The essentials of developmental apoptosis [version 1; peer review: 3 approved]

Anne K. Voss\(^1,2\), Andreas Strasser\(^1,2\)

\(^1\)Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia
\(^2\)Department of Medical Biology, The University of Melbourne, Melbourne, Victoria, Australia

Abstract

Apoptotic cells are commonly observed in a broad range of tissues during mammalian embryonic and fetal development. Specific requirements and functions of programmed cell death were inferred from early observations. These inferences did not hold up to functional proof for a requirement of apoptosis for normal tissue development in all cases. In this review, we summarize how the appraisal of the importance of developmental apoptosis has changed over the years, in particular with detailed functional assessment, such as by using gene-targeted mice lacking essential initiators or mediators of apoptosis. In recent years, the essentials of developmental apoptosis have emerged. We hypothesize that apoptosis is predominantly required to balance cell proliferation. The two interdependent processes—cell proliferation and apoptosis—together more powerfully regulate tissue growth than does each process alone. We proposed that this ensures that tissues and cell populations attain the appropriate size that allows fusion in the body midline and retain the size of cavities once formed. In addition, a limited number of tissues, albeit not all previously proposed, rely on apoptosis for remodeling, chiefly aortic arch remodeling, elimination of supernumerary neurons, removal of vaginal septa, and removal of interdigital webs in the formation of hands and feet.

Keywords

Embryo, fetus, development, programmed cell death, apoptosis, BIM, PUMA, BID, BMF, NOXA, BIK, BAD, HRK, BCL-2, MCL-1, BCL-XL, BCL-W, A1, BAX, BAK, BOK, APAF-1, caspases
Introduction
The observation that cells can undergo cell death during development was first made in the 1920s (reviewed in 1). The morphology of these dying cells was found to be distinctly different than that observed in necrotic cells (for example, cells subjected to injury). Isolated cells or at most small clusters of cells undergo cell death during normal development. Cells undergoing cell death during embryonic development shrink rather than rupture as seen in necrosis (Figure 1); cell nuclei condense in what is termed pyknosis (from the Greek pykno, meaning thick, compact, or dense) and ultimately fragment. The components of such dying cells are packaged into cytoplasmic membrane–enclosed vesicles awaiting phagocytosis by other cells. In contrast, during necrosis, which can occur during injury and inflammation, larger areas of tissue are usually affected, cells swell, the plasma membrane and nuclear envelope rupture, and cell contents spill out.

The predictable time course and location of the dying cells during development suggested that it was a regulated process and the term programmed cell death was used to indicate this concept. Based on the time course and location, the essential roles of programmed cell death during development—including requirement for tissue invagination and closure, union of body halves, lumen formation, bifurcation, regression of rudimentary organs, and cell differentiation—were inferred 1. Later, the electron microscopic characteristics of the dying cells were described and the term apoptosis (from the Greek apo + ptosis, meaning from + falling) was coined (reviewed in 2). The cell membrane–bound fragments of apoptotic cells were termed apoptotic bodies. Apoptosis is thought to drive morphogenesis by regulating cell number, tissue sculpturing, and deleting structures, including the conversion of solid structures into tubes and vesicles 3.

Regulation of apoptosis
In the following decades, a large number of researchers worked to decipher the molecular mechanisms that initiate and execute apoptosis during development; they also determined the role of apoptosis in disease conditions (reviewed in 4, 5). Two distinct but ultimately converging pathways initiate apoptosis 6: the mitochondrial, intrinsic, or B-cell lymphoma 2 (BCL-2)–regulated pathway and the extrinsic or death receptor pathway (Figure 2).

