Early descriptions of apoptotic cell death during tissue involution and embryogenesis

Variously called physiological cell death, programmed cell death, spontaneous cell death, chromatolysis.

Ovarian follicle, 1885 by Walther Flemming

Balance to mitosis (Graper, 1914)

Morphogenesis (Glucksmann, 1950)

“The initial stage, chromatopykcnosis, consists...in the appearance of a single chromatic mass sitting as a cap on the vacuole formed by the non-chromatic material.... Both the nucleus and the cytoplasm shrink by the loss of fluid... The granule loses its affinity for nuclear stains, becomes Feulgen-negative, breaks up and disappears: this is chromatolysis....The degenerating cell may be phagocytosed by a neighbor.”

Classic description of apoptosis

Apoptosis (a po to sis) Gr. fall + away

Described by Kerr, Wyllie and coworkers in 1972 (Br. J. Cancer 26, 239) as physiological cell death with characteristic features: contraction of cell volume, nuclear chromatin condensation, cell fragmentation, swift recognition and phagocytosis by adjacent cells. Differs from toxic cell death (necrosis) by preservation of cytoplasmic organelles and membranes. Single cell drop-out is characteristic.

Also observed in pathologic situations (usually at lower stimulus intensity), e.g. ionizing radiation, chemotherapeutic drugs, viral infections.

Necrosis

Typically seen following gross insults or membrane attack e.g. complement, severe hypoxia, hyperthermia, ischemia. Characteristic features are cell swelling, organellar swelling, and “flocculation” of nuclear chromatin. Early increased plasma membrane permeability. Mechanisms involve activation of calcium-dependent phospholipases, decreased ATP, lysosomal enzyme release, oxidative injury. Apoptotic cells may develop delayed necrotic appearance if not phagocytosed.
<table>
<thead>
<tr>
<th>Apoptosis</th>
<th>Necrosis</th>
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</thead>
<tbody>
<tr>
<td>Membrane blebbing</td>
<td>Loss of membrane integrity</td>
</tr>
<tr>
<td>Begins with shrinking of cytoplasm and condensation of nucleus</td>
<td>Begins with swelling of cytoplasm and mitochondria</td>
</tr>
<tr>
<td>End with fragmentation of cells into smaller bodies</td>
<td>Ends with total cell lysis</td>
</tr>
<tr>
<td>Release of various mitochondrial proteins into cytoplasm</td>
<td>Disintegration (swelling) of organelles</td>
</tr>
<tr>
<td>Tightly regulated signaling pathways</td>
<td>Ion pump failure</td>
</tr>
<tr>
<td>Energy (ATP)-dependent (stalled at 4°C)</td>
<td>No energy requirement</td>
</tr>
<tr>
<td>Non-random DNA fragmentation (oligonucleosomal ladder)</td>
<td>Random DNA digestion (smear)</td>
</tr>
<tr>
<td>Affects individual cells</td>
<td>Affects contiguous groups of cells</td>
</tr>
<tr>
<td>Induced by physiological stimuli</td>
<td>Induced by non-physiologic disturbances</td>
</tr>
<tr>
<td>Phagocytosis by adjacent cells or macrophages</td>
<td>Phagocytosis by macrophages</td>
</tr>
<tr>
<td>No Inflammatory response</td>
<td>Significant inflammatory response</td>
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</table>

Apoptosis should be viewed not as an intrinsically bad or good process, but rather as an fundamental cell activity that is highly organized and equivalent in complexity to other cell behaviors such as cell division and terminal differentiation. Whether occurring in the physiologic contexts of embryonic development and tissue renewal in the adult, or as a pathological response to cell injury and infectious pathogens, cell deaths are orchestrated for multiple purposes benefiting the organism (and thus altruistic). These include:

a) maintenance of epithelial barrier function

The apoptotic process for shedding of intestinal epithelial cells, as part of normal cell turnover, leaves intact an apical “bridge” between neighboring cells to preserve epithelial integrity.

b) destruction of microbes

Intracellular microbes, including M. tuberculosis, are killed as the host cell (macrophage) undergoes apoptosis, while necrotic death is ineffective (see J Exp Med 180: 1499, 1994 for early observations).
c) immune stimulation


d) recycling of material

Elegant study of Botryllus schlosseri, a marine urochordate, suggests that programmed cell death can be a recycling program for regeneration of organs and tissues (Dev Biol 249: 333, 2002).

