

Is caspase-dependent apoptosis only cell differentiation taken to the extreme?

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ABSTRACT The benefits of apoptosis for a multicellular organism are obvious and fit the current dogma that the maintenance and viability of such organisms are dependent on the selective elimination of unneeded or deleterious cell types. However, self destruction at the level of the individual cell defies the most basic precepts of biology (sustaining life). If apoptosis is viewed through this construct then one question becomes paramount, *i.e.*, why would an individual cell and its progeny develop, retain, or evolve a mechanism the sole purpose of which is to eliminate itself? In consideration of such a paradox, it is reasonable to postulate that prospective apoptotic pathways co-evolved with and or were co-opted from another basic cell function(s) that did not involve the death of the cell *per se*. In the following article, we present the hypothesis that the conserved biochemical pathways of apoptosis are integral components of terminal cell differentiation and it is the time of engagement and activity level of these pathways that ultimately determines the choice between cell death or cell maturation.—Fernando, P., Megeney, L. A. Is caspase-dependent apoptosis only cell differentiation taken to the extreme? *FASEB J.* 21, 8–17 (2007)

REGULATED SELF DESTRUCTION of a cell or apoptosis is a highly conserved phenomenon, moderating developmental processes and tissue homeostasis across the broad phylae of multicellular organisms. Apoptosis is governed by a complex array of proteins and integrated biochemical pathways that act to initiate and complete the destruction of a cell. In turn, the apoptotic phenotype is characterized by a number of general features that foreshadow cell death including mitochondrial membrane alterations, plasma membrane blebbing, cytoskeletal reorganization, DNA fragmentation, nuclear condensation, and dissolution (1–3). The apoptotic response leads to a definable cell outcome, yet the biochemical and morphological changes typical of the cell death process appear to be conserved features of other vital cell behaviors. Notable among these physiological responses is cell differentiation. Here we discuss the growing body of evidence that implicates the cell death machinery as vital components of the differentiation program and how these same observations may

alter our understanding on the origin and utility of cell death pathways and proteins.

Differentiation and apoptosis in multicellular organisms: twins separated at birth?

The defining hallmark of metazoan multicell life forms is cell specialization, an adaptation that results from the process commonly referred to as differentiation. Although the evolutionary record is not clearly defined, cell differentiation is an ancient feature and likely emerged within close temporal proximity to the development of programmed cell death/apoptosis (4, 5).

Nevertheless, despite the tendency to treat apoptosis and differentiation as divergent cell outcomes, there exists a high degree of morphological similarity for these contrasting events in complex animals. Indeed, to date vertebrate organisms have provided the most frequently examined model in this regard. For example, nuclear disruption is viewed as an irreversible step in the apoptotic process, a step that precedes the final physical destruction of a cell, yet terminal differentiation of many cell types including erythrocytes, keratinocytes, and lens fiber epithelial cells are characterized by complete removal of the nucleus (6). During erythropoiesis, the colony forming unit, erythroid (CFU-E) responds to stimulation by erythropoietin to give rise to proerythroblasts, the first distinguishable member of the erythrocyte lineage. Subsequently, the erythroblast expels its nucleus, resulting in the formation of the reticulocyte. Not surprisingly, the process to form mature erythrocytes has been shown to require extensive microtubule remodeling and membrane deformation (7). Although alterations to erythrocyte organelles and apparent membrane instability are parallel with apoptosis, the viability and function of these cells are maintained in the absence of the nucleus.

The formation of lens tissue also requires dramatic changes in cell structure and shape. During eye development, lens epithelial cells give rise to both primary and secondary lens fibers. Primary fibers form from the

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doi: 10.1096/fj.06-5912hyp

posterior half of the lens vesicle. These cells elongate and establish the core of the lens tissue. Secondary fibers form from the equatorial region of the lens vesicle. These cells extend to surround the core of primary lens fibers and results in the extrusion of their nuclei (8). Although lens epithelial cells maintain viability in the absence of nuclei and other organelles, it remains unclear whether these cells extrude their nuclei or target them for degradation. Nonetheless, the proper orientation of lens tissue requires active remodeling of membrane skeletal proteins that allow for cell migration and adhesion.

Although nuclear extrusion appears to be an integral component of differentiation in some cell types, other proapoptotic nuclear phenomena have been observed in nondying differentiating cell lineages. A number of studies have reported the appearance of transient DNA single strand breaks in non-dying vertebrate myoblast/muscle nuclei during early stages of the differentiation process (9–11) and *Drosophila* Schneider cells have been reported to undergo myogenic/muscle conversion and differentiation when subject to drugs that induce DNA double-strand breaks (12). Similarly, erythroleukemic differentiation as well as physiological monocyte and granulocyte differentiation is marked by the appearance of single and double DNA strand breaks and the differentiation process in these cells is attenuated by agents that inhibit the DNA ligation process (13, 14).

In addition to alterations in nuclear morphology, other typical apoptotic adaptations have been documented as conserved features of cell differentiation. Specifically, vertebrate skeletal muscle and neuronal cell lineages provide interesting models in this regard. At a gross level, neuronal differentiation is generally characterized by neurite outgrowth that is dependent on significant cytoskeletal rearrangements akin to the changes that often take place during apoptosis. In turn, these structural adaptations appear to originate from a common biochemical signal (see below). Pertaining to skeletal muscle, actin fiber disassembly/reorganization is a conserved feature of apoptosis (2) and differentiating myoblasts (15, 16). Second, the essential contractile protein myosin light chain kinase appears to be an indispensable feature of apoptotic membrane blebbing (17). Third, increased activity of matrix metalloproteinases is required for membrane fusion events associated with both apoptosis and myoblast differentiation (18, 19). Finally, the phospholipid reorientation that typifies apoptotic cells (phosphatidylserine exposure to the extracellular surface) also appears to be an integral component of myoblast fusion events that occur during formation of multinucleate myofibers (20).

Although certainly not conclusive, the degree of phenotypic overlap across such a broad range of cell types as described above is strongly suggestive of a common origin (or convergent adaptation) for cell differentiation and apoptosis as biological processes. Indeed, if we accept such a possibility, then the reasonable extension of this hypothesis would argue that

initiation and propagation of apoptosis and differentiation should be dependent on a similar cohort of factors and biochemical signals. Within this context, the choice between cell death or differentiation would ultimately reside with death or differentiation specific modifications to the same set of factors and signals.

Canonical apoptotic pathways: the common intermediate for conveying apoptosis and differentiation signals

Having provided the rationale for a phenotypic similarity between apoptosis and differentiation, the next challenge is to define an operative pathway common to each condition. One class of apoptotic proteins that may retain such a divergent function are the caspase proteases. It is well established that successful completion of apoptosis is largely dependent on the activity of this unique class of proteolytic enzymes (21). Activation of these enzymes usually represents the penultimate step in a hierarchical death pathway by cleaving vital protein substrates through aspartate directed targeting events (22). To date, four pathways leading to caspase activation have been characterized in vertebrate organisms and include the following: the receptor-mediated pathway, the mitochondria-mediated pathway, the granzyme B mediated pathway and the endoplasmic reticulum-mediated pathway. Of these, the receptor-mediated and mitochondria-mediated pathways are perhaps the best understood.

