Cell Death and Differentiation (2010) 17, 754–762

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Review

Apoptosis and the yeast actin cytoskeleton

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Actin represents one of the most abundant and extensively studied proteins found in eukaryotic cells. It has been identified as a major target for destruction during the process of apoptosis. Recent research has also highlighted a role for cytoskeletal components in the initiation and inhibition of apoptotic processes. The high degree of conservation that exists between actins from divergent eukaryotes, particularly with respect to those that contribute to the cytoskeleton, has meant that functional studies from the model yeast *Saccharomyces cerevisiae* have proven useful in elucidating its cellular roles. Within the context of apoptosis in yeasts, actin seems to function as part of the signalling mechanisms that link nutritional sensing to a mitochondrial-dependent commitment to cell death. Studies in yeasts have also shown that oxidative damage accrued by the actin cytoskeleton is closely monitored and is tethered to an apoptotic response. Strong, but as yet, undefined links between the actin cytoskeleton and apoptosis have also been described in studies from plant and animal systems. The widespread involvement of actin in apoptotic mechanisms from diverse eukaryotic organisms raises the possibility of conserved regulatory pathways, further strengthening the relevance of yeast research in this area.
Cell Death and Differentiation (2010) **17,** 754–762; doi:10.1038/cdd.2009.196; published online 18 December 2009

The ability to coordinate environmental sensing with appropriate cell-fate decisions is a pre-requisite for the success of both unicellular and multicellular organisms. Over the last decade it has become clear that the yeast, *Saccharomyces cerevisae,* is able to undergo programmed cell death that shows many of the hallmarks of apoptosis, including DNA fragmentation, Annexin V exposure, reactive oxygen species (ROS) build-up and the loss of mitochondrial membrane potential,[1] as reviewed in Buttner *et al.*[2] and Gourlay *et al.*[3] This finding has prompted the question, why should a unicellular organism possess the ability to undergo apoptosis, and to what benefit? Recent studies have highlighted the fact that yeasts, as is the case with a variety of prokaryotes, are capable of simple differentiation at the level of the colony.[4,5] Patterning within colonies is responsive to environmental sensing pathways that are linked to an apoptotic fate.[6] Within a colony, which can be described as an organized population of cells derived from a single progenitor, one could argue that the regulated death of specific sub-populations may benefit the survival of the clone. This may be of particular importance in the face of environmental challenge. In this short review, we will present evidence that the actin cytoskeleton forms part of the machinery that the yeasts have used to couple environmental sensing to programmed cell death at the level of both the single cell and the colony.

The Yeast Actin Cytoskeleton

In yeasts, the actin cytoskeleton has an essential role in the regulation of a number of processes, such as vesicle and organelle trafficking, cytokinesis, endocytosis and polarization of growth. Actin itself is encoded by a single gene, *ACT1*, whose product can exist in a globular monomeric (G-actin) or a filamentous (F-actin) form. F-actin filaments are assembled in a polarized manner with ATP-bound G-actin monomers preferentially added to fast growing (+) or barbed ends. The weak intrinsic ATPase activity showed by actin leads to the hydrolysis of ATP to ADP + inorganic phosphate. This is accompanied by a conformational change that is thought to destabilize the actin filament and promote monomer dissociation from the slow growing (−) or pointed end. The dynamic turnover of actin filaments and re-charging of filament-competent G-actin by the exchange of ADP for ATP is enhanced by actin-binding proteins to facilitate the cyclical dynamic process commonly called treadmilling (for a recent review see Pollard[7]). In yeasts, as in all eukaryotes, the plasticity of the actin cytoskeleton has been harnessed to facilitate cellular processes through the formation of higher-order structures, including cortical actin patches, cables and the cytokinetic ring (Figure 1).

During log growth, actin patches are found to gather at sites of intense growth and are numerous within the newly forming bud. Cortical actin patches are highly dynamic structures that show rapid movement and facilitate internalization of early endosomes.[8] Actin turnover drives patch motility, as demonstrated by the disruption of the cytoskeleton by addition of the monomer sequestering agent, latrunculin A (Lat-A). The polymerization of actin to form the patch ultrastructure is considered to be the driving force for generating endocytic

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Keywords: actin; mitochondria; apoptosis; ROS; Ras; ageing
Abbreviations: ROS, Reactive Oxygen Species; VDAC, Voltage Dependent Anion Channel; ActMAP, Actin Mediated Apoptosis; Lat-A, Latrunculin -A; PKA, Protein Kinase A
Received 17.8.09; revised 05.11.09; accepted 11.11.09; Edited by F Madeo; published online 18.12.09