e) intercellular signaling

Display of phosphatidylserine on surface of apoptotic cells is not only a label for phagocytosis (eat me), but triggers specific phagocyte responses mediated by a PS receptor (J Cell Biol 155: 649, 2001).

f) preservation of genomic integrity

Increasing viewpoint that p53 added to core DNA damage checkpoint pathways to eliminate mutated cells through apoptosis (see recent paper on Drosophila p53, PNAS 100, 4696, 2003)

Normal cell turnover

An adult human loses $10^{11}$ cells/day, with skin, intestine and hematopoietic tissues accounting for the majority. Normal cell death in the adult occurs in the context of continuously (skin, gut) or cyclically (endometrium, breast) renewing tissues. In most instances, homeostatic generation of new cells compensates for cell losses following terminal differentiation. An example is the intestinal epithelium, where one stem cell per epithelial crypt asymmetrically divides to produce a daughter cell that rapidly divides, terminally differentiates (coincident with cell cycle exit), migrates onto the surface epithelium, and undergoes a specialized form of apoptosis that leaves behind cytoplasmic bridges preserving epithelial barrier function, all within 2-3 days.
a) Neutrophils recruited to sites of inflammation undergo apoptosis synchronously with resolution of the inflammatory infiltrate. Apoptotic neutrophils have disabled degranulation capabilities and are silently phagocytosed by macrophages without activating a macrophage proinflammatory response. This clearance mechanism is specialized to apoptotic neutrophils, as neutrophils dying by necrosis or opsonized cells trigger macrophage secretion of inflammatory cytokines. Apoptotic neutrophils also stimulate production of anti-inflammatory cytokines such as TGF-beta by the engulfing cell.

b) Although a hallmark of apoptosis is the characteristic compaction of nuclear chromatin, enucleated cells are also subject to apoptotic mechanisms. Biochemical evidence suggests that RBC and platelet lifespans may also be curtailed through apoptotic processes. A general feature of apoptotic cells is a loss of the normal asymmetric distribution of plasma membrane phospholipids, with appearance of phosphatidylserine (PS) in the outer membrane leaflet. Loss of erythrocyte and platelet viability during aging in vitro is accompanied by PS exposure and activation of a family of apoptotic proteases, termed caspases. Increased PS content of the outer erythrocyte membrane has also been demonstrated in clinical diseases associated with shortened RBC lifespan, such as sickle cell anemia.

c) Physiologic cell death is also a mechanism that generates a reserve capacity for production of functionally mature cells. The glycoprotein hormone erythropoietin is produced by mesangial cells in the kidney and stimulates excess RBC production in proportion to the demand for oxygen-carrying capacity. The erythropoietin receptor (EpoR) is expressed on committed erythrocyte precursors (CFU-E and proerythroblasts). Growth factors, in general, mediate survival signals as well, as part of the “social” regulation of cell survival. The primary effect of Epo in vivo is to rescue erythroid precursors from death. The Epo-responsive erythroid compartment maintains a constant proliferation rate and size with varying demands (hypoxia, hypertransfusion), despite different rates of mature erythroid cell release. Thus, CFU-E's and proerythroblasts are overproduced in normal conditions, with excess cells removed prior to the erythroblast stage, in order to provide a rapidly accessible reserve in states of higher O2 demand. Similar cell kinetics of excessive production with apoptosis of maturing cells are found in small intestinal crypts and during spermatogenesis.

d) Another physiologic function for apoptosis is as a selection mechanism for specific cell phenotypes. The best known example occurs in the immune system following clonal diversification of T and B lymphocyte antigen receptors by gene recombination and error-
prone DNA replication. Positive and negative selection to match T cell receptors to Class I and Class II histocompatibility antigens expressed on accessory cells and eliminate cells reactive with self-antigens, takes place in the thymus. Affinity maturation of immunoglobulin-bearing B cells takes place in germinal centers of lymphoid organs. In each case, cells run a gauntlet of near-death experiences with death and survival signals directly linked to the binding properties of the antigen receptor on individual cells.