The mitochondrial pathway is initiated during development by limiting levels of growth factors, metabolic stress, lack of neurotrophic support, lack of blood flow, or loss of substrate adhesion (anoikis, from the Greek an + oikos, meaning without + home) and in disease or experimental settings by DNA damage and diverse cytotoxic agents. The response to these stimuli is regulated by BCL-2 protein family members 4. In response to death-inducing stimuli, pro-apoptotic members of the family (BIM, PUMA, BID, BMF, NOXA, BIK, BAD, and HRK; Table 1) activate a series of transcription factors, which leads to the expression of molecules that commit cells to death. These molecules include cytochrome c, which is released from the mitochondria into the cytoplasm, and other proteins that form complexes and initiate the execution of apoptosis.

| Characteristics     | Normal cell                                                                 | Apoptotic cell                                      | Necrotic cell                                      |
|---------------------|------------------------------------------------------------------------------|-----------------------------------------------------|---------------------------------------------------|
| Cell shape          | varies according to function                                                  | rounded and shrunk                                   | rounded and swollen                                |
| Chromatin           | well-defined domains of euchromatin and heterochromatin                       | Condensed, marginalized, later fragmented            | lighter, disorganized                              |
| Cell membrane       | intact                                                                       | intact, later apoptotic bodies                       | ruptured                                          |
| Nuclear envelope    | intact                                                                       | intact                                              | disintegrating                                    |
| Organelles (EM only)| well defined, numerous                                                        | shrunk                                              | not discernable                                   |
| Cytoplasm           | contained                                                                    | contained                                           | spilling out                                       |

Figure 1. The morphological distinction of apoptosis and necrosis. Schematic drawing and major characteristics. EM, electron microscopy.
collectively termed BH3 [BCL-2 homology domain 3]-only proteins) inhibit the anti-apoptotic BCL-2 family members (BCL-2, MCL-1, BCL-XL, BCL-W, and A1/BFL-1), which under steady-state conditions keep the pro-apoptotic effectors, the multi-BH domain proteins BAX and BAK, in an inactive state. In addition, some BH3-only proteins (for example, BIM and PUMA) have been reported to activate BAX and BAK directly. Once activated, BAX and BAK form pores within the mitochondrial outer membrane, which leads to mitochondrial outer membrane permeabilization (MOMP) and release of cytochrome C and other apoptogenic factors from the mitochondria into the cytoplasm (Figure 2).

Cytochrome C associates with APAF-1 and caspase-9 to form the apoptosome and this stimulates caspase-9 activation, which in turn cleaves and thereby activates effector caspase-3, -6, and -7. These effector caspses cleave hundreds of proteins and in certain cases proteolytically activate or inhibit other enzymes. This includes the activation of endonucleases that drive DNA fragmentation and the inhibition of flippases, which causes exposure of “eat me signals” on the plasma membrane. These processes lead to chromatin condensation and packaging of the cell content into apoptotic bodies and removal by phagocytosis.

In the death receptor pathway, apoptosis is initiated by the binding of certain members of the tumor necrosis factor (TNF) family to their cognate receptors that belong to the TNF receptor (TNFR) family (in particular, binding of FAS ligand [FASL] to its receptor FAS). This can set in train two different series of events: if available, pro-caspase-8, which directly activates effector caspase-3, -6 and -7, is activated. The pro-apoptotic BH3-only protein BID, after proteolytic conversion to tBID, can also activate the BAX/BAK-dependent mitochondrial apoptotic pathway to thereby amplify the apoptotic cascade (Figure 2). If caspase-8 is absent or inhibited (for example, by viral inhibitors of this protease, such as vFLIP), another form of regulated cell death, termed necroptosis, can be initiated via receptor-interacting serine/threonine-protein kinase 1 (RIPK1), RIPK3, and mixed lineage kinase domain-like pseudokinase (MLKL) (Figure 2). These alternative fates after the activation of FAS or certain other surface receptors (for example,
TNFR1) result in either apoptotic or necroptotic cell death with discernible histological morphologies (Figure 1). The form of cell death reported to occur during embryonic development resembles apoptotic cell death. Based on histological studies, necroptotic cell death, if it occurred during development, would not be common.