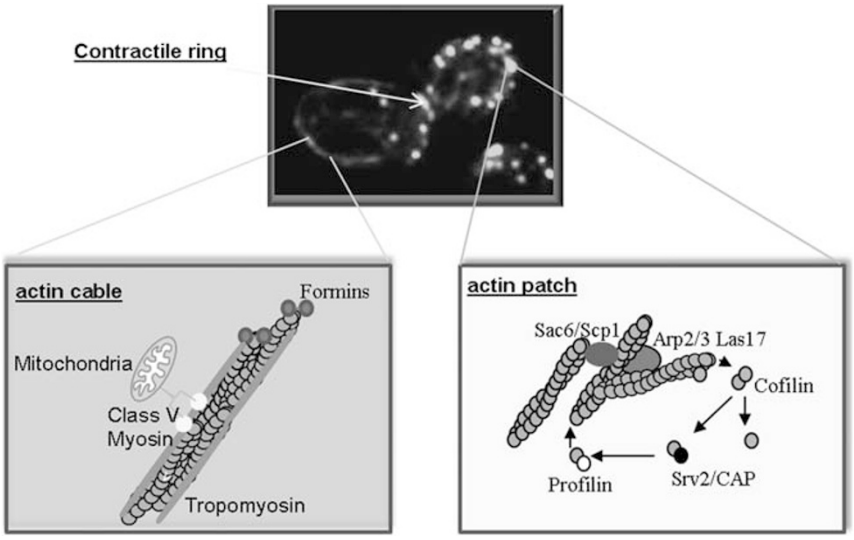

Figure 1 The F-actin cytoskeleton of *S. cerevisiae*. A dividing *S. cerevisiae* cell stained with Alex488-Phalloidin, which binds specifically to F-actin-containing structures, is shown. Three types of structure can be clearly visualized in yeasts using this technique: cortical actin patches that facilitate endocytosis and cortical membrane movement, actin cables that exert an effect as tracks for the movement of intracellular traffic and the contractile, or cytokinetic, ring

vesicles by pulling the membrane inwards, but the formation also serves well as a structural scaffold.[8–10] The release of vesicles requires the motor function of the myosins Myo3p and Myo5p, as mutants defective in the functions of these proteins can show an inability to achieve scission of endocytic vesicles from the cell cortex.[11] Once scission of an endocytic vesicle is achieved, the actin patch/vesicle structure is transported to endosomes. This is achieved by a passive transport system using the retrograde flow of actin cables from the cell cortex inwards.[10] Interestingly, actin patches are also highly responsive to cellular stress and will rapidly de-polarize in response to a signal cue such as heat shock or entry into the diauxic phase of growth. Upon release from stress, actin patches can be observed to rapidly polarize again as growth resumes. The responsiveness of actin to numerous environmental conditions indicates that it functions within signalling networks that are responsive to environmental conditions. However, little is known about how actin patches are regulated in response to environmental change.

Actin cables are composed of short overlapping filaments that are nucleated by the action of the formins, Bni1p and Bnr1p.[12,13] The F-actin found in cables and patches is manipulated by different regulators to produce scaffolds with different functions. Although patches use the Arp2/3 complex to facilitate branched actin structures, formins nucleate the straight actin filaments observed in cables. Real-time imaging studies using the actin-binding protein Abp140p fused to GFP revealed that one end of a cable is associated with budding tips or necks, whereas the other moves away towards the mother cell.[14] The force driving this movement of cables towards mother cells is maybe due to the polymerization of actin; however, recent studies have implicated myosins in actin cable dynamics.[15] The action of type V myosin coupled to actin cables is required for the segregation of organelles, including the vacuole, nucleus, cortical ER, mitochondria and peroxisomes during bud formation and growth.[16,17] Actin cables are targeted specifically to polarity sites, which are protein assemblies that direct new cell growth,[18] and which facilitate appropriate endocytosis and exocytosis in these regions.[19] Actin cables also show rapid turnover, and a current model proposes that the cables stabilizing protein tropomyosin, which readily decorates cables and influences turnover by stabilizing filaments, along with the actin regulators cofilin and actin interactin protein 1 (AIP1) cooperate to appropriately regulate cable filament turnover.[20,21]

During cytokinesis, bud separation is achieved through the action of an actomyosin contractile ring that is directed by the formation of a septum at the neck. The assembly of a septin ring, comprising Cdc3p, Cdc10p, Cdc11p and Cdc12p, generates a scaffold for actin ring formation.[22] This is achieved by septin-driven recruitment of the type II myosin, Myo1p, formins and IQGAP proteins that together promote actin ring formation.[23] Cytokinesis then proceeds when all essential components have been transported into the new daughter cell.[24] Constriction of the actomyosin ring and the formation of the septum provides a physical diffusion barrier that allows membrane and cell wall synthesis to be completed.

