Review

Rho GTPase signalling pathways in the morphological changes associated with apoptosis

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Abstract

The killing and removal of superfluous cells is an important step during embryonic development, tissue homeostasis, wound repair and the resolution of inflammation. A specific sequence of biochemical events leads to a form of cell death termed apoptosis, and ultimately to the disassembly of the dead cell for phagocytosis. Dynamic rearrangements of the actin cytoskeleton are central to the morphological changes observed both in apoptosis and phagocytosis. Recent research has highlighted the importance of Rho GTPase signalling pathways to these changes in cellular architecture. In this review, we will discuss how these signal transduction pathways affect the structure of the actin cytoskeleton and allow for the efficient clearance of apoptotic cells.

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Abbreviations: GDI, GDP-Dissociation Inhibitor; GEF, guanine nucleotide exchange factor; GAP, GTPase accelerating protein; MLC, myosin light chain

Introduction

Apoptosis is an evolutionarily conserved process in multicellular organisms that leads to the death and subsequent removal of redundant or excess cells. In the dying cell, a family of cysteine-proteases called caspases (reviewed in reference¹) are responsible for the 'execution' phase which is characterised by morphological changes, including cell contraction and dynamic membrane blebbing (Figure 1), one of the earliest described and most obvious aspects of apoptotic cell death.² Contractile force generated by actin-myosin II cytoskeletal structures has been implicated as the driving power behind cell contraction and the formation of

membrane blebs and apoptotic bodies (reviewed in reference³). Ultimately, the dead cell is packaged into membrane-clad apoptotic bodies that facilitate uptake by neighbouring cells or by specialised phagocytic cells. Dynamic rearrangements of the actin cytoskeleton in the phagocyte are required for the internalisation of apoptotic cell fragments. Recent research has revealed the importance of signal transduction pathways controlled by Rho family GTPases in regulating the marked changes in cell morphology observed in the processes of apoptosis and phagocytosis.

The Rho GTPases are a family of proteins (RhoA, RhoB, RhoC, RhoD, RhoE/Rnd3, RhoG, RhoH/TTF, Rnd1, Rnd2, Rac1, Rac2, Rac3, Cdc42/G25K, Wrch-1, TC10, TCL, Chp, Rif) that act as molecular switches in intracellular signal transduction pathways (reviewed in reference⁴). Rho activation results from a combination of reduced association with GDP-Dissociation Inhibitors (GDIs) and enhanced exchange of GDP for GTP promoted by Guanine nucleotide Exchange Factors (GEFs). Activated GTP-bound Rho proteins then transduce signals to downstream effector proteins and finally, through association with GTPase Accelerating Proteins (GAPs), return to the inactive GDP-bound form by hydrolysis of the bound GTP (Figure 2). One of the key functions of Rho proteins is to regulate the architecture of the actin cytoskeleton (reviewed in reference⁵). The best characterised proteins of this family are RhoA, which leads to the formation of actin stress fibres and actin-myosin II contractile force generation,^{6,7} Rac1, which promotes the formation of lamellipodia and membrane ruffles⁸ and Cdc42/G25K, which drives the formation of actin-rich filopodia.^{9,10}

One theory that attempted to explain the cell contraction, membrane blebbing and apoptotic body formation observed in apoptotic cells was that caspase-mediated proteolysis of structural and cell adhesion proteins (Table 1) leads to a release from points of cell attachment followed by collapse of the cell. However, numerous lines of evidence have demonstrated that the first phase of cell contraction and membrane blebbing (Figure 1) is a dynamic process associated with, and dependent upon, the presence of filamentous actin,^{11–23} increased myosin light chain (MLC) phosphorylation^{15,16,19,22,24} and myosin ATPase activity.^{15,16} After the initial phase of contraction and blebbing, a second phase of actin filament disassembly occurs via depolymerisation^{18,20} and possibly through caspase-mediated cleavage of actin monomers.^{25–27}

First phase: contraction and blebbing

Given the well-characterised effects of RhoA on promoting actin filament bundling and actin-myosin II contractile force generation, it was proposed that the activation of RhoA is responsible for the contraction observed in apoptotic cells.³ Consistent with this proposal, introduction of a constitutively-

Morphology Actin Cytoskeleton

Figure 1 Changes to cell morphology and actin cytoskeletal structures associated with apoptosis. 1 Normal; 2 Early apoptosis; 3 Blebbing; 4 Late apoptosis; 5 Engulfment of apoptotic bodies by phagocyte

active form of RhoA was shown to be sufficient for cell contraction and membrane blebbing.^{28,29}

Recent research, however, has shown that activation of RhoA is unlikely to be a general mechanism responsible for apoptotic contraction and blebbing. RhoA was not activated in response to a pro-apoptotic stimulus in NIH 3T3 or Swiss 3T3 cells (EA Sahai and MF Olson, unpublished observation and reference²³). In addition, inhibition of Rho with *C. botulinum* C3 toxin did not inhibit membrane blebbing^{23,24} or MLC phosphorylation.²⁴ Signalling downstream of Rho, however, was essential, as pharmacological inhibition of the Rho effector kinase ROCK prevented membrane blebbing in a range of cell types,^{23,24} which was accompanied by diminished MLC phosphorylation.²⁴ Active

ROCK I was shown to be sufficient for cell contraction and membrane blebbing in NIH 3T3 mouse fibroblasts, NIE-115 human neuroblastoma and Jurkat human T cells.^{23,24,30}

Taken together, these data are consistent with increased actin-myosin II contractile force being driven by Rho-independent ROCK activity that results in membrane blebbing during apoptosis.

In collaboration with other Rho effector proteins, ROCK contributes to agonist-induced changes to the actin cytoskeleton without necessarily producing dramatic contraction and blebbing.^{31,32} The ROCK I and ROCK II isoforms bind to Rho-GTP, which activates the ROCK catalytic domain by displacing the carboxy-terminal autoinhibitory domain (Figure 3).^{33–35} Deletion of the inhibitory region increases kinase

activity both *in vitro* and *in vivo*.^{34,36–38} During apoptosis, ROCK I, but not ROCK II, is cleaved by caspase-3 at a conserved sequence that removes the autoinhibitory do-

Figure 2 Regulation of Rho GTPase cycle. Cycling of Rho GTPase between inactive GDP-bound and active-GTP bound states. GDI, GDP-Dissociation Inhibitor; GEF, Guanine nucleotide Exchange Factor; GAP, GTPase Accelerating Protein

main.^{23,24} The truncated kinase has an eightfold higher specific activity *in vitro* relative to full-length protein in the absence of Rho.²³ The enhanced kinase activity is sufficient to drive caspase-independent cell contraction and membrane blebbing (Figure 3),^{23,24} consistent with a direct effect of ROCK on the development of the apoptotic morphology.

ROCK activation positively contributes to actin-myosin force generation through the phosphorylation of a number of downstream target proteins (Figure 3). ROCK-dependent activation of LIM kinases-1 and -2^{39–42} results in phosphorylation and inactivation of the actin-severing protein cofilin, thereby stabilising filamentous actin. ROCK also directly phosphorylates MLC,^{43–45} and phosphorylates and consequently inhibits MLC phosphatase.^{46,47} ROCK phosphorylation of calponin⁴⁸ and CPI-17⁴⁹ relieves their inhibition of myosin ATPase activity thereby promoting increased actin-myosin contractile force. Taken together, ROCK activation leads to a concerted series of events that promotes actin filament stabilisation, increased interaction with myosin and contractile force generation, which together drive cell contraction.

