Roles of mitochondria in apoptosis

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in *Mitochondria and diseases: from basis to bench*.

ROLES OF MITOCHONDRIA IN APOPTOSIS

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1. Programmed cell death is a fundamental process during development

Programmed cell death appears as a very early event in the course of evolution allowing to limit the size of cellular populations and to eliminate some undesirable cells (Ellis et al., 1991). This process is fundamental for the development of multicellular organisms, in the course of which many embryonic cells are brought to die. Programmed cell death, as does cell proliferation, intervenes in determination of the size and the form of organs, as well as in functional maturation of some systems. During the development of members, cell proliferation and differentiation allow the appearance and the growth of member buds, while the morphogenesis of fingers and toes invokes the death of cells initially located in interdigital positions. During the development of the nervous system, neurons that do not reach their target are eliminated by a process of programmed cell death (Thompson, 1995). This death allows to establish functions of the nervous system by playing on its plasticity. The functional maturation of the immune system also involves massive programmed cell death. The clones of self-reactive T lymphocytes are eliminated by a process of programmed cell death. In other cases, structures whose physiological role is only transitory are eliminated, for example during the metamorphosis of amphibians (tail of tadpoles) and insects (intersegmental muscles).

2. Apoptosis is a type of programmed cell death whose control is altered in several pathologies

What is the mechanism of programmed cell death? How is it controlled? Different types of programmed cell death can be defined, according to morphological criteria (Clarke, 1990; Schwartz et al., 1993). Apoptosis, defined in 1972 (Kerr et al., 1972), is the most frequently described type of programmed cell death. Apoptosis is often opposed to necrosis, which is a cellular death that results generally from massive aggressions of the external medium. A dysfunction of apoptosis or of its

control can be implicated in various pathologies (Thompson, 1995). A defect in the cell death program could be responsible for some autoimmune diseases (Tan, 1994). It is also implicated in oncogenesis. Indeed, apoptosis plays a fundamental role in different steps of carcinogenesis (Williams, 1991; Bursch et al., 1992). For example, in the course of hepatocarcinogenesis, initiated cells have an apoptotic activity that compensates the intense proliferative activity of the other cells. It results in elimination of most of the initiated cells. Apoptosis can also be observed at other stages of tumor progression as well as in other types of cancers. The control of cell proliferation by mitogens and apoptosis is always active in neoplasic cells and even in some malignant tumors with however an imbalance between the two phenomena. Some oncogens and oncosuppressor genes are now known for their action on apoptosis. The p53 oncosuppressor induces apoptosis in several transformed cell lines (Yonish-Rouach et al., 1991; Shaw et al., 1992) and the *c-myc* gene also seems to be involved in this process (Evan et al., 1992; Shi et al., 1992). On the contrary, the *bcl-2* oncogene can block apoptosis in different situations and this capacity is probably involved in the genesis of lymphomas and many cancers (Hockenbery et al., 1990; Sentman et al., 1991; Strasser et al., 1991).

Others pathologies can be due on the contrary to an ectopic induction of apoptosis. Some neuro-degenerative diseases, during which a massive neuronal death is observed, could involve apoptosis. This could be the case for Alzheimer and Parkinson diseases and for the amyotrophic lateral sclerosis (Thompson, 1995). This same phenomenon could also be involved in the pathogenesis of AIDS where it would lead to the elimination of T lymphocytes and then to immunodeficiency (Gougeon and Montagnier, 1993; Martin, 1993; Ameisen et al., 1995).

3. Programmed cell death is genetically controlled

The genetic approach, with the nematode *Caenorhabditis elegans*, has allowed to show that condemned cells express genes that participate actively in apoptosis (*ced-3*, *ced-4*). If the coordinated expression of these genes is disturbed, cells do not die. A gene, *ced-9*, capable of blocking apoptosis has also been found. *Ced-9* is the homologue of *bcl-2* that belongs to a family of genes that inhibits or

stimulates apoptosis. *Ced-3* is the homologue of a family of genes of cystein proteases (caspases) to which belongs the gene *ICE* that codes for the enzyme of conversion of interleukin-1β (table 1). More recently an homologue of Ced-4, called Apaf-1 has also been identified (Zou et al., 1997). These observations underline that this process appears early during evolution. The mode of action of genes of the *bcl-2* family is poorly understood and the substrates of the caspases really implicated in apoptosis remain to be ascertained (Kumar, 1995; Guénal et al., 1997a). In mammals, many other genes have been described as having a negative or positive action on apoptosis. Some of these genes are known to be involved in the control of cellular proliferation, this being in agreement with the idea that death by apoptosis allows to compensate cellular multiplication and that it is involved in protection against cancers. Furthermore, many viral gene products are inhibitors of apoptosis that act as antagonists of cellular proteins implicated in apoptosis (table 2). This observation shows that these different viruses have developed, in the course of evolution, different strategies to avoid the death of the infected cell before the end of the viral cycle (Gillet and Brun, 1996).