**Sculpting during embryogenesis**

During development, apoptosis is extensively used to sculpt the final shape of the embryo. This happens in macroscopic (phylogenetic, morphogenetic) and microscopic (histiogenetic) contexts. Regression of vestigial tails, interdigital webs, and pro- and mesonephros are accomplished by an autophagic type of cell death, which nonetheless biochemically resembles classical apoptosis. Certain anatomic structures, such as hollow viscus organs, are formed by apoptotic excavation of interior cell masses; the final form of other structures such as the forebrain, are shaped by apoptotic patterns within neuronal precursor cells. A prime example of histiogenetic death is the matching of the number of projecting neurons to the size of a target field, accomplished by apoptosis of surplus neurons. Excess or misdirected neurons fail to uncover caches of trophic factors produced by their designated targets.

**Cell damage control**

One of the central discoveries in cell death was the common phenotype of physiologic and pathologic deaths, since confirmed using biochemical and genetic approaches. Diverse forms of cellular damage trigger apoptotic death. DNA damage due to free radicals, alkylating agents, ultraviolet radiation as well as errors of replication (deficiency of nucleotide pools, topoisomerase inhibition, mismatch repair errors) trigger apoptosis via the operation of cell cycle checkpoints. Apoptotic cell death has been associated with numerous chemical toxins and idiosyncratic drug reactions, often attacking cells vulnerable to physiologic apoptosis.

Recently, there has been increased interest in the role of intracellular protein aggregates and misfolded proteins as a stimulus for apoptosis (Human Mol Genet 11: 1505, 2002). Experimental expression of aggregation prone proteins has been shown to inhibit the ubiquitin-proteasome system, leading to
accumulation of multiple proteasomal substrates. Ineffective erythropoiesis in beta-thalassemia major is caused by intramedullary apoptotic death of erythroblasts with aggregated alpha-globin chains, together with the erythrocyte membrane proteins spectrin and band 4.1.

**Governor of cell behavior**

Apoptosis has a tumor suppressor function to eliminate genetically unstable or mutated cells recognized by cellular checkpoints. It is also known that cells also detect supra-normal activation of dominant proto-oncogenes such as Myc and Ras, as apoptotic signals. Although many of these sensors act via induction of p19ARF (see below), whether proliferative or some other aspect of “oncogene stress” is monitored is not clear.

The list of triggers for apoptotic death seems endless. It appears that many aspects of cell behavior are closely monitored, with virtually any deviation from normal punished by apoptosis. For epithelia and other cell types attached to extracellular matrix or basement membranes, detachment triggers an apoptotic response, designated anoikis. Cellular differentiation also appears to have a fail-safe apoptotic response in many lineages, such that cells that do not execute a differentiation program successfully are eliminated. One of the most puzzling features of apoptosis is how such a multitude of stimuli can trigger a conserved response pathway.
## Assays for Apoptosis

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DNA fragmentation</strong></td>
<td>Physical separation of low and high molecular weight DNA with quantitation by colorimetric, radioactive, immunologic methods</td>
</tr>
<tr>
<td></td>
<td>Analysis by agarose gel electrophoresis</td>
</tr>
<tr>
<td><strong>DNA content analysis</strong></td>
<td>DNA content analysis by flow cytometry using fluorescent DNA binding dyes (DAPI, ethidium bromide, propidium iodide)</td>
</tr>
<tr>
<td><strong>DNA strand breaks</strong></td>
<td>Labeling of DNA strand breaks with modified nucleotides (TUNEL or ISNT)</td>
</tr>
<tr>
<td><strong>Altered nuclear staining</strong></td>
<td>Fluorescent dyes such as acridine orange, Hoeschst 33342 combined with membrane-impermeable dyes (propidium iodide, 7-AAD)</td>
</tr>
<tr>
<td><strong>Altered plasma membrane structure</strong></td>
<td>Labeling of externalized phosphatidylserine with Annexin V protein</td>
</tr>
<tr>
<td><strong>Protease activity</strong></td>
<td>Detection of caspase activity in cell extracts using fluorescent substrates</td>
</tr>
<tr>
<td></td>
<td>Immunodetection of processed caspase enzymes (immunoblot or in situ)</td>
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<tr>
<td></td>
<td>Cleavage of caspase substrate (PARP) by immunoblot</td>
</tr>
</tbody>
</table>
Genetic analysis of programmed cell death

Apoptotic cell death is prominent during embryologic development, e.g. in some areas of mammalian central nervous system, greater than 50% of cells born will normally die. “Programmed” terminology originated with embryologists and current usage represents the importance of developmental history and protein synthesis in many embryonic deaths.

