15. CELL DEATH AND DEVELOPMENT

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SUMMARY: In addition to proliferation, migration and differentiation, death of cells is an important element of the embryogenic process. Cell death plays a variety of important roles in development including sculpting of anatomical structures and organs, deletion of unneeded structures, regulating (by culling) appropriate cell numbers, eliminating abnormal, misplaced or harmful cells, and producing differentiated cells without organelles. Examples of these functions will be given along with information about how such death is regulated. Experimental studies over the past decade have revealed much about the molecular mechanisms that govern cell death during development. Much (but not all) developmental cell death occurs by a process designated as "apoptosis". Two major pathways of apoptotic death have been recognized: the "intrinsic" mitochondriadependent pathway and the "extrinsic" receptor mediated pathway. It appears that faulty cell death during development leads to embryonic lethality or to a variety of birth defects.

GLOSSARY:

Rhombomeres: transient segmented regions of the hind brain (rhombencephalon)
Syndactyly: fusion of digits, or bone elements of the digits or webbing of skin between digits.
Semicircular canals: bony walled tubes that lie at right angles to each other in the inner ear; contain sensors of angular movement.
Pronephros, mesonephros,Wolffian and Müllerian ducts: primordia of the embryonic urogenital system.

LEARNING OBJECTIVES:

You should be able to:
1) Discuss the functions of cell death in development.
2) Provide specific examples of the roles of cell death in development.
3) Discuss some of the tissue interactions that regulate developmental cell death.
4) Descirbe the basic molecular mechanisms that underlie the cell death process.

LECTURE NOTES:

I. Background

Soon after the realization that organisms are composed of individual cells, it was noted that cell death is a normal part of development. The first (circa 1840), reported example was cell death in the notochord and adjacent cartilage of metamorphic toads. It has since been recognized that death occurs during the normal development of a wide variety of tissues in metazoans, including humans. Moreover, it has become clear that in addition to proliferation, migration and differentiation, cell death plays an indispensable role in normal embryogenesis. Recent evidence also supports a role for misregulated cell death in a variety of human developmental defects.

II. Molecular Mechanisms of developmental cell death

A. Nomenclature and introduction:

Various terms have been used to describe the types of cell death that occur in development and under other conditions. The term “programmed cell death” is often used in the context of development and makes the point that genetic programs cause predictable death of specific groups of cells under physiologic circumstances. However, all mammalian cells appear to have the potential to die, and thus elaborate molecular mechanisms have evolved to regulate this process and to assure that the proper cells die at the right time and in the right place. In this section we will consider the “canonical” mechanisms that are triggered in cells that lead to their death during development.

Much of the death that occurs during development falls under the general mechanism of “apoptotic”. The term apoptosis was coined by Kerr, Wylie and Currie in 1972 to distinguish the morphologicfeatures of cells dying under a number of physiologic conditions from the features which occur (termed necrosis) when cells die in response to toxins or physical damage (Fig. 15-1). Typically, in apoptotic death, the nuclear chromatin condenses, the nucleus and cytoplasmic content of the cell become pyknotic, the DNA is digested by endonucleases (Fig. 15-2) and the cell breaks up into membrane limited fragments (Fig. 15-3) that are typically engulfed by macrophages (Fig. 15-4). Because the cytoplasmic contents are typically not released in apoptotic death, it generally occurs without inflammation and dead cells disappear from the tissue like “leaves falling from trees” (the meaning that Kerr, Wylie and Currie wished to convey in the term apoptosis). Another important feature of apoptotic death followed by phagocytosis is that cell components are not released into the circulation and are therefore not available to cause immune responses. This may be particularly important during development to avoid a maternal immune response to embryonic antigens. In contrast, necrotic cell death is distinguished by cell swelling, disintegration of cell membranes and loss of cytoplasmic contents, and random DNA digestion without chromatin condensation. Many cases of developmental cell death have features associated with apoptosis. However, there are also cases in which the morphologic features of dying cells are not necrotic, but also fail to fulfill all the criteria of apoptotic death.

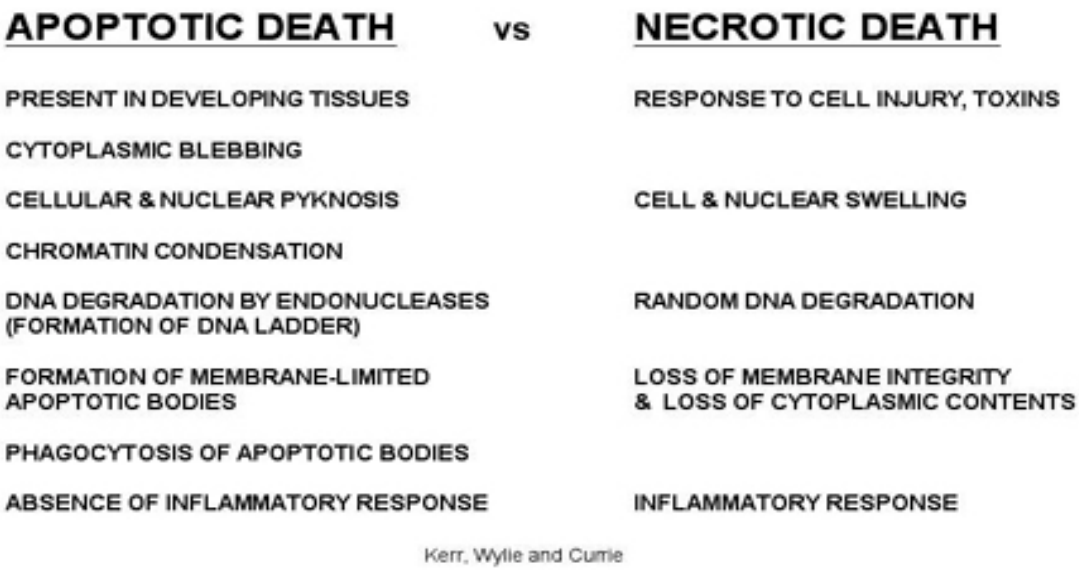

Fig. 15-1 Distinctions between apoptotic and necrotic death.

