CELL SUICIDE AND CELL DEATH

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The cells of an organism do not live indefinitely and their half-lives depend on cell type. There arecells whose lifespan is long, as muscle cells or neurons, while others have ephemeral lives, assome blood and epithelial cells, renewing from their progenitor cells. The number of cells composing a tissue in an adult organism remains, within certain limits, constant; dying cells are replaced by others, a process which is regulated and ensures the maintenance of a properbalance between loss, renewal and cell differentiation. It is estimated that the human body produces and eradicates every day billions of cells. Cell turnover in the tissues of an organism is based on maintaining a balance (homeostasis) between proliferation and cell death in order toensure adequate population at all times.

The normal or physiological state of an organism is achieved with cellular responses that allow cells and tissues to adapt and survive in the conditions of their environment and respond tostimuli. To this end, a variety of systems and processes are involved in the maintenance of cellintegrity, from the cell membrane (processes of endocytosis and exocytosis) and metabolic changes of gene expression, to defense mechanisms and repair systems. However, irreversibledamage can be done to reach a point of no return; irreversible morphological, functional andbiochemical changes prevent cells from performing vital functions and drag them to death. Cell death can be triggered by many causes: loss of function, mechanical damage, infection from microorganisms or viruses, toxic chemical action or lack of nutrients. Cell death, according to classical criteria can be divided into a death that runs through regulated mechanisms such as apoptosis, and unregulated (necrosis) [1].

Cell death by necrosis can occur when damage occurs that is lethal or produces an accidental death. Necrosis (from the Greek Nekros “death”) is the pathological death of cells or tissues of the organism. It is caused by an acute, irreversible, injuryresulting from a physiological situation or condition that can not be repaired by mechanisms of adaptation and resistance. This occurs due to harmful agents, or certain conditions or circumstances, such as an insufficient blood supply to the tissue (ischemia), lack of oxygen (hypoxia), trauma, exposure to ionizing radiation, chemical or toxic action or, for example, an infection or the development of an autoimmune disease. This form of cell death qualifies as a violent process as the cells swell and deteriorate cell structures, and critical functions are paralyzed for life. The viability loss is associated with disruption of the plasma membrane with consequent cell lysis and release to the exterior of cytoplasmic contents and organelles, damaging the tissue in which it is located. The release of cell contents in turn can cause inflammatory reactions. The morphological changes seen in necrotic cells are shown in Figure 1.

Figure 1. Schematic comparison of morphological characteristics of apoptotic cell death andnecrosis. The diagrams show the morphological changes that are observed in apoptotic and necrotic

cells. The micrographs obtained by transmission microscopy electronics compare thenucleus of a normal cell (upper part) with that of a cell that has begun the process of apoptosis (bottom).

Other types of cell death involving the activation of specific mechanisms that dictate that a suicide or programmed cell death is produced: a series of events that culminate in cell death in a genetically regulated fashion. These physiological mechanisms of death are employed by multicellular organisms during development, morphogenesis and maintenance of tissue homeostasis in the adult organism as well as to control cell number and eliminate infected, mutated or damaged cells [1 , 2]. This type of cell death takes place in an orderly and quiet manner, and relates to the whole body benefits during its life cycle. To define the latter process, and as a synonym for programmed cell death, “apoptosis” was coined —the term taken from the Greek neologism (apo: “off” or “separation” and ptosis: “drop”) [3]. Death by apoptosis is cleaner than necrosis, particular morphological changes are detected and cell membrane, which is not destroyed, includes apoptotic bodies or cellular material (Figure 1). No swelling occurs as phagocytic cells recognize, capture, and eliminate apoptotic bodies. Apoptosis can be defined as “the set of biochemical reactions that occur in the cell and determine their death in a regulated in response to a number of physiological or pathological events.” In this case, a series of stimuli or signals causes the cell to decide its own death, is what has been qualified as death that allows survival. This type of death has been conserved throughout the evolutionary scale between organisms as diverse as nematodes and mammals [4,5]. The self-destruct program is complex and requires a precise coordination between activation and execution of several programs of the sub-death machinery. The general characteristics of cell death by apoptosis and necrosis are given in Table I.

These two types of programmed cell death do not account for other mechanisms known today. There are intermediate processes that can hardly be classified into either group because they may share some of the characteristics of apoptosis and necrosis; in other cases, death mechanisms do not meet either of these two. In addition to apoptosis other types of programmed cell death have been described, which are considered in the last section, which may overlap in some characteristic or be mutually exclusive. [1] Autophagy is among them, and has been classified as one of these alternative forms of programmed cell death [6-10]. The execution of apoptosis, as we will subsequently describe, involves the participation of a group of proteases, caspases, so that this pathway of programmed cell death is dependent on their activation. In many organisms, including vertebrates, in some cases death occurs through physiological mechanisms that are independent of the involvement of caspases. For example, keratinocytes lose their nucleus as part of a normal differentiation program and detach from the skin, or the red cells survive 120 days after the loss of their nuclei and, finally, are phagocytosed [2] .

TABLE I General characteristic of death by apoptosis and necrosis

APOPTOSIS	NECROSIS
Physiological death. Highly regulated and controlled process	Not physiological; traumatic, accidental death. Process not regulated
Occurs during development, maintains tissue homeostasis and eliminates damaged cells	Does not occur during development
Energetically active process that requires biosynthesis of proteins [note by translator: synthesis is not necessarily immediately preceding death]	Energetically passive process

Follows a specific order of events. Condensation of chromatin and internucleosomal fragmentation of genomic DNA. Structural integrity of organelles is maintained	The cell swells, subcellular organelles lyse and disintegrate in a disorganized manner
Maintenance of integrity of the plasma membrane. The cell contents are enclosed in apoptotic bodies. Cell contents are not released. No inflammation is produced.	Rupture of cell membrane leads to liberation of cell contents into extracellular space, associated with inflammation
Active participation of cell components. Degradation mediated by caspases	Passive process
Phagocytosis of apoptotic bodies	Cell lysis and damage to neighboring cells.

CELL DEATH, DEVELOPMENT, AND CELL HOMEOSTASIS

Programmed cell death, as a cell controlled self-destruction process, allows the organisms propermorphogenesis and renewal and the elimination of cells that threaten their survival. Organisms as diverse as nematodes and humans have retained the genes encoding for the core of the cell death machinery [2, 4, 5]. In embryonic and fetal development, and in some cases later, apoptosis and autophagy are involved in the remodeling and elimination of numerous structures in SuperProcontrol of superproduction of cells, in removing defective cells and in the specific differention ofcells (Figure 2) [7]. All these processes are regulated by a combination of positive and negative signals that the cells receive.

In development, sometimes structures form that must be removed after formation that, after longevolution, have outlived their usefulness, or those that are only required in certain stages or may be required only for one of two sexes. A simple example of the remodeling of different structures, which highlights the consequences of the process of cell death and that shows that it isnecessary during ontogeny, is that humans have five individualized digits on each limb. Fingerformation occurs during fetal development by removal of interdigital areas. By contrast,programmed cell death is reduced in the extremities of the ducks so that they maintain theircharacteristic webbed foot. The loss of structures, like the tail of tadpoles during metamorphosisof amphibians in response to increased blood thyroid hormone, the loss of the tail of thedeveloping human embryo, or the metamorphosis of insects, are other cases where the importance of apoptosis is evident. Other examples are Müllerian ducts, forming the uterus andthe fallopian tubes in female mammals and which are removed by apoptosis in males. Similarly,testosterone in males directs cell death in mammary glands [7].

Figure 2. Cell death in development and cellular homeostasis. Programmed cell death is involvedin numerous physiological processes: remodeling of structures (such as the formation of the digitsof the limbs), developmental removal of structures that are no longer required (metamorphosis of insects and amphibians), control number of cells and removal of abnormal cells.

A notable example in relation to cell death, received special interest in the early twentieth century, is the development of the nervous system. During development neurons are formed in excess, controlling the excessive production of the same with a readjustment of their number by cell death, allowing further refinement of innervation so that those neurons die which are less able, those unable to establish a connection interstitial suitable. In addition, apoptosis has been assigned a role in the

control of immune system events, such as selection of T and Blymphocytes, cytotoxic death of damaged cells or apoptosis induction by cytokines. The immunesystem cells have the ability to discriminate between self and foreign, so that agents recognizemolecules foreign to our body and eliminate them. Those clones of cells that have developed the ability to recognize the body's own tissues are removed, there is a negative selection of cell repertoire that defend the body against all that is foreign to it. In the thymus autoreactive cells are removed by apoptosis. Thus tolerance is established which prevents the response to self-antigens and therefore not triggering autoimmune reactions [11].

Within the process of programmed cell death are also included other types of cell death, such asthat associated with aging or senescence. This process is related to an accumulation of damage to their genetic material and a reduction in cellular DNA telomeres throughout the life of the cells has been described. This results in a loss of stability of the chromatin, which ultimately causescell death [12]. Also, the process of terminal differentiation is another example. In various tissues, such as the intestinal epithelium, the cells proliferate and differentiate. The differentiated cells do not proliferate, reaching a state in which the cell does not respond to growth factors. Finallyterminal differentiation is associated with apoptosis and extrusion of the cell into the intestinallumen.

Abnormal or defective cells in which there has been DNA damage are also eliminated by apoptosis, these being replaced by new cells. A signal that can trigger apoptosis in a cell is the loss of adhesion to the surrounding extracellular matrix. This type of apoptosis is known as "anoikis".

Apoptosis is also a mechanism for elimination of virus-infected cells, a defense against viral infection. Eukaryotic cells have developed several routes to minimize damage produced by viral infection and eliminate the pathogen; cell death is one of them. However, some viruses have developed a defense against this removal giving them the ability to control the survival and death of the cell they infect. In such cases, to ensure an efficient propagation in the viral genome are encoded molecules that inhibit apoptosis. Thus, if the cell extends its life, the virus has more time to multiply. Thus, they inhibit capasases and prevent generation of inflammatory cytokines which influence the immune response [13].