In the death receptor-mediated pathway, a typical death stimulus results in ligand binding and activation of cell surface receptors of the tumor necrosis factor (TNF) superfamily (23). Subsequently, a death-inducing signaling complex (DISC) is assembled consisting of the ligand-receptor complex and intracellular adapter proteins such as Fas associated death domain (FADD) or TNF associated death domain (TNFADD) and procaspase-8 (23). Active caspase-8, formed by autoproteolytic cleavage of procaspase-8, then activates procaspase-3. A similar result is attained through the mitochondria-mediated apoptotic pathway. In this instance, an apoptotic stimulus results in the release of the respiratory chain molecule cytochrome *c* from the mitochondrial membrane. In an ATP-dependent manner, cytochrome *c* associates with apoptotic peptidase activating factor-1 (Apaf-1) and procaspase-9 to form the apoptosome (24–26). Formation of the apoptosome results in cleavage and activation of procaspase-9. Subsequent activation of effector caspases (caspase-3, -6, and -7) by caspase-9 results in the proteolytic processing of downstream target proteins, leading to the classical apoptotic phenotype.

Despite a definitive role for caspase proteins in cell death, complimentary lines of evidence suggest that some of these proteases may also function as regulators of cellular differentiation. For example, inhibition of caspase 3 activity limits nuclear condensation and extrusion in lens fiber epithelial cells, keratinocytes, and erythrocytes, attenuating or inhibiting the differentia-

tion of these cell types (27–31). In lens fiber epithelial cells, the increased caspase 3 activity originates from elevated cytosolic cytochrome *c* levels, suggesting that engagement of the mitochondrial-dependent apoptotic pathway is the trigger for differentiation in this cell type (32).

These observations have led to the proposition that cell differentiation may be a modified or curtailed form of cell death (28, 29). This death centric model of the differentiation process reinforces standard assumptions regarding apoptotic pathways and proteins, yet caspase proteases appear to modulate differentiation across a wide spectrum of cell types. As noted previously, skeletal muscle myoblast differentiation displays many apoptotic features and is in turn critically dependent on the cell autonomous activity of caspase 3 (33, 34). Arguably, the dependence on caspase 3 and the morphological changes associated with the differentiation process in skeletal muscle could be viewed as an attenuated form of apoptosis. However, caspase 3 appears to regulate differentiation in many other cell lineages that have less pronounced or limited apoptotic like changes in cell morphology. Cytokine-mediated differentiation of monocytes to activated macrophages is generally devoid of apoptotic cell rearrangements and has been demonstrated to be dependent on mitochondrial cytochrome *c* release and effector caspase activation (35). More recently, the cell autonomous prodifferentiation effects of caspase 3 have been noted in other diverse cell types that display minimal apoptotic like features and include differentiating osteoclasts (36), bone marrow stromal cells (37), neurons (38), and neural and glial progenitor cells (39, 40), *i.e.* chemical or genetic blockade of caspase 3 activity in all these cell types leads to an inhibition of differentiation.

As with caspase 3, caspase 8 dependent regulation of cellular differentiation has also been demonstrated. Monocytic differentiation into the macrophage lineage was disrupted using the pan caspase inhibitor p35, a dominant negative caspase 8 mutant and the cowpox virus caspase inhibitor CrmA (41). The specification of macrophages by caspase 8 was a result of proteolytic cleavage of select cellular proteins. Interestingly, these included proteins that mediate cell adhesion and migration and proteins that act to stabilize the cytoarchitecture. A lack of monocytic differentiation and macrophage formation was also found in a conditional *caspase 8* null mouse model (42). Moreover, deletion of *caspase 8* in the bone marrow resulted in a reduced number and function in hemopoietic progenitor cells *in vitro*.

Expression and activity of caspase 8 are also necessary for the syncytial fusion of human trophoblast cells in placental development. During pregnancy, trophoblast precursor cells separate from the inner cell mass and form a bilayer consisting of an inner cytotrophoblast layer with single nucleated cells and an outer syncytial trophoblast layer. Syncytiotrophoblasts form through fusion of cytotrophoblast cells to emerge as large multinucleated sheets of cells (see 43). Black and

colleagues found that either antisense or peptide inhibition of caspase 8 attenuated cytotrophoblast fusion with the syncytiotrophoblast (44). Not surprisingly, the lack of syncytial fusion by caspase 8 blockade prevented the activation of downstream effector caspases. In another model of differentiation, BMP-4 treatment resulted in the growth arrest and differentiation of mouse osetoebasts (36). BMP-4 also led to the ordered activation of caspase-8, -2, and -3 without stimulating apoptosis or necrosis. Inhibition of these caspases using peptide inhibitors blocked BMP-induced cell cycle arrest and osteoblast differentiation. In this study, the select inhibition of caspase-8, -2, or -3 each significantly reduced the degree of osteoblast differentiation to relatively similar levels compared to BMP-4 stimulated cells. Although a combinatorial inhibition of these caspase proteases was not demonstrated, it is tempting to speculate that the inhibition of caspase -8, -2 or -3 in combination may have additive effects on the inhibition of osteoblast differentiation.

Recently, members of the TNF family have emerged as apoptotic-associated proteins that operate in differentiation-related processes. Although this cytokine is typically considered a catabolic factor and has been associated with muscle wasting via cancer cachexia, current experiments suggest that lower concentrations of TNF can elicit myoblast differentiation and engage behaviors associated with activated muscle stem cells such as enhanced chemotaxis (45, 46). In addition, the TNF responsive FAS receptor has been linked to induction of cardiomyocyte hypertrophy, a postnatal adaptation that recapitulates many of the molecular signatures associated with cardiomyocyte differentiation (47). Lymphocyte activation has also been demonstrated to involve caspase mediated pathways (48, 49). In these studies, caspase 8 was enzymatically active after signaling by the T cell receptor, resulting in the cleavage of select downstream caspases and substrates without inducing apoptosis. *In vitro* this response is likely mediated through a mechanism involving the cellular FADD-like interleukin (IL)-1 β converting enzyme (FLICE) inhibitory protein (cFLIP) such that the active caspase 8 signal is diverted away from a prodeath pathway (49, reviewed in 50).

In addition to these aforementioned experiments that directly test the role of caspase proteases in cell differentiation, a number of other studies provide strong correlative support for caspase induced cell differentiation. Specifically, murine gene targeting of numerous death receptor signaling components (FADD, TRAIL) and various caspase proteases (caspase 8 and combined caspase 3 and 7 double null animals) have demonstrated profound deficiencies in myocardial development leading to embryonic lethality (51–54). Although the myocardial defects in these models have not been established to originate from a lack of caspase controlled cardiomyocyte differentiation, the limited ventricular maturation is consistent with such a probability. The apoptotic-inducing factor (AIF) is an apoptotic effector associated with the inner mitochon-

drial membrane. Surprisingly, deletion of *Aif* in cardiac tissue resulted in dilated cardiomyopathy and heart failure. In addition, loss of *Aif* in skeletal muscle resulted in atrophy and lactic acidemia (55). This outcome was attributed to deficiencies in mitochondrial respiration thus implying that an alternate function of AIF is to mediate aerobic energy metabolism. Furthermore, recent evidence also suggests that components of the TNF/TRAIL dependent pathways stimulate differentiation in a variety of cell types (56, 57). Taken together, these experiments provide a persuasive argument that both the intrinsic/mitochondrial apoptotic cascade and the death receptor mediated apoptosis pathway are indispensable components of normal cell differentiation programs. What remains to be defined is the relative contribution of each pathway to the differentiation process and what variation (if any) may exist across different cell lineages.

What are the factors that determine cell death vs. differentiation during activation of caspase/apoptotic pathways?