4. The different ways of induction of apoptosis seem to converge to a common effector phase

Figure 1 presents a simplified model of events occurring during apoptosis. Three phases can be defined. The induction phase can proceed through various ways depending on the cellular type and the nature of the induction signal: default in survival signals, death signals (TGF-β, TNF-α), viral infection, contradictory signals for proliferation (example: presence of c-myc and absence of growth factor), damage in DNA. During apoptosis induced by CD95-like surface receptors, an activation of caspases is observed during the induction phase. At the exit of this induction phase, cells undergo a series of physiological modifications (effector phase) that is going to lead them irreversibly to death. Then, cells are committed to the degradation phase where they present morphological (condensation of the cell, the nucleus and the chromatin) and molecular (fragmentation of the DNA in oligonucleosomal fragments) characteristics of apoptosis. In some cases, the phagocytosis of

apoptotic cells occurs before these modifications become perceptible. Products of *ced-3*, *ced-4* and *ced-9* as well as of their mammalian homologues intervene during the effector phase. Several proteins involved in the phase of degradation (ubiquitin, transglutaminase, nuclease) have also been identified.

5. A drop of the mitochondrial membrane potential is a universal and early event of apoptosis

What is the place of mitochondria in apoptosis? The mitochondrial membrane potential ($\Delta\Psi_m$) can be estimated with fluorochromes that incorporate into mitochondria as a function of their potential, according to the Nernst equation (table 3). The commitment to apoptosis is accompanied by a drop of the $\Delta\Psi_m$ that occurs before the fragmentation of DNA in oligonucleosomal fragments. At least in the case of apoptosis of fibroblasts immortalized by a temperature sensitive mutant of SV40 LT antigen, where apoptosis is mediated by the p53 protein (Zheng et al., 1994), this drop of $\Delta\Psi_m$ is responsible for a defect of maturation of mitochondrial proteins synthesized in the cytoplasm (Mignotte et al., 1990), cessation of mitochondrial translation and an uncoupling of the oxidative phosphorylations (Vayssière et al., 1994). The drop of $\Delta\Psi_m$ is detectable whatever the apoptotic induction signal, physiological (absence of growth factor, glucocorticoids, TNF) or non-physiological (irradiation, chemotherapy) (Petit et al., 1995; Zamzami et al., 1995a; Zamzami et al., 1995b; Marchetti et al., 1996; Sidoti-de Fraisse et al., 1998). Therefore, it seems that the drop of $\Delta\Psi_m$, an event that can be slowed down by cyclosporine A, is a universal characteristic that accompanies apoptosis, independently of the induction signal and of the cellular type (Kroemer et al., 1995). These data show on the one hand that the nuclear fragmentation is a late event as compared to the drop of the $\Delta\Psi_m$ and, on the other hand, that this drop marks the point of no-return of a cell condemned to die. The measure of $\Delta\Psi_m$ allows identification of cells in "pre-apoptosis", including in circulating T lymphocytes from human immunodeficiency virus-1 (HIV-1) carriers (Macho et al., 1995).

A kinetic analysis of splenocyte, T cell hybridoma and pre-B cell apoptosis realized with the help of hydroethidine (table 3) has permitted to show a reactive oxygen species (ROS) production

consecutive to the drop of $\Delta\Psi_m$ (Zamzami et al., 1995a). This step can be selectively inhibited by rotenone (inhibitor of the complex I of the respiratory chain) and by ruthenium red (inhibitor of the uniport of calcium of the inner mitochondrial membrane). The increased production of ROS seems to be accompanied by local effects on the cardiolipins of the inner mitochondrial membrane. The alteration of the cardiolipins is detectable by labeling cells with the probe 10-nonyl-acridine orange (table 3) that binds in a stoechiometric manner to the intact cardiolipins of the inner mitochondrial membrane. At this stage, cells possess a normal size and a plasma membrane whose permeability is not altered. These changes are visible only in an ulterior step coincident with a diminution of the cell size, an increase of membrane permeability and also degradation of the nuclear DNA (Kroemer et al., 1995). This last step can be delayed by compounds that eliminate free radicals, which suggests the implication of ROS in the execution of the apoptotic process.