MORPHOLOGICAL CHANGES IN APOPTOSIS

The first descriptions of programmed cell death by microscopy were performed by observing the morphological changes that occurred in cells and then changes at the ultrastructural level were described by electron microscopy. The latter technique allows visualizing cellular swelling, formation of membrane vesicles in the cell, changes in the size of mitochondria, endoplasmic reticulum dilatation, the number of lysosomes or the state of chromatin. However, given the importance of apoptosis by its relationship with certain pathologies, techniques for evaluating this process have proliferated and evolved [14]. Thus, today, to identify the type of cell death by morphological methods in addition a number of specific tests can be considered as functional or biochemical, which will be discussed later.

The apoptotic cell undergoes a series of morphological changes that define the process (Figure1). Cell adhesion and intercellular contacts diminshi and a loss of specialized structures of the cell surface (eg, the microvilli). In the plasma membrane, which initially remains intact, changes in the distribution of phospholipids and protrusions are formed, known as the bubbling process. Cell volume decreases and the cytoplasm is condensed. The nucleus shrinks and chromatin compacts, acquiring a marginal distribution around the nuclear envelope, with aggregation of the nuclear pore and focal dissolution of the nuclear lamina. The cytoskeletal proteins are disassembled and mitochondrial function is reduced. Finally, the cell collapses, causing a split into multiple structures, called apoptotic bodies, constituted by parts of the cytoplasm andorganelles surrounded by plasma membrane. This entire process occurs without release ofcytoplasmic contents or subcellular organelles to the external environment because, unlike cell death by necrosis, no breakage occurs in the plasma

membrane. In the physiologicalenvironment, pyknotic or apoptotic bodies are removed from the extracellular space byphagocytic cells and therefore do not cause any process of response to cell damage orinflammation [15].

Elimination of apoptotic bodies

Death by apoptosis is effective if the cell debris (apoptotic bodies) are removed through a complex and highly regulated process. Cleaning this cell debris can be performed by "professional"macrophages or by neighboring cells, "semi-professional" phagocytes which express primitive phagocytic potential [14]. In C. elegans multiple gene products (nuc-1, ced-1, -2, -5, -6, -7, -10 and -12) hav been described in the clearance of apoptotic cells. Since the worm lacks specialized phagocytes, this function is performed by the neighboring cells [7, 16, 17]. It is proposed that if apoptosis occurs in tissues with a low rate of apoptosis, phagocytosis may be provided by neighboring cells, but in those tissues with a high rate of apoptosis phagocytic cells, usually macrophages, perform this function.

How are apoptotic bodies recognized to proceed to their elimination? Phagocytic cells recognizeand discriminate clearly between an apoptotic cell and a viable cell. The specific and effectiveelimination is based on the existence of systems of recognition (receptors) located on the plasma membrane of phagocytes and in the signals called "eat me" that apoptotic cells carry, molecules indicating their status [15]. In the early stages of apoptosis the cell that has been preparing to die releases a number of factors or signals ("find me") that facilitate the recruitment of phagocytes.Among these signals, in order to locate the apoptotic cell, is the lysophosphatidylcholine, a lipid that is secreted, although phagocyte receptor type that recognizes is unknown [18]

A recognition mechanism is based on one of the early events occurring in the apoptotic cell,which serves particularly as a marker of apoptotic bodies, and which is related to the asymmetryof the plasma membrane. The distribution of phospholipids in the plasma membrane lipid bilayercomposition and precise orientation of phospholipids is asymmetrical, although a dynamic orphospholipids exchange between both sides exists. The maintenance of asymmetry involves several enzymatic activities. The exteriorization of phosphatidylserine (a phospholipid normallyconfined to the inner face of the plasma membrane) is the result of a balance is regulated byintracellular calcium levels, including the activity of the translocase and the aminophospholipid scramblase . In apoptotic cells is translocase inhibited and aminophospholipid scramblase is activated, producing a loss of plasma membrane asymmetry. This causes the display on the outer face of the membrane of phosphatidylserine molecules associated in patches: one of the signals"eat me". Phosphatidylserine is recognized by a specialized receptor of macrophages.

The recognition of apoptotic bodies also been associated with other changes that are produced in apoptotic cells. One of them affects carbohydrated proteins and lipids on thecell surface. This is described the loss of terminal sialic acid residues exposing normally masked regions; in this case, they can be recognized by lectins on the surface of macrophages. Also, other elements to function as candidates for signals are sites that can bind to the molecules of the extracellular fluid (such as the C1q component of complement or thrombospondin, among other molecules) which, upon binding to phosphatidylserine possibly would mark the apoptotic cell. Finally, in the phagocyte, a repertoire of receptors recognizes the set of signals. In additon to lectins, some of the subfamilies β1 integrins, β2, β3 and β5, cleaning receptors (scavenger), the C1q receptor and CD14 receptor, seem to be involved in the recognition of apoptotic bodies [15.18].

It is noteworthy that Santiago Ramon y Cajal, in 1911 describes the reactions of early degenerative Purkinje cells after trauma. Referring to necrotic cells he indicated: "Trauma rapidly mortifies Purkinje cells near the wound. At twelve hours after the operation, often at the edges of the lesion sets of six, eight or

more corpuscles of this kind are missing. And the curiosity is that it is impossible to recognize even their relics. An active movement of autolysis and reabsorption seems to have used up the remains of protoplasm "[19]. Reading this paragraph with a current perspective relates the Ramón y Cajal observation with the clean disposal of the remains of an apoptotic cell.

APOPTOSIS: SOME HISTORICAL BACKGROUND

Studies of programmed cell death are recent and it was in the mid-twentieth century that most attention was directed to this process. Several key issues have been resolved that now make many research groups interested in this topic. The historical background also includes previousevents, such as observation of Robert Hooke in 1665 that cork and other plant materials consist of small cavities or polyhedral cells. //He coined the term cell, although in fact it was the nichefound the bodies of the cells had died physiologically. In this context it should also be notedstudies in the nineteenth century by, among
others, Johannes Evangelista Purkinje, JohannesMüller, or Theodor Schwann, Matthias Schleiden and Rudolf Virchow, th allowed to build in the first half of that century theory cell. The development of the concept of death has earlier roots, from Hippocrates (470-377 a. C), which recounts the destruction of tissues in gangrene, thecentury in which Galen (129-201) begins to develop the concept
of necrosis and R. Virchowdefines the concept in 1858. The latter describes two types of cell death, necrobiosis (ambiguous term referring to natural death as opposed to the violent death or mortification) and necrosis(tissue degeneration, gangrene synonymous).

TABLE II. Programmed cell death, a recent history

XIX CENTURY	
1842	Carl Vogt, studying the evolution of the notochord and development of cartilage during the metamorphosis of amphibia observes that cells are "resorbed", for which he postulates that a physiological mechanism must exist [20]
1864	August Weismann describes the massive cell death in insect pupae, introduces the term "histolysis" and defines the cytological aspects of dying cells [21].
1872	Ludwig Stieda observes the death of chondrocytes in endochondral ossification: the cartilage is replaced by tissues that the osteoblasts form, that are not derived from chondrocytes, and that the cartilage cells die [22].
1883	Elie Metschnikoff (Ilya Ilych Mechnikov) who, along with Paul Ehrlich, received the Nobel Prize for Physiology and Medicine in 1908 in recognition of their work on immunity, analyzes cell death by histolysis in muscles during the metamorphosis of amphibians, which is caused by the accumulation of phagocytes in regressing muscles [23].
1885	Walther Flemming (creator of the terms chromatin and mitosis) working with ovarian follicle of rabbits, establishes that cell death (which he called "chromatolysis") is not produced by a mechanical rupture; rather a physiological lysis [2]
1886	Franz Nissen, utilizing Flemming's fixation solution and the nuclear stain hematoxylin, documents the chromatolysis occurring in the lactating mammary

	glands of dogs, rabbits, and cats [25].
1889	W. Felix Analyzes the death of myocytes and myofibers in muscles of mammals. He observes that the morphology of innervated muscle fibers is different from those that lack innervation, and that the latter die. He interprets the results as indicating a “proportionate action” and an “energy to create” that favors survival [26]
1889	John Beard observes the programmed loss of a population of sensory neurons in fish embryos. The cells shrink, become more glassy, lose their nuclei and nucleoli, and disappear [27].
1890	G. Arnheim describes the margination of chromatin [28]
	XX CENTURY
1906	R. Collin analyzes fate of motor and sensory neurons in chick embryos. He describes morphology of the neurons that die.[29 }
1910	Friedrich D. von Recklinghausen proposes the term “oncosis” for the cell death that is produced by ischemia or toxic agents, characterized by a swelling of the plasma membrane
191?	Viktor Hamburger proposes that “agents” (which were later designated as NGF (*Nerve Growth Factor*) control the development of neural centers [ganglia] and can quantitatively regulate the number of neurons [32].
194?9	Viktor Hamburger and Rita Levi-Montalcini who, along with Stanley Cohen received the Nobel Prize in Physiology and Medicine in 1986 for the discovery of growth factors, observe that cell death of neurons is a phenomenon that takes place during embryonic development, including the central nervous system [33].
1951	Alfred Glucksmann reviews cell death during ontogeny in vertebrates and establishes that it is a normal component of animal development [3].
1959	Christian de Duve, who along with Albert Claude and George E. Palade received the Nobel Prize in Physiology and Medicine in 1974 for their discoveries relating to the functional organization of the structure of cells, suggests that cells can die from within by bursting of lysosomes that act as suicide bags. {35].
1961	Ruth Bellairs determines the ultrastructural characteristics of the process of cell death during development of embryonic chicks [36].
1965	Richard A. Lockshin and Carroll M. Williams introduce the concept of programmed cell death by describing cell death observed during the process of the metamorphosis of insects (37].
1966	John W. Saunders suggests that during embryogenesis death is “suicide, not a murder” [38].