The weight of evidence demonstrates that caspase integrated apoptotic pathways regulate both cell differentiation and cell death. What remains more speculative is the mechanism(s) by which these same pathways elicit such a divergent response. One explanation is that the choice between death or differentiation derives largely from the caspase targeting and activation of substrates or cofactors that are unique to each event. Interestingly, although “differentiation only” caspase substrates have yet to be documented, examples of “death only” substrates do exist. The DNA damage repair protein poly(ADP) ribose polymerase 1 (PARP-1) is the most compelling example of a “death only” caspase substrate. PARP-1 is subject to caspase directed cleavage inactivation during early stages of apoptosis and the consequent rapid loss of this repair enzyme is then believed to hasten apoptotic nuclear disruption (58). However, although some observations suggest a nondeath role for PARP (29, 59), the majority of studies have indicated PARP as a death exclusive event. In addition to PARP, other “death only” caspase substrates are notable and include the MADS box transcription factors *mef2a* and *mef2c*. *Mef2* transcription factors are integral components of differentiation and survival in numerous cell lineages (60) and have been shown to be cleavage inactivated by caspases during apoptosis induction, specifically in differentiated neurons (61, 62). The large number of caspase substrates that have been identified to date (>300, 63) suggest that death and differentiation specific caspase responsive substrate pools may exist, although a comprehensive examination of caspase substrates in this regard has yet to be undertaken. Nevertheless, the existing experimental data indicate that caspase proteases may actually target an overlapping substrate pool when conveying death or differentiation signals in addition to targeting cell fate specific substrates.

To die or differentiate: timing and intensity make the difference

An example of such convergent death and differentiation signaling is the caspase activation of protein kinases. Caspase 3 is capable of targeting several protein kinases and rendering them active, usually through cleavage and removal of their C-terminal regulatory domain (64, 65). Of particular interest is the caspase activation of the p38 MAPK kinase family. Caspase directed activation of p38 typically occurs through the cleavage activation of an intervening kinase, which then targets and activates p38 itself (64, 66, 67). Once activated, p38 MAPK is capable of promoting either apoptosis or differentiation (by phosphorylating and enhancing prodifferentiation transcription factors such as the *mef2* proteins) in a plethora of cell types such as hematopoietic cells, neurons, cardiac and skeletal muscle (68). The dichotomous outcome evoked by activated p38 is also evident with other caspase targeted kinases such as the *ste-20* like MST1, a kinase that promotes apoptosis or myoblast differentiation following cleavage activation by caspase 3 (33, 67). PKC- δ is similarly cleavage activated by caspase 3, which is then indispensable in conveying the differentiation signal in keratinocytes as well as cell death across a number of cell types (30); and ROCK1, a caspase-targeted kinase equally adept at inducing neuronal apoptosis or differentiation (69). Finally, a recent report has also established that FAS exposure leads to a caspase 3 dependent differentiation signal in erythroid cells, a signal characterized by targeted activation of an ASK1/p38 kinase module (70).

Importantly, nonkinase caspase substrates have also been reported to maintain a critical role in the regulation of cell death and differentiation. The bHLH protein *Twist* has been characterized as a caspase 3 substrate, and its cleavage by caspase 3 leads to a loss of function followed by proteasome-mediated degradation (71). Interestingly, *Twist* expression is synonymous with a blockade of both apoptosis and differentiation in mesodermal cell lineages, suggesting that caspase cleavage of this protein is a prerequisite for effective completion of either program (71). The class II histone deacetylase HDAC4 has also been reported to be a caspase 3 substrate (72). This is an interesting observation given that HDACs are known to interact with and inhibit the prodifferentiation activity of *mef2* proteins (60, 73). Caspase activation of these targets provides a compelling example of the similarity between the death and differentiation signals, yet these proteins represent only a small number of the probable caspase substrates that have been identified to date (63). Clearly, a systematic investigation of each caspase substrate is needed to clarify whether these proteins maintain a regulatory role in both apoptosis and cell differentiation.

In the absence of substrate selectivity, a plausible explanation for the death *vs.* differentiation phenotype may originate with the timing and intensity of signal

pathway activation. Indeed, a simple inspection of the dynamics of the intrinsic death pathway suggests that the activities of the respective components are subject to rigid temporal control during a growth to differentiation transition. Both keratinocyte differentiation and monocyte to granulocyte conversion are marked by a slow elevation in cytosolic cytochrome *c* levels, followed by the requisite activation of caspase 3 (32, 35). In addition to these examples, a transient and biphasic caspase activity pattern has been noted to be a hallmark of the differentiation process in numerous cell types including skeletal myoblasts, osteoclasts, bone marrow stromal cells, and neurons (33, 36, 37, 39). These observations are in contrast to the pattern of caspase activity that typifies death associated signal events, a pattern that displays rapid and excessive protease activation to a level of activity that far exceeds that observed during differentiation (32, Fernando and Megeney, unpublished observations).

Presumably, the quantity of apoptotic-like stimuli is important for determining the cellular response to either differentiate or undergo apoptosis. The Ras GTPase activating protein (AP) (RasGAP) mediates both Ras- and Rho-dependent signaling pathways and is able to respond to varied degrees of caspase stimuli. A low level of caspase activity resulted in a partial cleavage of RasGAP without the apoptotic phenotype. However, with strong apoptotic cues that lead to high levels of caspase activity, RasGAP promoted apoptosis (74, 75). In another example, a brief period of MST1 activity accelerated the differentiation of myoblasts, yet when these same cells were exposed to prolonged MST1 stimulation, an accelerated apoptosis was observed (33). More recently, a reduced level of caspase activity was observed in differentiating lens cells. Specifically, the degree of caspase activity required to effectively act on differentiation signals was lower than that observed to induce apoptosis (32). Moreover, stimulation of apoptosis associated factors resulted in the controlled release of cytochrome *c* that did not lead to apoptosis. This is a particularly salient issue in that complete mitochondrial depletion of cytochrome *c* would result in an inability to generate ATP, a signal that itself could precipitate an irreversible initiation of programmed cell death. Therefore, stoichiometric activation of apoptotic factors during cellular development may be critical for the cell to either stage an apoptotic response or prompt further cellular commitment and specialization (see Fig. 1).

In addition to temporal kinetics, the final choice between death and differentiation may also depend on the subcellular localization of activated caspases and their constituent regulatory pathways. For example, it is reasonable to suggest that separate caspase signaling complexes may exist within a cell and that these complexes may be uniquely devoted to a death or differentiation response, presumably by targeting death or differentiation specific substrates. Although this hypothesis has never been directly tested (*i.e.*, by altering caspase localization and testing cell fate choice),

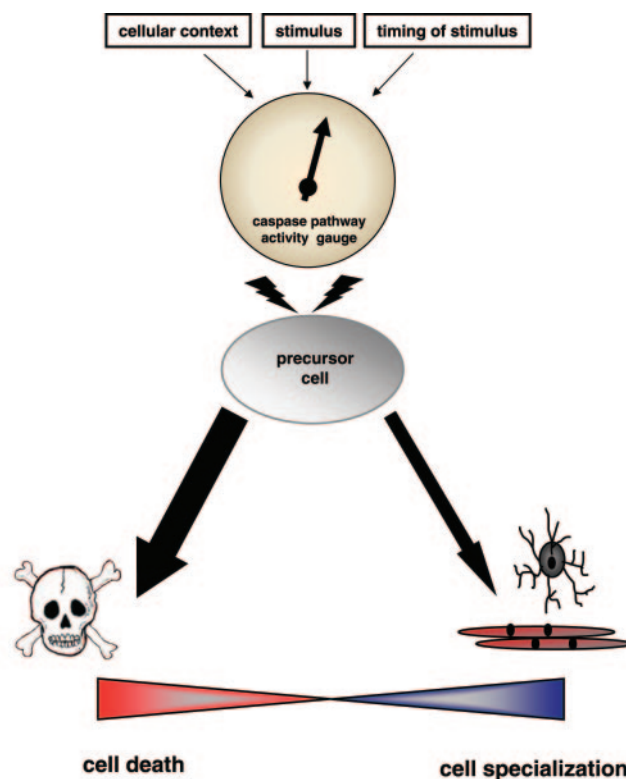


Figure 1. Regulating the caspase pathway. A model depicting the apoptosis-associated regulatory events during cellular differentiation and specialization. During a specific period of development (cellular context), a precursor cell receives a stimulus or multiple stimuli which are gauged according to the timing and intensity of the signal. These variables together elicit the appropriate caspase-associated cellular response which leads to either apoptosis or differentiation/cell specialization.