Yeast and the Intrinsic Pathway of Apoptosis

In addition to their role as key players in energy production and core metabolism, mitochondria are now known to have a pivotal role in the regulation of programmed cell death, the so-called intrinsic pathway of apoptosis. As mitochondria are thought to have arisen from a bacterial progenitor, as endosymbionts of a primitive eukaryote,[25] it follows that divergent lineages may show common activity. An example of this exists within theintrinsic pathway of cell death that has been observed to contribute to programmed cell death in yeasts,[26] humans[27] and plants.[28–30] During apoptosis driven by the intrinsic pathway, permeabilization of the mitochondrial outer membrane leads to the release of pro-apoptotic, factors such as cytochrome *c*, apoptosis-inducing factor 1 (AIF1) and

endonuclease G (Endo G). Although a role for cytochrome *c* release in yeast apoptosis is still to be established, both AIF1 and Endo G have been shown to translocate from the mitochondria to the nucleus upon apoptosis stimulation to facilitate DNA fragmentation.[26,31,32] A primary function of the mitochondria lies in the production of ATP through oxidative phosphorylation. The electron transport chain at the centre of the process generates reactive oxygen species as a by-product of this metabolic activity, which if allowed to accumulate, or if generated in excess, can cause sufficient damage to macromolecules to cause cell death. The release of ROS is a pivotal event that has been reported to occur in all reported cases of yeast apoptosis[26,33] and is also an important component of apoptosis/PCD in animals[34] and plants.[35–37] The participation of the intrinsic pathway of apoptosis is therefore an important component of eukaryotic cell death that has been harnessed throughout the eukaryotic kingdoms.

Linking Actin Dynamics to Apoptosis

The dynamic nature of the actin cytoskeleton has been appropriated by a number of cellular processes. Interestingly, recent evidence from fungal, plant and animal systems has added apoptosis/programmed cell death to this list. The use of drugs that manipulate actin has been instrumental in establishing a link between actin and apoptosis in diverse eukaryotes. For example, the stabilization of F-actin by addition of the drug, jasplakinolide, was shown to induce apoptosis in Jurkat T cells, accompanied by an increase in caspase-3 activation.[38,39] Entry into an apoptotic cell death was also observed to occur in yeast cells when grown in the presence of jasplakinolide.[40] Apoptosis can also be triggered by the destabilization of F-actin structures; for example, the application of cytochalasin D was shown to induce rapid cytochrome *c* release from mitochondria, caspase activation and apoptosis in murine cell lines.[41] From such studies, only a few of which are mentioned in this study (for a full review, see Franklin-Tong and Gourlay[42]), it would seem that apoptotic pathways found in diverse eukaryotes are responsive to the availability of a dynamic actin cytoskeleton. To serve as an example, the studies involving *Papaver* pollen showed that a short period of reduced actin availability was sufficient to commit pollen to programmed cell death.[43] Studies using actin-manipulating drugs have revealed that actin stabilization or de-stabilization may induce, or protect from, apoptosis. It would seem, therefore, that the availability of an appropriate amount of dynamic actin is an important factor in the commitment to apoptosis. In all cells a dynamic equilibrium exists between monomeric G-actin and F-actin, and this balance is tightly controlled. In *Saccharomyces cerevisiae*, it has been suggested that most of the total actin pool is held in the F-actin form.[44] In stark contrast, pollen from plants show a much higher ratio of G- to F-actin and are thought to hold approximately 10% in the filamentous form.[45,46] Similar results are reported in mammalian cells.[47] The regulation of actin pools is a consequence of the action of a number of actin-binding proteins, a number of which have been implicated in apoptosis regulation in both mammalian and yeast cells. The available data linking actin-regulatory proteins to apoptosis in mammalian cells is increasing at a pace (for a recent summary of this area of research, see Franklin-Tong and Gourlay[42]). The best-studied examples of actin regulatory proteins and their role in apoptosis in mammalian cells are gelsolin and cofilin. Gelsolin, an actin-binding protein capable of severing and capping actin filaments,[48,49] may protect cells from apoptosis by regulating voltage-dependent anion channels (VDACs), a mitochondrial membrane pore that regulates the release of pro-apoptotic factors and that is important for maintaining mitochondrial homoeostasis.[50] Along similar lines, the actin-binding protein cofilin, which promotes the depolymerization and severing of actin filaments and which is involved in the recycling of the G-actin monomers,[51,52] has been shown to be targeted to mitochondria after initiation of apoptosis.[53] Following this cytochrome *c* leakage occurs that triggers apoptosis. These studies suggest that actin/mitochondrial interactions have a crucial role in apoptosis initiation. An interesting possibility is that such a role may be conserved among divergent eukaryotes. In line with this, interactions between actin and the mitochondria also seem to regulate apoptosis in yeasts (discussed in detail below). Actin has also been identified as a substrate for caspase cleavage during the process of apoptosis at a proteolytic cleavage site (YELPD)[54,55] that has been conserved within yeast actin (our observations). The conservation of a caspase cleavage site within actin may suggest that the proteolytic cleavage of actin has a regulatory function. In support of this, the action of caspase activity on actin after apoptosis induction can lead to the production of an N-terminal 32-kDa product named Fractin and a 15-kDa fragment denoted as tActin.[56] The available evidence suggests that N-myristoylation of tActin can target this fragment to the mitochondria and promote morphological changes associated with apoptotic cells.[57]

