureus* may protect the human host from *Cryptococcal* invasion is an interesting proposition. It may also be the case that the corruption of actin and subsequent triggering of an apoptotic response is more widely used by bacterium within the world of microbial warfare.

5. Conclusions

We now know that actin plays many more roles in the cell beyond a structural scaffold or force generating machine that can manipulate membranes. Indeed, as outlined in this review, actin participates in the regulation of a number of signal transduction pathways and cellular events that are essential for a cells ability to retain adaptability and homeostatic control. We therefore propose that actin plays a conserved role in linking the ability of a cell to respond to environmental cues to promote a life/death decision. Our vision as to how actin may be integrated in this way in a budding yeast cell is presented in Figure 2. However actin also appears to be strongly connected to the control of apoptosis in diverse eukaryotic systems. For example actin dynamics are known to regulate the control of apoptosis during the self incompatibility response that is found in a number of plant species (Franklin-Tong & Campbell W Gourlay 2008). In mammalian cells the regulation of actin appears to be involved in the control of death receptor clustering, mitochondrial outer membrane permeability and the process of membrane blebbing that facilitates apoptotic body formation (Franklin-Tong & Campbell W Gourlay 2008; David Amberg et al. 2012). As such the actin cytoskeleton may fulfil a general function as an active biosensor of cell health whereby cytoskeletal damage that prevents an accurate response to environmental change triggers an apoptotic response and the removal an unhealthy cell from the population. Further research in this area will reveal the extent to which actin is involved in the integration of cell fate

pathways and offers exciting new avenues for therapeutic intervention. In line with this recent research has identified that specific actin functions may be targeted to develop new and specific anti-cancer therapeutics (Stehn et al. 2013).

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Figure legends

Figure 1 – The actin cytoskeleton participates in the control of signal transduction pathways that mediate adaption to environmental change

Shown are the known links between actin regulation and major signalling pathways that monitor environmental change. Experimental evidence suggests that actin is involved in the control of signalling through the RAS/cAMP/PKA pathway, pheromone and cell wall integrity MAPK pathways and TOR pathways. In this respect a functional actin cytoskeleton is essential to ensure that yeast cells are able to respond and adapt to a range of stresses. Coupled to this is the fact that the corruption of actin leads to the induction of apoptosis via the hyper-activation of RAS signalling. This combination leads us to suggest that actin mediated apoptosis serves as a mechanisms by which yeast cells that are unable to respond to a number of stresses can be removed from a population.

Figure 2 – Actin aggregation - a general mechanisms by which unresponsive cells can be removed from a population

The actin cytoskeleton is embedded within a number of systems that allow cells to respond to environmental change, stress conditions and the maintenance of homeostasis. The maintenance of a healthy actin cytoskeleton is therefore essential for cell adaptability and perpetuation of a population. Studies to date indicate that cells unable to respond to environmental cues as a result of corruption of the actin cytoskeleton are targeted for destruction via apoptosis. The role of actin in cell killing would appear to have been harnessed by certain microbes that induce aggregation of the cytoskeleton to trigger cell death. It may be the case therefore that the irreversible stabilisation of actin functions as a biosensor within yeast cells. In this respect actin mediated cell killing may provide an advantage to yeast cell populations.