limited circumstantial evidence suggests that qualitative differences may exist for the location of active caspase enzymes during death or differentiation. We have noted that the low level of active caspase 3 in growing C2C12 myoblasts is evenly distributed throughout the cell. However, within 12 h of low serum induction of differentiation, active caspase 3 concentrates in a perinuclear region of nonapoptotic myoblasts, eventually dispersing by 36 h. In contrast, active caspase 3 appears to be dispersed throughout the cell after exposure to apoptotic signals (Fernando and Megeney, unpublished observations). The molecular basis of this variable localization remains unknown and it may simply reflect the variable level of caspase activation that accompanies death (high levels of activation) *vs.* differentiation (lower levels of activation). Alternatively, each cell fate choice may be dependent on specific protein/protein interactions that position the protease in the required subcellular locale. Clearly, delineation of this issue will depend on a thorough characterization of all caspase scaffolding/interacting proteins.

Evidence for the apoptosis/cell differentiation paradox in nonvertebrate models

Much of what we know today regarding the hierarchical control and structure of apoptotic pathways have been

gleaned from intensive study in tractable invertebrate animals. *C. elegans* has been the foremost model studied in this regard, yet little to no investigation has taken place in this organism to explore alternative non death roles for the apoptotic machinery. The study of alternative roles for cell death mechanisms and proteins in *Drosophila* development has yielded striking parallels to the behavior of homologous proteins in vertebrates. The cell death cascades in *Drosophila* terminate with caspases, of which seven have been identified to date (DCP-1, drICE, Dredd, DRONC, Decay, DAMM, and STRICA) and these caspases are subject to upstream regulation provided by activating proteins homologous to the vertebrate apaf-1 protein (Dapaf-1/Dark) and bcl-2 family members (Debc1/dBorg-1 and Buffy/dBorg2) (76, 77). *Drosophila* also express homologues to the mammalian inhibitors of apoptosis (IAPs) and the *Drosophila* proteins (DIAP1 and DIAP2) directly inhibit caspase activity. Moreover, the DIAP proteins appear to be direct targets of a series of proteins (Reaper, Hid, and Grim) that actively degrade DIAPs, thus permitting apoptosis to proceed (78–80).

The most studied example of a non-death role for apoptotic proteins in *Drosophila* is sperm individualization/differentiation. *Drosophila* sperm differentiation is characterized by individualization and encapsulation of the haploid spermatid from a larger tissue syncytium, which requires removal of bulk cytoplasm from the developing spermatids. This cytoplasmic removal during individualization is dependent on cytochrome *c* release and consequent caspase activation (81), a process that is reminiscent of the cytochrome *c* activation of caspase activity to promote nuclear extrusion during differentiation of mammalian erythrocytes and lens fiber epithelial cells (29, 32). Interestingly, the *Drosophila* spermatid differentiation program appears to be dependent on multiple caspase activities (Dronc/caspase9, Dredd/caspase8, and drICE/caspase3) and DIAP regulation, all of which act at different times and locations within the developing spermatid (82).

The cell death machinery also appears to regulate cell differentiation/maturation in other *Drosophila* cell types that do not replicate the death like phenotype typical of spermatid individualization. DIAP1 and Dark mediated inhibition and activation of Dronc/caspase 9 has been observed to alter Rac-dependent cell migration in stationary ovary epithelial cells (83). More recently, one study has demonstrated that activity of the Dark-dependent caspase Dronc was required for the development of neural sensory organ precursor (SOP) cells (84). Here, the authors describe a mechanism in which activated Dronc targets and cleavage activates the Shaggy kinase (a homologue of mammalian GSK-3 β), the activated kinase then antagonizes wingless signals allowing for the formation of SOP cells (84). Clearly, SOP cell development does not have a direct corollary in mammalian systems; however, the requirement for caspase-mediated activation of kinase activity and differentiation of *Drosophila* SOPs bears a strong resemblance to the role of caspase activated kinases in

mammalian cell differentiation (*i.e.*, skeletal muscle and neural precursor cells, see above and 33, 39).

As an addendum to these *Drosophila* studies that directly implicate a role for apoptotic proteins in a limited number of differentiating cell types, a closer examination of murine null phenotypes for the same proteins are suggestive of widespread perturbations in cell differentiation. Targeted deletion of Dronc/caspase 9 leads to supernumerary neuronal cells and hyperplastic hematopoietic tissues (85). Remarkably, the targeted deletion of intrinsic cell death pathway components in murine models (caspase 3 and the caspase 9 activator Apaf-1) leads to very similar neuronal phenotypes characterized by brain overgrowth and retinal perturbations (86, 87). The traditional interpretation of these results has been that the lack of apoptotic machinery leads to a reduced cell death with a consequent expansion in cell numbers. However, the hyperproliferative outcomes in both model systems may also originate from a separate cell autonomous deficit in differentiation (as discussed above).

Death and maturation/differentiation in the single cell organism: are the origins of these adaptations rooted in the same biochemical pathways?

The accumulation of evidence demonstrating non-apoptotic outcomes for proapoptotic pathways has begun to generate a paradigm shift away from the belief that such mechanisms exist for the sole purpose of self destruction. Support for this concept has been and continues to be judiciously documented, yet significant questions remain unanswered. Paramount among these questions is the evolutionary intent of apoptotic proteins and pathways. Prevailing opinion suggests that apoptosis evolved in unicellular life forms as a means to eliminate individual cells for the benefit of the larger colony (88). Although such an apoptotic response is beneficial to the colony as a whole, the selective pressure to curb or eliminate a mechanism solely devoted to cell death should be significant. This perceived selective pressure could be mitigated by coupling the death pathways to other similar yet vital cell functions, such as differentiation. Alternatively, what we conceive to be pro-death proteins may have originated as a means to spur differentiation and were later co-opted as a programmed cell death mechanism. Clearly, acceptance of this divergent viewpoint will depend on establishing the non-death role of probable apoptotic proteins in single cell organisms.

As noted, apoptosis and cell differentiation are believed to be ancient features of cell life and likely appeared within a similar evolutionary timeframe (4, 88). Single cell eukaryotes such as yeast display behaviors similar to a regulated cell death and also demonstrate cellular adaptations that arguably are akin to a differentiation process (reviewed in 89). Although debate still continues regarding the physiological relevance of an apoptotic response in yeast, model yeast strains such as *Saccharomyces cerevisiae* encode for pro-

teins that are remarkably similar in form and function to known apoptotic factors. For example, the yeast Yca1 protein possesses caspase like cleavage activity and deletion of this factor reduces apoptotic like cell death following exposure to insults such as hydrogen peroxide (90, 91). The mitochondrial death protein apoptosis inducing factor (AIF) also appears to have a yeast orthologue (Aif1p) that translocates to the nucleus and engages apoptosis when stimulated by a death signal such as oxygen stress (92). However, despite the ability of Yca1 and Aif1p to mediate an experimental death response, neither protein appears to be essential for physiological cell death that is associated with ammonia exposure/colony aging (89). If Yca1 and Aif1p are not exclusively death oriented, then a reasonable assumption is that each protein retains another role within yeast, perhaps as regulators of differentiation/maturation.

