A role for actin in regulating apoptosis/programmed cell death: evidence spanning yeast, plants and animals

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Achieving an understanding of how apoptosis/PCD (programmed cell death) is integrated within cellular responses to environmental and intracellular signals is a daunting task. From the sensation of a stimulus to the point of no return, a programme of cell death must engage specific pro-death components, whose effects can in turn be enhanced or repressed by downstream regulatory factors. In recent years, considerable progress has been made in our understanding of how components involved in these processes function. We now know that some of the factors involved in PCD networks have ancient origins that pre-date multicellularity and, indeed, eukaryotes themselves. A subject attracting much attention is the role that the actin cytoskeleton, itself a cellular component with ancient origins, plays in cell death regulation. Actin, a key cellular component, has an established role as a cellular sensor, with reorganization and alterations in actin dynamics being a well-known consequence of signalling. A range of studies have revealed that actin also plays a key role in apoptosis/PCD regulation. Evidence implicating actin as a regulator of eukaryotic cell death has emerged from studies from the Animal, Plant and Fungal Kingdoms. Here we review recent data that provide evidence for an active, functional role for actin in determining whether PCD is triggered and executed, and discuss these findings within the context of regulation of actin dynamics.

Key words: actin, apoptosis, mitochondrion, programmed cell death (PCD), reactive oxygen species (ROS).

INTRODUCTION

Apoptosis/PCD (programmed cell death)

The fact that cells are selectively executed during normal development has been recognized and revisited a number of times over the last century [1]. However, it was not until the recent discovery of regulators of the most studied form of PCD, apoptosis (reviewed in [2]), that this became a ‘red hot’ field of study. PCD/apoptosis is an essential process in the development and maintenance of multicellular organisms. Although the terminology is increasingly complex and contentious, for simplicity’s sake we will use the term ‘apoptosis’ for animal and yeast studies and PCD in relation to an analogous process in plants, as these are generally accepted and used terms within the respective fields.

Cell death regulation is required during a wide range of processes in animal cells, such as embryogenesis [3], tissue maintenance of somatic cell populations [4] and immune system regulation [5], to name but a few examples. The importance of PCD/apoptosis is highlighted by the fact that de-regulation of apoptosis is associated with a host of diseases such as cancer (reviewed in [6]), autoimmune diseases (reviewed in [7]) and neurodegenerative disorders [8]. In plants, PCD plays a key role during embryogenesis, development and senescence, sculpting organ shape and enabling differentiation of specific cell types. Examples include the formation of xylem vessels, which are highly specialized organs involved in the transport of water and solutes [9], leaf patterning [10], embryogenesis [11], PCD of the aleurome surrounding the seed endosperm during germination [12] and senescence in leaves and flowers [13]. PCD in plants is also triggered in response to biotic and abiotic stimuli, such as pathogens or environmental stresses, where it is responsible for the removal of damaged or infected cells. Examples include UV-C light [14], temperature stress [15], pathogen attack [16,17] and SI (self-incompatibility) ‘self’ interactions [18,19]. In yeast, apoptosis is triggered in response to oxidative stress [20] and ageing [21], a variety of environmental and intracellular triggers [22,23], and plays an important role in the differentiation of colonies [24].

Compelling evidence that PCD and apoptosis have common origins comes from the observation that some of the molecular components of death mechanisms are highly conserved [25,26]. Some genes involved in regulating apoptosis have homologues in plants, such as *Bf-1* (Bax-1 inhibitor gene) [27-29] and *dad1* (Defender Against Death gene) [30,31]. Other genes, such as homologues of the Bcl-family genes, such as *Bcl-2* or *BAX*, are not present in plants or yeast. However, several proteins that regulate apoptosis in animals, such as the BCI2 family of pro- and anti-apoptotic proteins, display the expected function (survival/death) if expressed in plants or yeast (*Saccharomyces cerevisiae*), even though their homologues are not present (see, for example, [14,28,32-36]).

We now know that many of the factors involved in the regulation of apoptosis and PCD have ancient origins and can be found in the genomes of unicellular eukaryotes and prokaryotes, with...
evidence that even the simplest cells have the capacity to be programmed to die [2,26]. For example, at its simplest level, a programme of cell death could arise via the engagement of a single protein, for example a nuclease, which, when diverted from its 'normal' cellular function under certain circumstances, adopts an unregulated and destructive pattern of activity.

Mitochondria and the intrinsic pathway of apoptosis

The mitochondria represent a major force in the apoptotic machinery of eukaryotic cells. Mitochondria are thought to have evolved from a bacterial progenitor as endosymbionts of a primitive eukaryote [37]. A logical progression of this theory follows that modern-day eukaryotes of divergent lineages, but derived from a common ancestor, may exhibit common mitochondrial activity. In support of this notion, the mitochondrial, or intrinsic, pathway of cell death has been observed as an active component of apoptotic/PCD pathways in yeast [38], humans [39] and plants [40,41].

During apoptosis/PCD driven by the intrinsic pathway, the reception of an apoptotic stimulus leads to an increase in the permeability of the mitochondrial outer membrane, allowing pro-apoptotic molecules such as cytochrome c, AIF1 (apoptosis-inducing factor 1) and Endo G (endonuclease G) to be released from the intermembrane space into the cytoplasm. Cytochrome c release from the mitochondria regulates the formation of the apoptosome in mammalian cells. However, although cytochrome c release may play a role in apoptosis in other organisms, such as in the yeast S. cerevisiae [38], apoptosome-like structures are yet to be described outside of mammalian systems. Nevertheless, the conservation of other regulators of mitochondrial function in apoptosis has been clearly demonstrated. For example, AIF is present in all eukaryotes, and both AIF1 and Endo G release from the mitochondria and translocation to the nucleus during H2O2 induced apoptosis occurs during apoptosis in animals [42] and yeast [38,43,44].

The production of ROS (reactive oxygen species) from the electron transport chain of the mitochondria is also a key element of many apoptosis/PCD pathways. Loss of mitochondrial function, involving a decrease of mitochondrial transmembrane potential and ROS production, is a central component of apoptosis/PCD in animals [45], plants [46,47] and yeast [20,38]. ROS are produced as an inevitable by-product of an aerobic metabolism and have themselves been incorporated as important signalling molecules [48]. As ROS accumulation can be damaging, cells regulate their production and removal using powerful antioxidant machinery [45,49]. ROS production and release has also been employed within the mechanisms of cell death, as overproduction, which swamps the cells' antioxidant systems during PCD, leads to cellular damage from which they cannot recover [49].

Thus conservation of the mitochondrial/intrinsic pathway of apoptosis, may go some way to explain why regulated pathways of cell death can be found in highly divergent eukaryotes. However, it is noteworthy that a key downstream component of the mammalian intrinsic pathway, the apoptosome does not appear to be conserved, highlighting the fact that there are also likely to be crucial differences in how these signalling cascades operate.

Caspases

A good example of appropriation into PCD mechanisms lies in the key regulators of apoptosis in animals, the caspases. These proteins, when activated, cleave substrates following an aspartate residue and accelerate the progression of cell death [50]. Interestingly, a number of caspases do not have a role in the execution of apoptosis, whereas others function in both apoptotic and non-apoptotic signalling [50]. This suggests that, in some cases at least, caspases may have been 'hijacked' from their original cellular function to participate within apoptotic pathways. However, at a genetic level, some key differences between apoptosis/PCD in animal cells, yeast and plant cells exist. Although caspases do not appear to be present within the plant or fungal genomes investigated to date, both fungal and plant apoptosis/PCD have been shown to be associated with the induction of caspase-like activities (for examples, see [19,51–53]), and it has been demonstrated that caspase inhibitors can block PCD in plants and yeast [53].