In the following section, we summarize originally proposed roles of apoptotic cell death during development and more recent detailed functional studies that have identified the much more restricted gambit of processes that critically depend on developmental apoptosis. In the conclusion, we propose why developmental apoptosis occurs in many tissues, seemingly without being essential.

Apoptosis during embryonic development

Histological analyses of developing embryos discovered that pyknotic (dying/dead) cells were present in certain locations at given stages of development. Diverse animal models, from Caenorhabditis elegans to Mus musculus, were used. In C. elegans, specific individual cells destined to undergo cell death were identified\(^{8-10}\). In contrast, clusters of cells undergoing cell death in mouse embryos were noted at specific stages in specific regions, but death did not appear to be pre-programmed to target specific individual cells (Table 1). In mice, pyknotic cells were detected in a range of tissues, and it was inferred that their death was required for a range of developmental processes, including cavitation, vesicle and tube formation (lens, neural tube, and intestine), fusion of epithelial sheets (neural tube formation, midline body wall, and palate), removal of vestigial tissue (pro-nephros, parts of meso- and meta-nephros, notochord, and Müllerian duct in males and Wolffian duct in females), and tissue cellular homeostasis (summarized in 1).

Subsequent reports proposed that apoptotic cell death was essential for normal development in regions where pyknotic cells were observed (Table 1), including for the elimination of redundant cells from the inner cell mass of the embryonic day 3 (E3.5) blastocyst\(^ {11}\), and during the development of the epiblasts at E6 to E7\(^ {12}\); neural crest cells\(^ {13}\), lens vesicle\(^ {14}\), and the pro-amniotic cavity\(^ {15}\), for digit separation by removing interdigital tissue at E13.5\(^ {16}\), shaping the limbs\(^ {17}\), neural tube closure\(^ {18}\),

| Year | Proposed roles of apoptosis in development | Reference |
|------|------------------------------------------|-----------|
| 1951 | Table of incidences of programmed cell death during development Including vesicle formation (optic vesicle, lens) Tube formation by invagination and detachment from epithelial sheets (neural tube, intestine) Lumen formation (salivary gland, duodenum, colon, vagina) Fusion of epithelial sheets (midline body wall, palate) Removal of vestigial tissue (pro-nephros, parts of meso- and meta-nephros, notochord, and Müllerian duct in males Wolffian duct in females) | Glücksmann\(^ {1}\) |
| 1972 | Morphological characteristics of developmentally programmed cell death | Kerr et al.\(^ {2}\) |
| 1980 | Shaping of the epiblast | Poelmann\(^ {12}\) |
| 1989 | Elimination of redundant cells from the inner cell mass of the blastocyst | Pierce et al.\(^ {13}\) |
| 1992 | Kidney development | Koseki et al.\(^ {13}\) |
| 1993 | Kidney developing | Coles et al.\(^ {20}\) |
| 1993 | Elimination of neural crest from odd-numbered rhombomeres | Graham et al.\(^ {13}\) |
| 1994 | Lens vesicle development | Morgenbesser et al.\(^ {14}\) |
| 1995 | Lens vesicle development | Pan and Griepp\(^ {15}\) |
| 1995 | Pro-amniotic cavity formation | Coucouvanis and Martin\(^ {16}\) |
| 1996 | Digit separation by removing interdigital tissue | Jacobsen et al.\(^ {16}\) |
| 1997 | Inner ear morphogenesis | Fekete et al.\(^ {22}\) |
| 1997 | Shaping the limbs | Macias et al.\(^ {27}\) |
| 1997 | Chondrocytes undergo hypertrophy and apoptosis during enchondral bone development | Amling et al.\(^ {23}\) |
| 1997 | Neural tube closure | Weil et al.\(^ {28}\) |
| 2000 | Palatal shelf fusion | Martinez-Alvarez et al.\(^ {24}\) |
| 2015 | Epithelial folding | Monier et al.\(^ {25}\) |
and elimination of supernumerary neurons during early postnatal development of the brain.