C. elegans

Transparency allows cell divisions and cell deaths of individual cells to be observed and fate maps constructed. Out of 1090 somatic cells formed in development of adult hermaphrodite, 131 undergo programmed cell deaths.

Under Nomarski optics, these cells appear condensed, rounded, refractile. Rapid phagocytosis by neighboring cells. Genetic screens are performed in mutant ced-1 or ced-5 backgrounds that are defective in phagocytosis – which accumulate cell corpses.
Phenotypes of EMS (ethane methyl sulfonate) mutants with fewer programmed cell deaths

2 cell killing genes (ced-3, ced-4)

7 engulfment genes, 2 complementation groups (ced-1,2,5-8,10)

1 nuclease gene (nuc-1)

3 cell specification genes (ces-1,2,egl-1)

One dominant mutant allele, ced-9 n1950, prevents cell deaths. Null mutants have excess cell deaths and are embryonic lethal.
CED-9


<table>
<thead>
<tr>
<th>Genotype</th>
<th>Corpses in head</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.0</td>
</tr>
<tr>
<td>Ced-1 (engulfment deficient)</td>
<td>28</td>
</tr>
<tr>
<td>Ced-1; Ced-9 (n1950 = gain of function)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Failure of 131 cells to die has no glaring adverse consequences, although there are subtle effects on growth, fertility and chemotaxis. Abnormal survivors adopt specific, usually appropriate cell fates (resemble sisters or aunts). Egl-1 (gain of function) mutants have abnormal cell death of HSN neurons controlling egg-laying. Egl-1 (gf)/Ced-9 n1950 double mutants lay eggs normally.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Egg-laying defective %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.4</td>
</tr>
<tr>
<td>Egl-1</td>
<td>99</td>
</tr>
<tr>
<td>Ced-9 (n1950); Egl-1</td>
<td>0</td>
</tr>
</tbody>
</table>

Epistasis analysis: mutations in ced-3 and ced-4 suppress ced-9(lf) defects

CED-3

Member of ICE (interleukin-1-beta converting enzyme)/Caspase (cysteinyI aspartate-specific protease family (Cell 75: 641, 1993)

28% homologous to ICE, identical active site (QACRG)

Transient transfection of ICE in rat fibroblasts resulted in apoptosis, while mutants without protease function did not. Cell death blocked by co-
transfection with bcl-2 or crmA, a cowpox viral gene encoding an ICE inhibitor (Cell 75: 653, 1993).

Transient transfection of primary neuronal cultures with crmA protected against apoptosis induced by withdrawal of nerve growth factor (Science 263: 826, 1994).

CED-4
CED-4 protein binds to both CED-3 and CED-9 and is required for the processing of pro-CED-3 to active enzyme. CED-9 and prodomain of CED-3 compete for site on CED-4 (J Biol Chem 273: 17708, 1998).

EGL-1
BH3 containing homolog of Bax-like pro-apoptotic proteins (Cell 93: 519, 1998). Gain of function mutant causes death of HSN neurons, loss of function prevents all somatic deaths.

CED-8

CES-2

CES-1

CED-5
CED-6


CED-7

Engulfment gene similar to ABC transporters, required in both engulfing and target cell (Cell 93: 951, 1998).

CED-1

Cell surface receptor for CED-7, related to human SREC (Scavenger Receptor from Endothelial Cells) and EMILIN protein family of secreted glycoproteins (Cell 104: 43, 2001).

DAD-1

Originally described as ts mutant allele in hamster BHK21 cell line which undergoes apoptosis at restrictive temperature (Mol Cell Biol 13:6367, 1993). C. elegans homolog isolated and overexpression shown to prevent programmed cell deaths (EMBO J 14: 4434, 1995). Both genes are homologous to OST2 in Saccharomyces cerevisiae, subunit of enzyme required for protein glycosylation in ER. Homologs also found in plants.