6. Direct interventions on mitochondrial permeability transition modulate apoptosis

The permeability transition (PT), phenomenon inhibited by cyclosporine A, is characterized by the opening of mitochondrial megachannels that allows permeability to compounds of molecular weight < 1500 Da, a reduction of the $\Delta\Psi_m$ and the arrest of ATP synthesis (Bernardi et al., 1992; Petronilli et al., 1994). Interestingly, permeability transition has properties of self-amplification. Indeed, the drop of the $\Delta\Psi_m$, that is linked to depletion of non-oxidized glutathione (Macho et al., 1997) and that result from the opening of the PT pores would increase the permeability transition in a retrograde manner (Ichas et al., 1997). We have therefore proposed that the opening of the PT pore may constitute an irreversible state of the effector phase of apoptosis and could account for the apparent synchronization in the drop of $\Delta\Psi_m$ that takes place simultaneously in all the mitochondria of a same cell (Kroemer et al., 1995). The molecular composition of these megachannels is not entirely known. The peripheral benzodiazepin receptor, that has recently been implicated in the protection against ROS (Carayon et al., 1996), and the translocase of adenine nucleotides (ANT) are components of this channel. Indeed, among compounds described as being able to regulate the PT are

two specific inhibitors to the ANT: atractyloside and bongkrekic acid (BA). These two molecules could favor different conformations of the ANT: a closed conformation induced by BA and cyclosporine and an open conformation induced by atractyloside. The drop of $\Delta\Psi_m$ induced by the protoporphyrine IX, a ligand specific of the peripheral benzodiazepin receptor (that binds to ANT), entails apoptosis (Zamzami et al., 1996a). On the contrary, N-methyl Val-4-cyclosporin A (a derivative of cyclosporine that is not immunosuppressor) and bongkrekic acid, prevent the drop of the mitochondrial potential and the consecutive fragmentation of the DNA. These data show that the induction of PT provokes apoptosis while its inhibition provides a protection against it. These results sustain the hypothesis that permeability transition is involved in the drop of $\Delta\Psi_m$ observed during the effector phase of apoptosis (Kroemer et al., 1995; Petit et al., 1996; Mignotte and Vayssière, 1998).

7. Mitochondria can control nuclear apoptosis

Direct alterations of mitochondria can induce apoptosis (Hartley et al., 1994; Wolvetang et al., 1994). The links between mitochondrial perturbations and nuclear alterations can be studied by means of an acellular system where purified nuclei and purified mitochondria are confronted (Newmeyer et al., 1994). Such a system allows on the one hand to study reciprocal and direct effects of one organelle on another and, on the other hand, to characterize at the biochemical level the factors involved. The result of such a manipulation has shown that normal mitochondria have no effect on isolated cell nuclei, while the processing of mitochondria with compounds capable to induce the permeability transition (PT) is sufficient to provoke nuclear apoptosis (condensation of the chromatin and fragmentation of the DNA) (Zamzami et al., 1996b). A strict correlation between induction of the PT and nuclear apoptosis has been observed by using a variety of known inductors of the PT such as atractyloside, pro-oxidants, calcium, protonophores and substances that provoke linkage of thiol groups such as diamide. These compounds, that have no direct effect on nuclei in absence of mitochondria, confer pro-apoptotic properties upon mitochondria. The pro-apoptotic character (induction of nuclear apoptosis) of the mitochondria treated with atractyloside is altered by inhibitors

of the PT such as bongkrekic acid, cyclosporine A and compounds like monochlorobimane that block the cross-linking of the thiols. Cyclosporine A can be replaced by its non immunosuppressor analogue, N-methyl Val-4-cyclosporine A, which shows that its inhibitory effect on PT and nuclear apoptosis is independent of its calcineurine activity. These results suggest the implication of the PT in the regulation of apoptosis induced *via* the mitochondria.

Finally, mitochondria isolated from apoptotic cells *in vivo* are capable of inducing nuclear apoptosis in the acellular system. Indeed, the induction of mouse hepatocyte apoptosis *in vivo* by a combination of D-galactosamine and lipopolysaccharide entails the reduction of $\Delta\Psi_m$. The mitochondria isolated from these cells provoke apoptosis of HeLa cell nuclei *in vitro*. A similar result has been obtained with mitochondria isolated from cells of spleen processed with dexamethasone. These results show that mitochondria can effectively control nuclear apoptosis (Zamzami et al., 1996b). The mitochondria that undergo the PT would liberate a protein capable of inducing nuclear apoptosis. Some works suggest that the cytochrome c, by dissociating from the mitochondria, activate proteases implicated in nuclear apoptosis (Liu et al., 1996; Kluck et al., 1997; Yang et al., 1997). Other results suggest that the "apoptogenic" protein derived from the mitochondria is a protease of approximately 50 kDa, whose activity is sensitive to the tri-peptide Z-VAD.fmk (a caspase inhibitor) (Susin et al., 1996).