Single cell prokaryotes also display considerable phenotypic similarities between apoptosis and differentiation. A notable example is the sporulation process of *Bacillus Subtilis*. During adverse conditions that are not conducive to survival, *B. Subtilis* undergoes an asymmetric cell division giving rise to a daughter cell and a mother cell. The daughter cell is a long-lived spore capable of repopulating a bacterial colony, while the mother cell initiates a process of differentiation, ultimately leading to cell death (93). Other bacteria engage a less extreme differentiation adaptation in response to nutrient starvation, the best example being the stationary phase of *E. Coli* (94). In addition to nutrient-induced cell fate decisions, other prokaryotes such as actinomycetes, cyanobacteria, and alpha-proteobacteria are characterized by complex developmental programs that have hallmarks of both apoptosis and differentiation (95, 96). Interestingly, a wide variety of these bacteria possess orthologues of key apoptotic proteins such as caspase proteases (from the related paracaspase and metacaspase protease family), the mitochondrial OMI (HtrA-like) protease, and AIF (97). Despite the expression of these presumptive death proteins, the experimental evidence to support a role for these same factors in bacterial apoptosis is limited. Given this ambiguity, it is tempting to speculate that the true functional significance of these proteins may reside in an ability to regulate differentiation-like behaviors that act as a prelude to death (sporulation) or transcend the death process entirely (stationary phase).

The inhibitors of apoptosis: what is their prospective role within the context of cellular differentiation?

The BCL-2 family of proteins can act as both pro- and antiapoptotic regulators. The mammalian prosurvival members of the BCL-2 family including BCL-2, BCL-X_L, MCL-1, and A1 share homology through their BCL-2 homology (BH) domains to the antiapoptotic CED-9 found in *C. elegans* (98). These BCL-2 members function through the intrinsic apoptotic pathway at the level of the mitochondria (discussed above). In contrast to

the BCL-2 family of apoptotic inhibitors, the inhibitors of apoptosis proteins (IAPs) elicit their effects directly on caspase proteases.

IAPs are a family of structurally related proteins that was first identified in baculovirus (99) and later in *Drosophila* and mammals. The antiapoptotic activity of IAPs is conferred via the baculovirus IAP repeat (BIR) motif, an ~70–80 amino acid region of 1–3 repeats found at the IAP N terminus (99, 100). IAPs are known to bind directly to active caspase proteases thereby regulating their apoptotic activity (99), although this has only been demonstrated under physiological conditions for the X-linked IAP (XIAP; ref 99–101).

Under the prescribed context of IAPs as inhibitors of caspase activity, it is reasonable to assume that their function may suppress the differentiation program. Indeed, overexpression of cIAP-1 was shown to inhibit the differentiation of monocytic THP1 cells into macrophages (102). A role for the human neuronal IAP (NAIP) in cellular differentiation was also observed in differentiating PC12 cells (103). Specifically, overexpression of NAIP impaired neuronal differentiation and was correlated with reduced caspase 3 activity. Overexpression of DIAP also inhibited ovary epithelial cell migration in *Drosophila* (83). Given the stringent control of cellular protein synthesis, an over-accumulation of IAP under normal biological conditions is unlikely. Therefore, IAP function is presumably more complex than simply serving to inhibit apoptosis. The multitude of evidence demonstrating how IAPs themselves are regulated through transcriptional and post-translational mechanisms is support for such a presumption (reviewed in 99).

Subcellular localization may be another mechanism whereby IAP function is regulated. The cellular IAP-1 (cIAP-1) was shown to translocate from the nucleus to the cytosol during monocytic differentiation and translocation of cIAP-1 was not observed when differentiation was blocked (102). In the same study, XIAP was also shown to have cytoplasmic accumulation after differentiation in U937 human leukemia cells, although caspase activity was not measured. As mentioned above, XIAP is the only member of the IAP family that has been demonstrated to inhibit specific caspase activity *in vivo* through direct protein-protein interaction. More recently, IAPs have been demonstrated to bind caspases but not inhibit their proteolytic activity (104). Despite these results, however, a considerable amount of evidence suggests that IAPs can offer protection against fatal apoptotic consequences (99, 160, 101). One plausible interpretation with respect to its putative role in cellular differentiation is that *in vivo*, the IAPs, aside from XIAP, behave as sinks or holding vessels for active caspases thereby regulating stoichiometric concentrations of these proteases (99, 104). This would seem appropriate at a time when perhaps a very finite level of caspase activity is required to initiate differentiation specific signals. It is therefore likely that these proteins are more complex in their regulation and activity than originally believed. Clearly, the evolu-

ing dogma of caspase proteins and their role in non-apoptotic events, particularly cell differentiation, will ensure that cellular apoptotic inhibitors provide exciting investigations in the near future.

Summary

Individually, these various observations arouse a level of curiosity, yet when considered as a collective body of work these same observations imply that cell death pathways are much more than their name sake evokes. If we accept the weight of evidence that these same pathways are intrinsic features of cell differentiation, then it is a logical outcome that we must redefine our understanding of apoptosis. The latter suggestion should not be misconstrued, apoptosis is a defined process with a final outcome. However, it is clear that the machinery of death is also the machinery of life. Therefore, the challenge before us will be to identify the mechanisms and nuances that ultimately guide this same machinery to death or differentiation. **[FJ]**

The authors thank Ruth Slack and Valerie Wallace for thought provoking discussions. Work in the laboratory of L. A. M. is supported by the Canadian Institutes of Health Research, Muscular Dystrophy Association (USA) and the Heart and Stroke Foundation of Canada. L. A. M. holds the Mach-Gaensslen Chair in Cardiac Research at the Ottawa Hospital Research Institute.

