HOW DEATH SHAPES LIFE DURING DEVELOPMENT

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The formation of an adult animal from a fertilized embryo involves the production and death of cells. Surprisingly, many cells are produced during development with an ultimate fate of death, and defects in programmed cell death can result in developmental abnormalities. Recent studies indicate that cells can die by many different mechanisms, and these differences have implications for proper animal development and disorders such as cancer and autoimmunity.

Animals begin life as a single cell that progresses to a fully formed individual. Although it is obvious that cells need to divide and take on specific fates to form a complex animal, it is counterintuitive that millions of cells die during development and life. Why waste all these cells?

The destruction of cells was first observed by developmental biologists during the 1800s, but cell death was not clearly recognized as having a normal, formative role in developing animals until this was articulated by Glücksmann[1] in 1951 (BOX 1). Several terms have been used to describe the morphology and biochemistry of physiological cell death[2–5], all of which fit under the more global term 'programmed cell death', which was originally defined as a series of events that culminate in the death of a cell[6]. These genetically regulated programmed cell deaths are distinct from necrosis in response to insult, which results in leaking of the cell contents and inflammation.

Elegant studies of isolated cells have provided detailed mechanisms for the biochemical pathways that regulate programmed cell death[5,7]. However, recent studies of developing animals indicate that cells with extremely different dying-cell morphologies use common mechanisms for destruction[8]. This fact, coupled with the desire to develop therapies to control the cell death that is associated with disorders such as cancer, autoimmunity, neurodegeneration and myelodysplastic syndromes[9–14], has revitalized the desire to understand how cells die in the context of a developing organism.

In this review, I summarize the importance of cell death in development, the diversity that exists in dying cells and the importance of understanding the mechanisms of cell death in the context of an intact organism. I emphasize the differences between dying cells *in vivo*, and how differences in morphology might or might not reflect differences in cell-death mechanisms.

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Death is a normal component of development

Organisms as different as worms and humans have conserved the genes that encode the core cell-death machinery[15]. Furthermore, genetic studies of worm, fly and mouse models indicate that physiological cell death is essential for normal development. Studies of developing animals illustrate many reasons for cells to die[1,16–18]; for instance, during the formation and deletion of structures, to control cell numbers and to eliminate abnormal cells[16].

Forming structures. Programmed cell death is important in the formation of structures such as fingers and toes of the hand and foot[19] (FIG. 1a). It is also observed when epithelia invaginate (as in the formation of the neural tube), evaginate (as in the formation of the optic vesicle) and fuse (to form structures such as the palate)[1]. Both apoptotic and autophagic cell morphologies (BOX 1) are observed in cells that die in association with forming structures such as mouse digits[20] and palate[21,22]. Although the core cell-death machinery (BOX 2), including the caspases (TABLE 1), is involved in the elimination of cells during the formation of structures such as digits[20], this cell death can also occur in a caspase-independent manner[23].

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Box 1 | **Historical observations and definitions of cell death**

Walther Flemming was among the first to observe dying cells during animal development[84]. Although several other accounts were made of dying cells in association with growing and forming tissues, Glücksmann was the first to state clearly that cell death is a normal component of animal development[1].

In 1973, Schweichel and Merker[3] defined three types of physiological cell death, based on their morphological studies of developing vertebrate embryos: heterophagy, autophagy and non-lysosomal death. The distinction between these types of cell death was based on the location and role of lysosomes. Heterophagy, which had been previously described and is now widely known as apoptosis[2], is found in isolated dying cells that show condensation of the nucleus and cytoplasm, followed by fragmentation and phagocytosis by cells that degrade their contents.

Autophagic cell death is often observed when groups of associated cells or entire tissues die, and these dying cells contain 'autophagic vacuoles' in the cytoplasm that degrade cell components. Autophagic cells therefore seem to contain the machinery that is needed both to activate cell death and to degrade the dying cell (a process that largely occurs in the phagocyte during apoptosis). Non-lysosomal cell death has not been commonly observed in developing embryos. This type of cell death is characterized by swelling of cavities with membrane borders, followed by degeneration without lysosomal activity.

Deleting structures. Programmed cell death also deletes structures that are no longer needed (FIG. 1b). During the development of amphibians such as frogs, for example, the tadpole is remodelled to form an adult and structures such as the tadpole tail and intestine are deleted[24]. Metamorphosis is even more extreme in insects, in which many cells of the larvae are destroyed, while cells that will become the future adult undergo differentiation and morphogenesis[25]. In higher animals, such as mice and humans, testosterone triggers the death of mammary cells in males[26]. The deletion of structures is usually associated with an autophagic cell-death morphology[4], although at least some of these cells seem to be destroyed by using the core cell-death machinery[8].