The nature of caspase-like proteases in plant cells is currently the subject of considerable debate. Good candidates have arisen in the discovery of a caspase-related family of proteases, termed the metacaspases, in plant and fungal genomes [54]. Metacaspases structurally and functionally resemble animal caspases [55] and have been identified in yeast, fungi, protozoa and plants [54–57] and their involvement in PCD has been demonstrated. However, they do not cleave authentic caspase-specific substrates [58,59], and there are clearly several further caspase-like activities with activities similar to those found in animal cells (e.g. caspases 1, 3 and 6) identified in plants and yeast for which genes have not been identified. YVADase (caspase-1-like; enzyme cleaving the substrate Tyr-Val-Ala-Asp), VEIDase (caspase-6-like) and DEVDase (caspase-3-like) activities have been relatively well characterized and shown to regulate PCD in plant cells [18,19,60–63]. Recently a LEVDase (caspase-4-like) has been identified [19]. VPEs (vacuolar processing enzymes), which exhibit YVADase activity, represent the first cloned putative caspase-like genes in plants with an established role in PCD [61–64]. Caspase-3-like (DEVDase) activities, which are closest to the key caspase involved in the execution of animal apoptosis, have been identified in several plant systems using fluorescent caspase-3-specific substrates [14,19,66], and DEVD tetrapeptide inhibitors have been shown to alleviate PCD [18,19,51], implicating this type of activity in mediating PCD in several higher-plant systems. However, the gene encoding this caspase-3-like/DEVDase activity remains elusive and, for obvious reasons, its discovery remains a key goal.

The actin cytoskeleton

The actin cytoskeleton plays an essential role in a plethora of cellular functions, including endocytosis, motility, organelle and vesicle trafficking, cytokinesis and signal–response coupling. The appropriation of actin into these processes reflects the plasticity that exists within the systems of assembly and disassembly of filaments, and the range of actin binding and regulatory molecules. In a healthy cell, actin filaments are assembled in a polarized manner with ATP-bound actin monomers preferentially added to fast-growing (+) or barbed ends. Actin possesses a weak intrinsic ATPase activity, and the hydrolysis of ATP to ADP + P, is accompanied by a conformational change that destabilizes the actin filament and promotes monomer dissociation from the slow growing (−) or pointed end. Released ADP-bound actin monomers then undergo nucleotide exchange, which is facilitated by ABPs (actin-binding proteins), enabling re-charged ATP-bound actin to re-enter the cyclical process commonly called 'treadmilling' (for recent reviews, see [67]). A huge number of ABPs have evolved to facilitate and manipulate actin filament formation and turnover, a subject that has been reviewed in detail and will not be discussed here (for recent reviews on the topic, see [67–71]). Some of the central orchestrators of eukaryotic actin assembly are also well conserved. For example, the actin-nucleating Arp2/3 (actin-related protein 2/3) complex has been
shown to regulate the formation of branched filament networks in fungal [70] and animal cells [67]; however, although it is present in plants [72,73], whether it nucleates or makes branched networks has not yet been established. In yeast, Arp2/3 function regulates cortical actin patch assembly [70], whereas in mammalian cells it is required for the branching filamentous networks underlying the leading edge of motile cells [74]. In higher plants the ARP2/3 complex has emerged as a pivotal player in determining cell shape [73], although there are notable exceptions to this rule (see [68]). Other examples of ABPs with conserved functions in filament bundling, severing, capping, cross-linking and monomer sequestration are also evident and beyond the scope of the present review (see [68–70,76] for reviews). ABPs known to play a role in apoptosis in animal cells include gelsolin, coflin/ADF (actin-depolymerization factor), coronin and β-thymosins, and are discussed below.

The regulation of actin assembly and disassembly is under the control of complex signalling systems that link external signals to remodelling events, which result in altered cellular activities that adapt cell shape and/or behaviour to suit new environmental conditions. In the last decade, evidence that actin plays an important role in regulating apoptosis/PCD has emerged. Here we review data from yeast, plants and animal cells, which places actin firmly as a key player at the interface between environmental sensing mechanisms, controlling the cell death decision apparatus in each of these groups, despite their divergence.

**Actin and apoptosis in mammalian cells**

In mammalian cells, shape and organization is facilitated by the cytoskeleton, which typically comprises filamentous networks of actin, microtubule and intermediate filaments. The assembly of actin into higher-order structures in response to environmental cues is co-ordinated members of the Rho family of GTPases, the best studies being Rho, Rac and Cdc42 (cell division cycle 42), which regulate stress fibres, lamellipodia and filopodia formation respectively when activated (reviewed in [77]). These actin-dependent structures are essential for cellular processes such as the regulation of motility, cell shape and plasma-membrane integrity. Another more recently discovered role for actin in mammalian cells lies in the regulation of some of the morphological features associated with apoptosis [78], as well as in the regulation and triggering of apoptosis itself (see below).

**Actin dynamics and apoptosis**

The use of drugs to manipulate actin dynamics has been a useful tool to investigate links between actin and apoptosis signalling cascades. Although the drugs used in these studies are widely accepted to influence the dynamic capability of the actin cytoskeleton, it is worth highlighting the possibility that indirect or uncharacterized actin-independent effects may also occur. As drug treatment that indiscriminantly disrupts actin structures will disrupt a host of cellular processes, the interpretation of such data in isolation must be viewed cautiously. However, results produced from studies involving a number of actin regulatory proteins, as discussed below, support the results generated by the use of actin-disrupting drugs. Taken together, the results of these studies convincingly place actin within pathways that regulate a cells commitment to apoptosis in mammalian cells.

The addition of the F-actin-stabilizing drug Jasp (jasplakinolide) to Jurkat T-cells rapidly induced apoptosis, accompanied by an increase in caspase-3 activation [79,80]. In CTLL-20 cells [an IL-2 (interleukin-2)-dependent T-cell line that shows early commitment to apoptosis after IL-2 removal], Jasp enhanced apoptosis when added during IL-2 deprivation [80]. This could be inhibited by over-expression of the anti-apoptotic protein Bcl-xl, which functions at mitochondria [80]. In MCF10A cells, inhibition of actin depolymerization stimulated apoptosis and provided evidence for a novel role for Bcl-2 in cell death induced by direct disruption of the actin cytoskeleton [81]. In articular chondrocytes, disruption of F-actin by CytD (cytochalasin D) inhibited apoptosis [82]. Actin stabilization by Jasp has also been observed to induce apoptosis in HL-60 cells [83,84] Thus, although the exact mechanisms are unknown, these data suggest that actin stabilization can induce mitochondria-dependent apoptosis (see Figure 1).

The link between apoptosis triggering and actin dynamics is not restricted to scenarios in which F-actin structures are stabilized, as there is also evidence for a similar effect in some animal cells when actin is actively depolymerized (see Figure 1). In T-cells, for example, CytD treatment resulted in elevated caspase-3 activity, suggesting that actin depolymerization can regulate apoptosis [85]. More recently, it has been shown that CytD induced rapid cytochrome c release from mitochondria and consequent caspase activation in murine cell lines [86]. Apoptosis in ishaemic kidney cells, which involves actin depolymerization and can be stimulated by the actin depolymerizing drug LatB (latrunculin B) can be alleviated by Jasp [87]. Phalloidin, another actin-stabilizing drug, with the same binding site as Jasp, also prevents both cisplatin-mediated apoptosis and actin depolymerization in porcine kidney proximal-tubule cells [88]. In Jurkat T-cells undergoing Fas-mediated apoptosis, CytD enhanced the execution phase of apoptosis [89]. As the authors had also shown that Jasp can stimulate apoptosis in this system, they suggested that this might hint that alteration of actin dynamics might be responsible for modulating the apoptotic signal [89]. Thus both actin stabilization and depolymerization can stimulate apoptosis in animal cells.