1966	Jamshed R. Tata observe that inhibitors of the synthesis of RNA and proteins block death during amphibian metamorphosis, indicating that it is an active process [39].
1972	John F. Kerr, Andrew H. Wyllie and Alistair R. Currie coin the term “apoptosis” to differentiate this cellular death from necrosis, as a biological phenomenon with major implications on tissue kinetics [3].
1973	Jorn-Uwe Schweichel and Hans-Joachim Merker define three types of physiological cell death (heterophagy, autophagy and non-lysosomal death) based on morphological studies of vertebrate development [6].
1976	Miroslav Skalka, Jitka Matyasova and Milena Cejkova describe obtaining regular fragments of DNA from the chromatin of irradiated tissue [40].
1979	Emmanuel Farber and Murray M. Fisher identify another cellular suicide mechanism in hepatic cells induced by the intracellular liberation of free radicals [41].
1984	Andrew H. Wyllie and colleagues analyze the rupture of chromatin in apoptosis associated with chromatin condensation and dependent on synthesis of macromolecules [42].
	The nematode Caenorhabditis elegans and programmed cell death.
1974	Sydney Brenner publishes his first article on the new experimental model of the nematode Caenorhabditis elegans [43] and, with John E.Sulston, another referring to the DNA of this worm [44]. These studies were followed by work related to different cell lineages and mutants. Molecular studies are initiated on the process of programmed cell death during development of C. elegans.
1976	John E. Sulston describes the first death gene, nuc-1 (abnormal nuclease) which controls the activity of a DNA endonuclease; the mutants are defective in the degradation of DNA in dying cells [45).
1982	H. Robert Horvitz, Hilary M. Ellis And Paul W. Steinberg, in their work on programmed cell death nematode development, describe the genetic pathway involved in cell death [46].
1983	Edward M. Hedgecock, John E. Su1ston and J. Nichal Thomson establish for the first time mutants of C. Elegans(Ced-1, ced-2; abnormal cell death) in which the process of cell death is altered. These genes are necessary for the elimination by phagocytosis of cell bodies resulting from cell death (17).
1986	Hilary M. And H. Ellis Robert Horvitz describe mutants ced-3 and ced-4, the first assassin genes required for programmed death, the loss or the reduction of function of which changes the fate of the cells; those that would normally die survive [47].
1992	Michael 0. Hengartner, Ron E. Ellis and H. Robert Horvitz identify the ced-9 gene

	whose product has effects opposite those of ced-3 and ced-4. The function of ced-9 gene is to negatively regulate those activities required for programmed cell death. Mutations that activate ced-9 prevent cell death mutations that inactivate it cause cell death in cells that normally live, with lethal results [48].

Programmed cell death, a recent history	
1992	David L Vaux, Irving L. Weissman and Stuart K Kim show that the human protein Bcl-2 can inhibit programmed cell death in *C. elegans* [4].
1993	Junying Yuan and collaborators describe that the transcription of ced-3 is more abundant during embryogeneis, the stage during which most programmed cell death occurs. The protein CED-3 resembles the human protein ICE (*interleukin-1-beta-converting enzyme)* [49].
1994	Michael O. Hengartner and H. Robert Horvitz publish the sequence of the protein CED-9 and its homology with Bcl-2; they suggest that the molecular mechanisms of programmed cell death have been conserved from nematodes to mammals [5].
1998	Luis del Peso, Victor M González and Gabriel Nuñez describe the regulatory protein EGL-1, which binds to and inhibits CED-9; the protective effect of CED-9 is antagonized by EGL-1. This latter blocks the interaction of CED-9 with CED-4, producing the activation of CED-3 [50].
2002	Sydney Brenner, H. Robert Horvitz and John E. Sulston receive the Nobel Prize for Physiology and Medicine for their discoveries on “the genetic regulation of development of organs and programmed cell death”.

A summary of some key studies on knowledge of programmed cell death is shown in Table II [20-50] and can be extended in several literature reviews [51-54]. Given the copiousness of publications, we have selected a limited number of initial milestones (XIX century) and some of the twentieth century, focusing especially between 1950 and the end of the nineties. As a special mention are some of the work done in *Caenorhabditis elegans* on programmed cell death, as they led to the Nobel Prize in Physiology or Medicine to Sydney Brenner, H. Robert Horvitz and John E. Sulston for their discoveries concerning “genetic regulation of organ development and programmed cell death” (Figure 3).

The first data, the prelude to the death of cells as a critical physiological process, are obtained in the mid-nineteenth century by studies on the development of organisms. One of the pioneering studies was done by Carl Vogt [20], interested in the metamorphosis of amphibians, he maintained that the death of the notochord cells was followed by the formation of new cartilage by new cells. Although not using the term cell death, he refers to the “absorption”, destruction or disappearance of cells. At this time, in which people analyzed the regression of tissues in the metamorphosis of amphibians and insects, the use of the first and primitive microscopes and microtomes as well as developing methods of tissue fixation and different histological methods permitted them to see what happens in individual cells. Thus, detailed descriptions of dead cells made by Walther Flemming [24] are obtained by a fixative preparations made with his invention, much used later, which comprised acetic acid, chromium oxide and osmium tetroxide . W. Flemming uses the term “chromatolysis” to refer to cell death, a process that would coincide with what is now defined as apoptosis.

Figure 3. Winners of the Nobel Prize for Medicine and Physiology in 2002.

In the twentieth century, adding to several important points in the previous work can be done byhighlighting the Alfred Glücksmann [34] regarding the ontogeny in vertebrates, establishing that cell death is a normal component of animal development. In 1964 Richard A. Lockshin and CarrollWilliams introduced the concept of **programmed cell death** by [37]. They, at a time that research on the topic was scarce, reported that during the development of an organism cell death occurs at certain times and places through a number of scheduled events. John W. Saunders [38], a year later, in relation to the death in embryonic systems indicates: ".. an abundant cell death, often with a cataclysmic force, is part of early development in many animals, is the usual method to removeorgans and tissues that are functional only during embryonic or larval life….. "and concludes that during embryogenesis, death is a" suicide, not murder ".

The term apoptosis, from the Greek for leaves falling from the trees or the petals from flowers, is coined in 1972 by John F. Kerr, Andrew H. Alistair Wyllie and R. Currie [3] to differentiate death that occurs naturally or physiologically during development and pathological death by necrosiscaused by acute injury. From morphological evidence obtained by electron microscopy of liver tissue exposed to toxins or lymphocytes lymphocytes treated with hormones, the differenceswere established between two types of cell death. They also describe the structural changescharacteristic of apoptosis in cells that will die in particular tissues to maintain the balancebetween cell proliferation and death. They propose that death by apoptosis responds tointracellular death program that can be activated or inhibited by both physiological andpathological stimuli.

The first component that described the molecular mechanism of cell death in mammals was Bcl-2 protein [4], but the first evidence for the existence of a genetic program underlying physiological cell death was obtained from the studies on the development of the nematode Caenorhabditis elegans [46,47]. Selected experimental models in various areas of research are very useful if applicable or generalizable to other systems and if they provide a wealth of information quickly. One of the features that they should have is to be easily propagated in the laboratory and can be manipulated genetically to elucidate the range of genes involved in the process selected for the study. Among these models are bacteria, used in many fields, among other reasons, for their rapid growth and variety of regulatory mechanisms in response to different experimental conditions, the small size of their genomes, and the ease of obtaining mutants. Unicellular yeasts have the additional advantage that their cellular organization, subcellular organelles, and nucleus are similar to those of the eukaryotic cell and therefore their metabolic functions and and compartmentalized processes. Other experimental models, although more complex, have also been successfully used in many research areas, such as the plant Arabidopsis thaliana, Drosophila melanogaster or fruit fly, zebrafish or mice as well as propagation systems in vitro such as numerous cell lines.

The "worm" *C. elegans* has been an excellent experimental model and, in relation to apoptosis, made possible the identification of the mechanisms that regulate it. Furthermore, it should be noted that homology exists not only between genes involved in apoptosis in C. elegans and higher organisms, but also among others involved in various processes, making it possible to extrapolate the data obtained in the worm to other systems. C. elegans is a transparent worm (which allows scientists to follow the fate of each cell individually), about 1 mm long, with a smooth skin and long body, with a life cycle of 3 ½ days. Different lineages can easily be obtained, and it has a genome consisting of 17,800 genes that have been sequenced. It Is maintained and propagated in the laboratory on agar plates with bacteria, from which it feeds. It was selected as a new experimental model in the 60s by Sydney Brenner to study how genes direct cell division, differentiation and development of the nervous system, studies that led to the analysis of cell fate. In fact, the observation that to generate an adult worm always 1090 cells are produced by successive divisions, , but the adult worm has 959 cells, opened the door to research on the genetic basis

of programmed cell death. The 131 cells produced in excess (12% of total cells) are removed during development of a controlled-form.

Figure 4. Apoptotic pathway of programmed cell death in C. elegans and vertebrates. In programmed cell death, wherein the genes are conserved throughout evolution, are required: a) proteins that regulate (stimulate or suppress) apoptosis, b) adapter proteins that interact with regulators and effectors c) the effector proteins (cysteine proteases), the activation of which leads to degradation of intracellular substrates leading to cell death. All proteins described in C. elegans implicated in the execution of apoptosis have multiple homologs in mammals, some of which are shown in the figure.

Today C. elegans remains a widely used experimental model' from the 60s to now over 13,000 scientific articles have been published using this model. John E. Sulston joined the group of S. Brenner in 1969 and focused on the study of the control of the cell lines and cell suicide, showing in the 1970's that iC. elegans follows rigid guidelines. In the same vein, knowledge of the molecular and genetic basis of the apoptotic process began in 1982 with studies in the group of H. Robert Horvitz [46], which describes genes involved in the control and execution of apoptosis in C. elegans showing that they basically are the same in all animals [55]. A collection of studies are included in the acceptance speech of the Nobel Prize to H. R. Horvitz titled "Worms, life and death" (December, 2002).

In 1986, the Horvitz group started the study of genes which appear altered in mutants; thesegenes were named ced (cell death abnormal). They identified the genes ced-3 and ced-4, which activated the process of cell death, and gene ced-9, which inhibited it. Therefore, these genes are responsible for whether a cell lives or dies and constitute the execution complex (Figure 4). If a cell expresses the three genes it survived, but if it does not express the death inhibitor gene ced-9, it commits suicide by apoptosis. Knowledge about the behavior of these genes has allowed us to establish the "central dogma of apoptosis". The protein EGL-1 (which contains only BH3domain) is induced in cells that are destined to s die and interacts with the regulatory proteinCED-9. This interaction displaces adaptor protein CED-4, a protease activating factor, which promotes the activation of CED-3. The latter has protease activity and is one of the effectors andexecutors of apoptosis, which degrade their target substrates, dismantling the complex structureand triggering cell death. In vertebrates a similar route is described, by molecules homologous tothose described in C. elegans (Figure 4). However, the picture is more complex, the number ofregulatory and effector molecules is higher and, furthermore, additional regulatory mechanisms exist [56].