Membrane blebbing occurs where the strength of interactions that tether the plasma membrane to cytoskeletal structures is exceeded by the hydrodynamic force within the cell.^{50,51} Application of negative pressure to the exterior of cells allows bleb protrusion powered by the positive interior pressure,⁵² indicating that a pressure gradient between inside and outside of a cell is sufficient for blebbing. Alternatively, a reduction in the strength of interaction between the plasma membrane and the cytoskeleton may promote membrane blebbing, for example melanoma cells deficient in filamin, which links actin filaments to plasma membrane-associated proteins, bleb continuously.⁵³ During apoptosis, caspase-mediated proteolysis of structural and

Table 1 Cytoskeletal, structural and regulatory proteins cleaved by caspases during apoptosis

Protein	Function	Caspase	References
Actin	Polymerised into filaments	3	26
Gelsolin	Actin-severing	3	140
Gas 2	Actin filament binding	3,7	141
Fodrin	Actin filament binding	3	142,143
Beta II Spectrin	Actin filament binding	3	144
Filamin	Actin filament binding	?	145
α -adducin	Actin capping	3	146
FAK	Regulates cell adhesion	3,6	147,148
p130 ^{Cas}	Regulates cell adhesion	3	149
Plectin	Microfilament cross-linker	8	150
E-Cadherin	Cell-Cell adhesion	3	151,152
N-Cadherin	Cell-Cell adhesion	3	153
P-Cadherin	Cell-Cell adhesion	?	151
β -catenin	Cell-Cell adhesion	3	154
γ -catenin	Cell-Cell adhesion	3	155
Keratin-14	Intermediate Filament	?	156
Keratin-15	Intermediate Filament	?	157
Keratin-17	Intermediate Filament	?	157
Keratin-18	Intermediate Filament	3,6,7	158
Keratin-19	Intermediate Filament	?	159
Vimentin	Intermediate Filament	3	143,160
Tau	Neuronal microtubule-associated protein	3	161,162

Figure 3 ROCK activation and phosphorylation of downstream targets. Loss of autoinhibition by Rho binding, or caspase cleavage results in kinase activation. ROCK phosphorylates a number of substrates that regulate actin-myosin contractile force generation. MLC, Myosin Light Chain

regulatory proteins (Table 1) diminishes the interactions that tethers the plasma membrane to the cytoskeleton and allows the increased hydrodynamic forces generated by ROCK-induced cell contraction to drive bleb protrusion at points of weakness (Figure 1). Although required for apoptotic body formation,^{11,23} blebbing can be sustained for prolonged periods of time and will not directly lead to a breakdown of the cell.^{15,23,24,27,30} Therefore, additional events are required for the blebbing cell to be broken down into apoptotic bodies.

Second phase: breakdown of actin structures and apoptotic body formation

After the initial phase of cell contraction and membrane blebbing driven by ROCK-mediated actin-myosin contractile force generation, there is a second phase in which actin filaments are depolymerised.^{18,20} Signalling downstream of Rho GTPases may contribute to both phases of actin reorganisation. During apoptosis, caspase-mediated cleavage of the Rho effector kinases PRK1 (also known as PKN)⁵⁴ and PRK2⁵⁵ as well as the Rac/Cdc42 effector kinase PAK2 (also known as γ -PAK)^{56,57} releases constitutively active kinase fragments. Catalytically active PRK1/PKN has

been reported to promote actin stress fibre disassembly,⁵⁸ possibly resulting from the phosphorylation of the actin binding protein α -actinin as well as the phosphorylation of monomeric actin.⁵⁹ A C-terminal fragment of PRK2 released by caspase-mediated proteolysis may feed forward to promote apoptosis by inhibiting the catalytic activity of the anti-apoptotic protein kinase Akt/PKB and reduce its inhibitory phosphorylation of the pro-apoptotic Bcl-2 family member Bad.⁶⁰ PAK isoforms also have been shown to lead to actin stress fibre disassembly,^{23,61–63} possibly through the phosphorylation of the myosin-II heavy chain,⁶⁴ which is thought to promote actin-myosin destabilisation,⁶⁵ and through the phosphorylation and consequent inhibition of the MLC kinase.^{66,67} Overexpression of a dominant-negative form of PAK2 blocked Fas-induced apoptotic body formation in Jurkat T cells suggesting that PAK-mediated effects on actin filament depolymerisation and dismantling of cytoskeleton structures may be required for the final breakdown of the apoptotic cell.⁵⁶ Interestingly, PAK1 and PAK2 isoforms have been shown to protect cells from apoptosis through the phosphorylation of the pro-apoptotic Bad protein.^{68–70} Thus, in some situations the activation of PAK2 by caspase-cleavage may antagonise both the morphological and biochemical events in a cell not fully committed to apoptosis.

Caspase-cleaved Rho GTPase signalling proteins

In addition to the proteins listed above, four additional proteins involved in Rho GTPase signalling have been identified as being caspase-cleaved in apoptotic cells. Given the importance of the Rho GTPase family in the regulation of the actin cytoskeleton, any significant changes to their signalling activities would likely influence the apoptotic morphology.

CDC42/Rac1 The Rho family GTPases Cdc42 and Rac1 were shown to be cleaved by caspases-3 and -7 in a variety of cell lines during Fas-induced apoptosis.⁷¹ The cleavage occurs at a position that separates the N-terminal portion of the protein containing the principal effector-interaction domain from the C-terminal portion responsible for the essential localisation of the protein to membranous structures. Therefore, the most likely outcome of Cdc42 and Rac1 cleavage is the termination of downstream signalling. It has been suggested previously that Cdc42 and Rac1 signalling promote cell survival through the PAK-mediated phosphorylation of Bad^{68–70} and through the activation of NF- κ B.⁷² Therefore, the cleavage of Cdc42 and Rac1 may further the apoptotic process by eliminating their normal function as pro-survival signalling proteins. In addition, by eliminating the actin filament destabilising actions of Cdc42 and Rac1^{61,63,64} the effects of ROCK I on promoting actin-myosin II cell contractility would be accentuated.

D4–GDI In haematopoietic cells, Rho GTPases may be activated during apoptosis when they are released from the inhibitory actions of the D4–GDI protein, a GDI for Rho family proteins, which is cleaved by caspase-3 (Figure 2 and Table 2).^{73–75} Since RhoA, Rac1 and Cdc42 all bind to D4–GDI *in*

Figure 4 Mammalian homologues of the *Ced-2*, -5, and -10 engulfment genes form a signalling cassette that drives actin polymerisation at the site of cell corpse ingestion. Left, Cell undergoing apoptosis displays 'eat me' signal on outer leaflet of plasma membrane. Minimal actin structures in the resting phagocyte due to low levels of active Rac–GTP. Right, upon ligation of the 'eat me' signal to an engulfment receptor such as $\alpha_v\beta_3$ integrin expressed on the approaching phagocyte, a number of events occur that result in actin polymerisation. Integrin ligation results in tyrosine phosphorylation of the adaptor protein p130^{Cas}, which subsequently recruits CrkII, and the Rac-GDP binding protein DOCK180, to the plasma membrane. Now proximal to its GEF, Rac exchanges GDP for GTP resulting in actin polymerisation at the ingestion site. The newly polymerised actin filaments form pseudopods that migrate around the cell corpse and eventually fuse to complete the process of engulfment. GEF, Guanine nucleotide Exchange Factor

vitro, it is not clear what the effect of D4–GDI cleavage and potential simultaneous activation of these multiple pathways during apoptosis would be.

Vav1 The haematopoietic-specific RhoGEF Vav1, which is essential for normal T and B cell function,^{78,79} was shown to be cleaved by caspase-3 at a site that is also conserved in the related Vav2 and Vav3 proteins.⁸⁰ The consequences of caspase-mediated cleavage of Vav1 are not clear, however, ectopic expression of N-terminally deleted versions of Vav1 was sufficient to induce Rho-dependent actin stress fibres (MF Olson, unpublished observations and references^{81,82}). Thus, it is possible that cleavage of Vav1 serves to disrupt signalling from the T and B cell receptors and to promote rearrangements of the actin cytoskeleton that lead to the morphological changes in apoptotic immune cells.

Given the large and complex pattern of protein degradation during apoptosis (see Tables 1 and 2), some apparently opposing activities would seem to be simultaneously in effect, for example the increased actin-myosin interactions

and contractile force generation driven by cleaved ROCK 1 *versus* the actin-myosin disassembly and cell spreading from cleaved PAK2. These seemingly contradictory actions are likely influenced by differences in when and where a given protein is cleaved. In addition, signal intensity would determine which outcome predominates and the ultimate effects would change as relative signal strengths shifted over time. Many protein cleavage events probably do not contribute directly to the morphological changes or to the apoptotic process, rather these proteins are likely 'innocent bystanders' caught up in caspase-crossfire.