Evidence that the mitochondrial apoptotic pathway is essential for mammalian development

Mice lacking individual apoptotic regulators provided evidence for the requirements for specific regulators and suggested that developmental apoptosis was essential for mammalian development (Table 2).

Indeed, from the original large number of developmental processes proposed to require apoptosis to proceed normally (Table 1), knockout mouse studies in recent years have singled out those developmental processes that do and those that do not require apoptosis (Table 2). In particular, it has become clear that reducing apoptosis typically causes webbed digits, vaginal septa, and often lymphadenopathy; it commonly causes exencephaly and cleft face or palate and occasionally omphalocoe. Therefore, it can be said that the removal of the interdigital webbing, vaginal septa, blood cell homeostasis, neural tube, palate, and body wall closure depends critically on developmental apoptosis. However, whether development leading up to these events or the processes themselves require apoptosis was not discriminated by these studies. Indeed, the sequence of events can be complex. For example, developing neurons can undergo apoptosis before or after target innervation2,28. However, it appears that the lack or restriction of neurotrophic support may be the cause of neuronal apoptosis on both occasions2,23.

Technical difficulties in enumerating apoptotic cells during development

As a corollary of identifying developmental processes that require apoptosis, those processes that occur normally without apoptosis might also have been defined. However, to make such a claim with certainty, the possibility of residual apoptosis occurring would have to be excluded. The difficulties in assessing absolute numbers of apoptotic cells during development can be illustrated by using the example of embryos lacking the tumor suppressor p53, which in response to a number of stress stimuli can induce either cell cycle arrest or apoptosis3,31. Male mice lacking a functional Trp53 gene are born in the expected numbers, but female Trp53+/− mice are under-represented because of a partially penetrant failure of neural tube closure3,23. Originally, electron microscopy32 and terminal deoxynucleotidyl transferase dUTP nick end labeling of DNA fragments (TUNEL) (a technique used to identify cells undergoing apoptosis) on sections33 were employed to determine whether loss of p53 affected developmental apoptosis, but neither method detected a difference between wild-type and Bax−/−;Bak−/− double knockout (DKO) embryos (total absence of apoptosis)35 or Mcl1−/−;Bclx−/− double heterozygous mice (abnormally increased apoptosis)36. However, the low percentage of apoptotic cells in developing wild-type embryos (only about 1.5%34,35) does not provide a large dynamic range for detecting a gradual reduction in apoptosis. Indeed, although the more severe developmental abnormalities in mice with combined loss of pro-apoptotic BAX, BAK, and BOK compared with loss of only BAX and BAK suggested that the Bax−/−;Bak−/−;Bok−/− triple knockout mice (TKO) should have a greater reduction in developmental apoptosis than Bax−/−;Bak−/− DKO mice, flow cytometric analysis was unable to detect significant differences between these two genotypes35. Therefore, a claim of zero apoptosis is hard to support with the current methods. A more sensitive approach may be the use of mice carrying the CAG-sA5-YFP transgene, which encodes a fluorescently labeled, secreted form of annexin V. The fluorescently labeled annexin V accumulates to detectable levels when binding to phosphatidylserine exposed on the surface of apoptotic cells37. A caveat here may be that necroptotic cells also stain positive for annexin V38.