REFERENCES

- Kerr, J. F., Wyllie, A. H., and Currie, A. R. (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* **26**, 239–257
- Hall, A., and Nobes, C. D. (2000) Rho GTPases: molecular switches that control the organization and dynamics of the actin cytoskeleton. *Philos Trans. R Soc. Lond. B Biol. Sci.* **355**, 965–970
- Kroemer, G., and Martin, S. J. (2005) Caspase-independent cell death. *Nat Med.* **11**, 725–730
- Ameisen, J. C. (2002) On the origin, evolution, and nature of programmed cell death: a timeline of four billion years. *Cell Death Differ.* **9**, 367–393
- Furusawa, C., and Kaneko, K. (2002) Origin of multicellular organisms as an inevitable consequence of dynamical systems. *Anat. Rec.* **268**, 327–342
- Garrido, C., and Kroemer, G. (2004) Life's smile, death's grin: vital functions of apoptosis-executing proteins. *Curr. Opin. Cell Biol.* **16**, 639–646
- Chasis, J. A., and Schrier, S. L. (1989) Membrane deformability and the capacity for shape change in the erythrocyte. *Blood* **74**, 2562–2568
- Wride, M. A. (2000) Minireview: apoptosis as seen through a lens. *Apoptosis* **5**, 203–209
- Farzaneh, F., Zalin, R., Brill, D., and Shall, S. (1982) DNA strand breaks and ADP-ribosyl transferase activation during cell differentiation. *Nature* **300**, 362–366
- Dawson, B. A., and Lough, J. (1988) Immunocytochemical localization of transient DNA strand breaks in differentiating myotubes using in situ nick-translation. *Dev. Biol.* **127**, 362–367
- Coulton, G. R., Rogers, B., Strutt, P., Skynner, M. J., and Watt, D. J. (1992) In situ localisation of single-stranded DNA breaks in nuclei of a subpopulation of cells within regenerating skeletal muscle of the dystrophic mdx mouse. *J. Cell Sci.* **102**, 653–662
- Hossain, M. S., Akimitsu, N., Kurokawa, K., and Sekimizu, K. (2003) Myogenic differentiation of *Drosophila* Schneider cells by DNA double-strand break-inducing drugs. *Differentiation* **71**, 271–280
- McMahon, G., Alsina, J. L., and Levy, S. B. (1984) Induction of a Ca²⁺, Mg²⁺-dependent endonuclease activity during the early stages of murine erythroleukemic cell differentiation. *Proc. Natl. Acad. Sci. U. S. A.* **81**, 7461–7465
- Khan, Z., and Francis, G. E. (1987) Contrasting patterns of DNA strand breakage and ADP-ribosylation-dependent DNA ligation during granulocyte and monocyte differentiation. *Blood* **69**, 1114–1119
- Qu, G., Yan, H., and Strauch, A. R. (1997) Actin isoform utilization during differentiation and remodeling of BC3H1 myogenic cells. *J. Cell. Biochem.* **67**, 514–527
- Gallo, R., Serafini, M., Castellani, L., Falcone, G., and Alema, S. (1999) Distinct effects of Rac1 on differentiation of primary avian myoblasts. *Mol. Biol. Cell* **10**, 3137–3150
- Mills, J. C., Stone, N. L., Erhardt, J., and Pittman, R. N. (1998) Apoptotic membrane blebbing is regulated by myosin light chain phosphorylation. *J. Cell Biol.* **140**, 627–636
- Yagami-Hiromasa, T., Sato, T., Kurisaki, T., Kamijo, K., Nabeshima, Y., and Fujisawa-Sehara, A. (1995) A metalloprotease-disintegrin participating in myoblast fusion. *Nature* **377**, 652–656
- Powell, W. C., Fingleton, B., Wilson, C. L., Boothby, M., and Matrisian, L. M. (1999) The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. *Curr. Biol.* **9**, 1441–1447
- Van den Eijnde, S. M., van den Hoff, M. J., Reutelingsperger, C. P., van Heerde, W. L., Henfling, M. E., Vermeij-Keers, C., Schutte, B., Borgers, M., and Ramaekers, F. C. (2001) Transient expression of phosphatidylserine at cell-cell contact areas is required for myotube formation. *J. Cell Sci.* **114**, 3631–3642
- Utz, P. J., and Anderson, P. (2000) Life and death decisions: regulation of apoptosis by proteolysis of signaling molecules. *Cell Death Differ.* **7**, 589–602
- Nicholson, D. W. (1999) Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Differ.* **6**, 1028–1042
- Ashkenazi, A., and Dixit, V. M. (1998) Death receptors: signaling and modulation. *Science* **281**, 1305–1308
- Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S. M., Ahmad, M., Alnemri, E. S., and Wang, X. (1997) Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* **91**, 479–489
- Green, D., and Kroemer, G. (1998) The central executioners of apoptosis: caspases or mitochondria? *Trends Cell Biol.* **8**, 267–271
- Srinivasula, S. M., Ahmad, M., Fernandes-Alnemri, T., and Alnemri, E. S. (1998) Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. *Mol. Cell.* **1**, 949–957
- Ishizaki, Y., Jacobson, M. D., and Raff, M. C. (1998) A role for caspases in lens fiber differentiation. *J. Cell Biol.* **140**, 153–158
- Weil, M., Raff, M. C., and Braga, V. M. (1999) Caspase activation in the terminal differentiation of human epidermal keratinocytes. *Curr. Biol.* **9**, 361–364
- Zermati, Y., Garrido, C., Amsellem, S., Fishelson, S., Bouscary, D., Valensi, F., Varet, B., Solary, E., and Hermine, O. (2001) Caspase activation is required for terminal erythroid differentiation. *J. Exp. Med.* **193**, 247–254
- Okuyama, R., Nguyen, B. C., Talora, C., Ogawa, E., Di Vignano, A. T., Lioumi, M., Chiorino, G., Tagami, H., Woo, M., and Dotto, G. P. (2004) High commitment of embryonic keratinocytes to terminal differentiation through a Notch1-caspase 3 regulatory mechanism. *Dev. Cell.* **6**, 551–562
- Zandy, A. J., Lakhani, S., Zheng, T., Flavell, R. A., and Bassnett, S. (2005) Role of the executioner caspases during lens development. *J. Biol. Chem.* **280**, 30263–30272
- Weber, G. F., and Menko, A. S. (2005) The canonical intrinsic mitochondrial death pathway has a non-apoptotic role in signaling lens cell differentiation. *J. Biol. Chem.* **280**, 22135–22145
- Fernando, P., Kelly, J. F., Balazsi, K., Slack, R. S., and Megency, L. A. (2002) Caspase 3 activity is required for skeletal muscle differentiation. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 11025–11030
- Wedhas, N., Klamut, H. J., Dogra, C., Srivastava, A. K., Mohan, S., and Kumar, A. (2005) Inhibition of mechanosensitive cation channels inhibits myogenic differentiation by suppress-