Controlling cell numbers. The production of tissues and organs with the correct number of cells involves both cell division and programmed cell death (FIG. 1c). Excess cells are generated during the development of the nervous system in invertebrates and vertebrates[27,28], and during the development of the heart in chickens[29], and these subsequently die by programmed cell death. Although it is not clear why excess cells are produced and then removed, such cells might be required to establish appropriate patterns of cell migration and morphogenesis at one stage but then not be needed later. The cells that are removed to control cell number during development usually have an apoptotic cell morphology, although some of the lateral motor neurons that die during chick embryonic development do not[30].

Eliminating abnormal cells. During development — and also later in life — cells that harbour mutations can give rise to harmful characteristics such as uncontrolled growth. These cells are therefore eliminated by programmed cell death (FIG. 1d). One example from the vertebrate immune system is the removal of lymphocytes that produce self-reactive receptors during development[9].

Types of developmental cell death

There are likely to be many mechanisms for the regulation of cell death. Long ago, it was recognized on the basis of cell morphology during development that there are at least two forms of regulated cell death.

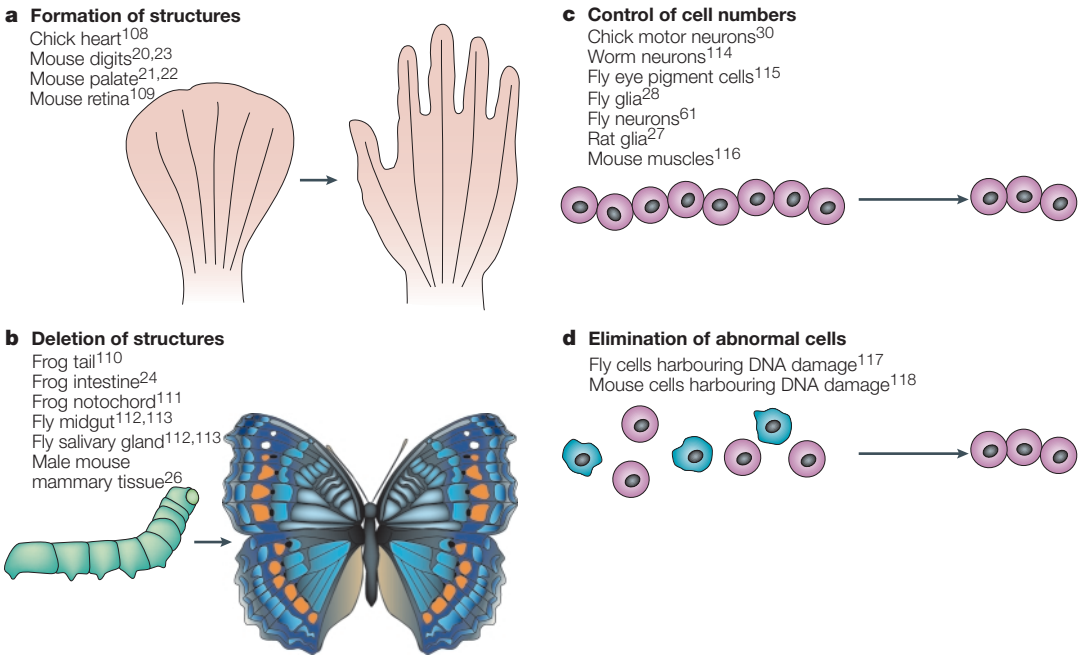

Figure 1 | **Programmed cell death during development.** Programmed cell death is involved in forming structures such as the digits of the hand (**a**), deleting structures such as nearly all of an insect's larval components (**b**), controlling cell numbers in, for example, the nervous system (**c**) and eliminating abnormal cells such as those that harbour mutations (**d**).

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Box 2 | **Core cell-death machinery**

Caenorhabditis elegans

Pioneering studies of the nematode *Caenorhabditis elegans* led to the isolation of the core cell-death genes *ced-3*, *ced-4* and *ced-9* (REF. 85) (TABLE 1). CED-3 is homologous to the mammalian caspase family, members of which are proteolytically activated and are crucial effectors of programmed cell death signalling[5,66,86,87]. CED-4 is homologous to mammalian Apaf-1, which is a caspase cofactor in the presence of cytochrome *c* and ATP[5,88,89]; this structure is now known as the apoptosome[7]. CED-9 is a member of the BCL-2 FAMILY of cell death regulators[90,91].