8. Bcl-2 inhibits permeability transition and the release of cytochrome c and AIF in the cytoplasm

Data concerning Bcl-2 underline the role of mitochondria in apoptosis (Kroemer, 1997; Reed, 1997; Mignotte and Vayssière, 1998). Bcl-2, and other related proteins, are localized in intracellular membranes and specially in the external membrane of mitochondria (Krajewski et al., 1993; Nakai et al., 1993; Nguyen et al., 1993; Akao et al., 1994; de Jong et al., 1994; Gonzalez-Garcia et al., 1994; Hickish et al., 1994; Janiak et al., 1994; Yang et al., 1995). Anti-apoptotic properties of Bcl-2 are affected in absence of its segment of anchorage to membranes (Tanaka et al., 1993; Nguyen et al.,

1994; Zhu et al., 1996). However, it has been shown that *bcl-2* can block apoptosis of cells devoid of mitochondrial DNA (ρ^0 cells) (Jacobson et al., 1993). These experiments show that, in these cells that have undergone a complex selection and that possess mitochondria that have a $\Delta\Psi_m$ close to the normal (Skowronek et al., 1992; Marchetti et al., 1996; Sidoti-de Fraisse et al., 1998), the anti-apoptotic activity of *bcl-2* can always be exerted. However, they exclude neither that the anti-apoptotic effect of *bcl-2* is linked to an activity that is always present in mitochondria of ρ^0 cells nor that its anti-apoptotic power exerts at several levels. It has effectively been shown that Bcl-2, in contrast to inhibitors of caspases, blocks the drop of $\Delta\Psi_m$ that occurs during the cellular death induced by respiratory inhibitors (Shimizu and Eguchi, 1996). The importance of the structural location of Bcl-2 in nuclei or mitochondria as compared to its anti-apoptotic function has been studied. These organelles have been purified from hybridomas of T cells transfected by *bcl-2* (Zamzami et al., 1996b). In acellular experiments, the treatment with atractyloside has shown that, in contrast to mitochondria purified from control cells, mitochondria purified from cells transfected by *bcl-2* do not provoke nuclear apoptosis. On the contrary, nuclei purified from cells transfected by *bcl-2* show a condensation of the chromatin and a fragmentation of the DNA when they are confronted to control mitochondria treated with atractyloside. Furthermore, *bcl-2* inhibits the induction of permeability transition (Decaudin et al., 1997) while *bax* promotes it (Pastorino et al., 1998). These results show that, even if Bcl-2 intervenes also during latter events (Guénal et al., 1997b), at least a part of its inhibitory activity on apoptosis is exerted by acting on the mitochondrial permeability transition.

Moreover, the structure of a protein of the Bcl-2 family (Bcl-x_L) has been recently established (Muchmore et al., 1996). It recalls that of bacterial toxins, especially the diphtheria toxin, that form a pH-sensitive transmembrane channel. Furthermore, the pro-apoptotic Bax protein can form channels (Antonsson et al., 1997), as reported also for the anti-apoptotic proteins Bcl-x_L (Minn et al., 1997) and Bcl-2 (Schendel et al., 1997). However, the intrinsic properties of Bax and those of Bcl-x_L and Bcl-2 reveal differences. The channel forming activity of Bcl-x_L and Bcl-2 is observed at highly acidic pH

while Bax forms channel in a wide range of pH including at pH=7, that found in cells. Furthermore, Bcl-2 can block the pore-forming activity of Bax. These results strengthen the hypothesis that these proteins are components of the mitochondrial PT pores. Bax might promote cell death by allowing the efflux of ions and small molecules across the mitochondrial membranes, thus triggering permeability transition, while Bcl-2 might counteract this effect.

It has been shown that Bcl-2 inhibits the release of cytochrome c (Kluck et al., 1997; Yang et al., 1997) and of AIF (Susin et al., 1996) outside the mitochondria. Thus, one level of action of Bcl-2 is to control the efflux of proteins from the mitochondrial intermembrane space through the outer membrane and into the cytosol where caspases are found. In the same way, Bcl-x_L inhibits AraC-induced preapoptotic accumulation of cytochrome c in the cytosol (Kim et al., 1997) and cells that overexpress Bcl-x_L fail to accumulate cytosolic cytochrome c or to undergo apoptosis in response to genotoxic stress. These findings support the hypothesis that Bcl-x_L, as well as Bcl-2, protect cells from apoptosis by inhibiting the availability of cytochrome c in the cytosol. The mechanism allowing for the release of AIF and cytochrome c needs further investigations. PT pores, which are thought to connect the mitochondrial matrix to the cytosol, within the contact sites between inner and outer mitochondrial membranes (Beutner et al., 1996) are only permeable to small compounds (molecular mass <1500 Da). Thus PT pores are probably not the structure directly responsible for the efflux of these intermembrane space apoptogenic proteins. Future works will have to determine the way used by AIF and cytochrome c to leave the mitochondria. Opening of the PT pore could be just a first step of a cascade of events causing an increase in the permeability of the outer mitochondrial membrane. Some results suggest that this permeability increase is due to mechanistic disruption secondary to an increase in matrix volume (Scarlett and Murphy, 1997; Van der Heiden et al., 1997).