- ing the expression of myogenic regulatory factors and caspase-3 activity. *FASEB J.* **19**, 1986–1997
35. Sordet, O., Rebe, C., Plenchette, S., Zermati, Y., Hermine, O., Vainchenker, W., Garrido, C., Solary, E., and Dubrez-Daloz, L. (2002) Specific involvement of caspases in the differentiation of monocytes into macrophages. *Blood* **100**, 4446–4453
 36. Mogi, M., and Togari, A. (2003) Activation of caspases is required for osteoblastic differentiation. *J. Biol. Chem.* **278**, 47477–47482
 37. Miura, M., Chen, X. D., Allen, M. R., Bi, Y., Gronthos, S., Seo, B. M., Lakhani, S., Flavell, R. A., Feng, X. H., *et al.* (2004) A crucial role of caspase-3 in osteogenic differentiation of bone marrow stromal stem cells. *J. Clin. Invest.* **114**, 1704–1713
 38. Rohn, T. T., Cusack, S. M., Kessinger, S. R., and Oxford, J. T. (2004) Caspase activation independent of cell death is required for proper cell dispersal and correct morphology in PC12 cells. *Exp. Cell Res.* **295**, 215–225
 39. Fernando, P., Brunette, S., and Megeney, L. A. (2005) Neural stem cell differentiation is dependent upon endogenous caspase-3 activity. *Faseb J.*
 40. Oomman, S., Strahlendorf, H., Finckbone, V., and Strahlendorf, J. (2005) Non-lethal active caspase-3 expression in Bergmann glia of postnatal rat cerebellum. *Brain Res. Dev. Brain Res.* **160**, 130–145
 41. Cathelin, S., Rebe, C., Haddaoui, L., Simioni, N., Verdier, F., Fontenay, M., Launay, S., Mayeux, P., and Solary, E. (2006) Identification of proteins cleaved downstream of caspase activation in monocytes undergoing macrophage differentiation. *J. Biol. Chem.*
 42. Kang, T.-B., Ben-Moshe, T., Varfolomeev, E. E., Pewzner-Jung, Y., Yogev, N., Jurewicz, A., Waisman, A., Brenner, O., Haffner, R., *et al.* (2004) Caspase-8 serves both apoptotic and nonapoptotic roles. *J. Immunol.* **173**, 2976–2984
 43. Gude, N. M., Roberts, C. T., Kalionis, B., and King, R. G. (2004) Growth and function of the normal human placenta. *Thrombosis Research Special Issue - State-of-the-Art 11th International Congress on Antiphospholipid Antibodies* **114**, 397–407
 44. Black, S., Kadyrov, M., Kaufmann, P., Ugele, B., Emans, N., and Huppertz, B. (2004) Syncytial fusion of human trophoblast depends on caspase 8. *Cell Death Differ.* **11**, 90–98
 45. Chen, S.-E., Gerken, E., Zhang, Y., Zhan, M., Mohan, R. K., Li, A. S., Reid, M. B., and Li, Y.-P. (2005) Role of TNF- α signaling in regeneration of cardiotoxin-injured muscle. *Am. J. Physiol. Cell Physiol.* **289**, C1179–1187
 46. Torrente, Y., El Fahime, E., Caron, N. J., Del Bo, R., Belicchi, M., Pisati, F., Tremblay, J. P., and Bresolin, N. (2003) Tumor necrosis factor- α (TNF- α) stimulates chemotactic response in mouse myogenic cells. *Cell Transplant.* **12**, 91–100
 47. Badorf, C., Ruetten, H., Mueller, S., Stahmer, M., Gehring, D., Jung, F., Ihling, C., Zeiher, A. M., and Dimmeler, S. (2002) Fas receptor signaling inhibits glycogen synthase kinase 3 β and induces cardiac hypertrophy following pressure overload. *J. Clin. Invest.* **109**, 373–381
 48. Alam, A., Cohen, L. Y., Aouad, S., and Sekaly, R.-P. (1999) Early activation of caspases during T lymphocyte stimulation results in selective substrate cleavage in nonapoptotic cells. *J. Exp. Med.* **190**, 1879–1890
 49. Kennedy, N. J., Kataoka, T., Tschopp, J., and Budd, R. C. (1999) Caspase Activation Is Required for T Cell Proliferation. *J. Exp. Med.* **190**, 1891–1896
 50. Siegel, R. M. (2006) Caspases at the crossroads of immune-cell life and death. **6**, 308–317
 51. Varfolomeev, E. E., Schuchmann, M., Luria, V., Chiannilkulchai, N., Beckmann, J. S., Mett, I. L., Rebrikov, D., Brodianski, V. M., Kemper, O. C., Kollet, O., *et al.* (1998) Targeted disruption of the mouse Caspase 8 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and is lethal prenatally. *Immunity* **9**, 267–276
 52. Yeh, W. C., Pompa, J. L., McCurrach, M. E., Shu, H. B., Elia, A. J., Shahinian, A., Ng, M., Wakeham, A., Khoo, W., *et al.* (1998) FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. *Science* **279**, 1954–1958
 53. Yeh, W. C., Itie, A., Elia, A. J., Ng, M., Shu, H. B., Wakeham, A., Mirtsos, C., Suzuki, N., Bonnard, M., Goeddel, D. V., and Mak, T. W. (2000) Requirement for Casper (c-FLIP) in regulation of death receptor-induced apoptosis and embryonic development. *Immunity* **12**, 633–642
 54. Lakhani, S. A., Masud, A., Kuida, K., Porter, G. A., Jr., Booth, C. J., Mehal, W. Z., Inayat, I., and Flavell, R. A. (2006) Caspases 3 and 7: key mediators of mitochondrial events of apoptosis. *Science* **311**, 847–851
 55. Joza, N., Oudit, G. Y., Brown, D., Benit, P., Kassiri, Z., Vahsen, N., Benoit, L., Patel, M. M., Nowikovsky, K., Vassault, A., *et al.* (2005) Muscle-specific loss of apoptosis-inducing factor leads to mitochondrial dysfunction, skeletal muscle atrophy, and dilated cardiomyopathy. *Mol. Cell Biol.* **25**, 10261–10272
 56. Chomarat, P., Dantin, C., Bennett, L., Banchereau, J., and Palucka, A. K. (2003) TNF skews monocyte differentiation from macrophages to dendritic cells. *J. Immunol.* **171**, 2262–2269
 57. Rimondi, E., Secchiero, P., Quaroni, A., Zerbinati, C., Capitani, S., and Zauli, G. (2006) Involvement of TRAIL/TRAIL-receptors in human intestinal cell differentiation. *J. Cell. Physiol.* **206**, 647–654
 58. Pirrotta, V. (2004) The ways of PARP. *Cell* **119**, 735–736
 59. Budihardjo, I. I., Poirier, G. G., and Kaufmann, S. H. (1998) Apparent cleavage of poly(ADP-ribose) polymerase in non-apoptotic mouse LTA cells: An artifact of cross-reactive secondary antibody. *Mol. Cell. Biochem.* **178**, 245–249
 60. McKinsey, T. A., Zhang, C. L., Lu, J., and Olson, E. N. (2000) Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature* **408**, 106–111
 61. Okamoto, S., Li, Z., Ju, C., Scholzke, M. N., Mathews, E., Cui, J., Salvesen, G. S., Bossy-Wetzel, E., and Lipton, S. A. (2002) Dominant-interfering forms of MEF2 generated by caspase cleavage contribute to NMDA-induced neuronal apoptosis. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 3974–3979
 62. Tang, X., Wang, X., Gong, X., Tong, M., Park, D., Xia, Z., and Mao, Z. (2005) Cyclin-dependent kinase 5 mediates neurotoxin-induced degradation of the transcription factor myocyte enhancer factor 2. *J. Neurosci.* **25**, 4823–4834
 63. Fischer, U., Janicke, R. U., and Schulze-Osthoff, K. (2003) Many cuts to ruin: a comprehensive update of caspase substrates. *Cell Death Differ.* **10**, 76–100
 64. Cardone, M. H., Salvesen, G. S., Widmann, C., Johnson, G., and Frisch, S. M. (1997) The regulation of anoikis: MEKK-1 activation requires cleavage by caspases. *Cell* **90**, 315–323
 65. Widmann, C., Gerwins, P., Johnson, N. L., Jarpe, M. B., and Johnson, G. L. (1998) MEK kinase 1, a substrate for DEVD-directed caspases, is involved in genotoxin-induced apoptosis. *Mol. Cell Biol.* **18**, 2416–2429
 66. Frasch, S. C., Nick, J. A., Fadok, V. A., Bratton, D. L., Worthen, G. S., and Henson, P. M. (1998) p38 mitogen-activated protein kinase-dependent and -independent intracellular signal transduction pathways leading to apoptosis in human neutrophils. *J. Biol. Chem.* **273**, 8389–8397
 67. Graves, J. D., Gotoh, Y., Draves, K. E., Ambrose, D., Han, D. K., Wright, M., Chernoff, J., Clark, E. A., and Krebs, E. G. (1998) Caspase-mediated activation and induction of apoptosis by the mammalian Ste20-like kinase Mst1. *EMBO J.* **17**, 2224–2234
 68. Zarubin, T., and Han, J. (2005) Activation and signaling of the p38 MAP kinase pathway. *Cell Res.* **15**, 11–18
 69. Riento, K., and Ridley, A. J. (2003) Rocks: multifunctional kinases in cell behaviour. *Nat. Rev. Mol. Cell Biol.* **4**, 446–456
 70. Rubiolo, C., Piazzolla, D., Meissl, K., Beug, H., Huber, J. C., Kolbus, A., and Baccarini, M. (2006) A balance between Raf-1 and Fas expression sets the pace of erythroid differentiation. *Blood*
 71. Demontis, S., Rigo, C., Piccinin, S., Mizzau, M., Sonogo, M., Fabris, M., Brancolini, C., and Maestro, R. (2006) Twist is substrate for caspase cleavage and proteasome-mediated degradation. *Cell Death Differ.* **13**, 335–345
 72. Liu, F., Dowling, M., Yang, X. J., and Kao, G. D. (2004) Caspase-mediated specific cleavage of human histone deacetylase 4. *J. Biol. Chem.* **279**, 34537–34546
 73. Mejat, A., Ramond, F., Bassel-Duby, R., Khochbin, S., Olson, E. N., and Schaeffer, L. (2005) Histone deacetylase 9 couples neuronal activity to muscle chromatin acetylation and gene expression. *Nat. Neurosci.* **8**, 313–321
 74. Yang, J. Y., and Widmann, C. (2001) Antiapoptotic signaling generated by caspase-induced cleavage of RasGAP. *Mol. Cell Biol.* **21**, 5346–5358