Actin and Mitochondrial Regulation in Yeast

Physical links between actin and the mitochondria. The mitochondria comprise a dynamic tubular network whose morphology and localization are kept under tight control. Many studies have shown that the regulation of mitochondrial integrity has a crucial role in apoptosis in yeast and mammalian systems. As an example, an increase in the fragmentation of mitochondria by the fission protein, Dnm1, is an important factor in apoptosis induced by treatment of yeast cells with acetic acid or hydrogen peroxide.[58] Links between the mitochondria and the actin cytoskeleton have been shown in divergent eukaryotic systems.[59] For example, actin is required to concentrate mitochondria in areas of cells with high energy demands,[60] and it facilitates distribution as cells undergo division.[61] In yeasts, mitochondria are thought to directly associate with actin cables through a protein complex termed mitochore, which comprises three mitochondrial transmembrane proteins (Mdm10p, Mdm12p and Mmm1p).[62] Loss of Mmm1p leads to the loss of association of mitochondria with actin.[63] Actin–mitochore attachments are thought to be important for the movement and inheritance of mitochondria. However, it may be the case that this interaction also has a role in the stability of mtDNA,

as a loss of Mmm1p leads to the collapse of mtDNA into aggregates and reduced inheritance.[62] Links to actin from the mitochore are facilitated by two accessory proteins, Puf3p and Jsn1p, that interact with the Arp2/3 complex.[64] Movement and inheritance of mitochondria is also facilitated by the action of the myosin, Myo2p, and its associated myosin light chain (Mlc1p).[65] Treatment of yeast cells with the actin depolymerizing drug, Lat-A, leads to significant disruption of mitochondrial morphology. This shows the importance of actin integrity on mitochondrial morphology and distribution.[66] A recent paper also identified a complex comprising several mitochore components, including Mmm1p, Mdm10p and Mdm12p, describing it as part of the molecular tether between ER and mitochondria, with a proposed role in phospholipid biosynthesis and calcium-signalling.[67] It may be the case that this newly described structure and the mitochore are separable, with roles in mitochondrial movement and homoeostasis. Further research will be required to clarify this.

Permeabilization of the outer mitochondrial membrane has an important role in the initiation of apoptosis.[68] The VDAC is a mitochondrial porin, encoded by POR1, which is localized in the outer membrane[69] that has a role in the regulation of maintenance of mitochondrial permeability. In mammalian systems the actin regulatory proteins, cofilin and gelsolin, are postulated to influence apoptosis through an actin-dependent interaction with the VDAC. Although such an interaction has yet to be given physiological relevance, evidence suggests that actin also influences VDAC function in fungal cells. Monomeric actin has been shown to bind to reduce the VDAC pore's conductance, reducing metabolic flux across the mitochondrial membrane in the filamentous fungus *Neurospora crassa*.[70] G-actin has also been shown to interact with the VDAC in *S. cerevisiae* using surface plasmon resonance.[71] The likelihood that actin interacts with VDAC in fungal cells gives rise to the possibility that such cytoskeletal–mitochondrial associations may be conserved, with actin potential acting to promote or stabilize a closed VDAC formation.

Actin and Mitophagy

The quality control of mitochondria has been shown to be a vital part of cellular homoeostasis and is monitored by a specific autophagic process named mitophagy. The process of mitophagy ensures the selective degradation of damaged mitochondria in mammalian cells and yeasts.[72] The selective degradation of mitochondria by this process has been shown to require a functional actin cytoskeleton. Cells carrying the *act1-159* allele, which stabilizes actin filaments by reducing monomer turnover rate, show the failure to appropriately localize crucial players in the process of mitophagy. The essential factor, Atg11p, was found to be mis-localized in *act1-159* cells. This was accompanied by defects in Atg9p cycling and the cytosol-to-vacuole targeting (Cvt) pathway that is essential for mitophagy.[73] It may be the case that components of the mitophagy machinery are mobilized by an actin-dependent process, as Atg9p, which localizes to the mitochondria and interacts with Atg11p, was found to colocalize with Arp2p. It was proposed that the Arp2/3 complex and actin are involved in the regulation of Atg9 transport for specific types of autophagy.[74] The studies outlined above show a role for actin in multiple functions of the mitochondria, namely transport, membrane permeability and mitophagy. It is highly likely that such physical interactions are of importance within the context of apoptosis regulation in yeasts.