Rho GTPases in phagocytosis

For normal tissue homeostasis to be maintained in the presence of cells undergoing apoptosis, the associated cell debris must be efficiently removed and destroyed. Therefore, programmed cell death *in vivo* must occur hand-in-hand with corpse clearance; for this reason phagocytosis is essential as the final step of the apoptotic process. Removal of dying cells

Table 2 Rho GTPase signalling proteins cleaved by caspases during apoptosis

Protein	Function	Caspase	References
ROCK I	Rho effector kinase	3	23,24
PRK1/PKN	Rho effector kinase	3	54
PRK2	Rho effector kinase	3	55
Pak2/ γ -PAK	Rac/Cdc42 effector kinase	3	56,57,163
D4-GDI	Rho GDP dissociation inhibitor	3	73–75
Rac1	Rho family GTPase	3,7	71
Cdc42	Rho family GTPase	3,7	71
Vav1	Rho family GEF	3	80

prior to their lysis prevents the exposure of surrounding cells and tissue to potentially toxic cell contents, thus protecting from inflammatory injury.^{83,84} Phagocytosis of cell corpses occurs by both 'professional' phagocytes such as macrophages and neutrophils and 'amateur' phagocytes that include epithelial cells and fibroblasts.

Actin cytoskeleton

A universal requirement for the process of phagocytosis is the integrity of the actin cytoskeleton. The requirement for polymerised actin is highlighted by the inhibition of phagocytosis by actin disrupting agents such as cytochalasins.^{85–87} Bound particles are surrounded by a phagocytic cup lined by newly polymerised actin microfilaments that provide the driving force for engulfment and subsequent phagosome formation (Figure 1).^{88,89} Following internalisation of the particle, F-actin is depolymerised and dissociated from the phagosome allowing subsequent endosomal fusion and lysosomal degradation of the ingested particle.⁹⁰

Labelling cell corpses and recognition by phagocytes

Efficient clearance of apoptotic bodies also depends upon their being labelled with 'eat me' signals, which appears to be an integral part of the apoptotic process.⁹¹ The best characterised engulfment signal is the appearance of phosphatidylserine on the outer leaflet of the plasma membrane, an event that may be a key determinant in phagocyte recognition through its binding to an evolutionarily-conserved receptor.⁹² Less well characterised phagocytic markers include sites that bind 'bridging' molecules from the extracellular fluid such as β_2 glycoprotein 1,⁹³ thrombospondin⁹⁴ and the complement factors iC3b and C1q.^{95–97}

Engulfment signals such as phosphatidylserine and bridging factors mediate phagocytosis through their interaction with specific receptors present on the approaching phagocyte. A variety of engulfment receptors involved in the clearance of dying cells have been identified including phagocyte lectins, integrins, scavenger receptors and macrophage receptors.^{89,98} Other factors may also influence the engulfment process, such as local changes in the lipid composition of the phagocyte plasma membrane. These

changes in lipid composition are controlled in part by the ABC1 transporter,⁹⁹ which is required for efficient recognition of apoptotic cells.¹⁰⁰

Genetic analysis of phagocytosis in *Caenorhabditis elegans*

ABC1 is the mammalian homologue of the *ced-7* gene from *Caenorhabditis elegans*, one of six genes identified in genetic screens for mutants defective in the clearance of apoptotic cells.^{101–103} These genes comprise two parallel pathways organised into the epistatic groups *ced-2*, *ced-5*, *ced-10* and *ced-1*, *ced-6*, *ced-7* (Table 3).¹⁰² Of the other components of the *ced-7* pathway, *ced-1* encodes a transmembrane receptor with homology to human SREC (Scavenger Receptor from Endothelial Cells)¹⁰⁴ and *ced-6* encodes a functionally conserved signalling adaptor molecule composed of a phosphotyrosine-binding domain and potential Src-homology domain 3 (SH3) binding sites.^{105,106} How the *ced-1/6/7* pathway conveys signals downstream of activated engulfment receptors to the subsequent internalisation of the apoptotic cell is poorly understood. This is in contrast to our relatively thorough understanding of the *ced-2/5/10* pathway, the components of which are not only required for cell corpse engulfment, but also for migration of the distal tip cells of the nematode gonad.^{107,108} This 'engulfment cassette' comprises a conserved signalling pathway previously implicated in regulation of the actin cytoskeleton and cell migration in mammalian cells.

Mammalian homologues of *ced* engulfment genes

Ced-2 encodes the homologue of the mammalian SH2/SH3-containing adaptor protein CrkII, *ced-5* is homologous to the large adaptor protein DOCK180, whilst *ced-10* is the nematode orthologue of Rac1 (Table 3).^{107,108} DOCK180 has been implicated in the activation of Rac since it has been shown to bind Rac–GDP, but not Cdc42 or RhoA, and overexpression of membrane-targeted DOCK180 increases Rac–GTP levels in 293T cells.^{109–111} Membrane targeting of DOCK180 induces spreading of NIH 3T3 cells¹¹² that is dependent on Rac function.¹⁰⁹ Although DOCK180 can increase Rac–GTP levels, it contains no discernible Dbl-homology GEF domain and is therefore unlikely to act as a Rac GEF itself.¹⁰⁹ Instead, DOCK180 may act as an adaptor that recruits Rac to the plasma membrane where it becomes activated (Figure 4). Although the Rac exchange factor(s) that mediates DOCK180-dependent Rac activation is unknown, it has been shown that DOCK180 can enhance the activation of Rac by the GEF Vav1.¹⁰⁹

Translocation of DOCK180/Rac to the plasma membrane is likely achieved through the interaction of DOCK180 with the amino terminal SH3 domain of the *ced-2* homologue, CrkII. CrkII localises to focal adhesions upon integrin stimulation, a process that is dependent on its interaction with a protein called p130Cas.^{113,114} Integrin stimulation results in tyrosine phosphorylation of

Table 3 *C. elegans* genes involved in phagocytosis of cell corpses and their mammalian homologues

Gene	Mammalian homologue	Possible function
<i>Ced-1</i>	SREC	Scavenger receptor ; may recognise 'eat me' signal on cell corpse
<i>Ced-6</i>	hCED-6	Signalling adaptor protein ; probably involved in relaying signal from engulfment receptor to phagocytic machinery
<i>Ced-7</i>	ABC1	Lipid transporter ; modulates lipid composition of plasma membrane
<i>Ced-2</i>	CrklI	Signalling adaptor protein ; binds DOCK180 and translocates to membrane
<i>Ced-5</i>	Dock180	Adaptor protein ; recruits Rac to CrklI and therefore plasma membrane for activation
<i>Ced-10</i>	Rac1	Rho GTPase ; promotes actin polymerisation required for cell corpse internalisation

p130^{Cas}^{115–117} and subsequent recruitment of the CrklI/DOCK180/Rac complex to focal adhesions^{109,110,118} via the CrklI SH2 domain (Figure 4).^{119,120} In addition, coexpression of p130^{Cas} and CrklI enhances the DOCK180-dependent activation of Rac and membrane spreading.^{109,110} It would also be intriguing to determine whether the MER receptor tyrosine kinase, which has been suggested to be essential for the phagocytosis and clearance of apoptotic cells by macrophages, signals to the CrklI/DOCK180/Rac pathway.¹²¹

The mammalian homologues of the *ced-2*, *-5* and *-10* genes previously implicated in adhesion-dependent signalling and migration as described above, have recently been confirmed as important players in the clearance of apoptotic mammalian cells.^{122–124} Interestingly, it would appear that integrin-mediated formation of the CrklI/DOCK180 complex and subsequent Rac activation are common features of both cell adhesion and phagocyte signalling. Integrins such as $\alpha_v\beta_5$ and $\alpha_v\beta_3$ have previously been implicated as engulfment receptors in professional and amateur phagocytes.^{125–127} For example, phagocytosis of cell corpses by dendritic cells is mediated by the $\alpha_v\beta_5$ integrin,¹²⁵ a process that was subsequently shown to depend on recruitment of the p130^{Cas}/CrklI/DOCK180 complex and subsequent Rac activation.¹²²

Other evidence exists that also implicates integrin-mediated Rac activation in the phagocytosis of dying mammalian cells. The engulfment of apoptotic Baf-3 cells by bone marrow-derived macrophages was dependent on the $\alpha_v\beta_3$ integrin and could be blocked by dominant negative versions of Rac and Cdc42.¹²⁴ Interestingly, whereas inhibition of Rac or Cdc42 signalling significantly blocked phagocytic uptake, inhibition of Rho actually enhanced the clearance of cell corpses.¹²⁴

There is growing evidence to suggest that members of the Rho GTPase family play a universal role in the reorganisation of the actin cytoskeleton during all forms of phagocytosis (reviewed in reference¹²⁸). For instance, particles opsonised by IgG are recognised by the Fc γ family of receptors for the constant region of immunoglobulin and are subsequently internalised in a Rac and Cdc42-dependent manner. Inhibition of Rac function prevents pseudopod fusion and phagosome closure during Fc ϵ RI-mediated phagocytosis, whilst inhibition of Cdc42 interferes with pseudopod extension.^{129–131} In contrast, internalisation of particles opsonised by complement fragments via binding to the $\alpha_M\beta_2$ integrin requires RhoA function, but not Rac and

Cdc42.¹³⁰ During both Fc γ - and $\alpha_M\beta_2$ -mediated phagocytosis, the actin-nucleating Arp2/3 complex accumulates at the ingestion site to promote actin neo-polymerisation.¹³²

Are Rho GTPases required downstream of the *ced-1/6/7* pathway?