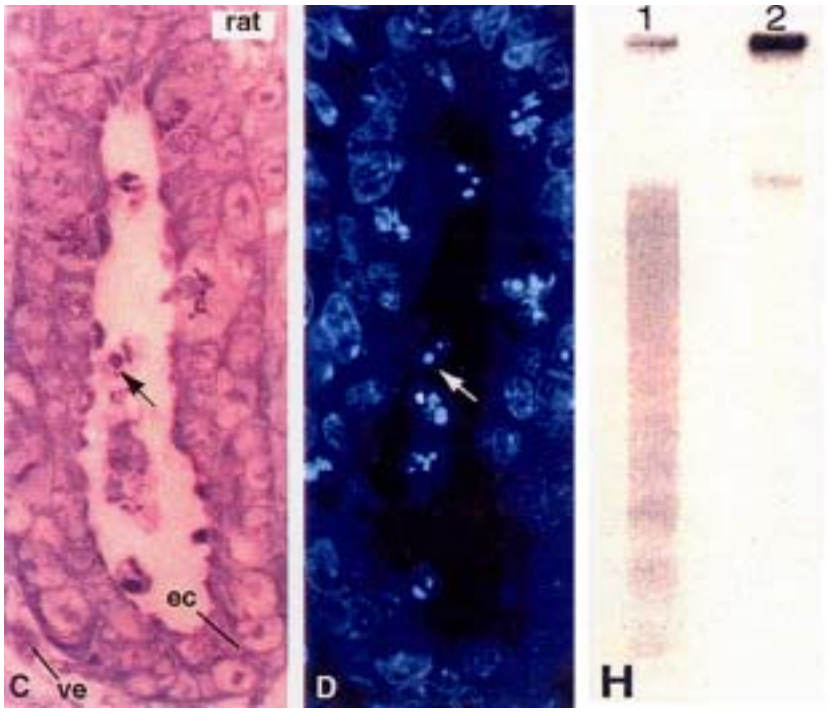

Fig. 15-2 Evidence of apoptotic death during formation of the proamniotic cavity. Left panels show section stained with the nuclear dye DAPI and examined by light microscropy (far left) and flurorescence (middle). DAPI staining shows nuclei with condensed chromatin, indicating apoptotic cells (one of which is indicated by the white arrow). Right panel shows analysis of DNA from dying (near right) and non-dying (far right) cells. DNA from dying cells shows fragmentation and appears as a typical apoptotic "ladder" in which the DNA has been fragmented by an endonuclease between nucleosomes to remove fragments about 200 base pairs in length. FROM: Coucouvanis and Martin. Cell 83: 279-287 (1995).

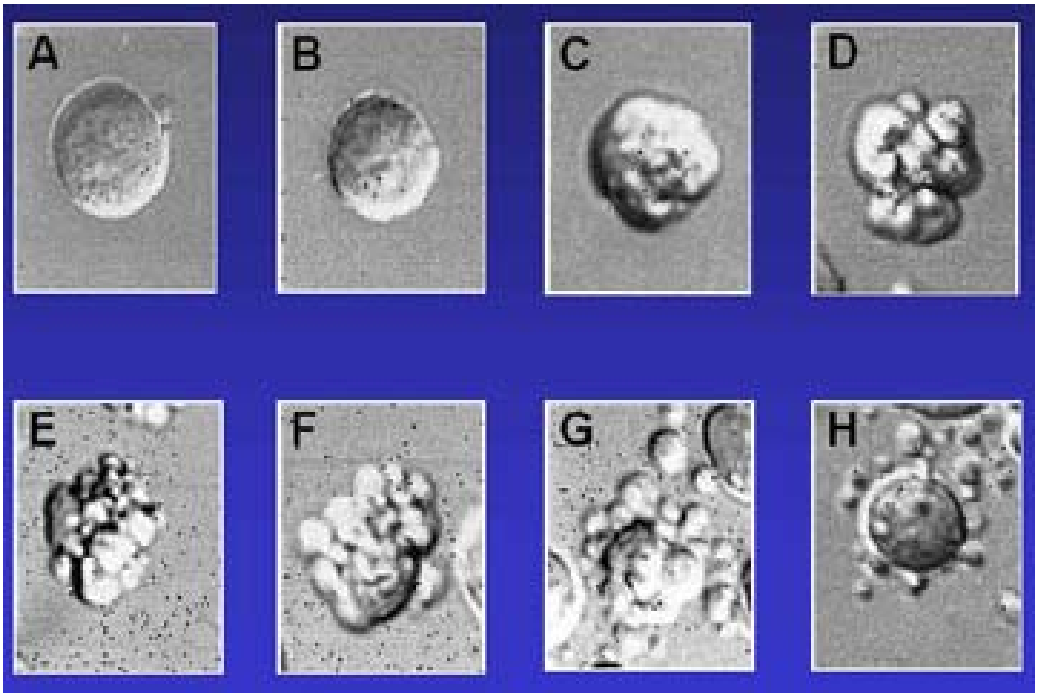

Fig. 15-3 Time lapse images of a cell undergoing apoptotic death. Note the blebbing (C-F) and formation of membrane-limited fragments (apoptotic bodies) (G,H).

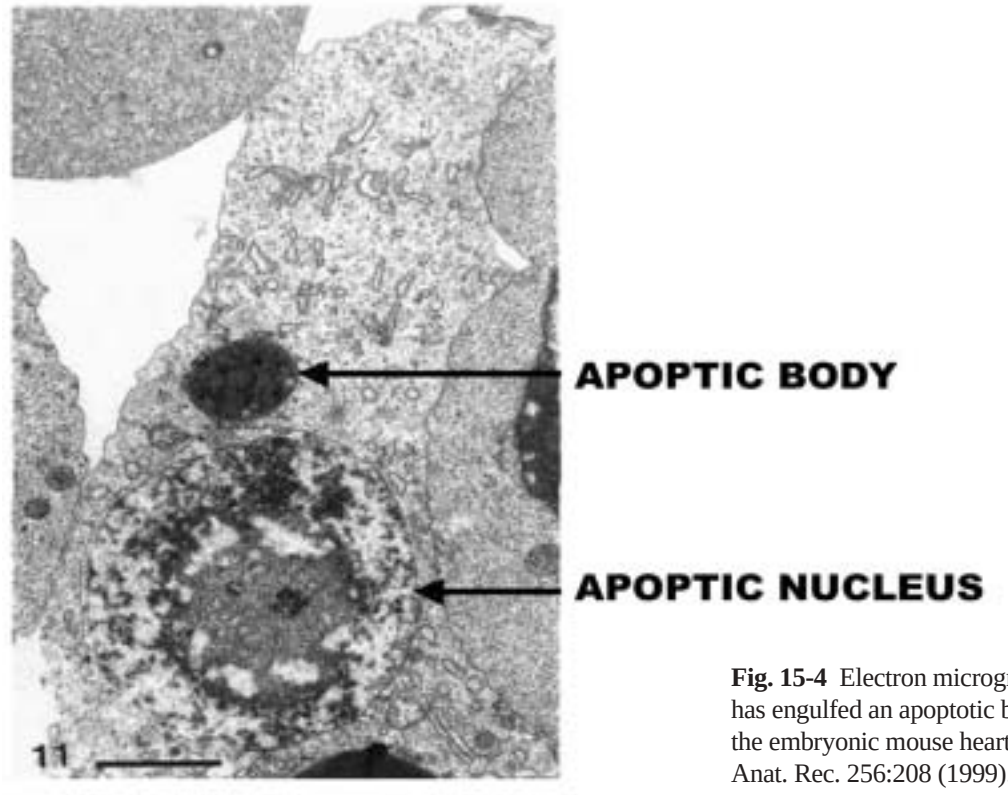

Fig. 15-4 Electron micrograph of a macrophage that has engulfed an apoptotic body and apoptotic nucleus in the embryonic mouse heart. From: Abdelwahid et al. Anat. Rec. 256:208 (1999).

B. Molecular mechanisms of developmental cell death

1. Introduction. A large number of studies carried out mostly within the last 15 years have revealed much information about the molecular mechanisms of developmental cell death. Even though several hundred different proteins have been implicated in mammalian cell death mechanisms and specific details appear to vary from cell type to cell type and from death stimulus to death stimulus, basic "canonical" death pathways have emerged.