ICD-1


Drosophila melanogaster

Ample cell death during embryogenesis and metamorphosis. Fields of cells easily seen by staining live embryos with acridine orange (AO). Steller screened 140 deletion mutants covering 50% of Drosophila genome for homozygous defects in cell death. One region, 75C, scored for lack of cell death. Homozygous deletion mutants have enlarged CNS due to excess cells. Embryos lack ectopic cell deaths following X-irradiation and when crossed to mutants strains with excess cell death.

Extremely high doses of X-rays cause some breakthrough apoptosis in these deletion mutants - argues for regulators, not effectors within deletion.

Three genes within deletion

Reaper

Induces apoptosis when ectopically expressed (wild-type or deletion background). Expression is restricted to cells that are going to die in embryos
and adults and is induced by irradiation (within 30 min) (Science 264: 677, 1994).

Hid (head involution defective)

Morphogenetic defect due to reduced cell death in head region during embryogenesis. 410 aa protein with N-terminal homology to reaper. Expression correlates with cell death, but also found in some living cells. Exhibits some synergy with reaper in ectopic expression experiments.

Grim

138 aa protein, also with N-terminal homology to reaper

N-terminal sequence of reaper, hid and grim is highly similar to the mammalian mitochondrial protein, SMAC/Diablo (more later).

Secondary screens use intermediate phenotypes of ectopic reaper or hid expression, e.g. in eye, to identify modifiers of reaper/hid-mediated cell death. Thread (DIAP1) mutants identified as enhancers of reaper/hid (Cell 83:1253, 1995). Overexpression suppresses cell death. Homologous to baculovirus IAP (inhibitor of apoptosis).

Reaper-interacting protein, Scythe, purified from Xenopus egg extracts. Scythe is related to Hip/Hop/Sti-1 co-chaperones for Hsp70, but like Bag-1 inhibits dissociation of Hsp70 clients. Reaper binding to Scythe reverses inhibition of Hsp70. Required for reaper-induced apoptosis.

Table 1. Conservation of key regulators of programmed cell death between species

<table>
<thead>
<tr>
<th></th>
<th>C. elegans</th>
<th>Drosophila</th>
<th>Mammals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2 family</td>
<td>3</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>CED-4/Apaf</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Caspases</td>
<td>4</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>BIR proteins</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Reaper, Hid and Grim</td>
<td>-</td>
<td>4</td>
<td>2-4</td>
</tr>
<tr>
<td>P53</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
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</table>

Caspases
The central effectors of apoptosis are a novel family of cysteine proteases, designated as caspases (cysteinyl aspartate-specific protease). All caspases are aspartases with a four residue recognition sequence P4-P1, based on substrate binding to the active site. Aspartases appear to be specialized for apoptotic pathways, with the only other known aspartase, granzyme B, a serine protease found in cytolysic T cell granules. Caspase cleavage motifs are found in proteins with low frequency, thus caspases produce limited digestion of substrates with several large proteolytic fragments. Approximately 280 substrates have been identified to date. These can be grouped in several categories.

Rather than identifying a critical life-sustaining protein that is a substrate for caspases, more progress has been made in understanding how the biochemical and morphologic features of apoptotic death are generated by cleavage of specific substrates. Proteolysis of structural elements of nuclear scaffold (lamins) and cytoskeleton (actin, fodrin, gelsolin) probably actuate membrane blebbing and packaging of chromatin and cytoplasmic material in apoptotic bodies. (lamins). DNA fragmentation is mediated by an endonuclease, CAD/DFF40, which is activated following caspase-mediated degradation of an inhibitory binding partner, ICAD/DFF45. Detachment of apoptotic cells from surfaces is correlated with cleavage of proteins involved in adhesion complexes (FAK).
Caspases also target proteins involved in energy-consuming cell processes. The classic example is polyADP ribose polymerase (PARP), a nuclear enzyme involved in DNA repair. PARP is activated by DNA damage and consumes NAD, and ultimately ATP, as a source of ADP ribose. Other caspase targets are involved in DNA repair (DNA-PKcs, Rad51, MCM3, DNA RFC140), ribosomal assembly (U1-70KsnRNP) and cell cycle (p21,p27). One rationale for this category may be that apoptosis itself requires energy in the form of ATP, and depletion of ATP can convert apoptosis into necrosis with accompanying pro-inflammatory effects.