In addition to the actin stabilization/depolymerization influencing entry into apoptosis debate, there are other apparent ‘anomalies’. In some cases, simply stimulating alterations to F-actin status is sufficient to induce apoptosis, whereas in other cell types, altering F-actin dynamics can only influence apoptosis if it has already been stimulated. These data argue that actin may influence apoptosis by different mechanisms in differing cell types. However, it is possible that it is the alteration of actin dynamics (i.e. the rate of actin polymerization/depolymerization) that modulates the signalling to apoptosis, with alterations to actin providing the sensory mechanism, involving either changes in polymer levels, changes in the flux of actin through the filament pool, or both [66]. One striking (and probably important) difference between different cell types is the ratio of G-actin to F-actin, where it has been measured. In budding yeast S. cerevisiae, the majority of the total actin pool is considered to be in the F-actin form [90]. By contrast, pollen from plants has high ratio of G- to F-actin, with as little as 5–10% of the total actin protein in the filamentous form [91,92]. Similarly high levels of G-actin are maintained under normal growth conditions in mammalian cells where this has been assessed [93]. Thus the endogenous balance between monomer and polymer in different eukaryotic cells is likely to vary considerably, depending on function and status, and this will reflect the nature of actin dynamics and their regulation in different cell types and species. Interestingly, actin is a known target of caspase activities during apoptosis, leading to the production of actin N-terminal 32 kDa (Fra31) and 15 kDa (Fra15) fragments. After cleavage, F-actin can undergo N-myristoylation, which targets it to mitochondria [94]. Moreover, expression of Tactin, but not Fra31, is sufficient to trigger morphological changes resembling those observed in apoptotic cells [95].
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Figure 1 Model outlining links between actin dynamics and apoptotic signalling in animal cells

Actin has been demonstrated to play a role at multiple stages of apoptosis in mammalian cells. Clustering of the CD95/Fas and CD44 death receptors has been shown to require actin; this action is important to elicit a full apoptosis response upon stimulation of these death receptors. Treatment of mammalian cells with actin-disrupting drugs such as Jaspl and CytD has been shown to affect the sensitivity of a number of different cell types to apoptotic stimuli. The regulation of actin by accessory proteins also plays an important role in the apoptosis response in mammalian cells. Actin monomer concentration, as regulated by the thymosins and the promotion of dynamic actin filaments by gelsolin, cofilin and coronin 1 have all been convincingly shown to regulate the commitment to apoptosis in response to stimuli. Both cofilin and gelsolin are known to regulate mitochondrial influence upon the commitment to apoptosis, probably by influencing membrane permambility via the VDAC. Mice lacking coronin 1, a haematopoietic specific isoform of coronin, display elevated levels of cell death displaying markers of apoptosis in cells of this lineage. After the initiation of apoptosis, membrane blebbing and the formation of apoptotic bodies is facilitated by the cytoskeleton, and actin is a key component in the manipulation of the plasma membrane required to elicit these phenotypes.

Actin regulatory proteins and apoptosis

A tightly controlled dynamic equilibrium between monomeric G-actin and F-actin exists within cells. An important part of this regulation lies within the activity of a number of actin monomer sequestration and regulatory proteins. Recent data suggests that the activity of actin regulatory proteins such as gelsolin, cofilin/ADF, coronin and β-thymosins, play a crucial role in the regulation of apoptosis in animal cells. The fact that an ever-increasing list of actin regulators are implicated in apoptosis regulation (see below) reflects the importance of cytoskeletal control in maintaining cellular homoeostasis in response to environmental change.

Gelsolin

Gelsolin is a member of the gelsolin superfamily, a conserved family defined by the presence of the gelsolin-like domain. Gelsolin itself is an ABP capable of severing and capping actin filaments in a manner which is responsive to changes in Ca\(^{2+}\), intracellular pH, tyrosine phosphorylation and phosphoinositides in vivo [96,97]. Of the seven members of this protein family, which includes adseverin, CapG, villin, advillin, supervillin and flightless, only gelsolin has been shown to play a role in the regulation of apoptosis to date. The overexpression of gelsolin has been demonstrated to inhibit the induction of apoptosis in Jurkat cells [98], most likely by blocking the loss of mitochondrial membrane potential. In another study the overexpression of gelsolin in HeLa cells, which do not normally produce gelsolin, led to an increase in susceptibility to apoptosis [99]. It has been proposed that gelsolin protects cells from apoptosis by regulating VDACs (voltage-dependent anion channels), mitochondrial membrane pores that regulate the release of pro-apoptotic factors and that are important for maintaining mitochondrial membrane potential [100], via an actin-regulatory mechanism (see Figure 1; [101]). One possibility is that a gelsolin–PtdInsP\(_{2}\) complex has the capacity to inhibit caspase-3 activity and so influence the execution phase of apoptosis [102]. The anti-apoptotic activity of gelsolin may also operate at the level of the mitochondria via a proposed role in the regulation of the VDAC. The overexpression of gelsolin in Jurkat cells was also found to impart resistance to Vpr (HIV viral protein R)-induced apoptosis [103]. This study and a previous study by the same author [104] showed that a specific C-terminally located domain of gelsolin, the G5 segment, was required for the anti-apoptotic effect. It was proposed that the G5 domain bound to the mitochondrial VDAC and prevented Vpr-induced loss of mitochondrial membrane potential [103,104]. A recent report convincingly demonstrated that gelsolin has an
anti-apoptotic pro-survival role in pancreatic β-cells [105]. Yermen and colleagues were able to demonstrate both a decrease in apoptosis in gelsolin-overexpressing primary mouse β-cells and an increase in apoptosis when gelsolin levels were depleted by RNA interference [105]. It has also been suggested that a newly established N-RAS (neuroblastoma RAS viral oncogene homologue)–gelsolin complex may play a role in the promotion of cell survival [106]. Interestingly gelsolin-knockout mice are more susceptible to brain injury following ischaemia [107], an effect that has been attributed to the gelsolin/actin-mediated regulation of Ca2+ channels. As the regulation of Ca2+ is known to play an important part in the regulation of apoptosis, it is tempting to speculate that a loss of apoptosis/PCD regulation may play a part in the reduced ability of gelsolin knockout mice to recover from injury.

Gelsolin is targeted and cleaved by caspases during apoptosis to yield an N-terminal product [99]. This accelerates apoptosis-associated morphological changes by severing actin filaments in an unregulated manner [99]. A recent in vitro study provided evidence that the pro-apoptotic N-terminal gelsolin fragment can compete or inhibit DNase 1 (a well known G-ABP and one of the principle nucleases involved in nuclear fragmentation during apoptosis) binding to actin [108]. The authors proposed an interesting positive-feedback mechanism, suggesting that the cleavage of actin and gelsolin by caspase activity during apoptosis will promote terminal DNA destruction, as DNase 1 is released from actin and activated [108]. Gelsolin is cleaved during apoptosis to yield both the pro-apoptotic N-terminal fragment and a C-terminal fragment (tGelsolin) that is N-myristylated and exhibits anti-apoptotic activities [101,109]. Although tGelsolin is N-myristylated, this post-translational modification was not found to target the C-terminal fragment to the mitochondria, as is the case for the N-myristylated tActin fragment also generated by caspase cleavage during apoptosis [94,109]. The authors established that overexpression of tGelsolin significantly reduced apoptosis when COS-1 cells were treated with etoposide in a manner that was dependent on its N-myristylation [109]. Thus it is becoming increasingly apparent that gelsolin is a key regulator of cell survival in mammalian cells, operating at multiple points within the regulation of apoptosis pathways.