2. Caspases. The major "executioners" of apoptotic death are a group of proteases known as "caspases." In mammals, there are about a dozen members of the caspase family. These proteases act via an active cysteine residue and selectively cleave their cellular protein substrates after aspartyl residues (Fig. 15-5). [The term caspase comes from a contraction of cysteine aspartase]. In healthy cells, capsases exist as catalytically inactive pro-forms. Once activated in response to apoptotic

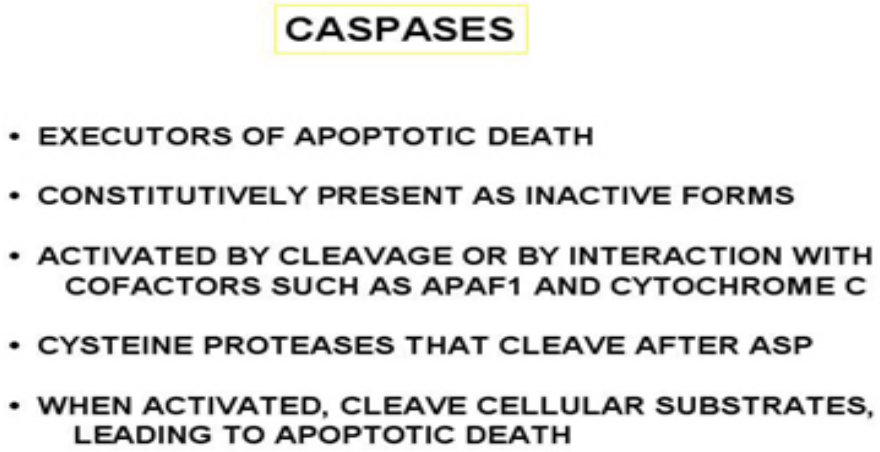

Fig. 15-5 Properties of the caspases (executioners of apoptotic death).

stimuli, caspases function as rapid and efficient executioners of apoptotic death. Their proteolytic activity results in digestion of many cellular proteins leading to their functional inactivation. In other cases, caspase cleavage leads to activation of pro-apoptotic molecules such as other caspase family members and of an endonuclease that degrades DNA). We will consider below two major canonical pathways that lead to caspase activation.

3. The mitochondrial pathway and caspase activation (Fig. 15-6). Mammalian cell death signals in many instances converge on the mitochondrion. The integrity of mitochondria is regulated in part by a family of proteins known as the BCL2 family. The lead member of this family, BCL2, was first discovered to be over-expressed in certain B cell tumors and to block cell death by stabilizing mitochondria. In addition to possessing anti-apoptotic members, the BCL2 family also possesses members that promote cell death. When imported into mitochondria, these proteins have destabilizing actions. BAX is a major example of such pro-apoptotic proteins. Another class of BCL2-related proteins share one domain in common with the four domains present in BCL2 and are known as BH3-domain proteins. These also promote death. One example is a protein designated BIM. Another class of pro-apoptotic BCL2 family members appear to be resident to mitochondria (one example being "BAK")

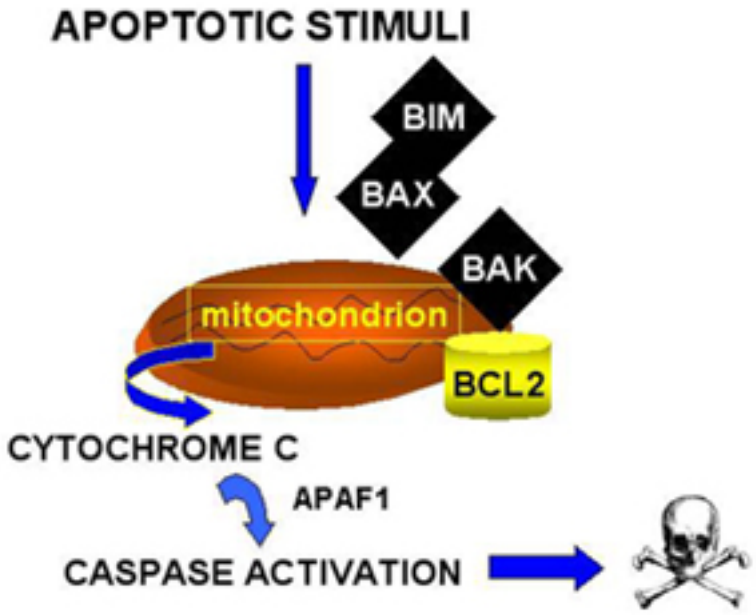

Fig. 15-6 Scheme of a canonical molecular mechanism of mitochrondrial dependent apoptotic cell death. Text describes the various molecules involved.

In response to a death stimulus (such as loss of trophic support by a growth factor), BH3 proteins and proapoptotic proteins such as BAX, move to mitochondria where they counteract the stabilizing actions of antiapoptotic proteins such as BCL2. The mechanisms that govern such mitochondrial translocation are not well understood, but in some cases involve upstream transcriptional pathways. Destabilization of mitochondria by pro-apoptotic proteins causes release of mitochondrial proteins into the cytoplasm. Among these is cytochrome C. Cytochrome C interacts with a cytoplasmic protein called APAF1 which also binds a member of the caspase family designated caspase 9. This interaction with Cytochrome C/APAF1 leads to activation of the pro-form of caspase 9. Once activated, caspase 9 in turn cleaves and activates other pro-caspases including caspase family members 3, 6 and 7. The latter, when activated, act as executioners and cause rapid apoptotic cell death. In addition to cytochrome C, destabilized mitochrondria release other agents that can mediate death. One example is the apoptosis inducing factor (AIF). This induces death that lacks some of the

typical morphologic features of apoptosis. One established paradigm in which AIF appears to be key is in formation of the proamniotic cavity.

4. The Receptor-mediated death pathway (Fig. 15-7). A second general apoptotic mechanism for activating caspases involves binding of ligands to specific cell surface receptors. A major example are members of the TNFα (tumor necrosis factor alpha) family which act as ligands for TNFα receptors. Binding of TNFα causes its trimerized receptors and permits the latter in turn to bind (via a sequence called the "death domain") a series of cytoplasmic adapter proteins (an example of which is designated "FADD") that act as scaffolds for binding of particular members of the caspase family (pro-caspases 8 and 10). Under such conditions, pro-caspases 8 and 10 become enzymatically activated. Once these are activated, there are two routes by which apoptotic death can be mediated. In the "direct" route, caspases 8 and 10 cleave and activate downstream pro-caspases such as pro-caspase 3, and together these promote apoptotic death. In the "indirect" route, caspases 8 and 10 cleave a BH3-domain member of the BCL2 family designated as BID, causing it to translocate to mitochondria along with other pro-apoptotic BCL2 family members such as BAX. This then triggers the mitochondrial death pathway described above. It appears that the "indirect" route amplifies weak caspase 8/10 signals that would otherwise be too weak to promote death by the "direct" route. In addition to TNFα and its receptor, other prominent death ligands and receptors include FAS/FAS ligand and TRAIL/TRAIL Receptor.