Developmental processes less likely to require apoptosis

It was surprising that apoptosis, given its stereotypic occurrence, was not essential for C. elegans development39 and from time to time it was speculated that apoptosis in mammalian development, too, might be less critical than its prevalence might suggest40. Throughout the decades of intense research on the role of apoptosis in a number of developmental processes, information surfaced indicating that, despite previous inferences, apoptosis was not required for specific processes. An example is the role of apoptosis in the formation of the pro-amnion cavity, which was viewed as a model of cavity formation/tube formation by apoptosis and supported by work in in vivo and culture models35,40,41 but was later called into question by the development of embryos past this point in the absence of detectable apoptosis35. Similarly, apoptosis had been observed in the ridge of the closing neural folds and therefore it had been proposed that neural tube closure required apoptotic cell death at the point where the two neural folds met. This was supported by the observation that the application of caspase inhibitors at a critical time point blocked neural tube closure46. On the surface, this conclusion appears to be amply supported by the many knockout mouse mutants of apoptotic regulators that display exencephaly or spina bifida or both (Bax;Bak;Bok TKO35, Trp53 KO32,34, Apaf1 KO32,42, Casp9 KO44,45, and Casp3;Casp7 DKO46). However, one study presented evidence that apoptosis is not actually required for the specific time of neural tube closure and that lack of apoptosis did not affect tissue remodeling and separation of the neural tube from the surface ectoderm at the fusion site47. Similarly, it is not clear whether apoptosis is required at the specific time of palate fusion35,48,51.

The studies showing that neural tube closure and palate fusion can occur without developmental apoptosis raise the question...
Table 2. Examples of developmental anomalies observed in mice after deletion of genes encoding regulators of apoptosis.

| Gene deletion | Major developmental phenotype (major adult phenotype) | Reference |
|---------------|------------------------------------------------------|-----------|
| A1a           | Developmentally normal (accelerated neutrophil apoptosis) | Hamasaki et al., 1998 |
| Apaf1         | Neural tube closure defect, delayed removal of interdigital webs | Cecconi et al., 1998; Yoshida et al., 1998 |
| Bak           | Developmentally normal (platelet accumulation)          | Lindsten et al., 2000; Mason et al., 2007 |
| Bax           | Developmentally normal (lymphocyte accumulation, male infertility) | Knudson et al., 1995 |
| Bax;Bak DKO   | Defective removal of interdigital webs, vaginal septa, cell homeostasis in the forebrain neurogenic region and in the hematopoietic system | Lindsten et al., 2000 |
| Bax;Bim DKO   | Webbed feet, male infertility (lymphocyte accumulation) | Hutcheson et al., 2005 |
| Bax;Bok DKO   | Developmentally normal (in addition to Bax SKO phenotype, number of oocytes increased) | Ke et al., 2013 |
| Bax;Bak;Bok TKO | Midline fusion defects (exencephaly, spina bifida, omphalocele, cleft face/palate/lip), webbed feet, vaginal septa, aortic arch defects, renal pelvis encroached by excess tissue, (supernumerary neurons and hematopoietic cells) | Ke et al., 2018; Carpio et al., 2016 |
| Bad           | Developmentally normal                                 | Ranger et al., 2003; Kelly et al., 2010 |
| Bclx          | Lethality at about E13.5, increased apoptosis of hematopoietic and neuronal cells (decreased platelet half-life) | Motoyama et al., 1995; Mason et al., 2007 |
| Bclx;Bim DKO  | Loss of BIM rescues hematopoietic and germ cells but not neuronal cell apoptosis in Bclx−/− mice | Akhtar et al., 2008 |
| Bcl2          | Developmentally normal (polycystic kidney, loss of lymphocytes) | Veis et al., 1993 |
| Bcl2;Bim DKO  | Developmentally normal (all defects caused by the loss of Bcl2 are rescued by the concomitant loss of Bim, including lymphocyte, kidney, and melanocytes) | Bouillet et al., 2001 |
| Bik           | Developmentally normal                                 | Coultas et al., 2004 |
| Bim           | About 50% of Bim−/− mice are lost during embryogenesis prior to E10; others are developmentally normal and fertile (hematopoietic cell accumulation, autoimmune kidney disease). | Bouillet et al., 1999 |
| Bim;Bmf DKO   | In addition to the Bim−/− phenotype, webbed feet, vaginal aplasia | Labi et al., 2014 |
| Bim;Bik DKO   | In addition to the Bim−/− phenotype, DKO display (male sterility) | Coultas et al., 2005 |
| Bim;Bad DKO   | In addition to the Bim−/− phenotype, slightly more lymphocyte accumulation | Kelly et al., 2010 |
| Bmf           | Vaginal atresia (lymphoid cell accumulation)            | Hubner et al., 2010; Labi et al., 2008, 2014 |
| Bok           | Developmentally normal                                 | Ke et al., 2012 |
| Casp3         | Reduced neuronal apoptosis, neural hyperplasias, pre- and postnatal lethality | Kuida et al., 1996 |
| Casp3;Casp7 DKO | Neural tube closure defect, heart anomalies            | Lakhani et al., 2006 |
| Casp9         | Neural tube closure defect                             | Hakem et al., 1998; Kuida et al., 1998 |
| Hrk           | Developmentally normal                                 | Coultas et al., 2007 |
| Mcl1          | Embryonic lethality at the blastocyst stage prior to implantation | Rinkenberger et al., 2000 |
| Mcl1;Bclx compound hets | Craniofacial anomalies, including cases of holoprosencephaly, which are rescued by loss of one copy of Bim | Grabow et al., 2018 |
| Mcl1;Bclx DKO | Nervous system–specific DKO, neuronal apoptosis         | Fogarty et al., 2019 |
| Puma          | Developmentally normal                                 | Villunger et al., 2003 |
| Noxa          | Developmentally normal                                 | Villunger et al., 2003 |
| Puma;Noxa DKO | Developmentally normal                                 | Michalak et al., 2008 |