Cofilin/ADF

Cofilin is a member of the cofilin/ADF family. It promotes the depolymerization and severing of actin filaments, and is involved in the recycling of the G-actin monomers [110,111]. The active (dephosphorylated) form of cofilin has been shown to be targeted to mitochondria after initiation of apoptosis [112]. A mitochondrial-targeted cofilin was able induce cytochrome c leakage and trigger apoptosis, an activity that requires the actin-binding domain of cofilin [112]. The association of cofilin and its effects on mitochondrial function have been further highlighted by recent work from the laboratory of James Bamberg on a stress-induced structure called the ‘ADF/cofilin rod’ [113,114]. ADF/cofilin rods are actin-containing structures that rapidly form in response to stresses, leading to ATP depletion as a result of increased phosphorylation of cofilin [113,114]. Cofilin rods have been shown to associate with mitochondria under conditions of stress and elicit a transient protective role in primary hippocampal neurons [114,115]. However, the persistence of ADF/cofilin rods was also shown to have a detrimental effect on mitochondrial function and to cause a loss of membrane potential [114,115]. This research is highly significant in the light of the role actin and cofilin have in apoptosis regulation, as actin-rich aggregates, such as Hirano bodies and ADF/cofilin rods, are associated with, and implicated in, many diseases (reviewed in [116]). Interestingly, a recent study in which β-actin was knocked down in HeLa cells using short interfering RNA, revealed a 2–5-fold increase in the level of phosphorylated cofilin [117]. However, cell death in HeLa cell cultures was not affected by β-actin knockdown in this study [117]. It may be the case that, as mentioned above, alterations to the actin cytoskeleton itself, or to key regulatory proteins such as cofilin, may not be sufficient to induce apoptosis in certain cell lines, or that additional initiating stimuli are required.

Coronin

Coronin is an important regulator of the evolutionarily conserved Arp2/3 complex, an important component of actin filament networks [67,68,70,72]. Arp2/3 filament nucleation activity is regulated by several proteins, one of which is coronin [118,119]. Of the seven mammalian coronin family members, coronin 1 is preferentially expressed in cells of haematopoietic origin, where it is co-expressed with other members (2, 3 and 7) [119]. A recent study in which coronin-1-knockout mice were generated suggested that coronin 1 exerted an inhibitory effect on cellular steady-state F-actin formation via an Arp2/3-dependent mechanism [120] (see Figure 1). The authors found that coronin 1 was also required for chemokine-mediated migration. Interestingly, actin dynamics, through a mitochondrial pathway, were linked to lymphocyte homoeostasis in this study. T-cells from the knockout mice had increased levels of annexin V (an indicator of apoptosis)-positive CD4+ and CD8+ thymocytes. An increased rate of in vivo apoptosis associated with caspase-3 and -9 cleavage, which could be reversed by caspase inhibitors, was also observed in these cells. Elevated cytochrome c levels in the cytoplasm and reduced mitochondrial membrane potential were also noted in coronin-knockout T-cells (see Figure 1). Moreover, actin stabilization was also linked to apoptosis, as it could be triggered in T-cells treated with Jasp [120] (see Figure 1).

β-Thymosins

A further example of the importance of regulation of the actin pool in the regulation of apoptosis has emerged from studies of the regulation of actin monomer sequestration by β-thymosin [121,122]. The β-thymosins are a conserved family of 5 kDa polypeptides that specifically bind monomeric G-actin, preventing its incorporation into filaments [123]. A key observation was that the overexpression of TB10 (thymosin B10) in ovarian tumour cells increased the rate of cell death [122]. TB10 and actin share the same binding site on E-Tmod (E-tropomodulin), a pointed-end actin-capping protein that modulates the length of the actin filament. Overexpression of E-Tmod was shown to block TB10-dependent apoptotic activity, supporting the hypothesis that a dynamic equilibrium among pools of actin, TB10 and E-Tmod regulates an apoptotic homoeostasis [122].

Signalling systems, actin and apoptosis

Actin and Fas-mediated apoptosis

The binding of a Fas ligand to the CD95 (APO-1/Fas) death receptor leads to clustering, subsequent DISC (death-inducing signalling complex) assembly and initiation of apoptosis (see Figure 1, where we attempt to draw together the links between actin and apoptosis in mammalian cells). The DISC comprises an adaptor molecule, FADD (Fas-associated death domain), and the initiator caspase 8 [124]. Two types of cells have been described in terms of CD95/Fas-induced apoptotic signalling. In cells classified as type 1, Fas activation leads to caspase 8.
recruitment to the DISC in quantities that result in the activation of caspase 3, resulting in an apoptotic response that does not require mitochondrial function [125]. In type II cells, however, DISC formation is less efficient, and small quantities of active caspase 8 are produced. In this case, the mitochondria are required to elicit a complete cell-death response [126–128].

A number of studies have highlighted the importance of actin filaments in regulating the susceptibility to stimuli that induce CD95/Fas-induced apoptosis [129–134]. For example, the disruption of actin filaments was shown to inhibit Fas-induced apoptosis in type I cells [129], as a result of DISC formation inhibition and reduction of caspase-8 activation [129]. Actin was also found to regulate Fas-induced apoptosis in activated human T-lymphocytes [131,132], an effect mediated by ezrin, a member of the ERM (ezrin–radixin–moesin) family [131,133]. Further evidence for the role of actin in Fas-mediated apoptosis has come from a recent study investigating the CD44–ezrin complex [135,136]. The transmembrane receptor CD44 also conveys extracellular signals to within the cell and binds to ezrin. Of the several isoforms of CD44 that exist, only CD44s (the CD44 standard form) modulates Fas-mediated apoptosis, forming a complex with ezrin and actin (see Figure 1). The authors suggested that the interaction between the cytoplasmic domain of CD44s and ezrin and the consequent organization of the actin cytoskeleton may function as a scaffold, which stabilizes DISC formation, thereby enhancing apoptotic sensitivity. As well as functioning within the process of DISC formation, actin is an important part of the endocytotic machinery that internalizes Fas receptor after induction [137].

Actin may not play such a major role in type II Fas-mediated apoptosis, as actin depolymerization did not affect CD95/Fas-induced apoptosis in a type II Jurkat cell line [89]. However, cytokine-withdrawal-induced apoptosis was enhanced in the same cell line when actin polymerization was inhibited [89]. In addition, disruption of actin by CytD or LatA reduced Fas-induced apoptosis of HIV-specific CD8+ T-cells [138]. CD95/Fas-induced apoptosis of HIV-specific CD8+ T-cells is regulated by mitochondria-related factors [139,140], which is recognized as a feature of type II cells. This adds weight to the idea that actin plays an important role in Fas-mediated apoptosis in both type I and type II cells.

**Actin, plasma-membrane stability and apoptosis**

Upon initiation of apoptosis, the cortical cytoskeleton is targeted by caspase activity (summarized in [78]), leading to a reduction in plasma-membrane integrity and blebbing (irregular bulges in the plasma membrane formed during apoptosis). Actin-dependent blebbing is promoted by myosin II-based contractile activity [141,142], which occurs as a result of the cleavage of ROCK1 (Rho-associated coiled-coil containing protein kinase 1), subsequent phosphorylation of its light chain MLC (myosin light chain), and activation of myosin II contractility [141,142]. The activity of caspase-cleaved ROCK I and a constitutively active MLC is necessary and sufficient for membrane blebbing [141–144]. The regulation of MLC phosphorylation during apoptosis has also been shown to involve the association of Par-4 (prostate apoptosis response-4), a protein known to regulate cellular response to apoptotic stimuli, with actin [145,146]. Deregulated expression of Par-4 has been observed to occur in a variety of tumour types [147,148], and it has been reported that down-regulation of Par-4 is essential for Ras-induced tumour progression [148]. Disruption of the actin cytoskeleton with CytD led to a significant decrease in Par-4-mediated apoptosis in rat fibroblasts [145,146]. Par-4 also recruits Dlk [DAP (death-associated protein)-like kinase], which belongs to the novel subfamily of DAP kinases, from the nucleus to the cytoplasm, and particularly to actin filaments [146,149]. Par-4-mediated recruitment of Dlk leads to enhanced phosphorylation of MLC and dramatic cytoskeletal rearrangements [146]. Par-4 has also been shown to interact with the Amida protein (named after Amida, a Buddhist god), identified as an interaction partner of Arc (activity-regulated cytoskeleton-associated protein) [150]. Co-expression of Par-4 and Amida led to the movement of Amida from the nucleus to cytoplasmic actin filaments, an increased MLC phosphorylation and enhanced induction of apoptosis. These studies highlight the importance of Par-4 recruitment of regulatory nuclear proteins to the actin filament system and suggest a mechanism through which apoptosis is triggered.