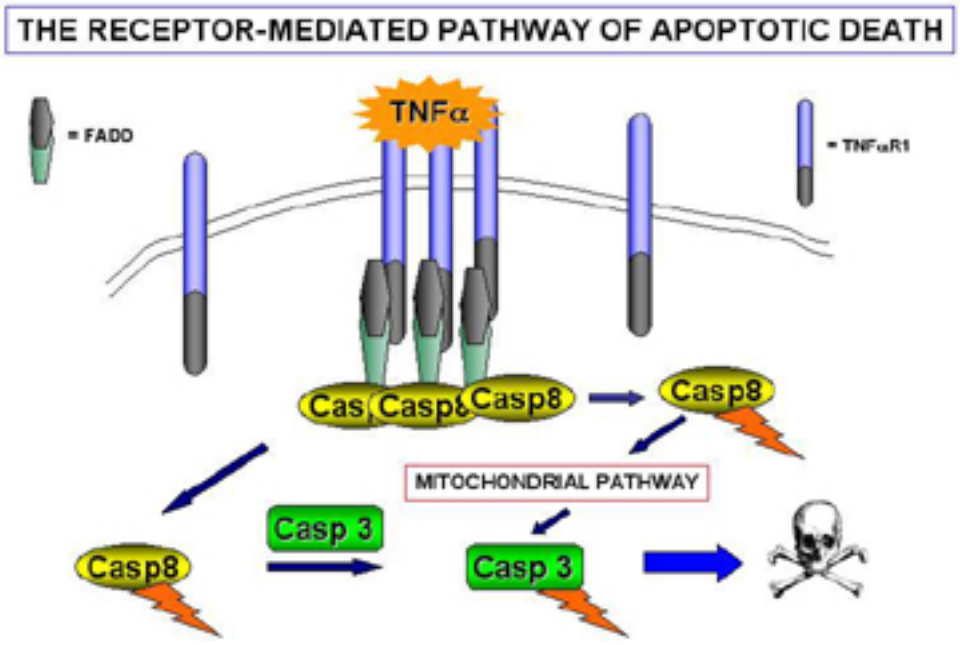

Fig. 15-7 Scheme for the mechanism of receptor-mediated apoptotic death. Details are discussed in the text.

III. Functions of developmental cell death

A. Sculpting/shaping structures/morphogenesis. Cell death plays a major role in morphogenesis. This ranges from contributing to creation of cavities and tubes from solid structures, to fusion of tissue masses (such as in formation of the palate, fusion of the neural tube), to sculpting of features such as digits.

B. Regulation of cell migration and pattern formation. Death of cells can affect the routes that cells take during migration and thereby affect pattern formation.

C. Deletion of unneeded structures. In some cases, vestigial structures are deleted by cell death(example, pronephros and mesonephros). In others, such as the case of the cortical subplate, structures play a transient role and are then eliminated. Cell death also functions in regulation of sexual differentiation by eliminating either the Wolffian or Müllerian ducts.

D. Regulation of cell numbers: culling. Particularly for cases in which structures that will eventually interact develop in isolation from one another, cell death functions to provide the appropriate numerical matchup between cell populations. The best defined example is in the nervous system in which development is characterized by large-scale death of neurons.

E. Eliminating abnormal, misplaced or harmful cells. DNA damage or problems with DNA replication that affect mitosis triggers cell death. This eliminates cells that may have a compromised genome and that might have the capacity to form tumors. Cell death also eliminates cells that have migrated to the inappropriate place (ectopic cells). Cell death mechanisms are also used to eliminate unneeded cells in the immune system.

F. Production of structures without organelles. In some instances, cell-death-like mechanisms function to create somata without nuclei or organelles. Examples include the formation of squamous epithelium from skin keratinocytes and the formation of clear lenses in the eye.

IV. Examples of developmental cell death and of mechanisms that regulate such death

A. Shaping structures/morphogenesis

1. Formation of the proamniotic cavity. Just prior to gastrulation, the solid embryonic blastocyst is transformed into a tube-like structure, the center of which is void of cells. The center of the blastocyst initially consists of a solid mass of pluripotent ectoderm surrounded by a basement membrane, outside of which there is a layer of endoderm. In the process of "cavitation", the inner ectodermal cells undergo cell death to form a hollow tube with a layer of "pseudostratified" columnar epthelium remaining on the inside of the basement membrane (Fig. 15-8). Recent studies indicate that this is achieved by release from the endodermal cells of a signal that promotes cell death of ectodermal

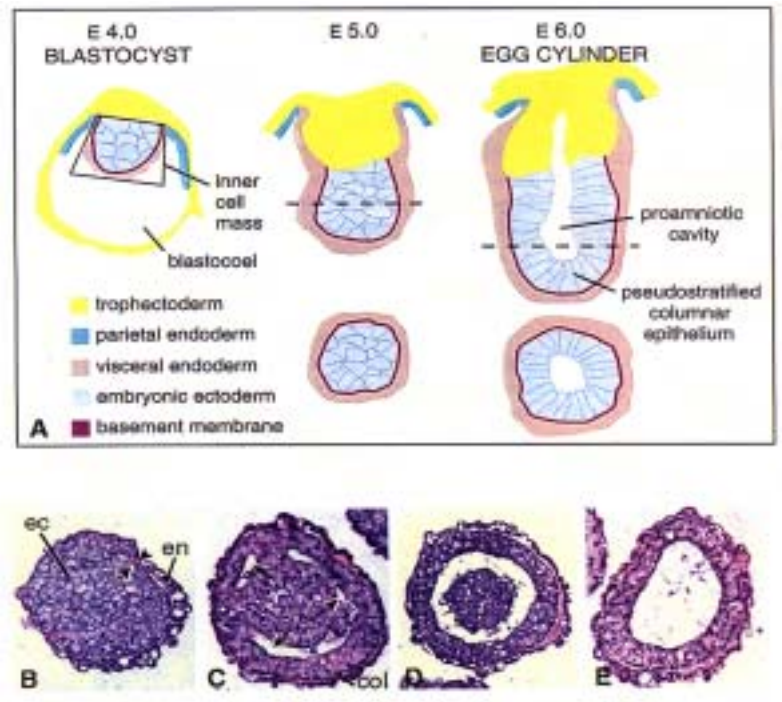

Fig. 15-8 Formation of the proamniotic cavity by the process of cell death. Upper panel shows schematic of changes at increasing times of development. Lower panel shows cross section of tissue showing formation of the proamniotic cavity in a culture system. The formation of the cavity from a solid tissue is achieved by death of ectodermal cells that are not in contact with the basement membrane. FROM: Coucouvanis and Martin. Cell 83: 279-287 (1995).

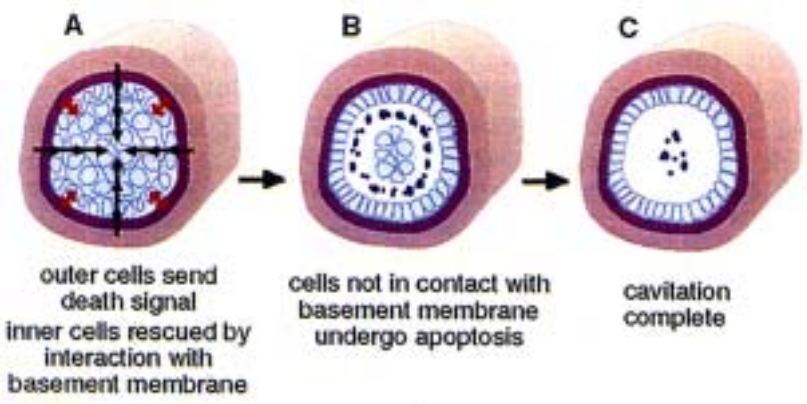

Fig. 15-9 Schematic model for formation of the proamniotic cavity and for cavitated (lumenal) structures during development. In panel A, long, black arrows represent a death signal produced by the outer (visceral endoderm) cells. The short red arrows represent a survival signal mediated by interaction between inner cells and basement membrane. Panel B shows an intermediate stage in lumen formation with dying cells represented by black dots. Panel C shows the completed process which results in formation of a hollow tube. FROM: Coucouvanis and Martin. Cell 83: 279-287 (1995).

cells, except for those in contact with the basement membrane (Fig. 15-9).