9. Bcl-2 family proteins relocalize cellular proteins to mitochondrial membranes

Bcl-2 has also been found to interact with several other cellular proteins that do not belong to the Bcl-2 related proteins family. These proteins include Nip1, Nip2, and Nip3, the function of which

is unknown (Boyd et al., 1994), the GTPase R-ras p23 (Fernandez and Bischoff, 1993), Raf-1 (Wang et al., 1994; Ali et al., 1997), BAG-1 (Takayama et al., 1995), the cellular prion protein (PrP) (Kurschner and Morgan, 1995), the p53 binding-protein p53-BP2 (Naumovski and Cleary, 1996), the protein phosphatase calcineurin (Shibasaki et al., 1997) and the mitochondrial membrane protein carnitine palmitoyltransferase I (Paumen et al., 1997). At least some of these interactions could reflect the ability of Bcl-2 to relocalize cellular proteins to mitochondrial membranes.

Indeed, a connection has been made between members of the CED-9/Bcl-2 family and caspases. Mutations that reduce or eliminate CED-9 activity also disrupt its ability to bind CED-4 (Spector et al., 1997). CED-9 (and Bcl-x_L) was found to interact with and inhibit the function of CED-4. Furthermore, CED-4 can simultaneously interact with CED-3 (Irmler et al., 1997) (or mammalian caspases (Chinnaiyan et al., 1997b)). Thus, CED-9 might control *C. elegans* cell death by binding to and regulating CED-4 and CED-3 activities. A similar mechanism could also exist in mammalian cells. Bcl-x_L interacts with caspase-1 (Chinnaiyan et al., 1997a) and inhibits the function of CED-4 (Chinnaiyan et al., 1997b). Furthermore, the human protease activating factor (Apaf-1), which participates in the cytochrome c-dependent activation of caspase-3, contains a caspase-recruitment domain (CARD). Altogether, these results suggest that Apaf-1, like the nematode CED-4, acts by activating caspases and could provide the physical link between Bcl-2 related proteins and caspases. (Zou et al., 1997). Since Bax and Bik can disrupt the association between CED-9 (or Bcl-xL) and CED-4 (Chinnaiyan et al., 1997b), it is tempting to speculate that Bcl-x_L and possibly other members of the Bcl-2 protein family inhibit apoptosis by maintaining the procaspases/Apaf-1 complexes associated to mitochondrial membranes and that Bax and Bik by dissociating the complexes permit the activation of procaspases.

10. Mitochondria participates in apoptosis signaling

Recently, it has appeared that mitochondria , in addition to their role during the effector phase of apoptosis, also intervene, in some cases, during the initiation phase of apoptosis. Much of the

available data converge to the hypothesis that when ROS are involved in apoptosis signaling, they are the consequence of an impairment of the mitochondrial respiratory chain (Schulze-Osthoff et al., 1992; France-Lanord et al., 1997; Gudz et al., 1997; Quillet-Mary et al., 1997; Sidoti-de Fraisse et al., 1998). What is the mechanism leading to the accumulation of mitochondrial ROS? Much of the available data converge to the hypothesis that ROS increases are the consequence of an impairment of the mitochondrial respiratory chain (Schulze-Osthoff et al., 1992; France-Lanord et al., 1997; Gudz et al., 1997; Quillet-Mary et al., 1997). Indeed, it was shown that an upstream inhibition, with chemical compounds acting on complex I (Schulze-Osthoff et al., 1992; Quillet-Mary et al., 1997), or an elimination of the electron transfer chain by depletion of the mtDNA prevent ROS accumulation and consequently protect cells against apoptosis induced by ceramide (Quillet-Mary et al., 1997) or TNF-α (Sidoti-de Fraisse et al., 1998). The ubiquinone site in complex III appears as the major site of mitochondrial ROS production as this site catalyzes the conversion of molecular oxygen to superoxide anion which can lead to the formation of other potent ROS such as hydrogen peroxide and hydrogen radicals. Such a model is supported by the observed potentiation of cell death processes in ROS-dependent apoptosis when electron flow was inhibited distal to the ubiquinone pool. However, the origin of these electron flow disturbances is not clear. The recently observed efflux of cytochrome c from mitochondria to cytosol in the early phases of apoptosis could provide a clue to resolve this question (Liu et al., 1996; Kluck et al., 1997; Yang et al., 1997). Indeed, the release of cytochrome c must lead to a breakdown of the mitochondrial electron flow downstream of the ubiquinone site which in turn would result in an increased generation of ROS. Such a model is supported by the described correlation between loss of cytochrome c and respiratory failure in a Fas-induced apoptosis model (Krippner et al., 1996).