Considering the apparent general requirement for Rho GTPase signalling during phagocytosis it seems likely that they also play a role downstream of the *ced-1/6/7* pathway. It is unlikely that *ced-1* itself signals through *ced-2/5* to *ced-10* activation and actin-re-organisation however, since mutations to any of the *ced-2*, *-5*, or *-10* genes significantly co-operate with *ced-1* mutations in the inhibition of engulfment.¹⁰² However, the possibility that components downstream of *ced-1* interact with *ced-10* was raised by the observation that overexpression of *ced-6* could partially suppress the engulfment defect of *ced-10* mutants.¹⁰⁶ Since *ced-6* has been proposed to act as an adaptor molecule and does not possess an obvious catalytic domain, it is unclear whether it acts upstream or downstream of *ced-10*.^{105,106,133,134} It is possible that the *ced-1* pathway signals through an alternative Rac-like GTPase even in the presence of a *ced-10* mutation since *C. elegans* express a Rac2 homologue¹³⁵ and a GTPase termed Mig-2 that is 64% identical to *ced-10*.¹³⁶ Interestingly, the scavenger receptor MARCO, which is involved in the macrophage clearance of bacteria, was recently shown to induce morphological changes associated with rearrangement of the actin cytoskeleton when overexpressed.¹³⁷ These MARCO-induced changes were partially inhibited by dominant negative Rac, but not Cdc42. Perhaps a similar pathway exists downstream of other scavenger receptors or the *C. elegans* homologue *ced-1* to regulate the actin cytoskeleton and phagocytosis of cell corpses via Rho GTPases.

Intriguing recent work suggests that besides their role in phagocytosis, the engulfment genes of *C. elegans* also may play an active role in the apoptotic process in the dying cell. A screen for genes that could synergise with a partial loss of function of the *ced-3* caspase identified all the previously characterised engulfment genes.^{138,139} A model has been proposed whereby activation of engulfment pathways in the phagocyte promotes a feed forward mechanism that ensures the demise of the associated cell. Since Rho GTPases such as Rac are important effectors in engulfment signalling pathways, this raises the remarkable possibility that Rho proteins may be involved in the

homicide of a cell previously thought to have committed suicide.

Conclusions

The actin cytoskeleton rearrangements in both the apoptotic and the phagocytic cell result from activation of signalling pathways associated with Rho GTPases. However, the mode of activation is entirely different in each case. During apoptosis, caspase-mediated cleavage of ROCK I gives rise to a constitutive Rho-independent signal that generates actin-myosin contractile force, membrane blebbing and formation of apoptotic bodies. In marked contrast, phagocytosis requires precise spatio-temporal regulation of Rac and Cdc42 to coordinate the dynamic actin remodelling necessary for the engulfment of the apoptotic cell.

It is evident that the actin rearrangements that accompany phagocytosis are critical for the removal of dying cells, thus preventing the exposure of surrounding cells and tissue to potentially toxic cell contents and an inappropriate immune response. However, it is not clear whether the membrane blebbing of an apoptotic cell plays an active part in its subsequent phagocytosis or if, in fact, it has any *in vivo* physiological purpose at all. Blebbing does appear to be essential for the formation of apoptotic bodies, which may facilitate recognition and clearance by phagocytes. Alternatively, the eventual breakdown of the cell into apoptotic bodies may aid the engulfment process. In addition, the contractile forces generated during the execution phase of apoptosis may be important for pulling adjacent cells with strong cell-cell contacts together, thus maintaining proper tissue organisation and integrity. Having identified the biochemical pathway responsible for the cell contraction and membrane blebbing during apoptosis, it will now be possible to finally determine the physiological function of these processes and answer the question 'What is blebbing for?'

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References

- Nicholson DW (1999) Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Differ.* 6: 1028–1042
- Kerr JF, Wyllie AH and Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* 26: 239–257
- Mills JC, Stone NL and Pittman RN (1999) Extranuclear apoptosis. The role of the cytoplasm in the execution phase. *J. Cell Biol.* 146: 703–708
- Takai Y, Sasaki T and Matozaki T (2001) Small GTP-binding proteins. *Physiol. Rev.* 81: 153–208
- Hall A (1998) Rho GTPases and the actin cytoskeleton. *Science* 279: 509–514
- Paterson HF, Self AJ, Garrett MD, Just I, Aktories K and Hall A (1990) Microinjection of recombinant p21rho induces rapid changes in cell morphology. *J. Cell Biol.* 111: 1001–1007
- Ridley AJ and Hall A (1992) The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* 70: 389–399
- Ridley AJ, Paterson HF, Johnston CL, Diekmann D and Hall A (1992) The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell* 70: 401–410
- Kozma R, Ahmed S, Best A and Lim L (1995) The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts. *Mol. Cell Biol.* 15: 1942–1952
- Nobes CD and Hall A (1995) Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* 81: 53–62
- Cotter TG, Lennon SV, Glynn JM and Green DR (1992) Microfilament-disrupting agents prevent the formation of apoptotic bodies in tumor cells undergoing apoptosis. *Cancer Res.* 52: 997–1005
- Laster SM and Mackenzie Jr JM (1996) Bleb formation and F-actin distribution during mitosis and tumor necrosis factor-induced apoptosis. *Microsc. Res. Tech.* 34: 272–280
- Vemuri GS, Zhang J, Huang R, Keen JH and Rittenhouse SE (1996) Thrombin stimulates wortmannin-inhibitable phosphoinositide 3-kinase and membrane blebbing in CHRF-288 cells. *Biochem. J.* 314: 805–810
- Pitzer F, Dantes A, Fuchs T, Baumeister W and Amsterdam A (1996) Removal of proteasomes from the nucleus and their accumulation in apoptotic blebs during programmed cell death. *FEBS Lett.* 394: 47–50
- Mills JC, Stone NL, Erhardt J and Pittman RN (1998) Apoptotic membrane blebbing is regulated by myosin light chain phosphorylation. *J. Cell Biol.* 140: 627–636
- Torgerson RR and McNiven MA (1998) The actin-myosin cytoskeleton mediates reversible agonist-induced membrane blebbing. *J. Cell Sci.* 111: 2911–2922
- Huot J, Houle F, Rousseau S, Deschesnes RG, Shah GM and Landry J (1998) SAPK2/p38-dependent F-actin reorganization regulates early membrane blebbing during stress-induced apoptosis. *J. Cell Biol.* 143: 1361–1373
- Rao JY, Jin YS, Zheng Q, Cheng J, Tai J and Hemstreet III GP (1999) Alterations of the actin polymerization status as an apoptotic morphological effector in HL-60 cells. *J. Cell Biochem.* 75: 686–697
- Hagmann J, Burger MM and Dagan D (1999) Regulation of plasma membrane blebbing by the cytoskeleton. *J. Cell Biochem.* 73: 488–499
- Suarez-Huerta N, Mosselmans R, Dumont JE and Robaye B (2000) Actin depolymerization and polymerization are required during apoptosis in endothelial cells. *J. Cell Physiol.* 184: 239–245
- Rentsch PS and Keller H (2000) Suction pressure can induce uncoupling of the plasma membrane from cortical actin. *Eur. J. Cell. Biol.* 79: 975–981
- Petrache I, Verin AD, Crow MT, Birukova A, Liu F and Garcia JG (2001) Differential effect of MLC kinase in TNF-alpha-induced endothelial cell apoptosis and barrier dysfunction. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 280: L1168–L1178
- Coleman ML, Sahai EA, Yeo M, Bosch M, Dewar A and Olson MF (2001) Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. *Nat. Cell Biol.* 3: 339–345
- Sebbagh M, Renvoize C, Hamelin J, Riche N, Bertoglio J and Breard J (2001) Caspase-3-mediated cleavage of ROCK I induces MLC phosphorylation and apoptotic membrane blebbing. *Nat. Cell Biol.* 3: 346–352
- Kayalar C, Ord T, Testa MP, Zhong LT and Bredesen DE (1996) Cleavage of actin by interleukin 1 beta-converting enzyme to reverse DNase I inhibition. *Proc. Natl. Acad. Sci. USA* 93: 2234–2238
- Mashima T, Naito M, Noguchi K, Miller DK, Nicholson DW and Tsuruo T (1997) Actin cleavage by CPP-32/apopain during the development of apoptosis. *Oncogene* 14: 1007–1012
- McCarthy NJ, Whyte MK, Gilbert CS and Evan GI (1997) Inhibition of Ced-3/ICE-related proteases does not prevent cell death induced by oncogenes, DNA damage, or the Bcl-2 homologue Bak. *J. Cell. Biol.* 136: 215–227
- Robertson D, Paterson HF, Adamson P, Hall A and Monaghan P (1995) Ultrastructural localization of ras-related proteins using epitope-tagged plasmids. *J. Histochem. Cytochem.* 43: 471–480