2. Formation of digits. Initially, the limb bud ends in a rounded mass without defined digits. Cell death occurs in the mesoderm between what will become the digits (Fig. 15-10). The regulation of death is promoted in part by the overlying epithelium which induces expression in the interdigital region of bone morphogenetic proteins (BMPs) and the transcription factors MSX1 and MSX2. The BMPs and MSX1/2 appear to play a required role in cell death in this region. Blockade of BMP function results in syndactyly.

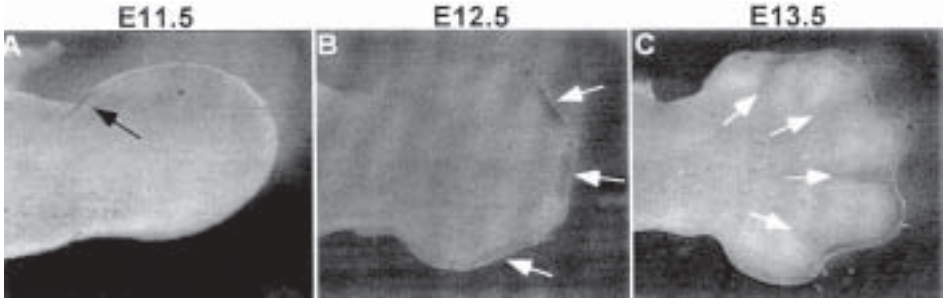

Fig. 15-10 Cell death in development of digits from limb bud. Dying cells are detected by staining with Nile blue. White arrows show areas of massive cell death. Embryonic stage is shown above panels (organism is mouse). FROM: Chen and Zhao, J. Exp. Zool. 282:691 (1998).

3. Formation of the semicircular canals. The semicircular canals arise by pinching off and evagination of a flattened pouch of cells from the otocyst. The opposing walls of the pouch appose and contact one another along the "fusion plate" to form fluid filled semicircular canals. A key element of this is extensive cell death along the fusion plate (Fig. 15-11). Interference with this death in experimental models results in defects in the morphology of the canals (Fig. 15-12).

4. Remodeling of the outflow tract (OFT) during transition from single to dual circulation in the developing heart. During cardiac development, the OFT both shortens and rotates to form the connection between the right ventricle and pulmonary artery. Its associated endocardial cushions remodel to form the pulmonic and aortic valves. These processes are marked by extensive levels of

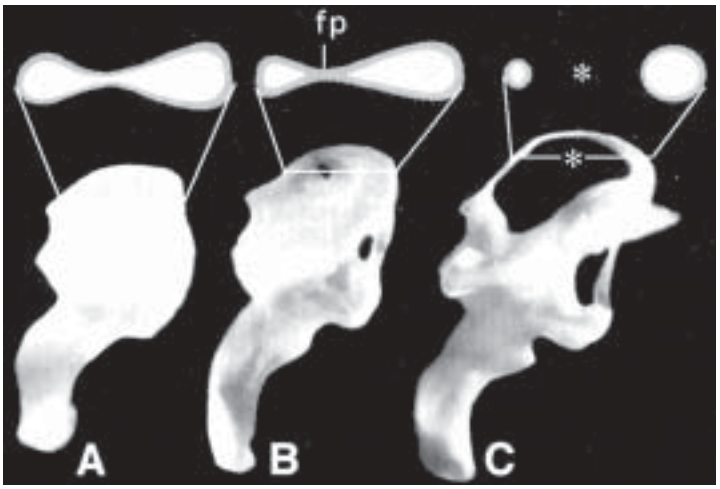

Fig. 15-11 Cell death in formation of the semicircular canals. Shown are fluid cavities of inner ears of chick embryos at various developmental stages. The canals form from a flattened pouch of epithelium that evaginates from the otocyst (A). The apposing walls of the pouch approach and contact each other over an extensive region called the fusion plate (B). Above each is a schematic cross section through the superior canal with the lumen in white and the epithelial cell layer in gray. The two sides of the canal pouch meet at the fusion plate (fp) which ultimately clears by cell death to leave the toroidal-shaped canal (C). FROM: Fekete et al., Development 124: 2451 (1997).

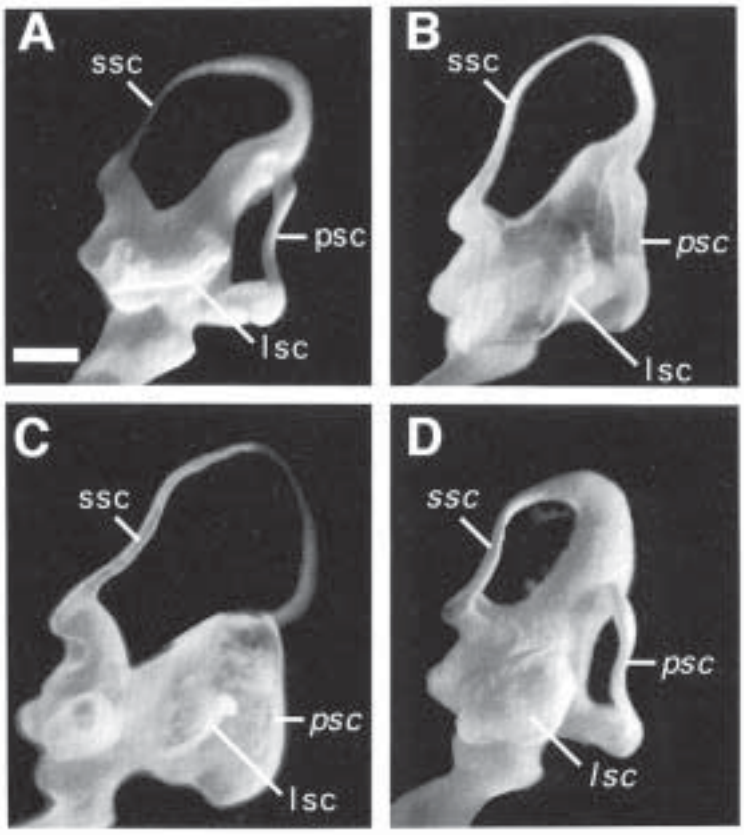

Fig. 15-12 Defects in semicircular canal formation induced by blockade of normal cell death. A shows normal control inner ear from E6.5 chick embryo. Ssc=superior semicircular canal; psc=posterior semicircular canal; lsc=lateral semicircular canal. B-D. Examples of canal phenotypes under conditions in which cell death has been partially blocked (by infection with a virus causing over-expression of the anti-apoptotic protein BCL2). FROM: Fekete et al., Development 124: 2451 (1997).

apoptotic cell death (Fig. 15-13). If such death is experimentally blocked in an animal model, by application of chemical caspase inhibitors, remodeling does not take place and major defects occur in ventriculo-arterial connections. For example, the aorta, which normally arises from the left ventricle, improperly connects with the right ventricle (Fig. 15-14). This is similar to the human congenital anomaly double outlet right ventricle (DORV).