Figure 5. Evolution of the number of publications on apoptosis and molecules involved in this process. The graph represents the annual change in the number of publications that refer to the terms "apoptosis", "Bcl-2" and "caspase". The database server used was PubMed of the U.S. National Library of Medicine and the National Institutes of Health (http://www.ncbi.nlm.nih.gov/pubmed/). It also indicates the year in which each term is first cited and groups the total number of publications over several years.

Another of the problems, which J. E. Sulston addressed refers to the fate of the corpses of dead cells, which also explains why no apoptosis was detected before despite the high number of cellsthat die daily in an organism. The cells kill themselves discreetly and their remains are removedcleanly without leaving traces of death, confirming that the process of clearance of apoptotic cellsis very effective. J. E. Sulston characterized other mutants *ced* in which the cells die, but there was an accumulation of corpses. From that moment he began to identify genes **involved** in this process.

The ability of Bcl-2, homologous to the anti-apoptotic gene ced-9, to prevent programmed cell death in *C. elegans* [4.5] and the existence of equivalent other genes that control apoptosis inmammals and *C. elegans* [49], shows that this process is highly conserved throughout evolution, and that it is basic and universal. All cells express the molecular

components that enable themmake it possible to “suicide” in response to signals from the environment of the cell or from within its interior.

From the beginning of the 1990s, research on the process of apoptosis has increased year by year in a dizzying and fascinating way. As seen in Figure 5, the apoptosis publications haveincreased exponentially; in the seven years of this century count more than 100,000 scientific articles. Those that refer to the protein Bcl-2 or its family members, as well as caspase, continuously accrue ever more numbers. The percentage of publications on apoptosis, compared to the total scientific articles in the area of health, now exceeds 2%.
Figure 6. Pathologies associated with alterations in the process of apoptosis. The table lists some of the pathologies in which there is direct correlation with changes in the balance between the processes of apoptosis and cell proliferation, either increase or inhibition of apoptosis.

Besides its importance as biological phenomenon, apoptosis has been associated with several diseases in which there is an imbalance between the balance of proliferation and cell death (Figure 6). Thus, the pathogenesis of various human diseases revealed that the apoptotic component may contribute to disease progression and even account for it. An excess of apoptosis-associated pathologies causes cell loss (degenerative), while a decrease causes an uncontrolled increase in cell number, modifying the cell turnover. Furthermore, alterations in the removal of apoptotic bodies can also contribute to an imbalance in cellular homeostasis [57]. For example, in neurodegenerative chronic diseases such as Alzheimer’s and Parkinson’s, cell death via an endogenous pathway suicide has been described [58]. Another aspect to consider is the response of different tumors to different therapies such as hormonal therapies, chemotherapy, radiotherapy or treatment with biologically active agents, which depend, at least in part on the ability to trigger apoptosis or other cell death. The acquisition of resistance of tumor cells not responding to therapy may reside in a deregulation of some stages of apoptosis [59]. It is desirable, and seems a promising future, that the basic knowledge of apoptosis and the pathways governing its implementation and removal of apoptotic bodies, can be applied to the design of drugs that selectively activate or inhibit cell death. On the website of the Spanish network of apoptosis (http://apored.bq.uam.es) can be found useful information on different lines of research related to apoptosis.

Molecules involved in the apoptotic process: intracellular effectors

Several families of molecules are involved in programmed cell death. The identification date of the first few molecules of these founder families or any key protein in this process, is collected in Table III [60-75]. The acronyms by which some of the molecules involved in apoptosis are recognized, are listed in Table IV.

Table III

TABLE III. Identification of some molecules implicated in programmed cell death	
1988	David L. Vaux, Suzane Cory and Jerry M. Adams describe the antiapoptotic and tumorigenic role of the human protein produced by the gene Gcl-2 [60].
1992	Two independent groups (from pharmaceutical companies) identify in human cells, and purify the protein ICE (*Interleukin-1-beta-converting enzyme*), a cysteinyl protease [61,62].
1989/1992	Several groups [63,64] obtain monoclonal antibodies that induce apoptosis, leading to the identification and cloning of the death receptor Fas/APO-1/CD95 [65].

1992	The observation that the exposure of phosphatidylserine on the surface of apoptotic cells could be a marker of apoptosis allows us to understand how dead cells are recognized before being phagocytosed [66].
1993	Junying Yuan and collaborators identify ICE as a protein equivalent to CED-3 [49].
1991/1994	A role is proposed for the protein p53 in the induction of apoptosis [67], from which later comes the argument that p53 causes apoptosis by means of a mechanism which can be blocked by Bcl-2 [68].
1993	The group of Shigekazu Nagata identifies the primary ligands of the receptor Fas [69,70].
1996	The term **caspase** is coined to group the family of proteases that are activated during apoptosis [73].
1996/1997	The relationship between the liberation of cytochrome c from the mitochondria and the process of apoptosis through the activation of certain caspases is recognized [74].
1997	Hou Zou and collaborators identify a protein similar to CED-4 and name it Apaf-1 (*Apoptotic protease activating factor*) [75].

TABLE IV: Relation of acronyms, definition and function in relation to apoptosis.

AIF	Apoptosis Inducing Factor	Mitochondrial oxidoreductase that induces apoptosis. Translocates to nucleus and activates deoxyribonuclease
ANT	Adenine Nucleotide Translocase	Transporter of adenine nucleotides through the permeability transition pore of mitochondria
Apaf-1	Apoptotic Protease Activating Factor	Cytosolic protein that interacts with cytochrome C, ATP, and procaspase-9 forming the complete apoptosome that activates executioner caspases
ATG	AuTophagy-related Gene	Genes implicated in survival and death of cells by autophagy
ATM	Ataxia Telangiectasia Mutated	Protein kinase that is activated in response to DNA damage; it is one of the kinases that phosphorylates p53.
Bcl-2	B-Cell Lymphoma-2	Antiapoptotic protein. Generic term that designates the family of proteins whose members can block or activate apoptosis,

		that regulate cell death by the intrinsic, mitochondrial route
BH	Bcl-2 Homology	Homologous regions of proteins of the Bcl-2 family
CARD	Caspase Recruitment Domains	Domain that can recruit caspases located in their prodomains and responsible for establishing interactions with other proteins that contain the same domain.
CARP	Cell cycle Apoptosis Regulatory Protein	Protein that inhibits caspases by blocking the death receptor pathway
Caspase	Cysteine-dependent ASPartate directed proteases	Term that groups the members of the ICE/CED3 family implicated in the effector and executioner phases of apoptosis
Ced	Cell Death abnormal	Genes implicated in the regulation of programmed cell death in *C. elegans.*
CrmA	Cytokine Response Modifier A	Inhibitor of caspases, of viral origin, which binds to the catalytic site of caspases
DD	Death Domain	Death domain that death receptors from the intrinsic pathway contain. Region responsible for the interaction with the same domains of adaptor proteins
DED	Death Effector Domain	Effector death domain localized in the prodomain of caspases; implicated in the interaction with proteins that contain the same domains.
DISC	Death Inducing Signaling Complex	Death signaling complex mediated by death receptors formed by FADD protein and caspases 8 and 10.
DNA-PK	DNA-Protein Kinase	Protein kinase dependent on DNA activated in response to damage to DNA, and is one of the proteins destroyed by caspases
FADD	Fas Associated Death Domain	Adaptor protein that interacts by means of its death domain with receptors for the extrinsic pathway. Possesses a death domain capable of interacting with caspases 8 and 10
FLIP	Flice-like Inhibitory Protein	Protein that contains a death effector domain that interacts with caspases, regulating their

		activity. Blocks apoptosis mediated by death receptors
IAP	Inhibitor of Apoptosis Protein	Member of mammalian protein family that inhibits caspases
ICAD/DFF45	Inhibitor of Caspase Activated DNase/DNA Fragmentation Factor 45	Protein that maintains the inhibition of the deoxnuclease responsible for the degradation of DNA during apoptosis. One of the proteins damaged by caspases
ICE	Inhibitor of Converting Enzyme	First cysteine protease described as implicated in apoptosis. Later renamed caspase-1; implicated in inflammation
MAPK	Mitogen Activated Protein Kinases	Family of protein kinases that phosphorylate serine/threonine residues and which respond to extracellular stimuli. Regulate various process such as proliferation, survival, and apoptosis
Mdm-2	Murine double minute-1	Protein with ubiquitin ligase activity; one of those that control the degradation of the protein p53. Interacts with p53, blocking its transcriptional activity; is a negative regulator
Omi/Htr A2	Oocyte Maturation Inhibitor/High Temperature Requirement protein A2	Mitochondrial protein that is released into cytoplasm and blocks protein inhibitors of caspases
PARP	PolyADP Ribose Polymerase	Poly ADP ribose polymerase, implicated in DNA repair; one of the proteins damaged by caspases
PI3K	Phosphatidyl Inositol 3 Kinase	Phosphoinositol 3 kinase, activated by a variety of growth factor receptors. The signaling pathway PI3K/Akt regulates cell proliferation, apoptosis, and contains transcriptional factors
RING	Really Interesting New Gene	Trivial name for structural domain or motif of Zinc Finger, $Cys_3HisCys_4$, which contains protein inhibitors of caspases. Implicated in the degradation by ubiquitinization of caspases
Smac/DIABLO	Second Mitochondria-derived Activator of Caspases/Direct IAP	Mitochondrial pro-apoptotic protein liberated into cytoplasm during apoptosis. Blocks endogenous caspase inhibitory proteins

	Binding protein with LOw isoelectric point	
ROS	Reactive Oxygen Species	Collective term for reactive oxygen species, including free radicals of oxygen (superoxide and hydroxyl radical) and a few non radical derivatives of molecular oxygen such as hydrogen peroxide. In mammalian cells they are produced as byproducts of normal metabolism and by enzyme systems attached to membranes such as the NADH oxidase complex, responding to exogenous stimuli
TNF	Tumor Necrosis Factor	Cytokine that participates in inflammation and is a ligand of one of the death receptors for the extrinsic pathway of apoptosis induction
TNFR	Tumor Necrosis Factor Receptor	Superfamily of the tumor necrosis factor receptors. Related to apoptosis, it is a cellular death receptor for signals from the extrinsic pathway
VDAC	Voltage Dependent Anion Channel	Component of the mitochondrial permeability transition pore, localized in the external membrane of the mitochondria, interacts with Bcl-2 family proteins.