This core cell-death machinery was placed into a regulatory hierarchy based on genetic EPISTASIS studies in *C. elegans*. Whereas mutations in *ced-3* and *ced-4* prevent the death of many cells, these animals seem to develop without major defects. By contrast, animals with mutations in the Bcl-2 family member *ced-9* die early in development because of ectopic cell death[92], and this study provided strong evidence that suppression of programmed cell death is crucial to the survival of many animal cells.

Drosophila melanogaster

Proper regulation of cell death is crucial in *Drosophila*. Flies with mutations in the caspase *dcp-1* die as larvae with tumours or are infertile because of defects associated with oogenesis[93,94]. Furthermore, flies with strong or NULL MUTATIONS in *dark* (the *Drosophila* homologue of *Apaf-1*) die during metamorphosis, have defects in nervous system cell death and often have tumours[95] (J. Abrams, personal communication). Mutations in the inhibitor of apoptosis (IAP) gene *diap1* result in massive ectopic cell death and embryonic lethality owing to the ectopic activity of caspases[49,50]. Although the IAP genes and their regulators are not generally considered to be part of the core cell-death machinery, these genes have a profound effect on caspase regulation and are conserved in a wide range of animals (TABLE 1). Recent genetic studies[96] have shown that *dark* suppresses this dramatic *diap1* phenotype, indicating that caspase-activated cell death requires input from both *dark* and *diap1*.

Mouse

Genetic studies of cell death in mice show similarities to the mutant phenotypes observed in *Drosophila*. Mutations in the mouse gene *Apaf-1* are lethal and have persistent cells that result in interdigital webs, severe craniofacial abnormalities and reduced cell death in the nervous system, which results in brain overgrowth[97,98]. Furthermore, animals with mutations in *caspase-3*, *caspase-8* and *caspase-9* die during either embryonic or perinatal development. Mice with *caspase-8* mutations die during embryogenesis with abnormalities in heart development[99]. Mice with *caspase-3* and *caspase-9* mutations also die early in development and show defects in programmed cell death in the nervous system, resulting in brain overgrowth[100,101]. Clearly, cell death is an important component of development of the nervous system and heart, but more detailed studies of cell biology in normal and mutant animals are required to understand the roles that dying cells have during the formation of these structures. Although mutations in most members of the Bcl-2 family do not seem to affect mouse development, mice with mutations in *BclX* die during embryogenesis with massive cell death in the nervous system[102]. Disruption of the Bcl-2 family member *Mcl-1* does not seem to affect cell death, but these mutants do die early during development[103].

Biochemical studies of dying cells have also led to the conclusion that there are at least two types of death — one that is activated via the mitochondria and a second that is independent of this organelle[5].

The most abundant morphological forms of programmed cell death in developing animals are apoptosis and cell death with autophagy[3,4]. The extreme differences between apoptotic and autophagic cells prompted investigations of their similar and distinguishing features[8,31]. The mechanisms of apoptosis have been extensively investigated, but those of autophagic cell death have been studied only recently. Moreover, although the mechanisms that underlie autophagy have been carefully studied in yeast, it is not yet clear whether this process is similar to the autophagy that is observed during cell death (BOX 3). Whereas many of the yeast genes that function during autophagy seem to have been conserved during the evolution of nematodes, flies and humans (TABLE 2), the roles of these genes in higher organisms remain uncertain.

Cells that show apoptotic and autophagic structures while dying clearly represent different forms of programmed cell death. Whereas apoptotic cells die in isolation and require a phagocyte for removal and degradation, cells that die with autophagy usually die in groups and contain the lysosomal machinery, which is needed for much of cell degradation during the last stage of cell death (FIG. 2). Reports of caspase protease activity and the occurrence of DNA fragmentation during apoptosis led to the belief that caspase activity and DNA fragmentation are synonymous with apoptosis. By contrast, insect muscles that die with autophagic morphology initiate a prominent UBIQUITIN-associated proteolysis and do not contain fragmented DNA[31].

Based on these observations, apoptosis and autophagic cell death were considered to be distinct. However, subsequent studies showed that autophagic midgut and salivary-gland cells show DNA fragmentation in *Drosophila melanogaster*[32,33], and it is now apparent that ubiquitin-mediated proteolysis is an important regulator of apoptosis as well[34,35]. Recent studies[36,37] of *Drosophila* indicate that ubiquitin-mediated proteolysis is used during apoptosis of eye pigment cells and sensory-neuron precursors during development.

BCL-2 FAMILY
Family of proteins that contain BH1–4 domains and regulate cell death.

EPISTASIS
Interaction between nonallelic genes such that the relationship within a hierarchy can be determined

NULL MUTATIONS
Mutations in genes that eliminate the protein's function.

UBIQUITIN
Polypeptide that is attached to proteins and targets them for degradation.