Together, these findings strongly implicate regulation of actin dynamics in playing a critical decision-making role in deciding whether cells go into apoptosis in animal cells. It seems likely that other ABPs responsible for regulating actin dynamics may also be involved in triggering apoptosis. Moreover, as a variety of cells exhibit this phenomenon, it is possible that this may be a widespread or universal feature.

**ACTIN AND APOPTOSIS IN YEAST**

Yeast cells have been shown to undergo regulated cell death that displays a number of the hallmarks of apoptosis, including DNA fragmentation, annexin V exposure, ROS build-up and the loss of mitochondrial membrane potential [53]. Apoptosis in the budding yeast *S. cerevisiae* can be triggered by a variety of exogenous stimuli or cellular dysfunctions, indicating that pathways that lead to death exist in these simple eukaryotes [38,151]. Importantly, it has been shown that, as has been found in many mammalian cells, the stabilization of cortical actin structures induces apoptosis in yeast [152]. Mutations in actin regulatory proteins that lead to the accumulation of aggregates of stabilized F-actin have been shown to trigger a process termed ‘actin mediated apoptosis’ (ActMAp) [152–154]. In actin-aggregating cell lines, ActMAp leads to a loss of mitochondrial membrane potential and the production and release of ROS into the cell which results in an apoptotic cell death (see Figure 2, where we attempt to draw together the links between actin and apoptosis in yeast). Interestingly, mutations that lead to an increase in the dynamic nature of the actin cytoskeleton were shown to result in reduced levels of ROS [152]. In addition, deletion of a gene encoding the actin bundling protein Scp1p, the yeast homologue of mammalian SM22/transgelin, which also destabilizes cortical actin structures, reduced ROS levels and led to a significant increase in replicative lifespan [152]. These data suggest that, in yeast, actin dynamics are linked to processes that regulate mitochondrial function and ROS regulation.

From the evidence available to date, ActMAp in yeast occurs as a result of an interaction that exists between the actin cytoskeleton and a key signalling pathway called the ‘Ras–cAMP–PKA (protein kinase A) cascade’ (see Figure 2). This signalling pathway is required for the co-ordination of cell growth and proliferation in response to the nutritional environment [155,156]. In yeast the regulation of Ras activity, like mammalian Ras, links extracellular signals to proliferation and growth [157]. When yeast cells experience nutritional depletion, the Ras–cAMP–PKA pathway activity is down-regulated, allowing effective cell-cycle exit and initiation of the cellular stress response. The Ras–cAMP–PKA pathway is linked to mitochondrial activity, as cells expressing the constitutively active ras<sup>11012al49</sup> allele exhibit elevated ROS levels [158,159]. Mutants that accumulate aggregates of F-actin were shown to trigger hyperactivity of the Ras–cAMP–PKA signalling pathway, which is responsible for the mitochondrial dysfunction...
Stabilization of the actin cytoskeleton, by treatment with drugs such as Jaspl or using strains carrying specific mutations, has been shown to lead to the apoptosis in the budding yeast S. cerevisiae. In actin-stabilized cells an inappropriate hyperactivation of the Ras–cAMP–PKA signalling cascade is observed when cells attempt to arrest their growth in the face of environmental stress. This results in severe mitochondrial dysfunction characterized by the loss of mitochondrial membrane potential and the production of ROS at a level capable of killing the cell. Actin is linked to cAMP signalling via the cyclase-associated protein Srv2p/CAP, which acts as a regulator of both actin dynamics and Ras–cAMP–PKA signalling. A consequence of ROS production is that the actin cytoskeleton can be further stabilized as a result of disulfide linkage between cysteine residues present within actin. Under normal circumstances the oxidoreductase, Oye2p, prevents actin stabilization under conditions of oxidative stress, and in this capacity possesses anti-apoptotic properties.

The formation of aberrant aggregations of F-actin was dependent on the activity of the protein Srv2p (suppressor of RasVal19)/CAP (cyclase-associated protein) [152]. Srv2p/CAP is a highly conserved actin regulatory protein that binds preferentially to ADP–G-actin via its C-terminal domain [160,161] and can associate with actin filaments through an interaction between a proline region and the SH3 domain of Abp1p (ABP 1) [162]. Srv2p/CAP is an attractive candidate to link Ras signalling to actin reorganization, as the N-terminus of Srv2p/CAP can bind to adenylate cyclase (Cyr1p) and facilitate cAMP/PKA activation [163,164]. Actin aggregation that triggered Ras–cAMP–PKA pathway activity was shown to require only the C-terminal actin-binding region of Srv2p/CAP; however, the presence of the N-terminal cyclase-binding domain enhanced PKA stimulation, leading to higher levels of ROS production [152]. The accumulation of ROS and subsequent death in actin-aggregating cells was demonstrated to require the activity of the PKA subunit Tpk3p, an enzyme known to play an important role in mitochondrial function (see Figure 2). The accumulation of ROS and subsequent death in actin-aggregating cells was demonstrated to require the activity of the PKA subunit Tpk3p, an enzyme known to play an important role in mitochondrial function (see Figure 2).

Yeast lacking Tpk3p exhibit altered mitochondrial enzymatic content, including decreased levels and activity of cytochrome c, a constituent of the electron-transport chain [165]. There is also evidence that the activity of Tpk3p within mitochondria can control transcription of mitochondrially encoded genes in a cAMP-dependent manner, by phosphorylation of mitochondria-located target proteins [166,167]. One possibility is that PKA activity is compartmentalized in yeast. This has been widely reported in higher eukaryotes, where PKA is sequestered to particular locations and cellular compartments by AKAPs (A-kinase anchoring proteins) [168].

Ras signalling may be a conserved mechanism by which yeasts are able to regulate cell death. Evidence to support this comes from data showing that the pathogenic yeast Candida albicans has also been shown to regulate apoptosis via Ras/cAMP signalling [169,170]. The significance of these findings also reinforces the potential for the study of yeast apoptosis within the field of medical microbiology, with respects to the development of new approaches to anti-fungal therapeutics.

Actin and mitochondrial regulation in yeast

The mitochondria are well established as important regulators of apoptosis/PCD [38,171]. The actin cytoskeleton has been demonstrated to be an important factor in the movement, deployment and function of mitochondria in a variety of systems [172]. Examples of this include the use of the actin cytoskeleton to ensure the concentration of mitochondria in areas of cells with high energy demands [173] and their correct distribution during cell division.
The mitochondrial network consists of a dynamic tubular network whose distribution throughout unicellular and multicellular organisms is tightly controlled. The maintenance of an appropriate balance between mitochondrial fission and fusion events is known to be an important factor in the regulation of PCD in yeast. The promotion of fragmentation, by the fission protein Dnm1 (dynamin 1), is a crucial element in the execution of apoptosis when yeasts are subjected to acetic acid or H$_2$O$_2$ treatment [175]. In *S. cerevisiae*, the mitochondria have been shown to physically interact with parallel bundles of F-actin cables. This occurs via a protein complex known as the mitochrome, which consists of three integral mitochondrial-outer-membrane proteins Mmm1p, Mdm10p and Mdm12p [176,177]. The force generated by the power of actin polymerization within cortical actin patches promotes mitochondrial movement through the cell [178,179]. The type V myosin Myo2 is also required to facilitate stable mitochondrial inheritance into newly emerging daughter cells [178,179].