Family of Bcl-2 protein

There are at least twenty members of this protein family in mammals [76,77]. The first to be described, and prototype structure that gives its name to this family, is the protein Bcl-2 (B-cell lymphoma-2) [60]. In *C. elegans*, a protein responsible for carrying out the program of apoptosis, CED-9, shows a very similar structural and functional homology with Bcl-2, preventing activation of the CED-4 protein CED-3 effector apoptosis (Figure 4).

Members of this family have opposing activities that mediate cell death and are also involved in other processes such as cellular response to tumorigenesis and tumor therapy. Proteins can be classified into subfamilies on the basis of structural and functional differences. Depending on how they affect the cell death process two types of proteins are described: anti-apoptotic, if they block apoptosis (for example Bcl-2, Bcl-XL, Bcl-w, Bcl-b, and Mcl-1), and pro-apoptotic (for example Bax and Bak), among which also are included those containing only a BH3 domain [1,78]. Figure 7 compares the structural domains of the proteins of the Bcl-2 family and are also included homologous proteins in C. elegans, CED-9 and EGL-1 in the corresponding groups,.

A common structural feature of members of this family is the presence of regions homologous to Bcl-2 called BH (Bcl-2 Homology; BH1-BH4). BH domains lacking enzymatic activity and are involved in the

processes of association of polypeptide chains; determine the ability of these proteins to interact between them to form homo-and heterodimers, or with other unrelated proteins. However, the effect exerted on the process of apoptosis appears not to correlate with the number and type of heterodimers that are members of this family, but with the free levels of Bcl-2 and Bax [79]. BH1 and BH2 domains are found in all these proteins and the BH3 domain sequence is different in pro-and anti-apoptotic proteins [77,80,81]. Moreover, BH4 is present exclusively in the sub-and anti-apoptotic family and is the domain responsible for interacting with megacanal components preventing mitochondrial alterations occurring in these organelles associated with apoptosis. In fact, caspases may act on the anti-apoptotic proteins, eliminating the BH4 domain structure.

Among the proapoptotic members are found proteins that have only the BH3 domain (Bad, Bid, Bik and Bim), some of which have a transmembrane domain (Figure 7). Except for the BH3 domain, they show no homology with Bcl-2 and form a subfamily or structurally diverse group. It has been described that, for regulating the onset of apoptosis, several of these proteins may be activated in different ways, as by proteolytic cleavage, phosphorylation and myristolation. These proteins can bind and inhibit the anti-apoptotic ones and therefore promote apoptosis. Ultimately, they act on mitochondria, inducing cytochrome c release of apoptogenic factors, eventually promoting apoptosis [78].

Bcl-2 has the four BH domains; it is an anti-apoptotic protein that is localized in the outer mitochondrial membrane and maintains the integrity of the mitochondria. Furthermore, exerts its activity interacting and thus inhibiting pro-apoptotic proteins. Furthermore, Bax is one of the more significant pro-apoptotic members of the family. It has the BH1, BH2 and BH3, but BH1 and BH2 are the ones that keep homology with Bcl-2. Bax is widely expressed in various tissues and its overexpression accelerates death in response to differing signals. The effects exerted by Bcl-2 preventing apoptosis and Bax promoting it are dependent on their interaction with membranes. Therefore, most of these proteins have carboxyl terminal transmembrane domains (except some BH3subfamily proteins such as Bid and Bad). This facilitates insertion into intracellular membranes (outer side of the mitochondrial membrane, the endoplasmic reticulum and the nuclear envelope). The structural similarity of Bcl-XL with the pore-forming domain of the toxin and diphtheria colicins A and E1 suggested that Bcl-XL and other members of the family may regulate apoptosis through pore formation [82]. In this regard, it has been reported that Bcl-2 can regulate the flow of protons to maintain the mitochondrial membrane potential in the presence of stimuli that elicit a decrease thereof [83]. Induction of apoptosis implies that cytoplasmic Bax protein migrates and joins mitochondrial membrane. Bax and Bak may act by interacting with mitochondrial megacanal components or may form an oligomeric channel leading to mitochondrial damage [84,85]. The formation of pores increases mitochondrial permeability and enables the release of apoptogenic factors [AIF (Apoptosis-Inducing Factor), cytochrome c, calcium, reactive oxygen species (ROS; Reactive Oxygen Species), etc.]. The mechanism mediating the effect of Bcl-2 probably derives from its location in the outer mitochondrial membrane where it binds to Bax preventing the formation of pores in the membrane and, therefore, the release of factors that trigger the apoptotic process [85,86].

Figure 7. Homologous regions of members of the Bcl-2 family and related proteins in *C. elegans*. The figure schematically representa proteins Bcl-2 family and their analogues in *C. elegans* CED-9 and EGL-1, grouped by subfamilies based on their anti-or pro-apoptotic activity. In addition, BH regions and transmembrane regions (TM) are indicated. In the Bcl-2 protein there exists a hydrophobic pocket (which encompasses BH3 regions, BH1 and BH2) responsible for interaction with Bcl-2 BH3 domains of pro-apoptotic proteins

The caspase family

The machinery for transmission of apoptotic signals is complex. You can start by different waysdepending on the stimulus received by the cell. After initiation of the signal, it is transmitted through various adapter proteins. However, all of the signals converge at the end in the activationof a family of cysteine

proteases, called caspases, which cut the target protein adjacent to a residue of aspartic acid, hence the name given (cysteine-dependent aspartate– directedproteases). The term caspase was introduced as a trivial name 1996 [73] to designate membersof the family of proteases ICE/CED3 and standardize nomenclature. Caspases are responsible, ultimately for executing the cell death process and activating other enzymes that degrade multipleproteins, thus dismantling the cellular architecture. The implication of these proteases in the processes of apoptosis is widely-documented [73, 77].

So far, in humans 11 members of this protein family are known [1]. The first described mammalianprotease was called ICE (Interleukin–1β–converting enzyme) or caspase-1. This is precisely one ofthe family members that has not been related to the process of apoptosis but with inflammation,as it is responsible for the breakdown and activation of interleukin 1β, a proinflammatory cytokine. Phylogenetic analysis reveals that there are two subfamilies of caspases [87]. The sub-family of caspase-1 (Caspases 1, 4, 5 and 11-14), which have been implicated in the control of inflammation, and the subfamily of caspase-3 (caspase 3 and 6-10), that specialize in triggeringand executing death by apoptosis. Caspase-2 is structurally similar to the subfamily of caspase-1 but its function is located in the subfamily of caspase-3.
Figure 8. Mechanism and inhibition of caspase activation. (A) The caspases are synthesized in the form of inactive proenzymes which generally require activation by proteolytic removal of theprodomain and separation of heavy (p20) and light (p10)subunits . This breakage always occursafter aspartic acid residues. After proteolytic digestion, the effector caspases (e.g. caspase-3) form an active heterotetramer composed of two heavy and two light subunits with two catalytic sites per tetramer. The effector caspase can be competitively inhibited by binding to the active sites of viral inhibitors (CrmA or p35), inhibitor of apoptosis proteins as XIAP, or inhibitorysynthetic peptides such as z-VAD-fmk. (B) Upon proteolytic activation, dimers of heavy and light chains are at conformational equilibrium and only one of the conformations is capable of formingactive tetramer. Some synthetic inhibitors, such as “compound 34", can exert allosteric inhibitionby binding to a cysteine residue at the interface, stabilizing the inactive conformation and preventing the formation of the tetramer.

The caspases are synthesized as zymogens (inactive proenzymes or pro-caspases). They are activated by limited proteolysis and association of subunits [88], acquiring their catalytic ability todegrade in turn their target substrates. In the structure of pro–caspases can be divided into threedomains: the amino terminal prodomain, large subunit (approximately 20 kDa) and small subunit(of 10 kDa). The length of the prodomain is highly variable and it is involved in regulation ofactivation. In this region is located a caspase recruitment domain (CARD, Caspase-Recruitment Domain) or death effector domain (DED; Death-Effector Domain), which typically facilitate interaction with proteins containing equivalent domains. Those caspases having a longprodomain appear to be involved in initiating the process of apoptosis and are called or activatoror initiator caspases (Caspases 2, 8, 9 and 10); they autoactivate and initiate proteolytic processing of other caspases). The prodomain of caspase-9 is involved in the formation of a multimeric complex called the apoptosome. A prodomain Caspases with a short prodomain are activated by initiator caspases to execute the apoptotic program and are known as effector caspases (Caspases 3, 6 and 7). These are, ultimately, the ones that degrade a wide range of substrates during apoptosis [87].

Caspases are activated once the various signaling pathways of apoptosis are initiated. The activation of each caspase requires a minimum of two proteolytic: one, which separate the prodomain from the rest of the molecule, and another which gives rise to large and small subunits (Figure 8A). These cuts, in which aspartic residues are involved occur in an orderly and are followed by the combination of two of each of

these subunits to form a tetrameric structure. This association may be homo-or heterodimeric, generating an enzyme with two active sites and absolute cutting specificity behind [C-terminal to] a residue of aspartic acid. This implies that these enzymes can be activated autocatalytically or as well by cascade of other enzymes with similar specificity. In healthy cells initiator caspases exist in monomeric form while the effectors appear as preformed dimers [77]. Furthermore, that some caspases can activated conformational changes has also been described.