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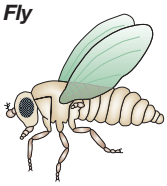
Table 1 | **Cell–death genes are conserved in different organisms**

Relationships are conserved within the generic signalling hierarchy

CED-9 ⊣ CED-4 → CED-3
Bcl-2 ⊣ Apaf-1 → Caspases

Gene sequences and functions are conserved

Gene family	*Worm*	*Fly*	*Mouse*
			
Caspases	*ced-3*, *csp-1*, *csp-2*	*Dredd*, *Dronc*, *Dream/Strica*, *Dcp-1*, *Drice*, *Decay*, *Daydream/Damm*	*Caspases 1–14*
Bcl-2	*ced-9*, *egl-1*	*Debcl-1/Drob-1/Dborg-1/Dbok*, *Buffy/Dborg-2*	*Bcl-2*, *Bcl-x*, *Bcl-w*, *Mcl-1*, *Al*, *Diva*, *Bax*, *Bak*, *Bok*, *Bik*, *Bik*, *Bid*, *Bad*, *Hrk*, *Bim*, *Bnip*, *Nix*
APAF-1	*ced-4*	*ark/dark/hac-1/dApaf-1*	*Apaf-1*
IAP	*bir-1*, *bir-2*	*diap-1*, *diap-2*, *dbruce*, *deterin*	*Xiap*, *c-iap1*, *C-ap2*, *hILP-2*, *ml-iap*, *naip*, *survivin*, *bruce*
RHG domain	?	*rpr*, *hid*, *grim*, *sickle*	*Smac/DIABLO* *Omi/HtrA2*

Although they are different, apoptotic and autophagic cell death also have several common features — for example, both are stepwise processes (FIG. 2)[8,38]. Furthermore, expression of the caspase inhibitor p35 prevents autophagic cell death in *Drosophila* salivary glands, and genes that are involved in apoptosis are induced immediately before the initiation of autophagy in this tissue[8,32,39,40]. However, cells with autophagic morphology can also die in a caspase-independent manner[41]. Thus, apoptosis and autophagic cell death might not be as distinct as suggested by cell morphology alone.

The simplest model to explain how and why cells die with autophagic morphology is that these cells contain the death and degradation machinery that, in the case of apoptosis, is contained in distinct apoptotic and phagocytic cells. Many models can be proposed to explain the observed differences in morphology that are seen during programmed cell death, and analyses of slight variations in apoptosis during development might be an easier way to identify the similarities and differences between dying cells. In support of this approach, there are reports of significant variations in the morphology of apoptotic cells[42]. These differences in morphology indicate that there are apparently distinct biochemical pathways for the activation of cell death. Given the limited number of detailed studies of dying cells in the context of a living organism, one can only speculate about how many biochemical pathways there might actually be, and how the treatment of disorders that are attributed to defects in cell death might affect non-target cells.

Box 3 | **Autophagy: same name, different processes?**

Autophagy was selected as a term to describe two independent processes — a type of programmed cell death in animals[3] and protein degradation in yeast and other eukaryotic cells under nutrient-limiting conditions[104]. In both cases, autophagy seems to involve vacuolar proteolysis. During autophagic cell death, this proteolysis presumably serves to degrade the cell and plays the part of the phagocyte lysosome during apoptosis.

Although autophagic vacuoles containing organelles such as mitochondria were observed in dying animal cells in the 1960s[4], it has not been clear how these structures form or which genes regulate this process. By contrast, the morphology of vacuole formation and the regulatory mechanisms of autophagy are well characterized in yeast[105,106]. Links have been made between yeast autophagy genes and human cell death[107], but their general roles in vacuolar protein sorting, protein degradation and cell signalling make it difficult to conclude that autophagy is the same in animal cell death and yeast protein degradation.

Genome sequence analyses indicate that worms, flies and humans have genes that are similar to the yeast genes that are involved in autophagy (TABLE 2). In addition, recent experiments indicate that several fly genes that are similar to yeast autophagy genes are transcribed in the salivary glands of *Drosophila* that die by autophagic cell death (C.-Y. Lee *et al.*, unpublished observations). Although we can speculate that protein-degradation mechanisms have been conserved in diverse organisms and that such mechanisms might be implemented under different physiological conditions, genetic and biochemical analyses are required to determine the similarities and differences between these autophagies.