Evidence to support the strong interaction between actin and the mitochondria comes from the use of either actin-depolymerizing drugs such as LatA [179] or actin mutants [180]. Both studies demonstrated that actin disruption leads to aberrant mitochondrial morphology and distribution. A connection between actin dynamics, mitochondrial function and the regulation of cell death came from studies using yeast cells expressing the actin allele *act1-159* [152]. Cells expressing *act1-159* form filaments that depolymerize slowly as a result of a failure to undergo a conformational change after P$_r$ release [181]. Under conditions of environmental stress, *act1-159*-expressing cells undergo mitochondrial-dependent apoptosis, characterized by the accumulation of high levels of intracellular ROS [152]. The relationship between actin dynamics, ROS production and the mitochondria was further highlighted by the fact that cells carrying the *act1-157* allele, which leads to increases in the dynamic capability of the cytoskeleton, exhibited reduced levels of ROS during growth [152].

The permeability of mitochondrial membranes is an important feature of apoptosis in yeast and other eukaryotes and is regulated in part by the VDAC [182]. Increasing evidence suggests that the actin cytoskeleton, and its accessory proteins, can influence VDAC function and so influence apoptotic pathway regulation. In the filamentous fungus *Neurospora crassa*, monomeric actin has been shown to bind to, and modulate, VDAC gating [183]. G-actin binding was found to significantly reduce the VDAC pore’s conductance, reducing metabolic flux across the mitochondrial membrane [183]. In addition, an interaction between G-actin and the VDAC was demonstrated using surface plasmon resonance, which showed physiologically relevant dose-dependent reversible binding between the *S. cerevisiae* VDAC and rabbit G-actin proteins [184]. Although the physiological relevance of actin binding to VDAC has not been demonstrated, it may be that this interaction contributes to VDAC channel regulation and mitochondrial membrane permability, with actin acting as a stabilizing molecule to maintain the closed conformation. The fact that actin regulatory proteins, such as coflin and gelsolin, are thought to influence apoptosis via an actin-dependent interaction with the VDAC in mammalian systems (discussed above) suggests that such a mechanism may be conserved within eukaryotes.

**Actin and oxidative stress in yeast: mechanisms of protection**

It has been known for some time that the actin cytoskeleton is sensitive to the oxidative status of cells [185]. A disulfide bond may be formed, under conditions of elevated ROS, between cysteine residues at positions 284 and 373 of actin [185–187]. The formation of such bonds is an important factor in the reduced flexibility observed in red blood cells from patients suffering from sickle-cell anaemia as a result of a decrease in the dynamic capability of the cytoskeleton [188,189]. The high degree of conservation observed for actin means that a similar disulfide bond can be formed in *S. cerevisiae* in response to oxidative stress. This promotes yeast as an attractive model with which to study the mechanisms involved in, and the effects of, actin stabilization as a consequence of oxidative stress (see Figure 2). A recent study has found that the oxidoreductase Oye2p, which is also known as OYE (‘old yellow enzyme’), serves to regulate oxidation between Cys285 and Cys374 on yeast actin [190]. The authors proposed that OYE can oppose the damaging effects of ROS by preventing disulfide-bond formation between these two cysteine residues [190]. Mutants expressing an *ACT1* gene in which Cys285 and Cys374 were replaced with the uncharged residue alanine were shown to be more resistant to oxidative stress than wild-type strains. In addition, these actin alleles could suppress the oxidative sensitivity displayed by cells lacking the OYE2 gene. As the literature points to a general phenomenon whereby stabilization of the actin cytoskeleton increases the likelihood of apoptotic cell death, the authors investigated whether the OYE2 actin protection mechanisms also protected cells from apoptosis [191]. As was expected, cells lacking OYE2 died, showing markers of apoptosis such as ROS accumulation and DNA fragmentation [191] which could be suppressed by the C284A and C374A mutations. These data add weight to the growing evidence that the dynamic nature of the actin cytoskeleton is tightly linked to the regulation of apoptosis in yeast cells.

**ACTIN AND PCD IN PLANTS**

There is considerable evidence that plants use the actin cytoskeleton as a biosensor to monitor the environment, translating this into alterations in polymerization status, which result in changes in morphogenesis (see [192]). Plants probably also use actin as a sensor to monitor stress, translating this into alterations in polymerization status, which, if large enough and held for a long enough period of time, triggers PCD. Figure 3 provides a model summarizing the evidence relating to the involvement of ROS, changes to actin dynamics and PCD. As this topic has not been reviewed before, we discuss specific examples in some detail below. Notably, as mentioned above, although there is extensive evidence that ROS plays a key role in PCD in plants, in contrast with the undisputed link in yeast and animal cells, there appears to be no existing evidence in the literature indicating a possible link between ROS and actin (see the right-hand side of Figure 3). This is likely to be due to a lack of investigation rather than negative evidence. This will be an interesting area to follow in the next few years, as it would be surprising, given the other similarities between these systems, if there were no involvement.

**Actin, PCD and plant–pathogen interactions in plants**

A classic example of PCD is the response of plants to pathogens. The plant HR (hypersensitive response) represents a rapid PCD that is initiated by the plant in response to pathogen attack. The HR occurs in a limited area around the pathogen entry site and often goes hand-in-hand with the activation of disease resistance. This has been intensely studied, and some of the most important findings relating to PCD execution have been established in this system. The reader is referred to [16,17,47,193] for reviews of some aspects of this huge topic.

Plant responses to pathogen attack are known to involve major reorganization of the actin cytoskeleton, with actin filaments bundling and aligning close to the site of infection [194–197]. A
Figure 3 Model outlining alterations to actin dynamics and ROS involvement in PCD in plants

A range of stimuli are known to trigger PCD in plants. This is known to involve mitochondria and ROS production. This is shown to the right of the Figure. Several key second messengers involved in signalling to PCD include Ca^{2+}, ROS and NO. Other components (not shown) include lipids, such as PtdA, are also known to act as signals involved in mediating HR and PCD; PtdA has been shown to decrease H_2O_2-promoted PCD [227]. Ceramides, important second messengers involved in signalling to apoptosis in animal cells, have recently been found also to play a role in signalling to PCD in plant cells [231,232]. Several of these components co-operate with ROS and/or NO to mediate PCD. See [47,192,233,234] for recent reviews. Early common PCD events in plants include changes to the mitochondria, such as loss of mitochondrial membrane potential (Δψ_m), cytochrome c leakage and increases in ROS. Although plants use ROS to regulate PCD, very little is currently understood about how ROS interacts with other signalling molecules during PCD. Crucially, whether this pathway interacts with actin (as in yeast and mammalian cells; see Figures 1 and 2) in plant cells is not known. Thus this pathway is depicted as separate from actin dynamics known to lead to PCD. However, recent data show a link between hexokinase (associated with mitochondria) and PCD [222]. Since association between F-actin and hexokinase has also been reported independently, there may be a link between these pathways. Involvement of F-actin depolymerization in PCD has been shown in several plant systems (shown in the left-hand side of the Figure). Embryogenesis in Picea involved actin depolymerization and PCD; CytD and LatB have been shown to induce PCD in this system [205]. In a cryptogein-induced HR, bistheonellide (Bis), which inhibits actin polymerization, further stimulated cryptogein-induced PCD [202]. In the Papaver pollen, SI stimulates actin depolymerization and, later, actin stabilization. Both actin depolymerization, using LatB, and stabilization, using Jasp, were sufficient to trigger caspase-like activities and PCD in pollen. Moreover, inhibiting SI-stimulated actin alterations using Jasp gave a significant alleviation of SI-induced PCD in incompatible pollen [66]. Mimicking SI-stimulated actin depolymerization by using LatB in combination with Jasp also alleviated PCD, providing firm evidence for actin polymerization status being crucial to initiating PCD in pollen. In NHR, although there is no direct link between actin and PCD, there is evidence that actin stabilization is involved in mediating preventing pathogen infection; CytB and CytE, which depolymerize actin, result in reduced resistance/increased infection in several systems. Though direct evidence linking phosphatidic acid to actin and PCD are lacking to date, PA triggers actin stabilization [228] and is known to signal to PCD, so may play a role in actin-mediated PCD.