Actin, Environmental Sensing and Apoptosis in Yeasts

The re-arrangement of actin structures in response to a cue had long been thought of as a signal transduction end point. However, it is now apparent that actin has an important role not only within signalling pathways themselves, but also in the regulation of key effector molecules and cell-fate decisions. An example of this was provided in the previous section with regard to the actin-dependent distribution of the autophagy regulator Atg9p. Another example has been provided by our recent work that has characterized the effects of actin disruption on the ability of a cell to navigate the diauxic shift. During this period of growth, cells must recognize changes in nutritional availability and elicit dramatic alterations to their transcriptome and proteome to cope with new metabolic demands. Yeast cells grown in media containing glucose obtain their energy primarily through fermentation during a rapid period of logarithmic growth. The production of ethanol through fermentation offers an alternative carbon source for the yeasts as they enter the diauxic shift. At this point yeast cells undergo a period of mitochondrial biogenesis to enable them to effectively use ethanol through oxidative phosphorylation. For successful transition from fermentative to respiratory growth, it follows that signalling mechanisms linking nutritional sensing to mitochondrial biogenesis must exist. We have discovered that molecules linking actin dynamics to the activity of an important glucose sensing pathway, the Ras/cAMP/PKA pathway, form one such link to mitochondrial function. The Ras/cAMP/PKA is an essential signalling mechanism that coordinates cell growth and proliferation in response to the nutritional environment.[75,76] As cells enter the diauxic shift Ras activity is downregulated, leading to a range of responses including cell-cycle exit, upregulation of stress response factors and the stimulation of carbohydrate storage. It is also clear that the activity of this pathway is linked to mitochondrial function as the constitutive activation of Ras in cells expressing the *ras*^{ala12val19} allele show elevated levels of respiration and accumulate ROS.[77,78] The dynamic status of the actin cytoskeleton was identified as an important factor in this pathway during studies that analysed mutations, leading to the build-up of stabilized aggregations of F-actin.[40,79] Mutations that accumulated actin aggregates during the diauxic phase of growth were shown to undergo a cell death that showed a number of apoptotic hallmarks. The ability of the Ras/cAMP/PKA pathway to regulate mitochondrial function and ROS production also seems to have been harnessed by a fungal apoptotic response. For example excessive Ras signalling has also been shown to lead to apoptosis in the fungal pathogen *Candida albicans*.[80,81] The prevention of aggregate formation by addition of the actin monomer sequestering drug Lat-A was sufficient to prevent apoptosis.[82] This strengthens the proposal that the formation of stable

F-actin structures, or aggregates, exerts an effect as the initiating factor within this apoptotic pathway. As a result, we have termed this phenomenon as *act*in-*m*ediated *ap*optosis (ActMAP). The formation of aberrant actin aggregations capable of initiating ActMAP was shown to require the activity of the protein Srv2p/CAP, a highly conserved actin regulatory protein that binds preferentially to ADP–G-actin through its C-terminal domain[83,84] and to actin filaments through a proline region that can interact with the SH3 domain of actin-binding protein 1 (Abp1p).[85] Srv2p/CAP provides a link between actin and Ras signalling as it binds to adenylate cyclase (Cyr1p) that catalyses cAMP production and PKA activation.[86,87] The accumulation of ROS and subsequent death in actin aggregating cells require the activity of the PKA subunit, Tpk3p. This PKA subunit has previously been shown to have a role in regulating mitochondrial function, as cells lacking Tpk3p show altered mitochondrial enzymatic content.[88] In addition, it has been suggested that mitochondrial-associated Tpk3p activity can influence the transcription of mitochondrial-encoded genes.[89,90]

A potential link between nutritional sensing machinery and ActMAp has recently been suggested. Loss of the *WHI2* gene was shown to lead to the accumulation of actin aggregates during diauxic shift and the triggering of cAMP/PKA-dependent apoptosis.[91] Whi2p has been shown to have a role in coordinating the cell-cycle and stress response to environmental sensing mechanisms.[92,93] Cells lacking Whi2p fail to exit the cell cycle under conditions of nutritional depletion and do not elicit an appropriate stress response. It was shown that *Δwhi2* cells also fail to downregulate Ras activity as actin aggregates appear. This study also showed that during the diauxic shift, Ras is removed from the plasma membrane and degraded within the vacuole.[91] The loss of Whi2p function instead leads to the accumulation of GTP-bound, or active, Ras at the mitochondria. The presence of active Ras at the mitochondria was sufficient to stimulate Tpk3p-dependent mitochondrial dysfunction and ROS production. The significance of the presence of active Ras at the mitochondria is currently unknown. However, in mammalian cells, the targeting of K-Ras to the mitochondria has been reported to occur in a PKC-dependent manner, and was sufficient to promote apoptosis attributed to an interaction with the anti-apoptotic factor Bcl-$X_{L.}$[94] Our preliminary data suggest however that PKC activity is not linked with targeting of Ras to the mitochondria in yeasts (data not shown). This does not rule out the possibility that phospho-regulation leading to mitochondrial targeting of Ras may not occur in yeasts.