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Table 2 | **Yeast proteins involved in autophagy and most similar proteins in worms, flies and humans**

Yeast protein	Worm	Fly	Human
APG1	NP_507869	CG10967	XP_008514
APG2	ns	CG1241	NP_060506
AUT1/APG3	NP_500024	CG6877	NP_071933
AUT2/APG4	NP_502208	CG6194	NP_116241
APG5	ns	CG1643	NP_004840
APG6/VPS30	T29537	CG5429	NP_003757
APG7	NP_502064	CG5489	NP_006386
AUT7/APG8	NP_495277	CG1534	NP_009216
APG9	NP_503178	CG3615	BAB15246
APG10	ns	ns	ns
APG12	NP_498228	CG10861	NP_004698
APG13	ns	ns	ns
APG14	ns	ns	ns
APG16	ns	ns	ns
APG17	ns	ns	ns
AUT10	T26730	LD38705p	AAH07596
AUT4	ns	ns	ns
CVT17	ns	ns	ns

Caenorhabditis elegans, *Drosophila melenogaster* and *Homo sapiens* genes were identified by using previously identified yeast proteins to conduct protein BLAST searches of the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/). Although these known and predicted proteins have been given other names, the names listed enable identification from this web site. BLAST values greater than 10^{-5} were considered insignificant (ns).

Activation of programmed cell death

Although the biochemical mechanisms that regulate programmed cell death have been extensively studied[5,7], cell death in developing animals has received less attention. Most studies of programmed cell death in developing animals have focused on the mechanisms of apoptosis, especially the identity and function of the core cell-death machinery. This has been conserved during the evolution of nematodes, flies, mice and humans (TABLE 1)[15], and is important for normal development (BOX 2). The core cell-death machinery is widely used in different types of programmed cell death. By contrast, there seem to be many mechanisms to regulate the activation — and possibly the removal — of dying cells.

The activation of programmed cell death is tightly regulated and ECTOPIC activation can be catastrophic. Several factors are involved in the activation process during development, including cell-lineage information, extracellular survival factors, steroid hormones, membrane-bound death receptors and DNA-damaging agents such as radiation (FIG. 3). Studies of cell lineage, survival factors and steroids have provided us with detailed mechanisms for how these signals activate cell demise during animal development.

Cell-lineage information. *Caenorhabditis elegans* is an ideal model for studies of cell death because of its nearly invariant cell lineage, which enables every cell to be followed during development. There are two forms of these simple nematodes — hermaphrodites and males. A few hermaphrodite-specific neurons die during the development of male worms. This programmed cell death in males depends on the function of the sex-determination gene *tra-1* (REF. 43), which encodes a ZINC-FINGER transcriptional regulator. The TRA-1A protein binds to a regulatory element in *egl-1*, represses the transcription of *egl-1* and so prevents the induction of cell death by EGL-1. EGL-1 is a BCL-HOMOLOGY-3 DOMAIN (BH3 domain) cell-death activator that interacts directly with CED-9. This interaction prevents CED-9 from doing its normal job, which is to suppress the activation of caspases[44]. So, this is an example in which a gene that functions in sex determination is involved in the death of a sex-specific neural-cell lineage. The conservation of EGL-1 with other members of the BH3 subfamily of pro-apoptotic Bcl-2-family members suggests that a similar mechanism might be widely used in apoptosis[45].

Extracellular survival factors. The withdrawal of TROPHIC SIGNALS has long been recognized to be an important stimulant of programmed cell death[46]. Growth-factor-mediated cell survival is involved in the development of GLIA and neurons in vertebrate nervous systems[47]. The mechanisms for trophic signalling have recently been dissected during the development of *Drosophila*[48]. The midline glial cells separate AXON TRACTS during embryonic development in *Drosophila*. Approximately ten midline glial cells are generated in each embryonic segment, but most of these glia die at a specific stage[28]. This programmed cell death depends on the loss of an interaction between the glia and neurons that secrete a protein called SPITZ. SPITZ is a ligand for the epidermal growth factor receptor, whose signals — via the Ras and

ECTOPIC
Event that occurs either in the wrong place or at the wrong time.

ZINC FINGER
Conserved protein domain that requires zinc nucleation to bind DNA and regulate RNA transcription.

BCL-HOMOLOGY-3 DOMAIN
Conserved domain within Bcl-2-family proteins.

TROPHIC SIGNALS
Molecules that are required for survival.

GLIA
Support cells of the nervous system.

AXON TRACTS
Group of neural-cell projections.