Execution caspases and biochemistry

How do the caspases execute the program of apoptosis? Caspases, though are extremely selective as proteases, exert their proteolytic activity on a large number of substrates. In these they break the peptide bond behind a residue of aspartic acid, but in them they recognize different tetrapeptide motifs (-DEVD-,-LETD-,-LEHD-are recognized by caspases 3, 8 and 9, respectively), conferring a certain substrate specificity. In turn, the inactive caspase can be the substrate and be activated, triggering a cascade of caspases a manner that activates other in a hierarchical order. [89]

Proteolysis of many caspase substrates is responsible for numerous metabolic and structural changes that are triggered during apoptosis. They act on a large number of proteins, both cytoplasmic and nuclear, with activities and participation in such diverse cellular processes such as DNA repair and metabolism, protein phosphorylation, signal transduction, cell cycle regulation and proliferation. They are related to proteins responsible for human genetic diseases and proteins directly involved in apoptosis [86]. Among the targets of caspases we can highlight ICAD/DFF45 (Inhibitor of Caspase Activated DNase/DNA Fragmentation Factor 45; molecule that maintains inhibited the deoxinuclease that is activated by caspase and is responsible for degradation of DNA during apoptosis), the related DNA repair and replication processes and transcription of DNA (DNA-PK), poly-(ADP-ribose)-polymerase (PARP: poly-(ADP) Ribose Polymerase) and structural proteins such as nuclear laminar cytokeratin 18.

Caspase inhibitors

Due to the lethal effects caused by caspases it is not surprising that their activities are subject to strict controls exercised at various levels. Thus, besides the subcellular compartmentalization, transcriptional regulation of various agents may occur , such as interferon γ, that affect gene expression of different caspases [90]. Furthermore, we have described various types of posttranslational modifications (nitrosylation, phosphorylation, etc.) that can modulate the activity of these proteases. [87] In addition, there are specific mechanisms of regulation such as the action of caspase inhibitors [91].

The first inhibitors described are of viral origin (Figure 8A). The inhibitor CrmA (cytokine response modifier A), a gene product of cowpox virus, inhibit caspases 1 and 8 and human caspase-10. In this manner it prevents activation of apoptosis by blocking TNF receptor (Tumor Necrosis Factor; death receptor pathway). The second inhibitor, a baculovirus product that does not infect human cells is p35, which has a broad spectrum of action: inhibiting the caspases-1, 3, 6, 7, 8 and 10. A proposed mechanism of action is that the inhibitors CrmA, p35 and other synthetic molecules, act as pseudosubstrates and bind to the catalytic center of the target caspases, inhibiting their catalytic activity. By contrast, other inhibitors such as compound 34 (synthetic inhibitor), bind to an allosteric center of the dimeric structure inactivating the caspase (Figure 8B) [92]. Furthermore, a family of mammalian proteins called IAPs (Inhibitor of Apoptosis Protein) that are able to bind to and inactivate various caspases (3, 7 and 9) has been described. We know several members of this family in mammals (cIAP-1, cIAP-2, XIAP, ILP-2, and NAIP survivin), although except for XIAP1, others seem to inhibit caspases at unphysiological concentrations [93]. In addition to these direct inhibitors other molecules are described, such as proteins and CARP2 CARP1 (CARP: Cell-cycle Regulatory Apoptosis Protein), which bind to caspase 8 and 10 and inhibit signal via Death Receptor. For structural domains that the protein inhibitors contain a mechanism of action has been proposed that would prevent dimerization of caspases, procaspases or binding to procaspases, eg procaspase-9,

preventing their activation. Yet another mechanism which is based on one of the domains, called RING (Really Interesting New Gene; RING-finger structural motif of zinc finger) may recruit conjugated to ubiquitin ligase enzyme E2, which catalyzes the transfer of ubiquitin to various substrates, including Caspase-3. These targets would be marked for degradation by the proteasome [94]. To this repertoire of inhibitors, and looking forward towards therapeutic treatments, must be added the development of synthetic inhibitors of various kinds. There have been described, among others, peptides bound to different groups which increase their stability and efficiency that are based on the sequence of the substrates of caspases (e.g. z-VAD-fmk) and acting as pseudosubstrates (Figure 8A and B) [91].

SIGNALING PATHWAYS OF THE APOPTOTIC PROCESS

Apoptosis, as an energy-dependent active process, may be initiated by a variety of stimuli, intracellular or extracellular, the cells participating in their own death in an organized and efficient manner. A mixed set of molecules that can be overexpressed or activated, or may be repressed or inhibited, are involved in apoptosis. The stimulation by pro-apoptotic molecules or inhibition by anti-apoptotic factors depends on the type of cellular insult. We have described an redundancy associated with functional specialization of the molecules constituting the different families involved in the development of the apoptotic program. Multiplication of cell death regulators allows members of a family to be able to perform their functions according to their sub-cellular location or, for example, depending on the protein-protein interactions that can be established. This division of labor allows multi-cellular organisms to detect and respond differentially to distinct stimuli. Thus, in humans, death induced via death receptors and genotoxic or endoplasmic reticulum stress involve caspases 8, 4 and 2, respectively.

The apoptotic program takes place in several stages. In the first, or effector phase determination, the cell reacts to a certain stimulus condition or to its absence (developmental signals, cellular stress, cell cycle alteration, etc.) by deciding to begin the process of apoptosis. In the second execution or degradative stage, the cell suffers a number of molecular alterations that declench cell death. To these must be added the cleanup phase or elimination of apoptotic bodies. The different routes via that regulate apoptosis and the molecules implicated in its activation and execution are shown in Figure 9 [77,78].

Via death receptor

A first signaling path of the apoptotic process has its origin in the cell membrane through what is known as extrinsic or death receptor path. These receptors belong to the TNF receptor superfamily (TNFR) whose members share a cysteine-rich extracellular domain, a transmembrane domain and a cytoplasmic domain sequence in the receptor to couple with the remainder of the apoptotic machinery. These receptors are also involved in the control of other processes such as immune and inflammatory, bone homeostasis and the development and differentiation of epithelial structures.

The signal is initiated upon binding of the corresponding ligands (pro-apoptotic and pro-inflammatory cytokines such as FasL, TNF, etc.). to their respective death receptors (Fas, TNFR1, TRAILR1, etc.) [95.96]. Ligand binding causes homotrimerization of the receptor and, thus, the death receptor is able to recruit adapter proteins to the cell membrane. This process involves the homophilic interaction between domains of the DD death (Death Domain) receptor with those of the adapter molecules (proteins bridging between the receptor and caspase) protein such as FADD (Fas Associated Death Domain). The adapter molecules possess death effector domains DEDs capable of homophilic interaction with members of the caspase family causing their activation. Thus is formed a death signaling complex (DISC; Death-Inducing Signaling Complex) containing the protein FADD and caspases 8 or 10. Activation of procaspase-8 requires its association with the adapter molecule FADD through DED domains, placing caspase-8 in the apoptotic pathway mediated by death receptors [97,98]. Caspase-8 activates itself, thus directing the execution of apoptosis and which, in turn, activates executioner caspases. Furthermore, caspase-3 can delete a domain of the protein Bid amplifiying the signal that causes cell death and damage to mitochondria.

Furthermore, via the signaling pathway of the death receptors, the activation of caspases can be regulated by protein FLIP (FLICE-like Inhibitory Protein). It contains the DED domain, allowing binding tothe prodomain of procaspase-8 blocking the interaction of the caspase-receptor complex with adapter protein and interfering with the apoptotic process.

Figure 9. Signaling pathways leading to death by apoptosis. Depending on the type of apoptotic stimulus, different routes can be activated initiating the signal cascade of the apoptotic program. The extrinsic pathway of receptor-mediated death begins in the plasma membrane upon binding of different ligands (for example, FasL, TRAIL or TNF) to their death receptors (Fas, TNFR1 or TRAILR1/R2, respectively) . This causes the receiver to homotrimerize through adapter proteins (such as FADD), activating the pro-caspase-8. In the intrinsic or mitochondrial pathway the family of Bcl-2 regulates the release of calcium, ROS (reactive oxygen species) and apoptogenic proteins (cytochrome c, Apaf-1 and AIF) which can activate the pro-caspase 9. In turn, the expression of the different members of the Bcl-2 family may be regulated by pro-apoptotic stimuli, such as DNA damage. In this case, the damage induce, by means of the ATM kinase or DNA-PK, activation of p53 which, in turn, promotes transcription of pro-apoptotic genes of this family (e.g. Bax) and represses the anti-apoptotic factors such as as Bcl-2 itself. Activation of pro-caspases prodomain involves the proteolytic release of and separating the large (purple) and small (pink) subunits, with the subsequent association of two large and two small subunits leading to the active species with two catalytic centers (green). Initiator caspases activate executioner caspases, which are triggers of a variety of cellular processes that ultimately lead to apoptotic cell death. Other modes of intrinsic activation of the apoptotic process, such as that produced by the p53-dependent activation of caspase-2, which in turn induces the mitochondrial release of mitochondrial oxidoreductase AIF and endonuclease EndoG , which are move to the nucleus and promote nucleosomal degradation of chromatin. Endoplasmic reticulum stress is another intrinsic pathways of induction of apoptosis , in which alteration in the homeostasis of calcium; caspase-12is implicated in this pathway.

Mitochondrial pathway

The central mediator organelle of the second route is the mitochondria, which may also be a mediator of necrosis and autophagy. The importance of these organelles is manifest as defects in the electron transport chain that can induce the formation of ROS damage resulting in lipid peroxidation and damage to the membrane [99]. In 199the mitochondria were proposed as one of the first targets during apoptosis. In addition to previous studies point to the relationship between apoptosis and mitochondria, at that time the changes observed in the initial stages of apoptosis were described: the mitochondrial membrane potential decreases associated with an uncoupling of the electron transport chain and ATP synthesis, and translation is reduced [100]. The mitochondrial membrane permeability is controlled by a complex known as the Megacanal pore or mitochondrial permeability transition pore in mitochondria, which maintains the homeostasis of the mitochondrial matrix. The opening of this polyprotein channel leads to an increase in the inner membrane permeability allowing entry or exit of different molecules, a process that is strictly controlled. The minimum pore components described are the inner membrane protein ANT (Adenine Nucleotide Translocator), VDAC (Voltage-Dependent Anion Chanel) and outer membrane of cyclophilin D from the mitochondrial matrix. Some members of the Bcl-2 regulate the activity of channel proteins ANT and VDAC [99,101].