11. Conclusions

In conclusion, mitochondria are involved in the decision of cells to survive or not at several levels (figure 2). At a first level, mitochondria can contribute to apoptosis signaling, as shown in

TNF-α- or ceramide-induced cell death during which increased mitochondrial ROS production appears as an early event of the induction phase. At a second level, mitochondria are involved in the control of the activation of the cell death machinery by docking at their surface, via Bcl-2 family proteins, execution caspases or by sequestering, in the intermembrane space, caspase activators as AIF or cytochrome c.

12. References

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Legende to figures

Figure 1: Simplified model of events occurring during apoptosis.

Several induction pathways (lack of survival signal, death signal, viral infection, contradictory signals for proliferation or DNA damage) lead to a series of events similar for all apoptotic deaths. There is firstly an induction phase leading to an effector (or execution) phase during which only few morphological changes are visible and then a degradation phase during which the morphological changes of apoptosis become evident.

Figure 2: Roles of mitochondria during apoptosis.

First,, some, apoptosis inducing signals involve an increased mitochondrial ROS accumulation. Second, after the induction phase, mitochondria participates in the activation of executing caspases responsible for the morphological changes in two ways. On the one hand, Ced-4/Ced-3 (Apaf-1/caspase) complexes dissociate from intracellular membranes and are released in the cytosol. On the other hand, the opening of PT pores is a first step of a cascade of events causing an increase in the permeability of the outer mitochondrial membrane, an efflux of proteins as AIF or cytochrome c, an increase in ROS production and an activation of executing caspases.

C. elegans	**Mammals**
CED-3	*Activation caspases*: caspase-1 (ICE), -4 (ICH-2), -6 (Mch2), -8 (MACH/FLICE)… *Execution caspases*: caspase-2 (ICH-1), -3 (CPP32), -4 (ICH-2), -7 (ICE-LAP3)…
CED-4	Apaf-1
CED-9	*Anti-apoptotic*: Bcl-2, Bcl-x_L, Bcl-w, Bfl-1, Brag-1, Mcl-1, A1, NR13… *Pro-apoptotic*: Bax, Bak, Bcl-x_S, Bad, Bik, Hrk…

Table 1: Programmed cell death in nematodes and mammals is controled by homologous genes. Mammalian caspases act either during the activation or the execution phase of PCD. In mammals some members of the Bcl-2 related proteins are death antagonists while other are death agonists.

Pro-apoptotic cellular proteins	**Anti-apoptotic viral proteins**
p53	Adenovirus E1B 55k
	SV40 large T
	HPV E6
FADD (adatator)	v-FLIPs
Caspases of the ICE/Ced-3 family	Cowpox virus Crm A
	Baculovirus p35
Pro-apoptotic members of the Bcl-2 family	Epstein-Barr virus BHRF1
	LMW5HL
	Adenovirus E1B 19k

Table 2: many viral genes products act as antagonists of pro-apoptotic cellular proteins.

Fluorochrome	**Specificity**
Annexine V	Binds to phosphatydilserine upon its exposure to the outer leaflet of the plasma membrane
Rhodamine 123 3,3' dihexyloxacarbocyanine iodide ($DIOC_6(3)$) 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1)	Accumulates in mitochondria as a function of the mitochondrial membrane potential.
Dihydroethidine (HE)	Reacts with reactive oxygen species (mainly superoxide anion), and is converted to ethidium bromide.
Dichlorofluorsceine-diacetate (DCFH-DA)	Reacts with reactive oxygen species (mainly hydrogen peroxide), and is converted to dichlorofluorescein.
Monochloraobimane	Forms a fluorescent GST catalyzed adduct with intracellular GSH
Nonyl acridine orange (NAO)	Bind stœchiometrically to cardiolipins of the inner mitochondrial membrane.
cis-parinaric acid	Peroxidation of membranous lipids.
Propidium iodide (PI)	Membranes from viable cells are not permeable to this fluorochrome that intercalates in DNA.
dUTP-FITC dUTP-biotine	Incorporates in DNA breaks by the action of DNA polymerase or terminal desoxyribonucleotidyl transferase.