DKO, double knockout; E, embryonic day; hets, heterozygotes; SKO, single knockout; TKO, triple knockout.
of why so many mouse mutants of critical constituents of the mitochondrial apoptotic pathway display exencephaly, spina bifida, and cleft palate (Table 2). A possible explanation is that it is not the process of tissue fusion that requires apoptosis but rather that apoptosis is important to arrive at body halves that are of a size compatible with the fusion process.

**Death receptor pathway–induced apoptosis and necroptosis**

Activation of the death receptor pathway can lead to apoptosis via caspase-8 activation or, when caspase-8 is inhibited, to necroptosis via RIPK1/RIPK3/MLKL activation (Figure 2). Death receptor pathway–mediated apoptosis is morphologically similar to mitochondrial apoptosis, but necroptosis is distinct, resembling necrosis. Disturbance of the normal regulation of death receptor–induced cell death pathways can result in developmental lethality. Mouse embryos lacking caspase-8 display prenatal lethality\(^8\) and mice lacking RIPK1 die shortly after birth\(^9\) whereas RIPK3-deficient mice develop normally\(^10\). However, if both death receptor–activated processes (apoptosis and necroptosis) are inactivated by simultaneously deleting genes necessary for each process, the resulting mice are viable (for example, Casp8;Ripk1/3 DKO\(^11\); Casp8;Mlkl or Casp8;Fadd DKO\(^12\); and Ripk1;Ripk3;Casp8 or Ripk1;Ripk3;FADD TKO\(^13\)). The studies using these compound mutant mice provided evidence that death receptor–mediated apoptosis is not required for normal mouse development; rather, a balance between caspase-8 and RIPK1/RIPK3 activity needs to be maintained to prevent MLKL activation and consequent necroptosis. The observation that mice lacking MLKL\(^14\), the essential mediator of necroptosis, develop normally reveals that this programmed cell death process is not required for embryogenesis. In agreement with these findings, early histological studies did not detect substantial involvement of necrotic processes in mammalian development.

**Other forms of cell death and cell removal mechanisms**

The most abundant form of developmental cell death appears to be apoptosis, and more specifically mitochondrial apoptosis, as the death receptor pathway does not appear to be essential for normal development (discussed above). Furthermore, reduction of mitochondrial apoptosis to below current detection levels by simultaneous deletion of Bax, Bak, and Bok did not appear to cause a compensatory increase in necroptosis, pyroptosis, or autophagy\(^15\), suggesting that these other forms of cell death may not be poised to compensate for a deficiency of the mitochondrial apoptotic pathway.