Role for the actin cytoskeleton in HR-mediated PCD and NHR (non-host resistance), which provides immunity against many pathogens, has been implicated by several studies. However, generally, actin filaments have been reported to bundle and persist until the onset of HR-mediated PCD. This hints that actin stabilization may play a role in mediating PCD. Although there is no direct link between actin alterations and PCD in this system, evidence for a crucial role of the actin cytoskeleton in cellular defence has been provided by several laboratories. Inhibitors of actin polymerization, such as cytochalasin, which causes depolymerization of actin microfilaments, have been demonstrated to prevent defence responses, allow enhanced penetration efficiency of non-host pathogenic fungi that normally cannot invade the plant. The existing data suggest that alterations in actin polymerization status may be a general mechanism used by plants to mediate PCD (see Figure 3). For example, potato (Solanum tuberosum) cells treated with CytB resulted in suppression of HR cell death triggered by the potato-blight fungus Phytophthora infestans [198], and the incidence of PCD in cowpea (Vigna unguiculata) by cowpea rust fungus (Uromyces phaseoli) infection was significantly reduced by CytE [195]. These data first suggested an involvement of alterations to the actin cytoskeleton in HR-mediated PCD and imply a possible causal relationship between the changes in actin cytoskeletal dynamics/organization and defence against fungal pathogens utilizing the PCD mechanism. However, later studies suggest that actin dynamics is not involved in R (race-specific resistance)-gene-mediated resistance in barley (Hordeum vulgare) [199].

There is considerably more evidence that the actin cytoskeleton is involved in NHR. Treatment of barley with cytochalasins significantly increased penetration of the non-pathogen [200]. Several other studies have recently added further information and, together, they provide compelling evidence for a role for actin dynamics in NHR. For example, a decrease in NHR was observed...
following treatment of *Arabidopsis* (thale cress) plants with CytE, and in *eds1* mutants, which are defective in host resistance. Use of CytE treatment to disrupt the actin cytoktoskeleton resulted in severely compromised NHR in *Arabidopsis* infected with wheat powdery mildew (*Blumeria graminis*), allowing infection [201]. This has helped establish a requirement for actin polymerization for NHR. Also, in *Arabidopsis*, cytE treatment significantly reduced callose papilla formation (a key feature of infection) and allowed pathogen penetration, suggesting that actin depolymerization is important for infection. Similarly, in barley, treatment with either CytE or ectopic expression of ADF in barley epidermal cells gave increased levels of infection [199].

Since actin-depolymerizing drugs generally seem to inhibit PCD and allow greater infectivity by the pathogen, this strongly suggests that actin stabilization (as in yeast) is an important trigger for PCD in some plant systems. However, a recent study of HR-mediated PCD in cryptogeen elicitor-induced tobacco (*Nicotiana*) BY-2 cells showed that the actin polymerization inhibitor bistheonellide significantly accelerated and increased the incidence of PCD [202], suggesting that actin depolymerization can also play a role in HR-PCD (see Figure 3). Together these studies provide strong evidence that the actin cytoskeleton plays a key role in plant–pathogen interactions and resistance. However, focus on what is exactly involved has not been thoroughly pursued to date, although it has been suggested that dynamic disassembly and reassembly of the actin cytoskeleton may play a role as well as its influence in organizing key cellular events in the HR [196]. The recent finding that cryptogeen-induced HR-mediated PCD is enhanced by effectively preventing actin stabilization substantiates this notion that either stabilization or depolymerization (or perhaps gross alterations in actin dynamics) can play important roles in regulating this phenomenon.

**Actin, PCD and embryogenesis in plants**

PCD involving metacaspases [111] plays an important role during embryogenesis in plants. PCD has at least two important functions during this early developmental phase, including elimination of the suspensor (the structure connecting the endosperm to the embryo), which is only required during early development [203] and also in selective embryo abortion [204]. A role for the actin cytoskeleton in PCD in embryogenic tissues in *Picea abies* (Norway spruce) has been implicated. Depolymerization of F-actin, using LatB or CytD, resulted in abnormal embryos, accompanied by a high incidence of cell death and DNA fragmentation [205] (see Figure 3). This provided the first hint that actin depolymerization might play a role in PCD, though the possibility that PCD might be induced indirectly, owing to development being disrupted as a result of cytoksetal collapse, could not be ruled out. The authors suggested that actin may be a negative regulator of PCD in the developing embryo and that preservation of thick longitudinal F-actin cables in the suspensor cells may regulate the timing of PCD-related events.

**Actin, PCD and the SI response**

Pollen–pistil interactions often involve SI, a key mechanism preventing self-fertilization, resulting in inhibition of incompatible pollen-tube growth and consequent inbreeding. Several distinct types of SI have evolved (see [206,207]). SI in *Papaver rhoes* L. (the field poppy) involves PCD of incompatible pollen [18]. SI triggers a Ca²⁺-dependent signalling cascade (see [208] for a review). PCD events include leakage of cytochrome c, dramatic morphological alterations to organelles [209], activation of a mitogen-activated protein kinase [210], activation of DEVDase, VEIDase and LEVDase caspase-like activities [18,19] and, later, DNA fragmentation [18]. In this SI system, extensive and sustained F-actin depolymerization, followed later by a striking stabilization of actin into punctate foci, is stimulated by SI interactions in incompatible pollen [92,211].

A causal link between actin depolymerization and PCD in pollen was established, using pretreatment with the actin-stabilizing drug Jasp prior to SI induction. Counteracting depolymerization in this way resulted in an alleviation of SI-induced PCD in incompatible pollen [66] and implicated SI-induced actin depolymerization in triggering PCD in *Papaver* pollen (see Figure 3). This represents the first report of a specific causal link between actin-polymerization status and initiation of PCD in a plant cell. This was confirmed by using the actin-depolymerizing drug LatB to mimic the levels of depolymerization stimulated by SI, establishing the notion that actin depolymerization is sufficient to trigger PCD (see Figure 3). A similar experiment using Jasp to counteract the effect of LatB-induced depolymerization demonstrated that Jasp could ‘rescue’ pollen from entry into PCD [66]. This demonstrated the involvement of actin polymerization status in initiating PCD in pollen. Surprisingly, it does not seem to be actin depolymerization *per se* that stimulates PCD in this system, as Jasp treatment also triggers PCD. Thus sustained changes to actin filament levels or dynamics appear to play a functional role in initiating PCD in *Papaver* pollen. Moreover, a relatively transient, but substantial, F-actin depolymerization (∼50% reduction for ∼10 min) can trigger PCD, which involves a caspase-3-like activity [66]. Whether the later formation of stabilized actin foci also play a role in regulating PCD is not yet known. However, the fact that actin stabilization can mediate PCD in this system suggests that it may. Moreover, it is well established that, in yeast, actin stabilization triggers PCD. Thus in *Papaver* it is possible that both of these F-actin alterations stimulated by SI play a key role in different phases of the PCD cascade, with sustained depolymerization being followed by reorganization and stabilization of F-actin.