75. Yang, J. Y., Michod, D., Walicki, J., Murphy, B. M., Kasibhatla, S., Martin, S. J., and Widmann, C. (2004) Partial cleavage of RasGAP by caspases is required for cell survival in mild stress conditions. *Mol. Cell Biol.* **24**, 10425–10436
76. Baehrecke, E. H. (2002) How death shapes life during development. *Nat Rev Mol. Cell Biol.* **3**, 779–787
77. Hay, B. A., Huh, J. R., and Guo, M. (2004) The genetics of cell death: approaches, insights and opportunities in *Drosophila*. *Nat. Rev. Genet.* **5**, 911–922
78. Yoo, S. J., Huh, J. R., Muro, I., Yu, H., Wang, L., Wang, S. L., Feldman, R. M., Clem, R. J., Muller, H. A., and Hay, B. A. (2002) Hid, Rpr and Grim negatively regulate DIAP1 levels through distinct mechanisms. *Nat. Cell Biol.* **4**, 416–424
79. Holley, C. L., Olson, M. R., Colon-Ramos, D. A., and Kornbluth, S. (2002) Reaper eliminates IAP proteins through stimulated IAP degradation and generalized translational inhibition. *Nat. Cell Biol.* **4**, 439–444
80. Ryoo, H. D., Bergmann, A., Gonen, H., Ciechanover, A., and Steller, H. (2002) Regulation of *Drosophila* IAP1 degradation and apoptosis by reaper and *ubcD1*. *Nat. Cell Biol.* **4**, 432–438
81. Arama, E., Agapite, J., and Steller, H. (2003) Caspase activity and a specific cytochrome C are required for sperm differentiation in *Drosophila*. *Dev. Cell.* **4**, 687–697
82. Huh, J. R., Verwooy, S. Y., Yu, H., Yan, N., Shi, Y., Guo, M., and Hay, B. A. (2004) Multiple apoptotic caspase cascades are required in nonapoptotic roles for *Drosophila* spermatid individualization. *PLoS Biol.* **2**, E15
83. Geisbrecht, E. R., and Montell, D. J. (2004) A role for *Drosophila* IAP1-mediated caspase inhibition in Rac-dependent cell migration. *Cell* **118**, 111–125
84. Kanuka, H., Kuranaga, E., Takemoto, K., Hiratou, T., Okano, H., and Miura, M. (2005) *Drosophila* caspase transduces Shaggy/GSK-3 β kinase activity in neural precursor development. *EMBO J.* **24**, 3793–3806
85. Chew, S. K., Akdemir, F., Chen, P., Lu, W. J., Mills, K., Daish, T., Kumar, S., Rodriguez, A., and Abrams, J. M. (2004) The apical caspase *dronc* governs programmed and unprogrammed cell death in *Drosophila*. *Dev. Cell.* **7**, 897–907
86. Kuida, K., Zheng, T. S., Na, S., Kuan, C., Yang, D., Karasuyama, H., Rakic, P., and Flavell, R. A. (1996) Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. *Nature* **384**, 368–372
87. Cecconi, F., Alvarez-Bolado, G., Meyer, B. I., Roth, K. A., and Gruss, P. (1998) *Apaf1* (CED-4 homolog) regulates programmed cell death in mammalian development. *Cell* **94**, 727–737
88. Ameisen, J. C. (1996) The origin of programmed cell death. *Science* **272**, 1278–1279
89. Vachova, L., and Palkova, Z. (2005) Physiological regulation of yeast cell death in multicellular colonies is triggered by ammonia. *J. Cell Biol.* **169**, 711–717
90. Madeo, F., Herker, E., Maldener, C., Wissing, S., Lachelt, S., Herlan, M., Fehr, M., Lauber, K., Sigrist, S. J., Wesselborg, S., and Frohlich, K. U. (2002) A caspase-related protease regulates apoptosis in yeast. *Mol. Cell.* **9**, 911–917
91. Khan, M. A., Chock, P. B., and Stadtman, E. R. (2005) Knockout of caspase-like gene, YCA1, abrogates apoptosis and elevates oxidized proteins in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 17326–17331
92. Wissing, S., Ludovico, P., Herker, E., Buttner, S., Engelhardt, S. M., Decker, T., Link, A., Proksch, A., Rodrigues, F., Corte-Real, M., et al. (2004) An AIF orthologue regulates apoptosis in yeast. *J. Cell Biol.* **166**, 969–974
93. Hengge-Aronis, R. (1993) Survival of hunger and stress: the role of *rpoS* in early stationary phase gene regulation in *E. coli*. *Cell* **72**, 165–168
94. Aizenman, E., Engelberg-Kulka, H., and Glaser, G. (1996) An *Escherichia coli* chromosomal “addiction module” regulated by guanosine [corrected] 3',5'-bispyrophosphate: a model for programmed bacterial cell death. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 6059–6063
95. Kaiser, D. (1999) Cell fate and organogenesis in bacteria. *Trends Genet.* **15**, 273–277
96. Koonin, E. V., and Aravind, L. (2002) Origin and evolution of eukaryotic apoptosis: the bacterial connection. *Cell Death Differ.* **9**, 394–404
97. Boyce, M., Degterev, A., and Yuan, J. (2004) Caspases: an ancient cellular sword of Damocles. *Cell Death Differ.* **11**, 29–37
98. Willis, S., Day, C. L., Hinds, M. G., and Huang, D. C. (2003) The Bcl-2-regulated apoptotic pathway. *J. Cell Sci.* **116**, 4053–4056
99. Salvesen, G. S., and Duckett, C. S. (2002) IAP PROTEINS: Blocking the road to death's door. *Nat. Rev. Mol. Cell Biol.* **3**, 401–410
100. Shi, Y. (2002) Mechanisms of caspase activation and inhibition during apoptosis. *Mol. Cell.* **9**, 459–470
101. Liston, P., Fong, W. G., and Korneluk, R. G. (2003) The inhibitors of apoptosis: there is more to life than Bcl2. *Oncogene* **22**, 8568–8580
102. Plenchette, S., Cathelin, S., Rebe, C., Launay, S., Ladoire, S., Sordet, O., Ponnelle, T., Debili, N., Phan, T. H., Padua, R. A., et al. (2004) Translocation of the inhibitor of apoptosis protein c-IAP1 from the nucleus to the Golgi in hematopoietic cells undergoing differentiation: a nuclear export signal-mediated event. *Blood* **104**, 2035–2043
103. Gotz, R., Karch, C., Digby, M. R., Troppmair, J., Rapp, U. R., and Sendtner, M. (2000) The neuronal apoptosis inhibitory protein suppresses neuronal differentiation and apoptosis in PC12 cells. *Hum. Mol. Genet.* **9**, 2479–2489
104. Eckelman, B. P., and Salvesen, G. S. (2006) The Human Anti-apoptotic Proteins cIAP1 and cIAP2 Bind but Do Not Inhibit Caspases. *J. Biol. Chem.* **281**, 3254–3260

Received for publication April 5, 2006.

Accepted for publication July 24, 2006.