Many of apoptotic stimuli (cellular stress, drugs, radiation, oxidizing agents, etc.) utilize this second signaling pathway, known as the intrinsic or mitochondrial pathway which is regulated by proteins of the Bcl-2 (Figure 9) [102]. Mitochondrial damage and the release of mitochondrial proteins amplify the apoptotic signal in mammalian cells; changes in permeability of the mitochondrial membrane causes the release into the cytoplasm of more than 40 molecules implicated in apoptosis. Among those released from the mitochondria into the cytoplasm are various apoptogenic proteins as cytochrome c, apoptosis

inducing factor AIF, endonuclease G, and Smac / DIABLO (Smac: Second mitochondria-derived activator of caspases; DIABLO: Direct IAP binding protein with low isoelectric point) and the serine protease Omi / Htr A2 (Oocyte Maturation Inhibitor / High temperature requirement protein A2). It also generates a flow of released calcium and ROS.

The release of cytochrome c is a critical event as it interacts with the cytosolic protein Apaf-1 (Apoptotic protease activating factor-1). The latter acts as an adapter molecule in this pathway, with dATP and subsequently with pro-caspase-9 [79,80]. It is known as the heptomeric mega complex known as the apoptosome, which executes the apoptotic program [103]. The complex formation leads to a conformational change and activation of pro-caspase-9, which in turn cuts the s prodomain from the effector caspases, such as caspases 3, 6 and 7, activating them. The overall process requires energy and that the machinery of the cell is not too damaged, if the damage reaches certain levels, the cell that initiated the early stages of apoptosis can proceed via necrotic death.

Another protein that is released from the mitochondria is AIF (Figure 9), a mitochondrial oxidoreductase, that, unlike cytochrome c, once in the cytoplasm translocates to the nucleus to activate PARP-I. As a result the DNA fragments in a fashio independent of the activity of caspases. When the pro-apoptotic protein Smac / DIABLO is released into the cytoplasm, it interacts with endogenous caspase inhibitors (IAP) [104] by neutralizing them and, therefore, preventing their blockage of apoptosis.

Mitochondrial damage can originate by different mechanisms. Pro-apoptotic proteins such as Bax and Bak, can interact with VDAC [84]. Thus, it has been reported that apoptotic signals in the absence of this protein interact with the outer membrane Bax thus controlling lethal effects exerted by it. However, if a death signal is recieved, family members Bcl-2 and Bid or Bad displace VDAC. Bax and Bak are activated and the mitochondrial membrane becomes permeable, releasing the apoptotic mitochondrial proteins that activate this pathway. Furthermore, Bax ANT can cooperate with forming a lethal oligomeric channel in the mitochondrial membrane. Other mitochondrial damage may also be caused by induction of an influx of potassium or by an interaction with the caspase-2 (which happens independently of caspase activity).

The intrinsic pathway is strictly controlled by members of the Bcl-2 family, and mainly leads to activation of caspase-9. However, in some cell types (eg, hepatocytes), this route can operate in the absence of caspase-9 or its activator Apaf-1. In this case the extrinsic pathway is interlinked with a loop forming the intrinsic signal amplification mediated by death receptors. In this loop involving the pro-apoptotic protein Bid is processed into two fragments by caspase-8. One of them, the C-terminal one (tBid) acts on mitochondria leading to the release of cytochrome c. Therefore, Bid functions as a bridge between the two tracks amplifying the activation of caspases.

Other ways of inducing apoptosis

The mitochondria, which play a central role in the intrinsic pathway of apoptosis, are not the only organelle involved in sub-cellular cell death. Lysosomes and endoplasmic reticulum play an important role in the release of other factors death as cathepsins, calpains and other proteases. Thus, endoplasmic reticulum stress (Figure 9) is considered as one of the intrinsic pathways inducing apoptosis; it is characterized by disruption of calcium homeostasis and the accumulation of incorrectly folded proteins. In this case, it triggers a specific cascade of initiation of apoptosis by activation of caspase-12 located on the cytosolic side of the endoplasmic reticulum [105.106]. Active caspase-12 causes, in turn, the activation of caspase-9 and from this caspase-3, but independently of cytochrome c pathway so that it does not require prior activation of the mitochondrial pathway. Moreover, the release of calcium also induces activation of calpains (calcium-activated neutral proteases) which are normally in an inactive form as zymogens.

Another means of induction of the process of apoptosis begins when DNA damage is produced. Cellular responses to DNA damageare mediated by kinases, of which two include: ATM (Ataxia Telangiectasia Mutated) and DNA-dependent protein kinase (DNA-PK) (Figure 9). Both direct a series of responses, such as cell cycle arrest, to repair the DNA or, if the damage is excessive, to induce apoptosis. The transcription factor p53 is involved in this control; it regulates the transcription of various genes in response to a variety of stress signals (DNA damage, nutrient deficiency, hypoxia, telomere shortening, activation of oncogenes, etc.). [107]. When there is genotoxic stress (DNA damage), p53 controls processes such as DNA repair, cell cycle arrest, senescence and apoptosis. P53 degradation is controlled by interaction with several proteins such as ubiquitin ligase Mdm-2 (Murine double minute-2). The p53 protein is regulated by post-translational modifications (acetylation, phosphorylation, etc.). A range of kinases, including ATM, phosphorylate p53 on certain residues or inhibit ubiquitination by Mdm-2; other subsequent modifications such as acetylation by acetyl-transferases and methylation by methyl transferases stabilize p53 and increase its specific binding to DNA. All these processes increase the half-life of p53. DNA damage induced phosphorylation of p53 and MDM-2 inhibits the interaction of both proteins, which leads to an activation and stabilization of p53. This activation leads tetramer binding of p53 to p53 response elements of target genes and recruits various coactivators and transcription factors that, together, modulate the expression of genes controlled by p53. The expression of certain genes involved in different processes, such as cell cycle arrest by induction of the p21 gene (an inhibitor of cyclin-dependent protein kinase) in DNA repair (GADD45α gene), or involved in apoptosis (induced repression of Bax and Bcl-2), trigger mechanisms through which the DNA is repaired or the cell enters into apoptosis [108]. Moreover, considering apoptosis, in some systems a relationship between p53 protein and activation of caspase-2 in response to DNA damage is described that leads to cell death (Fig. 9) [109].

The survival and death signals can also be transmitted by signaling pathways in which different protein kinases are involved [110]. One pathway is the PI3K (Phosphatidylinositol-3-kinase) with consequent activation of protein kinase B (PKB or Akt). This may influence the sensitivity of the cells to respond to cell death-inducing signals by controlling molecules that regulate apoptosis (for example caspase-9 and Bad) or by regulating the activity of transcriptional factors [111]. The activation of Akt produces the direct phosphorylation of Bad, which remains bound to 14-3-3 protein. In contrast, non-phosphorylated Bad binds to Bcl-XL (or Bcl-2), preventing it from exerting its survival action [112]. Moreover, the path of the MAPK (Mitogen Activated Protein Kinases) is involved in the control of cell proliferation, differentiation and apoptosis. At the end point of the cascade, three kinases (JNK, ERK and p38) are able to respond to multiple stimuli; the activation of these leads to phosphorylation of several target proteins thereby controlling the enabled or disabled status of these proteins and thus their biological activity.

TECHNIQUES TO ASSESS THE PROCESS OF APOPTOSIS

There is a diverse set of techniques that allow assessing different aspects of the process of apoptosis, such as changes in the plasma membrane, DNA fragmentation, caspase activation or degradation of substrates [14].

The plasma membrane of cells dying is permeable to certain reagents (or fluorochrome dyes) and can be dyed with them. However, living cells exclude so-called “vital dyes,” that do not penetrate the plasma membrane. One of these molecules is propidium iodide (PI), which after binding to DNA greatly increases its fluorescence upon excitation with UV light; the PI stains only non-viable cells. However, this compound can be used to detect the amount of DNA in viable cells by flow cytometry if the cells are permeabilized, fixed with ethanol and suitably processed prior to staining. Thus, the pre-apoptotic cells normally present two peaks in flow cytometry, which correspond to the G0/G1 phases of the cell cycle and G2 / M; S phase cells are located between the two peaks (Figure 10A), whereas during apoptosis DNA fragmentation that occurs in apoptotic cells can be detected as a peak corresponding to hypodiploid DNA (Figure 10B).

In biochemical analysis, early detection of apoptosis is based on the assessment of changes in the location of phosphatidylserine (PS) in the plasma membrane since, as mentioned, this phospholipid is normally located on the inner side of the same. In apoptosis plasma membrane asymmetry is lost leading to exposure of PS on the outer side, this translocation preceding apoptotic events [66]. This process can be quantified by flow cytometry using conjugated annexin A5-fluorochromes, as this protein has a high affinity for this type of phospholipid. Annexin-A5 can also mark necrotic cells, but if used in conjunction with PI, one can distinguish between apoptotic and necrotic cells (Figure 11).

Figure 10. Determination of apoptosis of cells in culture by assessing hypoploidy. Determinationof apoptosis of cells in culture can be performed by flow cytometry after cell permeabilization andstaining the DNA with propidium iodide. In normal cells (A), this method can be used to determinethe percentage of cells in different cell cycle phases: G1 (diploid), G2 / M (tetraploid) and S (intermediate). In apoptotic cells (B), wherein the fragmented DNA is characterized by thepresence of a peak corresponding to hypodiploid cells.

Figure 11. Determination of apoptosis by assessment of exposure of phosphatidylserine. One of the features of apoptotic cells is the reversion of the asymmetry of the membrane with theconsequent exposure of phosphatidylserine on the outer face of the plasma membrane (without allowing it to produce a permeabilization as in necrosis). This exposure can be quantified by flowcytometry using FITC-labeled Annexin A5 (Annexin has high affinity for this phospholipid acid) inconjunction with propidium iodide. Thus, cells staining strongly for FITC–annexin A5 and little little for propidium iodide (lower right quadrant) would be in the early stages of apoptosis whereas those mind stained with both markers (right upper quadrant) have plasma membrane damage as a result of a process of necrosis, either primary or as a lethal consequence of the evolution of the process of apoptosis. The figure shows the large difference in the stainings effected on normal cells (A) or in apoptotic cells (B).