The aggregation of actin can be observed within wild-type cells as they age (Figure 2). It has been shown that aged yeast cells undergo apoptosis.[95] With strong evidence pointing to the hypothesis that the accumulation of actin aggregates promote apoptosis in yeast cells, it is tempting to suggest that ActMAp also functions as a mechanism by which aged cells are pruned from a population. It may be the case that actin integrity is also crucial to the regulation of cell death within colonies. To examine this, we analysed cell death within colonies formed by wild-type cells and in the *act1-159* strain that shows reduced actin dynamics and accumulates aggregates of F-actin,[96] yeast colony using a phloxine B uptake method (Figure 3). In wild-type colonies, phloxine uptake appears in striking radial pattern, and is excluded from the colony centre and growing edge, reflecting the simple differentiation that occurs in even lab-cultured *S. cerevisae* colonies. However, in *act1-159* colonies, phloxine uptake occurs throughout the colony. The same result can be obtained in other mutant backgrounds that lead to ActMAP, such as in cells lacking Whi2p (Figure 3). Our interpretation of this is that a reduction in actin dynamics and propensity to

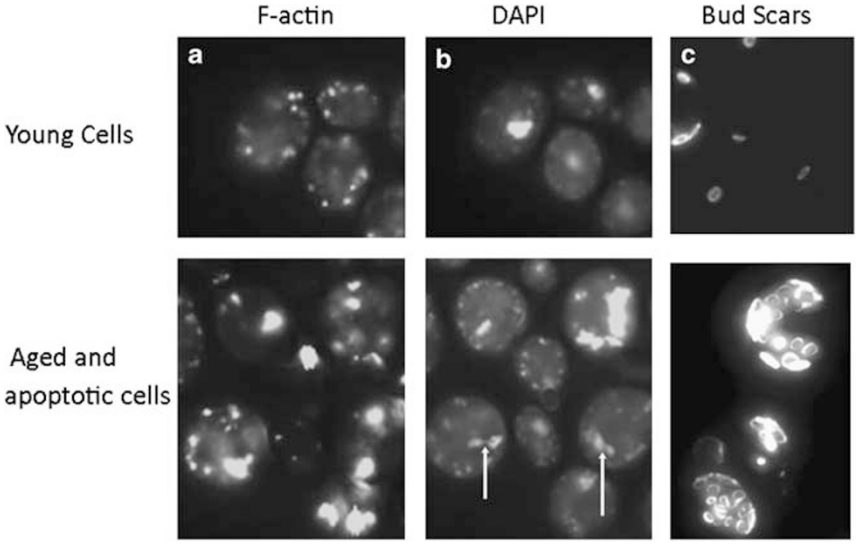

Figure 2 The actin cytoskeleton in young and aged apoptotic yeast cells. *S. cerevisiae* grown in liquid culture for 24 h were subjected to centrifugal elutriation, and fractions containing very young cells and old mother cells were collected on the basis of size and density. Cells from both populations were stained for F-actin with Alexa488-Phalloidin (**a**) and co-stained for DNA with DAPI (**b**). Yeast cells acquire a new chitin-rich bud scar with every cell division and hence the age separation was verified by staining these scars with fluorescently labelled wheat germ agglutinin (**c**). As can be observed, cells from the 'old' fraction contain many more bud scars than those in the 'young' sample. Young cells show punctate actin patches, a compact single nucleus and numerous mtDNA nucleoids. In contrast, aged cells show aggregated actin cytoskeletons, fragmentation of nuclear DNA, an indicator of apoptosis (arrows) and reduced mtDNA content