Table 3: Some fluorochromes used for the study of mitochondrial and nuclear alterations during apoptosis.

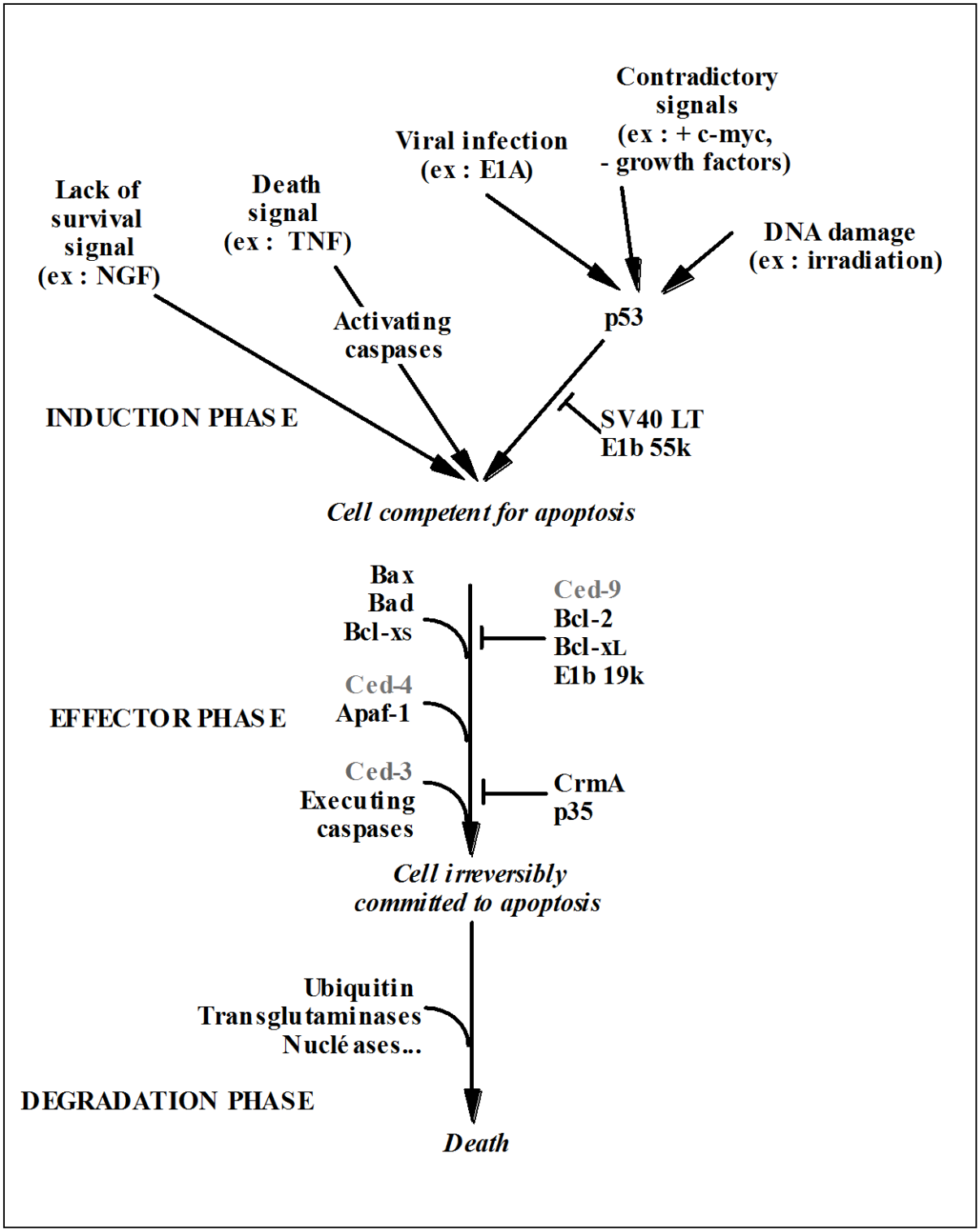
Contradictory signals (ex : + c-myc, - growth factors)
Viral infection (ex : E1A)
Lack of survival signal (ex : NGF)
Death signal (ex : TNF)
DNA damage (ex : irradiation)
Activating caspases
p53
INDUCTION PHASE
SV40 LT
E1b 55k
Cell competent for apoptosis
Bax
Bad
Bcl-xs
Ced-9
Bcl-2
Bcl-xL
E1b 19k
Ced-4
Apaf-1
EFFECTOR PHASE
Ced-3
Executing caspases
CrmA
p35
Cell irreversibly committed to apoptosis
Ubiquitin
Transglutaminases
Nucléases...
DEGRADATION PHASE
Death