A number of alternative cell removal processes have been proposed to play a role during development, including entosis\(^16\), autophagy\(^17,18\), cell extrusion from epithelia\(^19,20,21\), and cell senescence\(^22,23\). However, their prominence and roles during mammalian development remain to be determined. Autophagy is morphologically distinct from apoptosis\(^24\) and so could be assessed and its frequency could be compared to apoptosis. An overlap between apoptosis and entosis exists insofar as apoptotic cells are eventually engulfed by other cells. Whether mammalian cells are extruded from epithelia (for example, in the intestine) before or after they have initiated cell death appears contentious\(^24,25\), and the ultimate fate of senescent cells seems to be cell death, possibly mostly through apoptosis\(^26\).

Overall, the mitochondrial apoptotic pathway appears to be the major form of developmental cell death. However, it should be noted that low prevalence of a given process does not necessarily imply little importance. Accordingly, it is possible that in specific cases these alternative forms of cell death play an essential role during mammalian development, which remains to be functionally tested. This will probably require the generation and analysis of mice deficient in multiple cell death pathways and possibly even mice lacking several cell death pathways plus other processes (for example, cell senescence) to uncover redundancy.

**Open questions**

The following open questions exist in the field of developmental apoptosis.

1. The field might benefit from the results of a comprehensive, sensitive, and quantitative analysis of apoptosis throughout the sequence of mammalian development that either does not rely on tissue sections or uses sophisticated methods of three-dimensional reconstruction and numerical assessment.

2. Once a sensitive technical approach to (1) has been developed, the question of whether developmental apoptosis is dynamically regulated during development in response to disruptions (for example, of proliferation or cell survival) could be answered.

3. If similarly sensitive and quantitative detection methods were developed for other forms of cell death, their relative importance compared with apoptosis could be assessed.

4. The role of a number of BCL-2 family members in development remains to be determined, while single knockout of the genes encoding these does not result in overt phenotypic anomalies, which is possibly due to functional redundancies.

5. In addition, based on the theory of evolution, one would expect that even these BCL-2 family members have essential functions (that provide a selective advantage), which remain to be discovered.

**Conclusions**

The available data identify developmental processes that are clearly dependent on functional apoptosis, whereas other processes previously thought to depend on programmed cell death do not appear to be as reliant on apoptosis.

Processes apparently independent of apoptosis based on normal embryonic and fetal development in the combined absence
of BAX, BAK, and BOK include, for example, the forma-
tion of the pro-amniotic cavity, epiblast, intestine, and lens
development as well as enchondral ossification. (For more details,
see Table S2 in Ke et al., 2018.) However, the caveat that
residual apoptosis not detectable by current methods (discussed
in the preceding sections) needs to be kept in mind.

It is certainly curious that a complex and regulated process,
such as apoptosis, occurs in an evolutionary conserved man-
ner during development, including in places where it appears
to be largely or completely dispensable. One possibility is that
apoptosis occurs as a by-product of other processes; for
example, in the process of tissue remodeling, cells might
lose their contact to the extracellular matrix and, without this
survival signal, might die by apoptosis (anoikis).

Another possible explanation is that a twofold control of devel-
opping tissue size and cell population homeostasis by maintaining
a balance between proliferation and cell death affords not just
one but two cellular mechanisms of fine-tuning cell number.
This would provide a more robust regulation, less prone to
errors and variation in response to negative impact.

Indeed, mammalian and other embryos appear to have an
astounding capacity to compensate for the loss of cells, cer-
tainly by upregulating cell proliferation but perhaps also by
actively downregulating developmental apoptosis. It can be
assumed, on the basis of its evolutionary conservation, that
apoptotic cell death, even in regions where it is not absolutely
required, provides a competitive advantage.

Acknowledgments
The authors thank Leigh Coultas of the Walter and Eliza Hall
Institute of Medical Research, Australia, for critical reading
of the manuscript.

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