29. Jin S, Shimizu M, Balasubramanyam A and Epstein HF (2000) Myotonic dystrophy protein kinase (DMPK) induces actin cytoskeletal reorganization and apoptotic-like blebbing in lens cells. *Cell Motil Cytoskeleton* 45: 133–148
30. Hirose M, Ishizaki T, Watanabe N, Uehata M, Kranenburg O, Moolenaar WH, Matsumura F, Maekawa M, Bito H and Narumiya S (1998) Molecular dissection of the Rho-associated protein kinase (p160ROCK)-regulated neurite remodeling in neuroblastoma N1E-115 cells. *J. Cell. Biol.* 141: 1625–1636
31. Watanabe N, Kato T, Fujita A, Ishizaki T and Narumiya S (1999) Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. *Nat. Cell. Biol.* 1: 136–143
32. Tominaga T, Sahai E, Chardin P, McCormick F, Courtneidge SA and Alberts AS (2000) Diaphanous-related formins bridge Rho GTPase and Src tyrosine kinase signaling. *Mol. Cell.* 5: 13–25
33. Matsui T, Amano M, Yamamoto T, Chihara K, Nakafuku M, Ito M, Nakano T, Okawa K, Iwamatsu A and Kaibuchi K (1996) Rho-associated kinase, a novel serine/threonine kinase, as a putative target for small GTP binding protein Rho. *EMBO J.* 15: 2208–2216
34. Leung T, Chen XQ, Manser E and Lim L (1996) The p160 RhoA-binding kinase ROKalpha is a member of a kinase family and is involved in the reorganization of the cytoskeleton. *Mol. Cell. Biol.* 16: 5313–5327
35. Ishizaki T, Maekawa M, Fujisawa K, Okawa K, Iwamatsu A, Fujita A, Watanabe N, Saito Y, Kakizuka A, Morii N and Narumiya S (1996) The small GTP-binding protein Rho binds to and activates a 160 kDa Ser/Thr protein kinase homologous to myotonic dystrophy kinase. *EMBO J.* 15: 1885–1893
36. Ishizaki T, Naito M, Fujisawa K, Maekawa M, Watanabe N, Saito Y and Narumiya S (1997) p160ROCK, a Rho-associated coiled-coil forming protein kinase, works downstream of Rho and induces focal adhesions. *FEBS Lett.* 404: 118–124
37. Amano M, Chihara K, Kimura K, Fukata Y, Nakamura N, Matsuura Y and Kaibuchi K (1997) Formation of actin stress fibers and focal adhesions enhanced by Rho-kinase. *Science* 275: 1308–1311
38. Amano M, Chihara K, Nakamura N, Kaneko T, Matsuura Y and Kaibuchi K (1999) The COOH terminus of Rho-kinase negatively regulates rho-kinase activity. *J. Biol. Chem.* 274: 32418–32424
39. Amano T, Tanabe K, Eto T, Narumiya S and Mizuno K (2001) LIM-kinase 2 induces formation of stress fibres, focal adhesions and membrane blebs, dependent on its activation by Rho-associated kinase-catalysed phosphorylation at threonine-505. *Biochem. J.* 354: 149–159
40. Sumi T, Matsumoto K and Nakamura T (2001) Specific activation of LIM kinase 2 via phosphorylation of threonine 505 by ROCK, a Rho-dependent protein kinase. *J. Biol. Chem.* 276: 670–676
41. Ohashi K, Nagata K, Maekawa M, Ishizaki T, Narumiya S and Mizuno K (2000) Rho-associated kinase ROCK activates LIM-kinase 1 by phosphorylation at threonine 508 within the activation loop. *J. Biol. Chem.* 275: 3577–3582
42. Maekawa M, Ishizaki T, Boku S, Watanabe N, Fujita A, Iwamatsu A, Obinata T, Ohashi K, Mizuno K and Narumiya S (1999) Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. *Science* 285: 895–898
43. Amano M, Ito M, Kimura K, Fukata Y, Chihara K, Nakano T, Matsuura Y and Kaibuchi K (1996) Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). *J. Biol. Chem.* 271: 20246–20249
44. Kureishi Y, Kobayashi S, Amano M, Kimura K, Kanaide H, Nakano T, Kaibuchi K and Ito M (1997) Rho-associated kinase directly induces smooth muscle contraction through myosin light chain phosphorylation. *J. Biol. Chem.* 272: 12257–12260
45. Totsukawa G, Yamakita Y, Yamashiro S, Hartshorne DJ, Sasaki Y and Matsumura F (2000) Distinct roles of ROCK (Rho-kinase) and MLCK in spatial regulation of MLC phosphorylation for assembly of stress fibers and focal adhesions in 3T3 fibroblasts. *J. Cell Biol.* 150: 797–806
46. Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okawa K, Iwamatsu A and Kaibuchi K (1996) Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* 273: 245–248
47. Kawano Y, Fukata Y, Oshiro N, Amano M, Nakamura T, Ito M, Matsumura F, Inagaki M and Kaibuchi K (1999) Phosphorylation of myosin-binding subunit (MBS) of myosin phosphatase by Rho-kinase in vivo. *J. Cell Biol.* 147: 1023–1038
48. Kaneko T, Amano M, Maeda A, Goto H, Takahashi K, Ito M and Kaibuchi K (2000) Identification of calponin as a novel substrate of Rho-kinase. *Biochem. Biophys. Res. Commun.* 273: 110–116
49. Koyama M, Ito M, Feng J, Seko T, Shiraki K, Takase K, Hartshorne DJ and Nakano T (2000) Phosphorylation of CPI-17, an inhibitory phosphoprotein of smooth muscle myosin phosphatase, by Rho-kinase. *FEBS Lett.* 475: 197–200
50. Fedier A and Keller HU (1997) Suppression of bleb formation, locomotion, and polarity of Walker carcinosarcoma cells by hypertonic media correlates with cell volume reduction but not with changes in the F-actin content. *Cell Motil. Cytoskeleton* 37: 326–337
51. Dai J and Sheetz MP (1999) Membrane tether formation from blebbing cells. *Biophys. J.* 77: 3363–3370
52. Rentsch PS and Keller H (2000) Suction pressure can induce uncoupling of the plasma membrane from cortical actin. *Eur. J. Cell Biol.* 79: 975–981
53. Cunningham CC, Gorlin JB, Kwiatkowski DJ, Hartwig JH, Janmey PA, Byers HR and Stossel TP (1992) Actin-binding protein requirement for cortical stability and efficient locomotion. *Science* 255: 325–327
54. Takahashi M, Mukai H, Toshimori M, Miyamoto M and Ono Y (1998) Proteolytic activation of PKN by caspase-3 or related protease during apoptosis. *Proc. Natl. Acad. Sci. USA* 95: 11566–11571
55. Cryns VL, Byun Y, Rana A, Mellor H, Lustig KD, Ghanem L, Parker PJ, Kirschner MW and Yuan J (1997) Specific proteolysis of the kinase protein kinase C-related kinase 2 by caspase-3 during apoptosis. Identification by a novel, small pool expression cloning strategy. *J. Biol. Chem.* 272: 29449–29453
56. Rudel T and Bokoch GM (1997) Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of PAK2. *Science* 276: 1572–1574
57. Lee N, MacDonald H, Reinhard C, Halenbeck R, Roulston A, Shi T and Williams LT (1997) Activation of hPAK65 by caspase cleavage induces some of the morphological and biochemical changes of apoptosis. *Proc. Natl. Acad. Sci. USA* 94: 13642–13647
58. Dong LQ, Landa LR, Wick MJ, Zhu L, Mukai H, Ono Y and Liu F (2000) Phosphorylation of protein kinase N by phosphoinositide-dependent protein kinase-1 mediates insulin signals to the actin cytoskeleton. *Proc. Natl. Acad. Sci. USA* 97: 5089–5094
59. Mukai H, Toshimori M, Shibata H, Takanaga H, Kitagawa M, Miyahara M, Shimakawa M and Ono Y (1997) Interaction of PKN with alpha-actinin. *J. Biol. Chem.* 272: 4740–4746
60. Koh H, Lee KH, Kim D, Kim S, Kim JW and Chung J (2000) Inhibition of Akt and its anti-apoptotic activities by tumor necrosis factor-induced protein kinase C-related kinase 2 (PRK2) cleavage. *J. Biol. Chem.* 275: 34451–34458
61. Manser E, Huang HY, Loo TH, Chen XQ, Dong JM, Leung T and Lim L (1997) Expression of constitutively active alpha-PAK reveals effects of the kinase on actin and focal complexes. *Mol. Cell Biol.* 17: 1129–1143
62. Frost JA, Khokhlatchev A, Stippes S, White MA and Cobb MH (1998) Differential effects of PAK1-activating mutations reveal activity-dependent and -independent effects on cytoskeletal regulation. *J. Biol. Chem.* 273: 28191–28198
63. Zhao ZS, Manser E, Chen XQ, Chong C, Leung T and Lim L (1998) A conserved negative regulatory region in alphaPAK: inhibition of PAK kinases reveals their morphological roles downstream of Cdc42 and Rac1. *Mol. Cell Biol.* 18: 2153–2163
64. van Leeuwen FN, van Delft S, Kain HE, van der Kammen RA and Collard JG (1999) Rac regulates phosphorylation of the myosin-II heavy chain, actinomyosin disassembly and cell spreading. *Nat. Cell Biol.* 1: 242–248
65. Egelhoff TT, Lee RJ and Spudich JA (1993) Dictyostelium myosin heavy chain phosphorylation sites regulate myosin filament assembly and localization in vivo. *Cell* 75: 363–371
66. Sanders LC, Matsumura F, Bokoch GM and de Lanerolle P (1999) Inhibition of myosin light chain kinase by p21-activated kinase. *Science* 283: 2083–2085
67. Goeckeler ZM, Masaracchia RA, Zeng Q, Chew TL, Gallagher P and Wysolmerski RB (2000) Phosphorylation of myosin light chain kinase by p21-activated kinase PAK2. *J. Biol. Chem.* 275: 18366–18374
68. Schurmann A, Mooney AF, Sanders LC, Sells MA, Wang HG, Reed JC and Bokoch GM (2000) p21-activated kinase 1 phosphorylates the death agonist bad and protects cells from apoptosis. *Mol. Cell Biol.* 20: 453–461
69. Tang Y, Zhou H, Chen A, Pittman RN and Field J (2000) The Akt proto-oncogene links Ras to Pak and cell survival signals. *J. Biol. Chem.* 275: 9106–9109
70. Jakobi R, Moertl E and Koeppl MA (2001) p21-activated protein kinase gamma-PAK suppresses programmed cell death of BALB3T3 fibroblasts. *J. Biol. Chem.* 276: 16624–16634