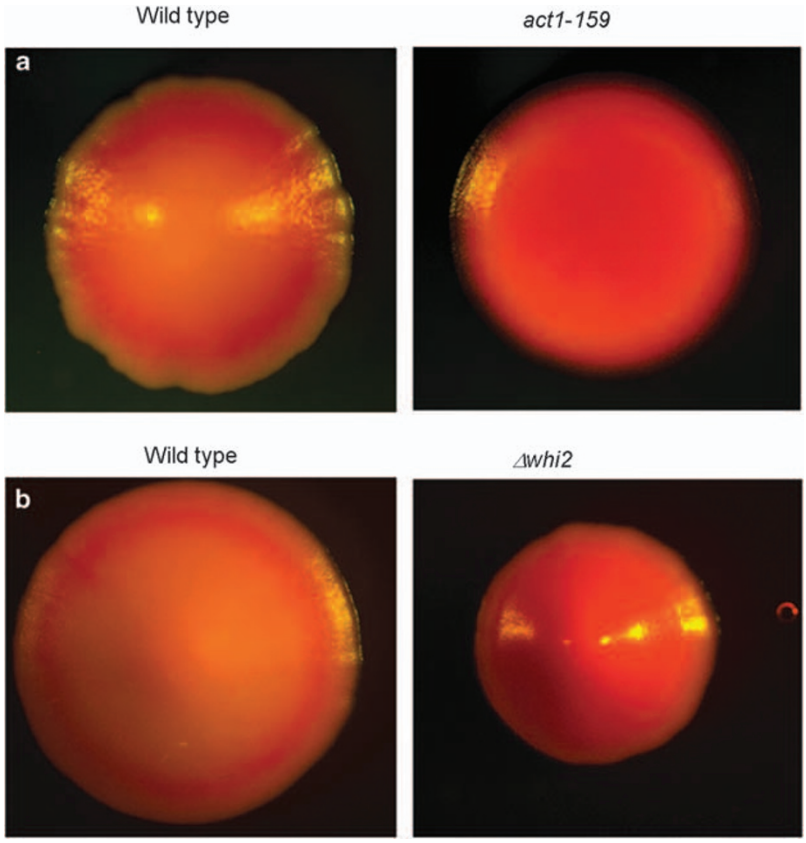

Figure 3 Actin and cell death in yeast colonies. Yeast colonies were grown on YPD agar plates containing 10 μM phloxine B for 6 days. Colonies formed by the actin mutant *act1-159* (**a**) and *Δwhi2* (**b**) strains are presented with their corresponding wild types. A GFP filter set was used to capture the fluorescence emitted by phloxine B under UV illumination. Pictures were taken using a Leica MZ FLIII binocular dissecting microscope and captured with a Leica DC300F digital camera (Central Imaging Facility, University of Kent, UK). Both the wild-type strains present a similar radial uptake of phloxine B, whereas in both cases the mutant strains that are prone to actin aggregation and apoptosis stain red throughout, indicating unregulated cell death within the colony

form actin aggregates promotes unregulated cell death throughout the colony.

The influence of actin assembly has also been shown to influence the formation of amyloid aggregates in yeast cells. Recent studies using *S. cerevisiae* to model polyglutamine aggregation showed that a fragment of human huntingtin, followed by an expanded 103-Q repeat region, readily aggregated and caused cell death that showed apoptotic phenotypes.[97] Disruption of the actin cytoskeleton with Lat-A was shown to lead to an increase in the presence of poly-Q aggregates in yeasts.[98]

Actin, Oxidative Stress and Ageing

The actin cytoskeleton is sensitive to increases in the oxidative status of cells.[99] This occurs principally through the oxidation of exposed cysteine residues.[100] Particularly noteworthy are the cysteines located at positions 285 and 374. These residues, although highly conserved among actins, seem to have no part in filament assembly or stability, as their mutation to alanine was shown to have little effect on normal growth.[101] The accumulation of ROS can lead to disulphide bond formation between the cysteine residues at positions 284 and 374.[99] The formation of a bond between C284 and C374 leads to reduced dynamic capability of the cytoskeleton and this is known to have a devastating effect upon the red blood cells in patients suffering from sickle cell anaemia.[102,103] Oxidative stress has also been shown to influence the dynamic nature of actin in *S. cerevisiae*. Recent research has shown that a disulphide bond is formed between C285 and C374 in response to oxidative stress. Yeasts therefore provide a simple model in which to assess the formation and consequence of oxidatively damaged actin-containing structures. The researchers were also able to show that a protective mechanism exists in yeasts that can shield actin from the ravages of oxidative stress. The oxidoreductase Oye2p, also known as old yellow enzyme, would seem to prevent excessive disulphide bond formation between C285 and C374 in response to oxidative stress.[104] By changing the C285 and C374 residues to alanine, the researchers were able to generate yeast strains that were more resistant to oxidative stress. The ablation of *OYE2* leads to increased sensitivity to oxidative stress and this could be rescued by mutating these exposed cyteines to alanine.[104,105] In line with our previous studies, the stabilization of actin elicited by the loss of Oye2p led to accelerated cell death that showed the hallmark features of apoptosis, including ROS accumulation and DNA fragmentation.[106] As was the case for oxidative