B. Regulation of cell migration and pattern formation

Emmigration of the cranial neural crest-formation of separate migratory streams. Cranial neural crest cells migrate from the area of the rhombomeres in separate streams that ultimately give rise to key structures in the head and neck. One mechanism that assures separation of the various streams is

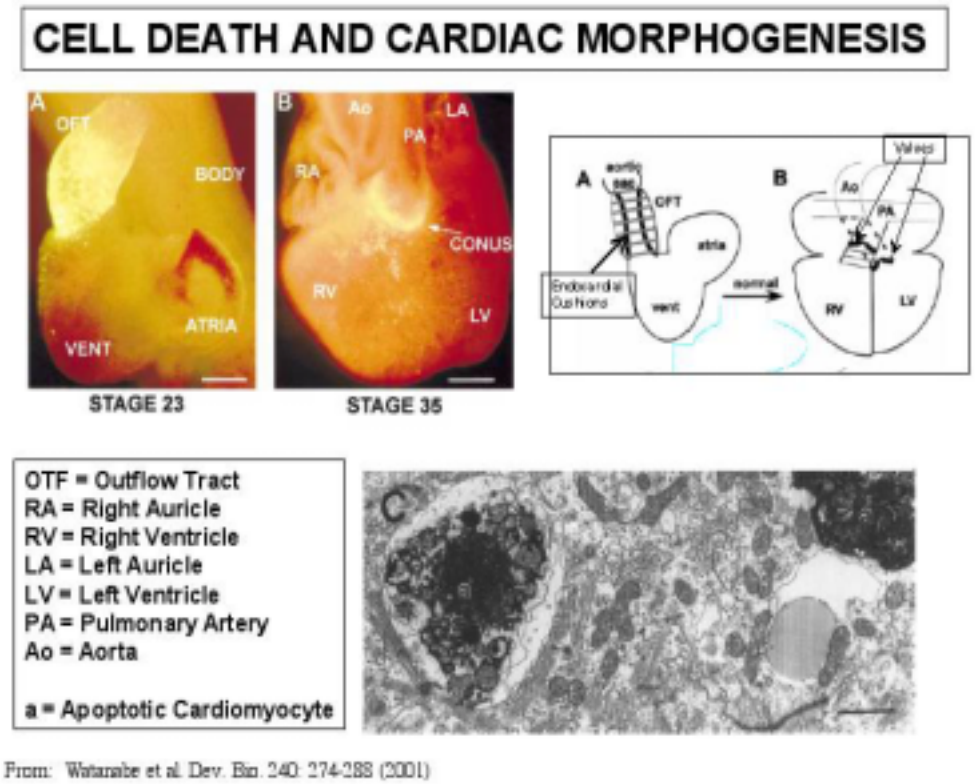

Fig. 15-13 Reorganization and cell death in the outflow tract of the developing heart during transition from single to dual circulation. A.B. Upper left and right: Shortening and rotation of the cardiac outflow tract during embryonic chick cardiac development to form the conus (or subpulmonic infundibulum) which connects the right ventricle with the pulmonary artery. The endocardial cushions of the outflow tract also remodel to form the pulmonic and aortic valves. C. Apoptotic cardiomyocytes in developing outflow tract (stage 30-31).

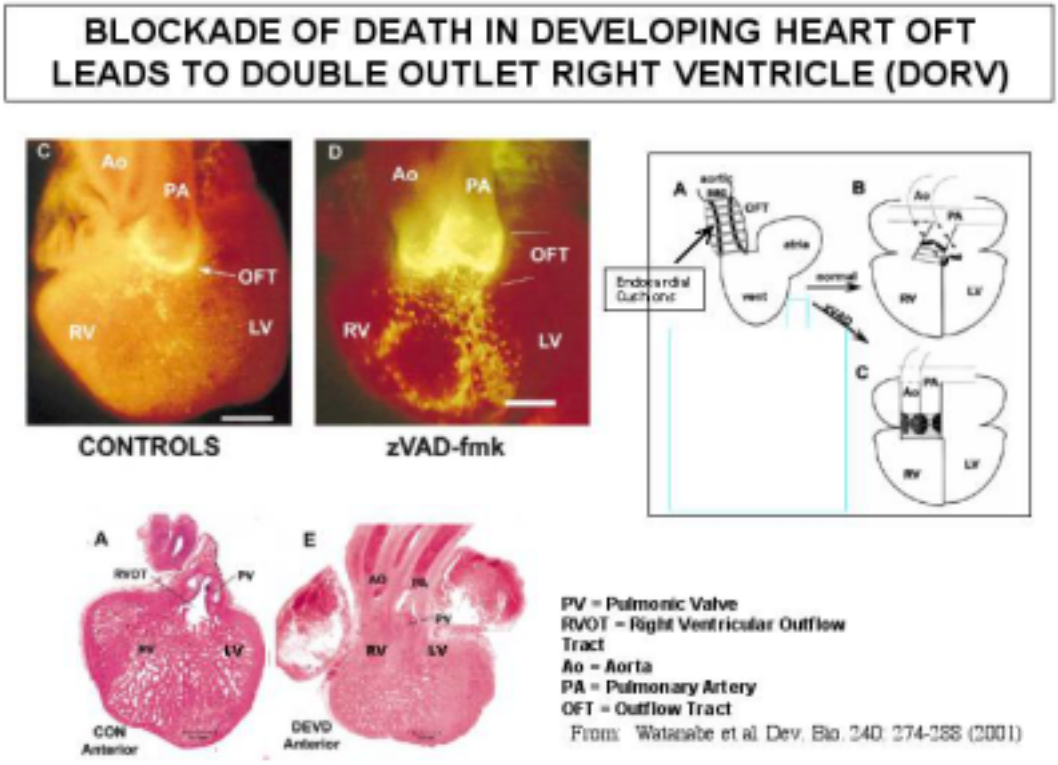

Fig. 15-14 Experimental blockade of normal cell death in developing chick embryo heart leads to defects in ventriculo-arterial connections similar to those in human double outlet right ventricle (DORV). Panels show effects of blocking normal apoptotic death with zVAD-fmk or DEVD, inhibitors of caspase activity. As a result of blocked death, the outflow tract is enlarged and improperly remodeled. Note that the aorta, which normally arises from the Left Ventricle, connects to the Right Ventricle in experimental organisms. In addition, the endocardial cushions showed abnormal valve formation with respect to morphology and position.

elimination by death of crest cells in alternate rhombomeres (Fig. 15-15A).
This selective death is triggered by release of death signals from adjoining rhombomeres. These death signals, as in the case of digit formation, include a BMP which elevates expression of the transcription factor MSX-2 (Fig.15-15B).