71. Tu S and Cerione RA (2001) Cdc42 is a substrate for caspases and influences fas-induced apoptosis. *J. Biol. Chem.* 276: 19656–19663
72. Perona R, Montaner S, Saniger L, Sanchez-Perez I, Bravo R and Lacal JC (1997) Activation of the nuclear factor-kappaB by Rho, CDC42, and Rac-1 proteins. *Genes Dev.* 11: 463–475
73. Na S, Chuang TH, Cunningham A, Turi TG, Hanke JH, Bokoch GM and Danley DE (1996) D4-GDI, a substrate of CPP32, is proteolyzed during Fas-induced apoptosis. *J. Biol. Chem.* 271: 11209–11213
74. Danley DE, Chuang TH and Bokoch GM (1996) Defective Rho GTPase regulation by IL-1 beta-converting enzyme-mediated cleavage of D4 GDP dissociation inhibitor. *J. Immunol.* 157: 500–503
75. Krieser RJ and Eastman A (1999) Cleavage and nuclear translocation of the caspase 3 substrate Rho GDP-dissociation inhibitor, D4-GDI, during apoptosis. *Cell Death Differ.* 6: 412–419
76. Scherle P, Behrens T and Staudt LM (1993) Ly-GDI, a GDP-dissociation inhibitor of the RhoA GTP-binding protein, is expressed preferentially in lymphocytes. *Proc. Natl. Acad. Sci. USA* 90: 7568–7572
77. Adra CN, Ko J, Leonard D, Wirth LJ, Cerione RA and Lim B (1993) Identification of a novel protein with GDP dissociation inhibitor activity for the ras-like proteins CDC42Hs and rac 1. *Genes Chromosomes Cancer* 8: 253–261
78. Zhang R, Alt FW, Davidson L, Orkin SH and Swat W (1995) Defective signalling through the T- and B-cell antigen receptors in lymphoid cells lacking the vav proto-oncogene. *Nature* 374: 470–473
79. Tarakhovskiy A, Turner M, Schaal S, Mee PJ, Duddy LP, Rajewsky K and Tybulewicz VL (1995) Defective antigen receptor-mediated proliferation of B and T cells in the absence of Vav. *Nature* 374: 467–470
80. Hofmann TG, Hehner SP, Droge W and Schmitz ML (2000) Caspase-dependent cleavage and inactivation of the Vav1 proto-oncogene product during apoptosis prevents IL-2 transcription. *Oncogene* 19: 1153–1163
81. Kranewitter WJ and Gimona M (1999) N-terminally truncated Vav induces the formation of depolymerization-resistant actin filaments in NIH 3T3 cells. *FEBS Lett.* 455: 123–129
82. Olson MF, Pasteris NG, Gorski JL and Hall A (1996) Fociogenital dysplasia protein (FGD1) and Vav, two related proteins required for normal embryonic development, are upstream regulators of Rho GTPases. *Curr. Biol.* 6: 1628–1633
83. Ren Y and Savill J (1998) Apoptosis: the importance of being eaten. *Cell Death Differ.* 5: 563–568
84. Savill J (1997) Recognition and phagocytosis of cells undergoing apoptosis. *Br. Med. Bull.* 53: 491–508
85. Castellano F, Montcourrier P and Chavrier P (2000) Membrane recruitment of Rac1 triggers phagocytosis. *J. Cell Sci.* 113: 2955–2961
86. Greenberg S, el Khoury J, di Virgilio F, Kaplan EM and Silberstein SC (1991) Ca(2+)-independent F-actin assembly and disassembly during Fc receptor-mediated phagocytosis in mouse macrophages. *J. Cell Biol.* 113: 757–767
87. Zigmund SH and Hirsch JG (1972) Effects of cytochalasin B on polymorphonuclear leucocyte locomotion, phagocytosis and glycolysis. *Exp. Cell. Res.* 73: 383–393
88. Allen LA and Aderem A (1996) Molecular definition of distinct cytoskeletal structures involved in complement- and Fc receptor-mediated phagocytosis in macrophages. *J. Exp. Med.* 184: 627–637
89. Kwiatkowska K and Sobota A (1999) Signaling pathways in phagocytosis. *Bioessays* 21: 422–431
90. Bengtsson T, Jaconi ME, Gustafson M, Magnusson KE, Theler JM, Lew DP and Stendahl O (1993) Actin dynamics in human neutrophils during adhesion and phagocytosis is controlled by changes in intracellular free calcium. *Eur. J. Cell. Biol.* 62: 49–58
91. Savill J and Fadok V (2000) Corpse clearance defines the meaning of cell death. *Nature* 407: 784–788
92. Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA and Henson PM (2000) A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* 405: 85–90
93. Balasubramanian K, Chandra J and Schroit AJ (1997) Immune clearance of phosphatidylserine-expressing cells by phagocytes. The role of beta2-glycoprotein I in macrophage recognition. *J. Biol. Chem.* 272: 31113–31117
94. Savill J, Hogg N, Ren Y and Haslett C (1992) Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J. Clin. Invest.* 90: 1513–1522
95. Mevorach D, Mascarenhas JO, Gershov D and Elkon KB (1998) Complement-dependent clearance of apoptotic cells by human macrophages. *J. Exp. Med.* 188: 2313–2320
96. Botto M, Dell'Agnola C, Bygrave AE, Thompson EM, Cook HT, Petry F, Loos M, Pandolfi PP and Walport MJ (1998) Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat. Genet.* 19: 56–59
97. Taylor PR, Carugati A, Fadok VA, Cook HT, Andrews M, Carroll MC, Savill JS, Henson PM (2000) A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo. *J. Exp. Med.* 192: 359–366
98. Platt N, da Silva RP and Gordon S (1998) Recognizing death: the phagocytosis of apoptotic cells. *Trends Cell Biol.* 8: 365–372
99. Marguet D, Luciani MF, Moynault A, Williamson P and Chimini G (1999) Engulfment of apoptotic cells involves the redistribution of membrane phosphatidylserine on phagocyte and prey. *Nat. Cell Biol.* 1: 454–456
100. Hamon Y, Broccardo C, Chambenoit O, Luciani MF, Toti F, Chaslin S, Freyssinet JM, Devaux PF, McNeish J, Marguet D and Chimini G (2000) ABC1 promotes engulfment of apoptotic cells and transbilayer redistribution of phosphatidylserine. *Nat. Cell Biol.* 2: 399–406
101. Hedgecock EM, Sulston JE and Thomson JN (1983) Mutations affecting programmed cell deaths in the nematode *Caenorhabditis elegans*. *Science* 220: 1277–1279
102. Ellis RE, Jacobson DM and Horvitz HR (1991) Genes required for the engulfment of cell corpses during programmed cell death in *Caenorhabditis elegans*. *Genetics* 129: 79–94
103. Chung S, Gumienny TL, Hengartner MO and Driscoll M (2000) A common set of engulfment genes mediates removal of both apoptotic and necrotic cell corpses in *C. elegans*. *Nat. Cell Biol.* 2: 931–937
104. Zhou Z, Hartwig E and Horvitz HR (2001) CED-1 is a transmembrane receptor that mediates cell corpse engulfment in *C. elegans*. *Cell* 104: 43–56
105. Su HP, Brugnera E, Van Crielinge W, Smits E, Hengartner M, Bogaert T and Ravichandran KS (2000) Identification and characterization of a dimerization domain in CED-6, an adapter protein involved in engulfment of apoptotic cells. *J. Biol. Chem.* 275: 9542–9549
106. Liu QA and Hengartner MO (1998) Candidate adaptor protein CED-6 promotes the engulfment of apoptotic cells in *C. elegans*. *Cell* 93: 961–972
107. Reddien PW and Horvitz HR (2000) CED-2/CrkII and CED-10/Rac control phagocytosis and cell migration in *Caenorhabditis elegans*. *Nat. Cell Biol.* 2: 131–136
108. Wu YC and Horvitz HR (1998) *C. elegans* phagocytosis and cell-migration protein CED-5 is similar to human DOCK180. *Nature* 392: 502–504
109. Kiyokawa E, Hashimoto Y, Kobayashi S, Sugimura H, Kurata T and Matsuda M (1998) Activation of Rac1 by a Crk SH3-binding protein, DOCK180. *Genes Dev.* 12: 3331–3336
110. Kiyokawa E, Hashimoto Y, Kurata T, Sugimura H and Matsuda M (1998) Evidence that DOCK180 up-regulates signals from the CrkII-p130(Cas) complex. *J. Biol. Chem.* 273: 24479–24484
111. Nolan KM, Barrett K, Lu Y, Hu KQ, Vincent S and Settleman J (1998) Myoblast city, the Drosophila homolog of DOCK180/CED-5, is required in a Rac signaling pathway utilized for multiple developmental processes. *Genes Dev.* 12: 3337–3342
112. Hasegawa H, Kiyokawa E, Tanaka S, Nagashima K, Gotoh N, Shibuya M, Kurata T and Matsuda M (1996) DOCK180, a major CRK-binding protein, alters cell morphology upon translocation to the cell membrane. *Mol. Cell Biol.* 16: 1770–1776
113. Vuori K, Hirai H, Aizawa S and Ruoslahti E (1996) Introduction of p130cas signaling complex formation upon integrin-mediated cell adhesion: a role for Src family kinases. *Mol. Cell Biol.* 16: 2606–2613
114. Nievers MG, Birge RB, Greulich H, Verkleij AJ, Hanafusa H and van Bergen en Henegouwen PM (1997) v-Crk-induced cell transformation: changes in focal adhesion composition and signaling. *J. Cell Sci.* 110: 389–399
115. Nojima Y, Morino N, Nimura T, Hamasaki K, Furuya H, Sakai R, Sato T, Tachibana K, Morimoto C and Yazaki Y *et al* (1995) Integrin-mediated cell adhesion promotes tyrosine phosphorylation of p130Cas, a Src homology 3-containing molecule having multiple Src homology 2-binding motifs. *J. Biol. Chem.* 270: 15398–15402