Chromatin condensation, and presence of apoptotic bodies can be assessed by fluorescence microscopy after staining of cells with fluorescent reagents that are incorporated by viable cells asacridine orange (NA), DAPI (4,6-diamidino -2-phenylindole) or Hoechst 33342 (Figure 12A). These reagents, used in conjunction with PI in vitro to distinguish necrotic cells (which will be positive for PI), and early apoptotic cells (positive for NA, PI negative) or late apoptosis (PI positive but with afragmented nucleus) . Late apoptosis is also known as secondary necrosis, and is detected invitro experiments with cultured cells as the disposal system for apoptotic bodies is not present.

Figure 12. Apoptotic bodies and degradation of chromosomal DNA [forming a] ladder. (A) In the micrograph obtained from apoptotic cells stained with DAPI nuclei can be seen with a normalaspect as well as apoptotic bodies or cell residues that have died by apoptosis. (B) The degradation of chromosomal DNA in apoptotic cells occurs preferentially at internucleosomalregions, which generates DNA fragments of approximately 200 base pairs or multiples thereof.For this reason, the electrophoretic analysis of DNA from apoptotic cells usually presents acharacteristic ladder striping which is very different from the random degradation seen in necrotic cells.

Nucleosomal fragmentation of genomic DNA [42] can be determined by various methods, some of which are noted below. Electrophoretic analysis of genomic DNA using electrophoresis gels consisting of 2% agarose allows the production of classic profiles “DNA ladder”, corresponding to the internucleosomal fragmentation by endonucleases produced at intervals of 180-200 bp (Figure 12B). The TUNEL assay (terminal transferase- deoxynucleotidyl -dUTP Nick End Labeling) is one *in situ* test based on specific binding of terminal deoxynucleotidyl transferase at the 3’OH ends of DNA fragment and the incorporation of labeled dUTP into the said end. Also, flow cytometry can analyze DNA fragmentation using antibodies

that detect internucleosomal ruptures, or ELISA (Enzyme-Linked Immunosorbent assay) to evaluate associated histone DNA fragments.

The measurement of caspase activation can be carried out by very different methods [113]. The most direct approach is to conduct activity assays with fluorescent substrates specific for each caspase. To complement these studies, we can determine whether there is activation of caspases by western blot by analyzing whether certain specific substrates are proteolytically processed (eg, PARP-1, topoisomerase I, cytokeratin-18 or laminins A and C). We can also assess whether caspases themselves have undergone the proteolytic activation process required for the formation of the subunits comprising the active enzyme. The detection of activation of several caspases can also be performed by immunofluorescence and flow cytometry using antibodies that recognize only the active conformation thereof. These antibodies, to be sensitive to conformation, cannot be used in western blots. Finally, we can also use markers that possess specific affinity for the active site of some caspases. These substrate analogs have an -inhibitor group and another group that allows detection (for example, biotin, fluorescein or 2,4 – dinitrophenol) either in cells (immunofluorescence or flow cytometry) or after transfer to membranes.

There are additional methods for detection of apoptosis to allow assessment of specific mediators in the plasma membrane (eg specific death receptors or ceramide), the mitochondrial dysfunction associated with a decrease in membrane potential (detectable by flow cytometry with specific fluorescent probes such as Rhodamine-123), mitochondrial membrane permeabilization with release of specific markers (such as cytochrome c, or mitochondrial protein Smac / DIABLO), or the detection of proteins or pro-or anti-apoptotic factors (such as different members of the Bcl-2, AIF, Apaf-1, heat shock proteins, etc.) using antibodies specific for immunohistochemistry or western blot.

TYPES OF PROGRAMMED CELL DEATH

Numerous experimental evidences show that apoptosis is not the only mechanism of cell suicide. Depending on cell type and stimulus, where the cells are ready to die these different death pathways may be selected . In Table V are collected various forms of these pathways, as have been classified by different authors, and we only comment on certain aspects of some of them.

Morphological studies on the development of vertebrates permitted in 1973 the definition three types of physiological cell death: heterophagy, autophagy and lysosomal death. These can be distinguished into three types depending on the location and role of lysosomes [6.7]. Heterophagy corresponds to what we now know as apoptosis. In a recent publication [1], in addition to apoptosis and programmed cell death autophagy, necroptosis and death mediated by PARP-1 are also considered.

Autophagy, the catalytic mechanism conserved throughout evolution and involved in tissue remodeling during development, is a route of cell degradation involved in the elimination of both subcellular organelles and protein, damaged or superfluous. This process is also used as a defense mechanism against viral and bacterial invasion. Autophagy as survival mechanism assures that cellular constituents are recycled, providing a source of alternative energy during periods of metabolic stress and participates in this way, in the maintenance of cell viability and homeostasis [8 Excessive degradation with signs of autophagy in dying cells has been observed, so autophagy (“self-eating”, from the Greek auto “act on itself” and phagos “eat”) is classified as an alternative form of programmed cell death. This occurs, for example, in some systems in nutrient deficient conditions so that groups of associated cells or tissues die. At the molecular level it has been proposed that there may be a response machinery with common paths between apoptosis and autophagy. Although the relationship between the two processes is complex, a cell, depending on the signals it receives, can die either of two ways or a combination thereof [10]. Moreover, autophagy could assume the role of suicidal path when a component of apoptosis fails. In fact, inhibition of apoptosis by blocking the activity of caspases leads to cell death by autophagy producing an accumulation of ROS due to degradation of the catalase, a lipid peroxidation and loss of plasma

membrane integrity [10]. Autophagy is characterized by the presence in the cytoplasm of double membrane vesicles that include cellular components, called autophagosomes, whose content is degraded by lysosomal enzymes once autophagosomes fuse with lysosomes. The molecular basis of this process is not known as exhaustively as apoptosis, but studies on the loss of gene function have allowed unlocking its secrets both as a mechanism of cell death and survival, and its relationship to illnesses (cancer , neurodegeneration, aging and immunity) [114,115].

One difference between apoptosis and autophagy lies in the implementation of these programs. Caspase activation and condensation and fragmentation of DNA are characteristic of apoptosis, while the autophagic passes through proteolysis associated with the ubiquitin pathway and DNA is not fragmented. [7] However, the differences are not as clear as, in some systems ubiquitin degradation is described as well as characteristics of apoptosis, and there are other similarities between the two processes, as the effect of caspase inhibitor p35 also blocks the autophagy. Although the molecular machinery is only partially elucidated, group of genes known as ATG (Autophagy-related) has been described, conserved from yeast to humans and involved in the survival and death. The product of one of these genes, the beclin-1, has a BH3 domain that interacts with the protein Bcl-2, which reflects a convergent regulation of apoptosis and autophagy [1].

Besides apoptosis and autophagy, another kind of death is the necroptosis, which progresses with the morphological characteristics of necrosis but on a programmed basis [1]. It has been described as occurring when caspases are blocked by inhibitors, or in the event that mutations have occurred in caspase genes or those of other proteins involved in apoptosis. It can be induced specifically through signals from the membrane by the binding of a ligand to TNFR death receptors, and is regulated by intracellular signaling mechanisms through a cascade of kinases. Among other processes, an adapter protein, RIP-1, is activated; this latter contains DD domains and has tyrosine kinase activity. This is translocated to the mitochondria interfering with ANT, mitochondrial Megacanal component, resulting in mitochondrial dysfunction, increased ROS production and activation of the JNK kinase. Although the order of the steps subsequent to the activation of RIP-1 is not well understood, various alterations have been described as an activation of phospholipase A2, lipoxygenases and acidic sphingomyelinase. Another type of programmed death is **mediated by PARP-1** [1] as part of the response to DNA damage produced by lack of nutrients or energy, or in response to pathogen infection. This pathway may develop as a complementary function or enhance apoptosis. PARP-1 participates in maintaining genomic stability and is activated when a break occurs in a strand of DNA. To repair this nick, this enzyme recruits factors responsible for DNA repair, joining ADP-ribose units to chromatin-associated proteins. Although the molecular mechanism is also not known too, if the repair fails, the end effects which lead to this type of cell death can be summarized in the activation of two paths: one produces a energetic collapse and the other via RIP and JNK, to mitochondrial dysfunction and release of AIF .

Depending on the involvement of caspases, other models of programmed cell death are described for those which, in any case, not much is known [116]. **Paraptosis** is characterized by the presence of vacuoles in the cytoplasm that begins with swelling of the mitochondria and endoplasmic reticulum. It is mediated by MAPK, by a member of the death receptors, and receptor for insulin type growth factor-1 and it is inhibited by molecules that do not inhibit apoptosis [117]. **Mitotic catastrophe** occurs by failure of mitosis, caused by a lack of control of the restriction point of the cell cycle. It is described as associated with a permeabilization of mitochondrial membrane and the involvement of caspases in this type of death is still debated.

Cell death programs play an essential role in the development and in adult life of an organism. The cell has a broad spectrum of possibilities to die, with a complex set of molecules involved collaborating in the process. Therefore, it is not surprising that often the concepts and nomenclature are not used correctly. To unify criteria, the Nomenclature Committee on Cell Death [118] has established a set of criteria to

define the different types of cell death (Table V), considering different mechanisms and morphologies, to be able to use correct and generalized terminology and generalized.

Table V

TABLE V: Types of cell death

References	Types of death
Degterev and Yuan [1] Classification as a function of regulation of process, of differences in induction of cell death, and in molecules implicated in its execution	Unregulated death: NECROSIS Regulated cell death: APOPTOSIS (Type I) AUTOPHAGY (Type II) NECROPTOSIS Death mediated by PARP-I
Bröker, Kruyt, and Giaccone [92] Classification according to the activation of caspases	Path dependent on caspases: APOPTOSIS Path independent of apoptosis NECROSIS AUTOPHAGY PARAPTOSIS MITOTIC CATASTROPHY SLOW DEATH
Bröker, Kruyt, and Giaccone [92] Types of cell death as a function of nuclear morphology	NECROSIS APOPTOSIS NECROTIC TYPE APOPTOTIC TYPE
Majno and Joris [51] Revision of evolution of concept of cell death	NECROSIS (accidental death) APOPTOSIS ONCOSIS
Kroemer and collaborators [118] Types of cell death based on mechanisms that lead to distinct morphologies	NECROSIS APOPTOSIS AUTOPHAGY MITOTIC CATASTROPHE ANOIKIS

	EXCITOTOXICITY
	WALLERIAN DEGENERATION
	CORNIFICATION

Bibliography