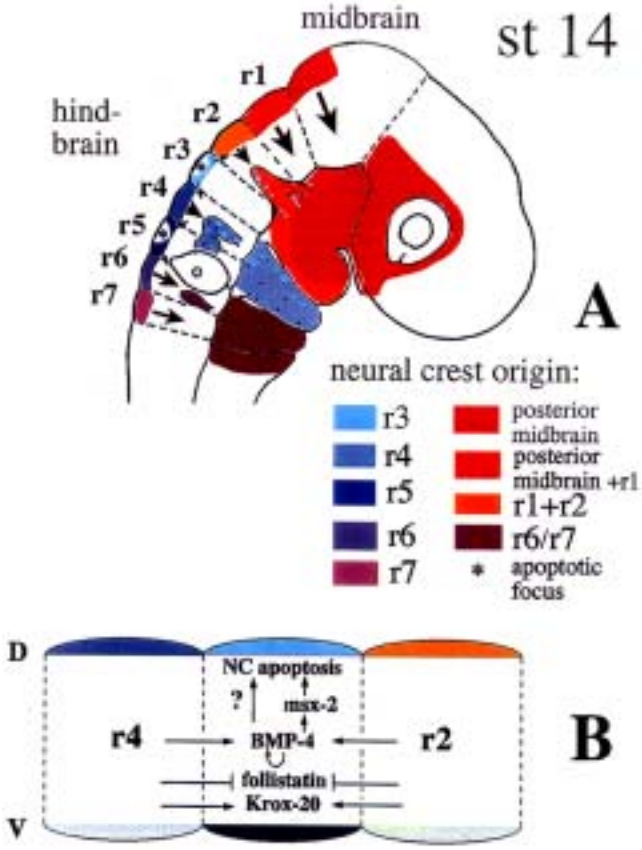

Fig. 15-15 Cell death in the cranial neural crest creates separate streams of migrating cells destined for specific branchial arches. A shows origin and fate of various cranial neural crest populations (chick embryo, stage 14) and their relationship to rhombomeres. Areas of massive cell death (apoptosis) in neural crest cells in contact with rhombomeres 3 and 5 permit formation of distinct streams of migrating cells. B shows an experimentally supported mechanism in which rhombomeres adjacent to rhombomeres 3 and 5 influence them to synthesize bone morphogenic factor 4 (BMP-4) which leads to up regulation of the transcription factor msx-2 and subsequent death of neural crest cells. From: Graham, Koentges and Lumsden, Mol Cell Neurosci 8: 76 (1996)

C. Deletion of unneeded structures

Reproductive tract development: Müllerian duct regression by cell death. Prior to initiation of sex determination, mammalian embryos possess anlagen for both male and female reproductive organs. The Wolffian duct gives rise to male organs and the Müllerian duct to female organs. During embryogenesis, the inappropriate duct is eliminated by cell death. In male embryos, the testes secrete a protein known as Müllerian inhibiting substance which induces mesenchymal cells surrounding the Müllerian ducts to secrete a signal that triggers death of the epithelial duct cells. Androgen secreted by the testes also promotes survival and maturation of the Wolffian ducts. In females, the Müllerian ducts survive and mature in the absence of Müllerian inhibiting substance while the Wolffian duct cells die in absence of testosterone (Figs. 15-16, 15-17).

D. Regulating cell numbers: culling

Neuronal cell death. A good example of this occurs in the nervous system. Cell counts have verified that in almost every part of the immature nervous system, there is over-production of postmitotic neurons and that about half of these die during development (Fig. 15-18).
The selection of those neurons that will live and those that will die is apparently stochastic and depends on interaction with targets. That is, neuronal targets (either peripheral organs such as muscle and glands, or other neurons) synthesize and secrete limited amounts of trophic factors that are

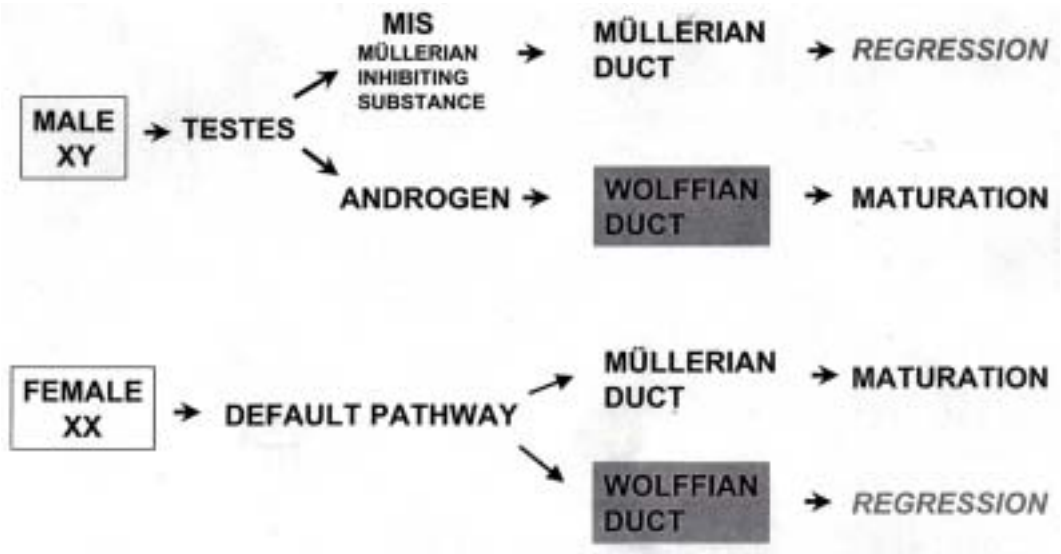

Fig. 15-16 Scheme for selective gender-dependent regulation of Müllerian and Wolffian duct maturation in embryos.

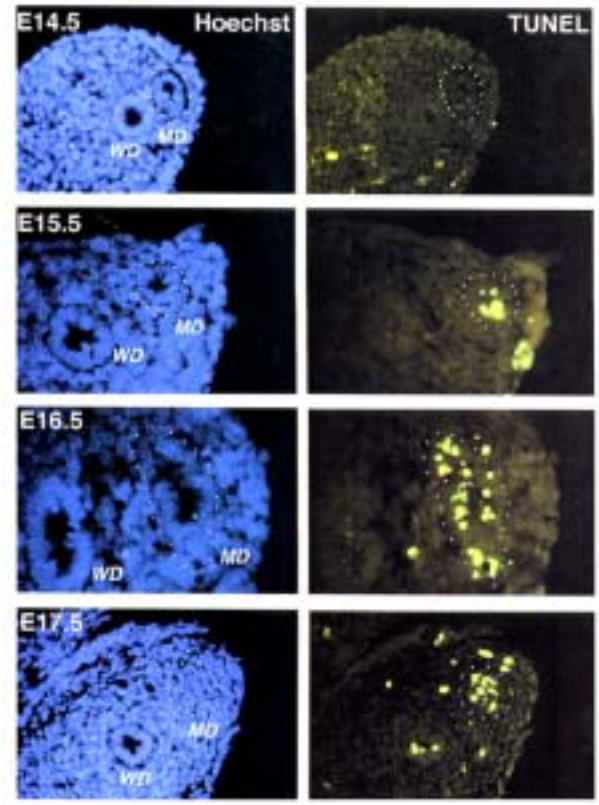

Fig. 15-17 Cell death during elimination of the Müllerian duct (dotted white line; MD) during embryonic development of the male mouse. WD=Wolffian duct. Hoescht staining (left) shows cell nuclei; TUNEL (right) shows dying cells. FROM: Roberts et al., Devel. Bio. 208: 110 (1999).

required for neuron survival. Those neurons that successfully compete for the limited trophic factor supply survive whereas those that do not undergo cell death. Presumably, this mechanism assures an appropriate match between targets and the required number of presynaptic neurons (Fig. 15-19).

E. Elimination of ectopic cells

Ectopic neurons. Because neurons require target-derived trophic factors for their survival, those that migrate to ectopic locations from which they cannot make appropriate contacts with targets, will be eliminated. Similarly, neurons that fail to make contacts or make inappropriate target contacts will be eliminated.