116. Vuori K and Ruoslahti E (1995) Tyrosine phosphorylation of p130Cas and cortactin accompanies integrin-mediated cell adhesion to extracellular matrix. *J. Biol. Chem.* 270: 22259–22262
117. Nakamoto T, Sakai R, Honda H, Ogawa S, Ueno H, Suzuki T, Aizawa S, Yazaki Y and Hirai H (1997) Requirements for localization of p130cas to focal adhesions. *Mol. Cell Biol.* 17: 3884–3897
118. Gu J, Sumida Y, Sanzen N and Sekiguchi K (2001) Laminin-10/11 and fibronectin differentially regulate integrin-dependent Rho and rac activation via p130Cas-CrkII-DOCK180 pathway. *J. Biol. Chem.* 276: 21
119. Feller SM, Ren R, Hanafusa H and Baltimore D (1994) SH2 and SH3 domains as molecular adhesives: the interactions of Crk and Abl. *Trends Biochem. Sci.* 19: 453–458
120. Matsuda M, Nagata S, Tanaka S, Nagashima K and Kurata T (1993) Structural requirement of CRK SH2 region for binding to phosphotyrosine-containing proteins. Evidence from reactivity to monoclonal antibodies. *J. Biol. Chem.* 268: 4441–4446
121. Scott RS, McMahon EJ, Pop SM, Reap EA, Caricchio R, Cohen PL, Earp HS and Matsushima GK (2001) Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature* 411: 207–211
122. Albert ML, Kim JI and Birge RB (2000) Alphavbeta5 integrin recruits the CrkII-Dock180-rac1 complex for phagocytosis of apoptotic cells. *Nat. Cell Biol.* 2: 899–905
123. Tosello-Trampont AC, Brugnera E and Ravichandran KS (2001) Evidence for a conserved role for CRKII and Rac in engulfment of apoptotic cells. *J. Biol. Chem.* 276: 13797–13802
124. Leverrier Y and Ridley AJ (2001) Requirement for Rho GTPases and PI3-kinases during apoptotic cell phagocytosis by macrophages. *Curr. Biol.* 11: 195–199
125. Albert ML, Sauter B and Bhardwaj N (1998) Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature* 392: 86–89
126. Finnemann SC and Rodriguez-Boulan E (1999) Macrophage and retinal pigment epithelium phagocytosis: apoptotic cells and photoreceptors compete for alphavbeta3 and alphavbeta5 integrins, and protein kinase C regulates alphavbeta5 binding and cytoskeletal linkage. *J. Exp. Med.* 190: 861–874
127. Savill J, Dransfield I, Hogg N and Haslett C (1990) Vitronectin receptor-mediated phagocytosis of cells undergoing apoptosis. *Nature* 343: 170–173
128. Chimini G and Chavrier P (2000) Function of Rho family proteins in actin dynamics during phagocytosis and engulfment. *Nat. Cell Biol.* 2: E191–E196
129. Cox D, Chang P, Zhang Q, Reddy PG, Bokoch GM and Greenberg S (1997) Requirements for both Rac1 and Cdc42 in membrane ruffling and phagocytosis in leukocytes. *J. Exp. Med.* 186: 1487–1494
130. Caron E and Hall A (1998) Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. *Science* 282: 1717–1721
131. Massol P, Montcourrier P, Guillemot JC and Chavrier P (1998) Fc receptor-mediated phagocytosis requires CDC42 and Rac1. *EMBO J.* 17: 6219–6229
132. May RC, Caron E, Hall A and Machesky LM (2000) Involvement of the Arp2/3 complex in phagocytosis mediated by FcgammaR or CR3. *Nat. Cell Biol.* 2: 246–248
133. Liu QA and Hengartner MO (1999) Human CED-6 encodes a functional homologue of the *Caenorhabditis elegans* engulfment protein CED-6. *Curr. Biol.* 9: 1347–1350
134. Smits E, Van Crielinge W, Plaetinck G and Bogaert T (1999) The human homologue of *Caenorhabditis elegans* CED-6 specifically promotes phagocytosis of apoptotic cells. *Curr. Biol.* 9: 1351–1354
135. Chen W, Yap SF and Lim L (1996) Isolation of the gene coding for *Caenorhabditis elegans* Rac2 homologue, a Ras-related small GTP-binding protein. *Gene* 180: 217–219
136. Zipkin ID, Kindt RM and Kenyon CJ (1997) Role of a new Rho family member in cell migration and axon guidance in *C. elegans*. *Cell* 90: 883–894
137. Pikkarainen T, Brannstrom A and Tryggvason K (1999) Expression of macrophage MARCO receptor induces formation of dendritic plasma membrane processes. *J. Biol. Chem.* 274: 10975–10982
138. Hoepfner DJ, Hengartner MO and Schnabel R (2001) Engulfment genes cooperate with ced-3 to promote cell death in *Caenorhabditis elegans*. *Nature* 412: 202–206
139. Reddien PW, Cameron S and Horvitz HR (2001) Phagocytosis promotes programmed cell death in *C. elegans*. *Nature* 412: 198–202
140. Kothakota S, Azuma T, Reinhard C, Klippel A, Tang J, Chu K, McGarry TJ, Kirschner MW, Kohts K, Kwiatkowski DJ and Williams LT (1997) Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis. *Science* 278: 294–298
141. Sgorbissa A, Benetti R, Marzintotto S, Schneider C and Brancolini C (1999) Caspase-3 and caspase-7 but not caspase-6 cleave Gas2 in vitro: implications for microfilament reorganization during apoptosis. *J. Cell Sci.* 112: 4475–4482