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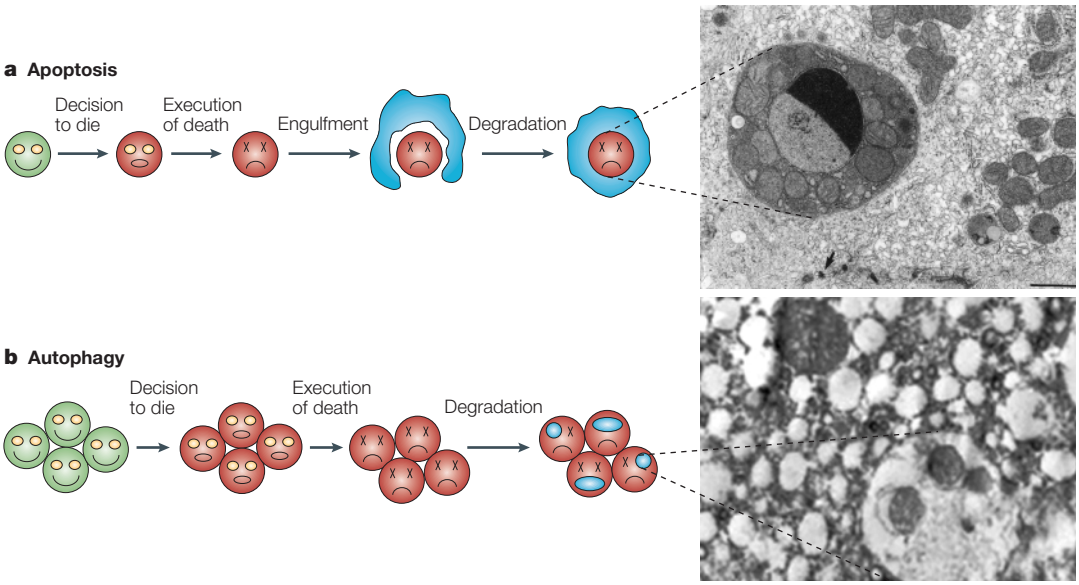

Figure 2 | **Apoptotic and autophagic cell death are regulated at distinct steps.** These include the decision to die, execution of death and degradation[38], and have two distinguishing features. **a** | Apoptosis occurs in isolated cells. These dying cells are ingested by phagocytes (blue cell), where they are degraded by lysosomes. The transmission electron micrograph shows an apoptotic cell that has been phagocytosed. **b** | Autophagic cell death usually occurs when groups of cells or entire tissues die during development. Autophagic cells are largely (if not completely) responsible for the generation of the lysosomal machinery (blue) for their degradation. The transmission electron micrograph shows an 'autophagic vacuole' containing mitochondria within a dying salivary-gland cell.

mitogen-activated protein kinase pathways — suppress a pro-apoptotic protein called HID[48]. Therefore, loss of the SPITZ signal enables HID to activate apoptosis by interacting with DIAP1 and relieving its inhibition of caspases[49,50].

The interaction of HID with DIAP1, with its consequent activation of caspases, is similar to the interaction in vertebrates of a protein called Smac/DIABLO with an 'inhibitor of apoptosis' (IAP) protein called XIAP[51]. This suggests that this mechanism of signalling has also been conserved through evolution. However, studies of how Smac/DIABLO is regulated after growth-factor withdrawal are needed to determine whether the entire trophic signalling pathway is conserved between invertebrates and vertebrates. It should also be realized that *hid* and *Smac/DIABLO* are not the only genes in flies and mammals whose products interact directly with IAPs, and there is evidence that *reaper* (*rpr*), *grim* and *sickle* in flies, and *Omi/HtrA2* in mammals also act through a similar mechanism[52–59].

Steroid hormones. Steroid hormones seem to regulate programmed cell death by both increases and decreases in hormone titre. Withdrawal of androgens triggers programmed cell death in the prostate gland[60], and withdrawal of the steroid hormone 20-hydroxyecdysone (ecdysone) induces neural cell death in insects[61]. By contrast, increases in steroids activate programmed cell death of *Drosophila* larval midguts and salivary glands during development[32].

Ecdysone triggers autophagic death of salivary gland cells by a two-step nuclear-receptor-activated gene-transcription hierarchy. The nuclear-receptor genes *EcR* and *usp* encode components of the hormone-receptor complex[62,63]. The ecdysone receptor complex and the COMPETENCE FACTOR βFTZ-F1 trigger the first step in this hierarchy by activating transcription of *BR-C*, *E74* and *E93* (REFS 64,65). The production of the BR-C, E74A and E93 transcription-regulating proteins in turn activates the second step in this hierarchy by regulating cell-death genes. Mutations in *BR-C*, *E74* and *E93* alter the transcription of the cell-death genes *rpr*, *hid*, *ark* (Apaf-1 related killer) and *dronc* (a caspase) during salivary-gland death[39,40]. Mutations in *BR-C*, *E74* and *E93* also prevent salivary-gland cell death, so the regulation of transcription of genes involved in cell death probably determines whether these cells will die.