Figure 1

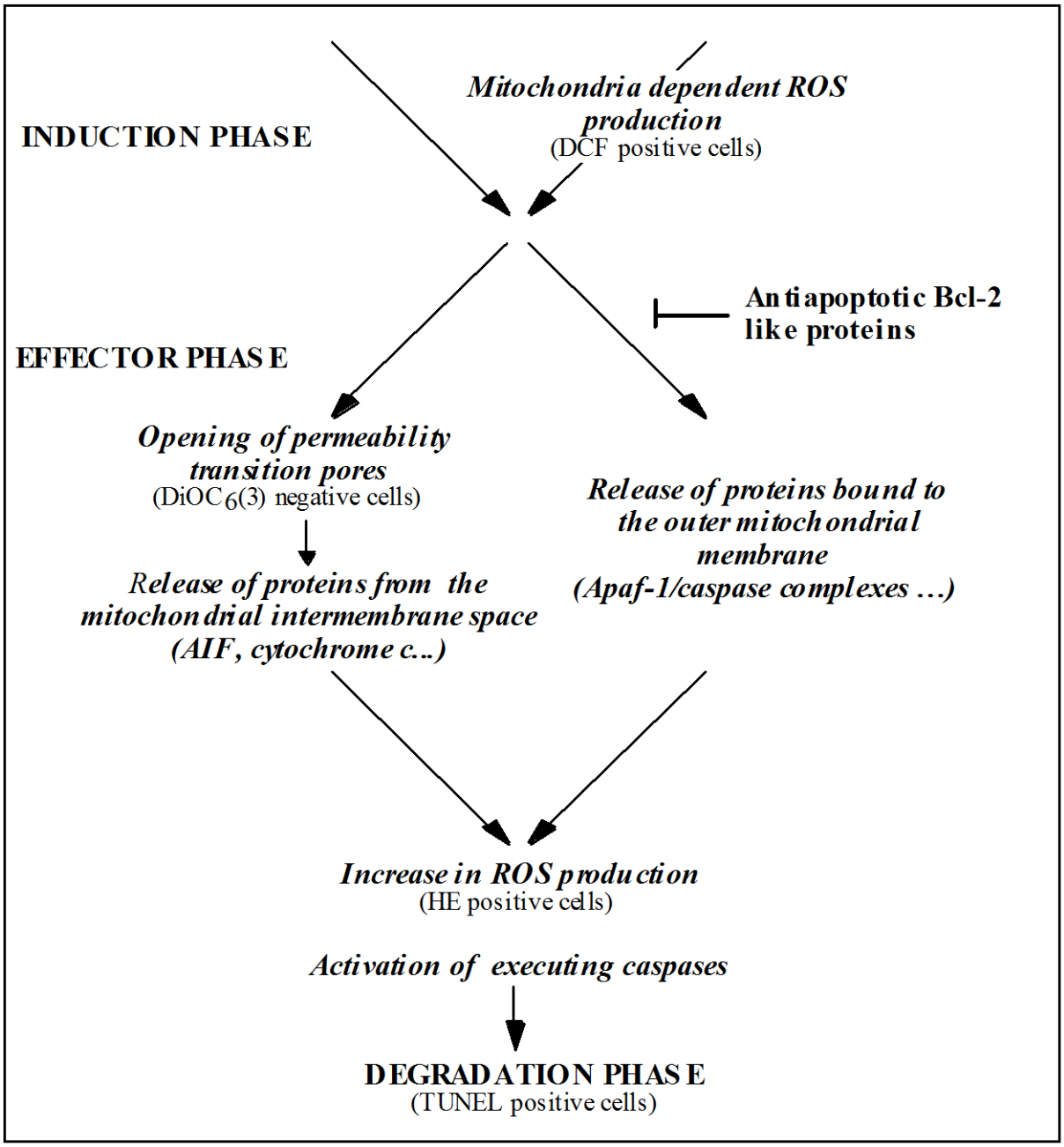
INDUCTION PHASE
Mitochondria dependent ROS production
(DCF positive cells)
Antiapoptotic Bcl-2 like proteins
EFFECTOR PHASE
Opening of permeability transition pores
(DiOC6(3) negative cells)
Release of proteins bound to the outer mitochondrial membrane
(Apaf-1/caspase complexes …)
Release of proteins from the mitochondrial intermembrane space
(AIF, cytochrome c..)
Increase in ROS production
(HE positive cells)
Activation of executing caspases
DEGRADATION PHASE
(TUNEL positive cells)

Figure 2