142. Martin SJ, O'Brien GA, Nishioka WK, McGahon AJ, Mahboubi A, Saido TC and Green DR (1995) Proteolysis of fodrin (non-erythroid spectrin) during apoptosis. *J. Biol. Chem.* 270: 6425–6428
143. Slee EA, Adrain C and Martin SJ (2001) Executioner caspase-3, -6, and -7 perform distinct, non-redundant roles during the demolition phase of apoptosis. *J. Biol. Chem.* 276: 7320–7326
144. Wang KK, Posmantur R, Nath R, McGinnis K, Whitton M, Talanian RV, Glantz SB and Morrow JS (1998) Simultaneous degradation of alphaIIb and betaIIb spectrin by caspase 3 (CPP32) in apoptotic cells. *J. Biol. Chem.* 273: 22490–22497
145. Browne KA, Johnstone RW, Jans DA and Trapani JA (2000) Filamin (280-kDa actin-binding protein) is a caspase substrate and is also cleaved directly by the cytotoxic T lymphocyte protease granzyme B during apoptosis. *J. Biol. Chem.* 275: 39262–39266
146. van de Water B, Tijdens IB, Verbrugge A, Huigsloot M, Dihal AA, Stevens JL, Jaken S and Mulder GJ (2000) Cleavage of the actin-capping protein alpha-actinin at Asp-Asp-Ser-Asp633-Ala by caspase-3 is preceded by its phosphorylation on serine 726 in cisplatin-induced apoptosis of renal epithelial cells. *J. Biol. Chem.* 275: 25805–25813
147. Gervais FG, Thornberry NA, Ruffolo SC, Nicholson DW and Roy S (1998) Caspases cleave focal adhesion kinase during apoptosis to generate a FRNK-like polypeptide. *J. Biol. Chem.* 273: 17102–17108
148. Levkau B, Herren B, Koyama H, Ross R and Raines EW (1998) Caspase-mediated cleavage of focal adhesion kinase pp125FAK and disassembly of focal adhesions in human endothelial cell apoptosis. *J. Exp. Med.* 187: 579–586
149. Kook S, Shim SR, Choi SJ, Ahnn J, Kim JI, Eom SH, Jung YK, Paik SG and Song WK (2000) Caspase-mediated cleavage of p130cas in etoposide-induced apoptotic Rat-1 cells. *Mol. Biol. Cell.* 11: 929–939
150. Stegh AH, Herrmann H, Lampel S, Weisenberger D, Andra K, Seper M, Wiche G, Krammer PH and Peter ME (2000) Identification of the cytolinker plectin as a major early in vivo substrate for caspase 8 during CD95- and tumor necrosis factor receptor-mediated apoptosis. *Mol. Cell Biol.* 20: 5665–5679
151. Schmeiser K and Grand RJ (1999) The fate of E- and P-cadherin during the early stages of apoptosis. *Cell Death Differ.* 6: 377–386
152. Steinhilber U, Weiske J, Badcock V, Tauber R, Bommer K and Huber O (2001) Cleavage and shedding of E-cadherin after induction of apoptosis. *J. Biol. Chem.* 276: 4972–4980
153. Hunter I, McGregor D and Robins SP (2001) Caspase-dependent cleavage of cadherins and catenins during osteoblast apoptosis. *J. Bone Miner Res.* 16: 466–477
154. Brancolini C, Lazarevic D, Rodriguez J and Schneider C (1997) Dismantling cell-cell contacts during apoptosis is coupled to a caspase-dependent proteolytic cleavage of beta-catenin. *J. Cell Biol.* 139: 759–771
155. Brancolini C, Sgorbissa A and Schneider C (1998) Proteolytic processing of the adherens junctions components beta-catenin and gamma-catenin/plakoglobin during apoptosis. *Cell Death Differ.* 5: 1042–1050
156. Ku NO and Omary MB (2001) Effect of mutation and phosphorylation of type I keratins on their caspase-mediated degradation. *J. Biol. Chem.* 276: 16
157. Badock V, Steinhilber U, Bommer K, Wittmann-Liebold B and Otto A (2001) Apoptosis-induced cleavage of keratin 15 and keratin 17 in a human breast epithelial cell line. *Cell Death Differ.* 8: 308–315
158. Caulin C, Salvesen GS and Oshima RG (1997) Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. *J. Cell Biol.* 138: 1379–1394
159. Prasad S, Soldatenkov VA, Srinivasarao G and Dritschilo A (1998) Identification of keratins 18, 19 and heat-shock protein 90 beta as candidate substrates of proteolysis during ionization radiation-induced apoptosis of estrogen-receptor negative breast tumor cells. *Int. J. Oncol.* 13: 757–764

160. Prasad SC, Thraves PJ, Kuettel MR, Srinivasarao GY, Dritschilo A and Soldatenkov VA (1998) Apoptosis-associated proteolysis of vimentin in human prostate epithelial tumor cells. *Biochem. Biophys. Res. Commun.* 249: 332–338
161. Canu N, Dus L, Barbato C, Ciotti MT, Brancolini C, Rinaldi AM, Novak M, Cattaneo A, Bradbury A and Calissano P (1998) Tau cleavage and dephosphorylation in cerebellar granule neurons undergoing apoptosis. *J. Neurosci.* 18: 7061–7074
162. Fasulo L, Ugolini G, Visintin M, Bradbury A, Brancolini C, Verzillo V, Novak M and Cattaneo A (2000) The neuronal microtubule-associated protein tau is a substrate for caspase-3 and an effector of apoptosis. *J. Neurochem.* 75: 624–633
163. Walter BN, Huang Z, Jakobi R, Tuazon PT, Alnemri ES, Litwack G and Traugh JA (1998) Cleavage and activation of p21-activated protein kinase gamma-PAK by CPP32 (caspase 3). Effects of autophosphorylation on activity. *J. Biol. Chem.* 273: 28733–28739