Cell removal and degradation

The regulated removal of cells in the absence of inflammation is a simple, inclusive definition of programmed cell death and clearly distinguishes this process from necrosis. Thus, the removal and degradation of dying cells during development by phagocytosis and autophagy is a crucial step downstream of the core cell-death machinery (FIG. 3). Although the mechanisms that govern the autophagic removal of dying cells remain mysterious, we know much about the phagocytosis and degradation of apoptotic cells.

The degradation of an apoptotic cell begins before phagocytosis. Early changes in apoptotic-cell

COMPETENCE FACTOR
Factor that enables a specific response to a stimulus at a specific location or time.

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morphology — such as cytoplasmic blebbing and DNA fragmentation — involve the regulated remodelling and proteolysis of cell substrates such as actin, lamins and tubulin[66]. Although many of these changes occur before an apoptotic cell is engulfed, several aspects of cell degradation require phagocytosis[67,68]. Considerable progress has been made in understanding how cells are removed by studying the surface of dying cells and phagocytes. The isolation of a phosphatidylserine receptor[69] helped to define a mechanism for phagocyte recognition of an apoptotic cell. This receptor recognizes phosphatidylserine on the surface of dying cells, where it serves as an 'eat me' signal to phagocytes. In addition, several scavenger receptors have been implicated in the recognition of dying cells[70,71]. The role of these receptors in corpse clearance is supported by genetic studies of *Drosophila*, in which the CD36 scavenger receptor gene *croquemort* is required for removal of apoptotic cells during embryogenesis[72].

The mechanisms that regulate the phagocytosis of dying cells have been most thoroughly studied in *C. elegans*. Genetic studies led to the identification of *ced-1*, *ced-2*, *ced-5*, *ced-6*, *ced-7*, *ced-10* and *ced-12* (REFS 73,74). These genes encode two REDUNDANT phagocytosis pathways — *ced-1*, *ced-6* and *ced-7* are involved in one pathway and *ced-2*, *ced-5*, *ced-10* and *ced-12* in the other. Mutations in any single gene within a pathway do not alter phagocytosis but mutations in genes in both pathways prevent the engulfment of dying cells. The relationships between *ced-1*, *ced-6* and *ced-7* are not completely understood: *ced-1* encodes a protein with similarity to human scavenger receptors that is active in phagocytes[75]; *ced-6* encodes an adaptor protein[76]; and *ced-7* encodes a protein that is similar to members of the ABC transporter family[77]. By contrast, *ced-2*, *ced-5*, *ced-10* and *ced-12* encode homologues of mammalian CrkII, DOCK180, Rac1 and ELMO, respectively[78–80]. CED-2 and CrkII contain SH2 AND SH3 DOMAINS, and interact with CED-5 and DOCK180, respectively. CED-5 and DOCK180 in turn interact with CED-12 and ELMO, respectively, and these interactions are thought to signal to CED-10 and Rac1 (respectively) to trigger cytoskeletal reorganization. Therefore, this conserved apparatus signals from the cell surface to the cytoskeleton and seems to trigger the migration and remodelling of the phagocyte that is associated with engulfment of dying cells.

Recent studies have provided insight into the mechanisms that underlie the recognition and engulfment of apoptotic cells by phagocytes. However, cells that die by caspase-independent mechanisms are also engulfed, and neither caspases nor apoptosis are required for phagocytosis[81,82]. Furthermore, little is known about the genetic mechanisms of cell degradation following apoptosis and during autophagic cell death. On the basis of morphological observations, similar lysosome-based approaches seem to be used to degrade both apoptotic and autophagic cells[4]. Once an apoptotic cell is ingested by a phagocyte, the engulfed cell fuses with a primary lysosome, in which degradation occurs by proteolysis. By contrast, autophagic vacuoles form by the fusion of an autophagosome with a lysosome in the dying cell, and the vacuole contents are then degraded by proteolysis. Although the mechanisms of subsequent steps in degradation are uncertain, they seem to be important for the removal of cells while still maintaining homeostasis.