In the intracellular balance between survival and pro-apoptotic factors, caspases can tip the balance or provide a feed-forward mechanism to guarantee irreversibility of the process. Both of the predominant mitochondrial protectors, Bcl-2 and Bcl-xL, are subject to N-terminal cleavage by caspases. Not only does this disable their survival activities, but experimentally the truncated versions behave as pro-apoptotic factors. Full activation of a Bcl-2 related pro-apoptotic factor, Bid, requires caspase-
mediated processing to generate a truncated form, tBid, which readily translocates to its mitochondrial site of action.

Caspases are expressed in normal, healthy cells as monomeric zymogens with low to absent protease activity, with strong activation dependent on proteolytic processing into large and small subunits that then assemble as tetramers (dimers of heterodimers). Caspases are themselves cleaved immediately distal to aspartates found within caspase recognition motifs. The specificity among caspases are distributed such that many caspase zymogens must be processed in trans by a distinct caspase, creating a hierarchy of proteolytic activation (amplification). Several caspases also have autocatalytic or self-processing activity when clustered (induced proximity model), and are held in check by the regulated assembly of self-activating complexes. These caspases are distinguished by the presence of a long pro-domain that serves as a docking site for recruitment into a catalytic complex. Protein associations within these complexes are built around homomeric interactions between three related binding domains, known as death (DD), death effector (DED) and CARD domains.

Two distinct caspase-activating assemblies are known that, although additional complexes are likely. Caspase-8 is engaged by a family of cell surface receptors known as death receptors, including TNF, Fas and TRAIL. Ligand binding induces trimerization of the death receptor. The cytoplasmic tail of the death receptor binds an adaptor protein, FADD/MORT1 (or TRADD), through homologous death domains. The second interaction domain in FADD/MORT1, a death effector domain, binds a similar DED in the pro-domain of caspase-8, leading to autocatalysis. The death receptor, FADD and caspase-8 complex is known as the death inducing signaling complex (DISC).
The second assembly, the apoptosome, is specialized for activating caspase-9, which has a CARD-type prodomain. Formation of the apoptosome is initiated by the translocation of the electron carrier, cytochrome c, from the mitochondria to the cytoplasm. Cytochrome c binds to an adaptor protein, APAF-1, which in the presence of ATP or dATP, uncovers a CARD domain binding site. Docking of caspase-9 facilitates its autocatalytic processing.

Recent studies indicate that processing of initiator caspases 8 and 9 is not required for activation, only adaptor-induced dimerization. Procaspase-8 dimers are selectively active in cross-cleavage of pro-caspase 8 dimers, such that processing or dissociation from the DISC shuts down the auto-catalytic cycle (EMBO 22: 4132-42, 2003). APAF-1 promotes dimerization of procaspase 9, with the dimer interface allowing a productive conformational rearrangement of one of the two active sites (PNAS 98: 14250, 2001). In both cases, protease activity appears to be regulated by allosteric mechanisms, rather than protein cleavage.

Due to its active site cysteine, caspases are also subject to regulation by thiol status. This residue appears to be particularly susceptible to stable S-nitrosylation mediated by nitric oxide in the presence of ROS and transition metals. This is a physiologically important mechanism in the maintenance of endothelial cell survival by shear-induced stress and prolongation of neutrophil survival by activation of NADPH-oxidase.

Although justifiably known for their apoptotic role, there is accumulating evidence that caspases also have roles in healthy cells. These
include sperm individualization, the process of separating mature spermatids from syncytia (Dev. Cell 4: 687, 2003), erythrocyte differentiation (Exp Cell Res 240: 206, 1998), lens fiber differentiation (J. Cell Biol 140: 153, 1998), macrophage differentiation (Blood 100: 4446, 2002) and keratinocyte differentiation (Cell Death Differ 7: 1218, 2000). The means of restricting caspase activity to certain substrates or subcellular structures are not well understood at present, although compartmentalized initiation and expression of inhibitors are likely to be involved.