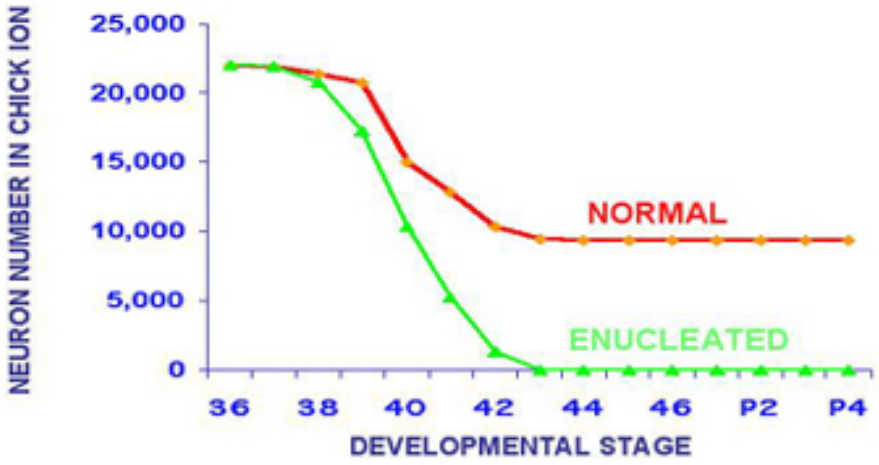

Fig. 15-18 Neuron number in the isthmo-optic nucleus (ION) of the chick embryo brain as a function of developmental stage. Figure shows neuron number during normal development and in embryos in which the target of the ION neurons has been removed. From: Clarke, Rogers & Cowan J. Comp. Neurol. 167: 125 (1976).

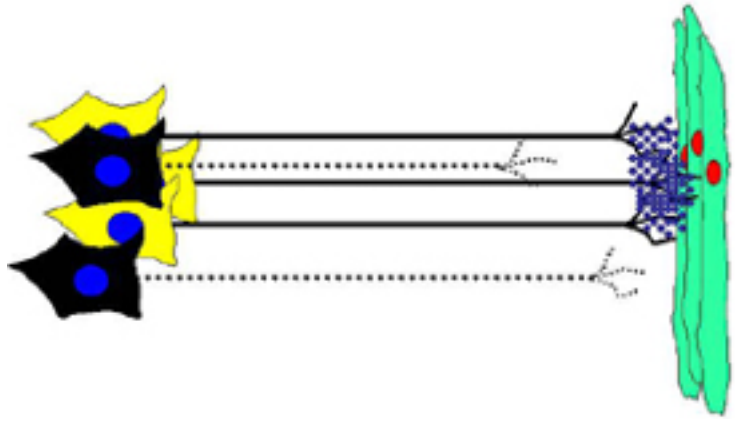

Fig. 15-19 Regulation of neuron survival and death during embryogenesis by competition for limiting supply of neurotrophic factor. Green cells at right depict target cells that release a limiting supply of trophic factor (blue dots). Those neurons that compete for the survival factor (depicted by yellow cell bodies) survive, while those that do not (black cell bodies) die.

F. Production of structures without Organelles

Lens production. Fiber cells in the developing lens lose their nuclei and other organelles by a mechanism similar to that of cell death. This ensures that an optically clear structure is created (Fig. 15-20).

V. Consequences of faulty cell death during development

Given the importance of cell death in development, it can be anticipated that genetic, viral or teratogenic influences that affect cell death programs will impact on the embryogenic process. Based on animal models, it would appear that mutations in general apoptotic mechanisms are embryonic lethal. One example is faulty regulation of the post-mitochondrial apoptotic pathway which results in massive over-production of neural tissue and embryonic lethality (Fig. 15-21). There is much circumstantial evidence that a variety of human congenital conditions such as syndactylyally, failure of neural tube closure, cleft palate, congenital heart defects involving alignment of valves and of cardiac blood vessels, etc involve defects in normal cell death mechanisms. As one interesting example, recent findings show that myeloid progenitor cells in patients with severe congenital neutropenia (Kostmann syndrome) have defective expression of Bcl-2, causing such cells to undergo excessive death.

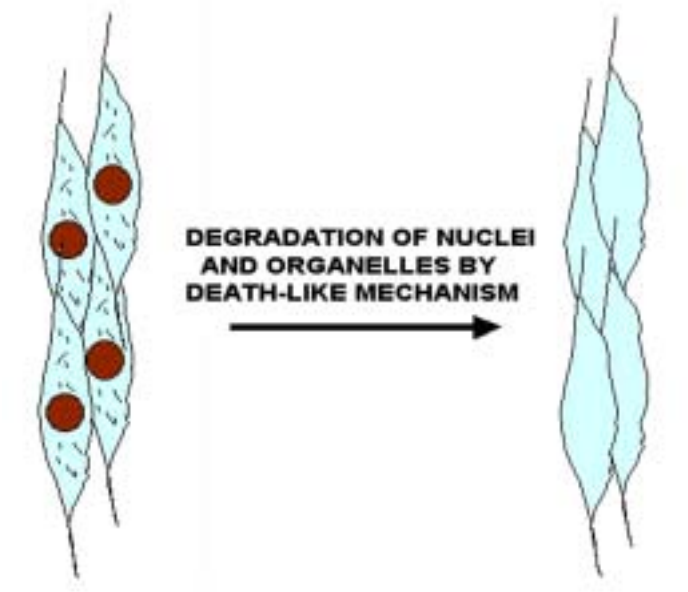

Fig. 15-20 Scheme depicting formation of the clear lens of the eye from lens fibre cells by "death-like" elimination of nucleus and organelles.

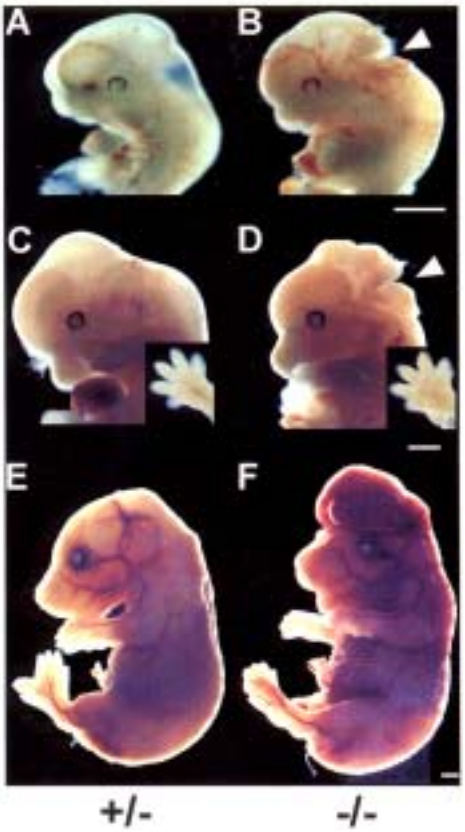

Fig. 15-21 Example of consequences of interfering with the cell death pathway during development.
Panels B, D and F show mouse embryos for which the expression of caspase 9 has been genetically deleted. Panels A,C and E show litter mates in which only one copy of the caspase 9 gene has been deleted and which are phenotypically indistinguishable from normal embryos. Note the formation of excess brain tissue (which protrudes through the skull in panel F) in the caspase 9 null animals. From: Kuida et a Cell 94: 325-337, 1998.

References and suggested reading:

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