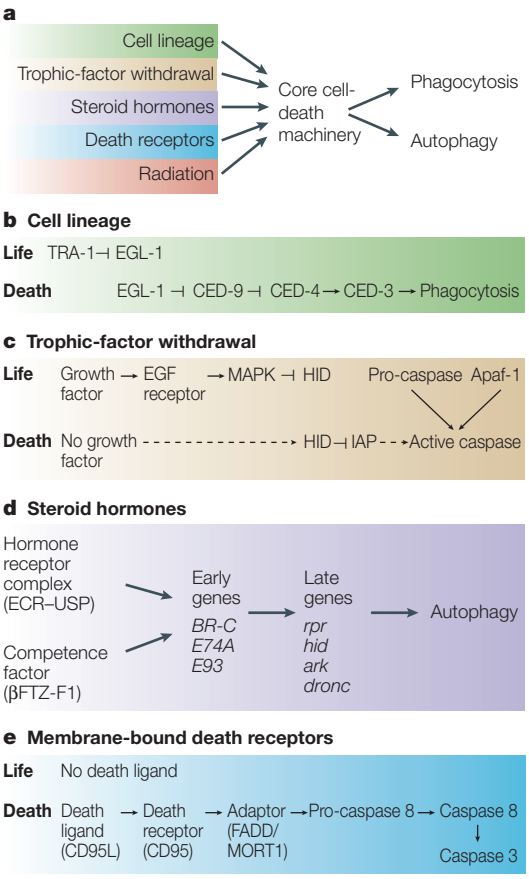

Figure 3 | **Pathways leading to programmed cell death.** **a** | Programmed cell death is activated by many stimuli, including cell-lineage information, extracellular survival factors, steroid hormones, membrane-bound death receptors and DNA damage caused by radiation. These death-activating signals integrate into the core cell-death machinery, which is upstream of the genes that are involved in phagocytosis and degradation or autophagic degradation of dying cells. **b** | Cell lineage signals the death of neurons in *Caenorhabditis elegans* by EGL-1 production and its interaction with CED-9. In turn, CED-4 can activate the CED-3 caspase, and this results in apoptosis followed by phagocytosis. **c** | Growth-factor withdrawal activates the core cell-death machinery in glia of the *Drosophila* nervous system. This is accomplished by HID activation and interaction with IAP, which enables caspase activation and death. **d** | Steroid hormones activate a two-step hierarchy composed of early-gene-transcription regulators and late core-cell-death genes, which trigger *Drosophila* salivary-gland autophagy. **e** | Ligand interaction with membrane-bound death receptors recruits proteins to the cell surface to activate the core cell-death machinery in the mammalian immune system. The interaction of death receptors with the adaptor FADD recruits pro-caspase 8, and the activation of caspase 8 results in the activation of a caspase cascade that results in immune-cell apoptosis. EGF, epidermal growth factor; IAP, inhibitor of apoptosis protein; MAPK, mitogen-activated protein kinase.

REDUNDANT
Gene or pathway that is duplicated; elimination of one therefore does not result in a defect.

SH2 AND SH3 DOMAINS
Conserved Src-homology-2 and -3 domains are found in signalling and cytoskeleton proteins, and are thought to mediate protein–protein interactions.

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TRANSCRIPTOME
All of the messenger RNA species that are present in a cell, tissue or organism at a point in time.

PROTEOME
All of the protein species that are present in a cell, tissue or organism at a point in time.

Conclusions

Programmed cell death, like cell division, is an integral component of normal animal development. Although some progress was made in understanding developmental cell death in the twentieth century, recent studies have focused on isolated cells to obtain elegant mechanisms for the death of a narrow, derived population of cells. These biochemical studies point to the genes that regulate apoptosis as possible targets for therapies both to activate and to inactivate cell death, depending on the physiological context[83]. Biologists and clinicians with an interest in therapies to regulate cell death because of its association with human syndromes should take special note of the possible diversity and relatedness of cell death during development. New strategies to kill cells will probably be identified in studies of diverse cell populations such as those in developing animals, but these approaches to modify cell death could also have profound effects on non-target cells.

The field of programmed cell death is rapidly moving towards integrated or systems approaches. This is commendable and is rooted in the original genomic approaches of Mendel, who allowed the intact organism to illustrate which genes were important. This approach was successfully implemented by Robert Horvitz and colleagues to isolate the core cell-death machinery from worms[38], and similar approaches continue to be used in other model systems.

Knowledge of the full genome sequences for humans and several model systems is enabling researchers to analyse the TRANSCRIPTOME and PROTEOME during cell death and, where there are mutations, to analyse the effects of mutations on biochemical changes during developmental cell death. These vast data sets will require sophisticated computational approaches to get overviews of the cell and to make rapid queries for biochemical details without losing the overall picture of what is going on in the developing organism.

Future studies of developmental cell death will determine the similarities and differences between the mechanisms that underlie the destruction of cells under physiological conditions. For example, studies of autophagy during cell death will determine the importance of the core cell-death machinery in this less-studied form of cell death, and whether the mechanisms of autophagy that have been identified in studies of yeast are active in dying animal cells. This detailed understanding of the mechanisms that regulate apoptotic and autophagic cell death will be useful in the diagnosis of abnormal cell growth and the design of rational therapies.

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