stress, the apoptotic cell death induced by actin stabilization in *Δoye2* cells could be suppressed by C285A and C374A mutations.[106] Interestingly, mutations that lead to an increase in the dynamic nature of the actin cytoskeleton have been shown to lead to reduced levels of ROS.[40] Deletion of *SCP1*, a gene encoding the actin-bundling protein Scp1p, the yeast homologue of mammalian SM22/transgelin that also destabilizes cortical actin structures, reduced ROS levels and led to a significant increase in replicative lifespan.[40] These data suggest that in yeasts, actin dynamics are linked to processes that regulate ROS accumulation as well as being a target of oxidative stress. An interesting possibility is that an actin redox cycle, such as that executed by Oye2p, when taken in consideration with the relative abundance of actin (approximately 1% of total protein), may exert an effect as a buffer against the accumulation of cellular oxidative stress. If this proves to be the case, such a mechanism may have important implications for cellular ageing. Evidence to support this comes from studies analysing the partitioning of oxidative stress damage between mother and daughter cell. Newly emerging daughter cells have been found to possess a greater ROS detoxification capacity than their progenitor mother cells.[107] This is thought to involve an actin-dependent mechanism, and an as-yet-undefined role for the Sirtuin family member, Sir2, a known factor in the regulation of cellular ageing.[108,109] Treatment of dividing cells with the actin-disrupting drug, Latrunculin-A, prevented the protection of newly forming cells from ROS accumulation, implicating actin in this process.[107]

Perspectives

Although actin functions within a number of essential cellular processes, it would also seem to have an active role in the regulation of cellular ageing and apoptosis. At this time, the pathways by which actin functions to regulate cell death in eukaryotic cells are only beginning to be unravelled. Future studies will determine whether conservation exists among eukaryotes with regard to specific actin-regulated cell death signalling mechanisms. We present a model outlining the current data available in yeasts in Figure 4. It has been proposed that the dynamic status of the actin cytoskeleton may exert an effect as a biosensor for cellular health. This idea is certainly worthy of consideration, as actin is not only responsive to the levels of ATP within cells, but also to the levels of oxidative damage. It may be the case that cells, such as those that are aged and whose cytoskeleton can no longer respond to extracellular cues as a result of a reduced dynamic capability, are diverted to an apoptotic cell fate. Such a hypothesis demands that mechanisms must exist that can sense levels of actin damage. The emerging evidence from studies in yeasts would support the existence for such a mechanism. In addition, environmental sensing systems that are linked to cell death may use actin to position or regulate key regulatory factors; evidence has arisen in this with respect to Atg9 and Ras. As the cytoskeleton is a crucial component of trafficking and membrane maintenance systems, it seems likely that more regulatory molecules are influenced by the dynamic nature of actin. Central to the role of actin in apoptosis is its close association with mitochondrial function.

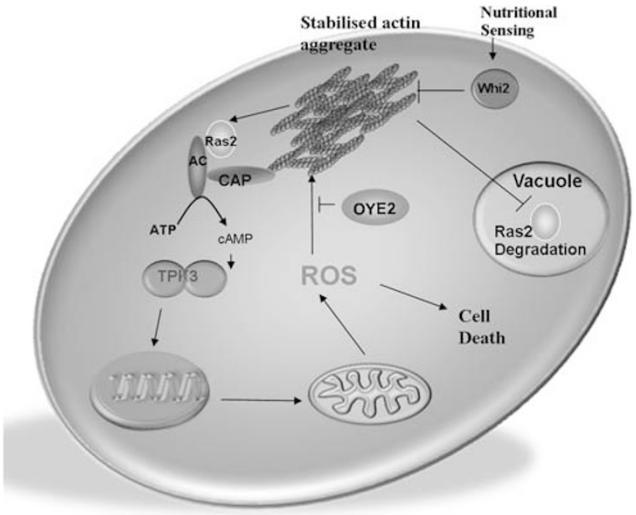

Figure 4 The killer actin cycle in *S. cerevisiae*. Yeast cells that show stabilization of the actin cytoskeleton have been shown to undergo apoptosis through hyperactivation of the Ras/cAMP/PKA signalling pathway. Actin is linked to the sensation of nutritional signals, a candidate molecule for this linkage being Whi2p. The overproduction of cAMP leads to PKA activation and mitochondrial dysfunction that leads to ROS accumulation that seems to function through the Tpk3p subunit. Evidence suggests that this occurs through transcriptional changes that disrupt the electron transport chain homoeostasis which results in massive ROS production. Actin is itself sensitive to the presence of high levels of ROS, and the formation of covalent cross-linkages between exposed cysteines as a result of oxidative stress also leads to the stabilization of F-actin. The stabilization of actin can therefore lead to a cyclical chain of events that culminate in cell death that shows markers of apoptosis

As the mitochondria have been consistently identified as an important factor in many apoptotic pathways, it will also be of great importance to elucidate the precise mechanisms by which actin influences the function of this organelle.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements. We are extremely grateful to Mark Rinnerthaler and Peter Laun from the laboratory of Professor Michael Breitenbach (University of Salzburg), who kindly gifted elutriated yeast cells for this work. This work was sponsored by a Medical Research Council (MRC) career development fellowship to CWG (ref no. 78573).

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