Together, these data imply a key role for the actin cytoskeleton as a sensor of cellular stress in pollen tubes. Although studies have not explored this aspect fully, candidate ABPs that could mediate this event potentially include PrABP80, a candidate SI-mediated ABP that exhibits potent Ca²⁺-dependent severing [212]. *In vitro* kinetic actin polymerization assays showed that PrABP80 has the properties of a gelsolin; MS of PrABP peptides confirmed this identification. It is thought that PrABP80 acts synergistically with profilin to mediate the Ca²⁺-dependent F-actin depolymerization stimulated by SI [212].

Interestingly, SI also triggers very rapid apparent depolymerization of cortical microtubules [213]. Moreover, actin depolymerization triggers apparent microtubule depolymerization [213], and recently obtained results show that, although disruption of microtubule dynamics alone does not trigger PCD, SI-induced PCD is alleviated by taxol. This suggests that signal integration between microfilaments and microtubules is required for triggering of PCD.

*Pyrus* (pear) uses a different SI mechanism, involving S-RNases (S-locus RNases) and F-box proteins as the pistil and pollen determinants respectively (see [206]). Interestingly, dramatic reorganization of the actin cytoskeleton in incompatible *Pyrus* pollen growing *in vitro* has recently been reported to be stimulated by S-RNases [214]. Because of the very different S-components and pollen-inhibition system, it is thought to use a completely different mechanism from *Papaver* to regulate incompatible pollen-tube growth [206,207]. Despite this, the punctate actin foci formed appear rather similar to those observed in the late...
Actin regulation of apoptosis/programmed cell death

Actin, PCD and environmental adaptation

A study on cold acclimation in olive tree (Olea europaea) protoplasts provides hints suggesting a role for changes in the actin cytoskeleton in acclimation-related PCD. Cold shock in non-acclimated wild-type protoplasts resulted in a decrease in actin signal, suggesting depolymerization, whereas after acclimation no cold-shock-induced change in F-actin signal was observed. The authors suggested a role for osmotin in influencing actin cytoskeleton organization, acclimation and PCD [218]. However, in this case it is unlikely that this signals to PCD, as it has been reported that incompatible pollen can be ‘rescued’ when moved on to a compatible stigma [217].

Links between actin, PCD and other components

Relatively little is known about links between the actin cytoskeleton with other components involved in PCD in plants, and this is an avenue that needs to be explored in the future. There are some components that may be involved in linking actin dynamics to PCD. A potential connection between hexokinase, PCD, mitochondria and actin dynamics has been implied by recent findings. Hexokinase is involved in sugar signalling and is involved in many aspects of plant growth and senescence [220]. Virus-induced gene silencing of a plant hexokinase, Hxk1 (hexokinase isozyme 1) in Nicotiana resulted in PCD involving caspase-9- and caspase-3-like proteolytic activities. Moreover, overexpressing HXXI and HXXII resulted in increased resistance to PCD. Interestingly, the Hxk1 was associated with the mitochondria [221,222], and addition of recombinant Hxk1 to mitochondria-enriched fractions prevented cytochrome c release and loss of mitochondrial membrane potential [222]. These data firmly suggest that hexokinase plays a key role in modulating PCD in plant cells. The mitochondrial localization fits nicely with recent findings suggesting a pivotal role for mitochondria-associated hexokinase in the regulation of apoptosis in animal cells by binding with BAD (Bcl-2/Bcl-XL antagonists, causing cell death) [223,224]. Moreover, actin has been shown to interact with mitochondrial VDAC in maize (Zea mays) [225]. Although a direct link between PCD, hexokinase and actin has not been made in plants yet, in other systems, it has been shown that hexokinase interacts with cortical actin filaments and that actin depolymerization prevents hexokinase translocation [226]. Recent data support the idea that this may be the case in plants too, as in Arabidopsis Hxk1 also interacts with F-actin [221]. Moreover, sugar signalling is compromised by disruption of F-actin, and addition of glucose to Arabidopsis seedlings rapidly disrupted F-actin [221]. Interestingly, although hexokinase and actin are functionally diverse proteins, they have a structurally similar ATPase domain with conserved residues. Thus these recent data clearly suggest a possible role for plant hexokinases in actin–depolymerization-mediated PCD (see Figure 3), although the connections remain indirect to date and mechanisms unknown. This certainly makes this an area to watch with respect to the unfolding story of actin–PCD links in plant cells.

A general role for actin alterations mediating PCD in plants?

From the relatively sparse, but emerging, data there seems no doubt that there exists a role for regulation of actin dynamics in regulating PCD in plant cells. Moreover, data suggest that the alterations in actin polymerization status may be a widespread or universal mechanism used by plants to mediate PCD, though details may vary. For example, it appears that, generally in the NHR, actin stabilization is important for regulating PCD, whereas in other systems, such as embryogenesis [205] and the SI response [66], it appears to be actin depolymerization that is the main physiological stimulus triggering PCD in incompatible pollen, though actin stabilization can also stimulate PCD (see Figure 3). Thus stabilization of actin during PCD may operate through this type of mechanism, thus hinting at a further possible involvement of alteration of actin dynamics in signalling to PCD in plant systems.

Perspectives: Actin as a common mechanism regulating apoptosis/PCD in eukaryotes?

The regulation of the cytoskeleton forms an essential link between signal and appropriate response in all eukaryotic cells. The overwhelming evidence suggests that cells from lower to more highly evolved systems have adopted the dynamic state of the
cytoskeleton as an indicator of the cell's overall health. It has been shown that, at least in some cases, actin polymerization status plays a role in mediating PCD, clear differences exist between plants, yeast and animal cells with respect to both components involved in actin and PCD regulation. For example, in yeast, actin stabilization triggers apoptosis [89,152]. In animal cells, depending on the cell type, either actin stabilization or depolymerization (see above) can stimulate apoptosis. In plant cells also, it seems that either depolymerization or stabilization of F-actin can stimulate PCD. Because of these differences, it has been proposed that the dynamics of actin polymerization may be responsible for modulating apoptotic signalling cascades, rather than absolute requirement for one mechanism or the other. This needs further investigation.

The question as to whether core interactions and regulatory mechanisms between cytoskeletal components and pathways that influence apoptosis remains unanswered. However, a strong body of evidence points to a close relationship between the actin and microtubule cytoskeletons and the regulation of mitochondrial function as a possible point of conserved apoptotic regulation. From the available data, the strongest conserved point of regulation may come from the regulation of mitochondrial membrane permeability, a prime candidate being the regulation of the VDAC. Mitochondrially derived apoptosis regulatory factors, such as ROS production, outer membrane permeability and mitochondrial morphology, have all been linked to cytoskeletal function in disparate systems, lending weight to the possibility that conserved regulatory mechanisms exist. Another interesting emerging theme is that aberrant actin formations appear to have detrimental effects upon downstream signalling mechanisms that retard a cell's ability to respond to environmental change.

Although the mechanisms involved will differ markedly in terms of signalling components between organisms, physiologically relevant links between environmental sensing, the actin cytoskeleton and apoptosis/PCD may be present in all eukaryotic systems. Although many actin-binding/regulatory proteins are conserved throughout the eukaryotes, there are notable exceptions known to play a role in apoptosis in mammalian cells that have not been identified in yeast and are not present in the Arabidopsis genome database. Notably, β-thymosin and gelsolin genes/sequences (see the section above relating to these proteins in animal cells) do not appear to be present in plants [75] or yeast, and coronins have not been found in plants. However, others, e.g. cofilin/ADF are present in both yeast and plants. Moreover, although a gelsolin sequence is not found in plants, a gelsolin-like activity has been measured [212]. Thus, it will be of considerable interest to explore whether these proteins or alternative proteins with similar activities also play a similar role in regulating apoptosis/PCD in these organisms, providing evidence for universal mechanisms in controlling apoptosis/PCD.

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