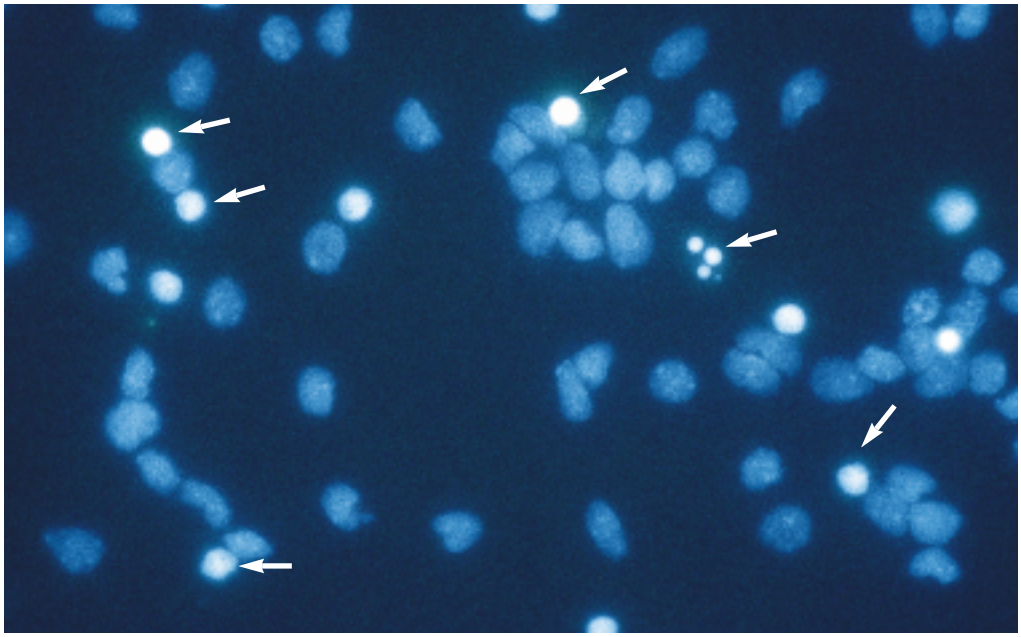
CELL SUICIDE

In the cell with DNA damages induced by genotoxic agents, and in particular by ionizing radiation, protein p53 plays a central role. It is involved in the control of cell proliferation and differentiation, but also in keeping the cell genome intact. It can induce the cells to commit suicide, i.e., undergo apoptosis, to avoid the risk of tumor growth, which would be deleterious to the whole organism. Conversely, tumor cells can develop if the cell suicide program is faulty. Thorough knowledge of the mechanisms of apoptosis, so that cell death can be manipulated, may one day lead to a breakthrough in cancer therapy.



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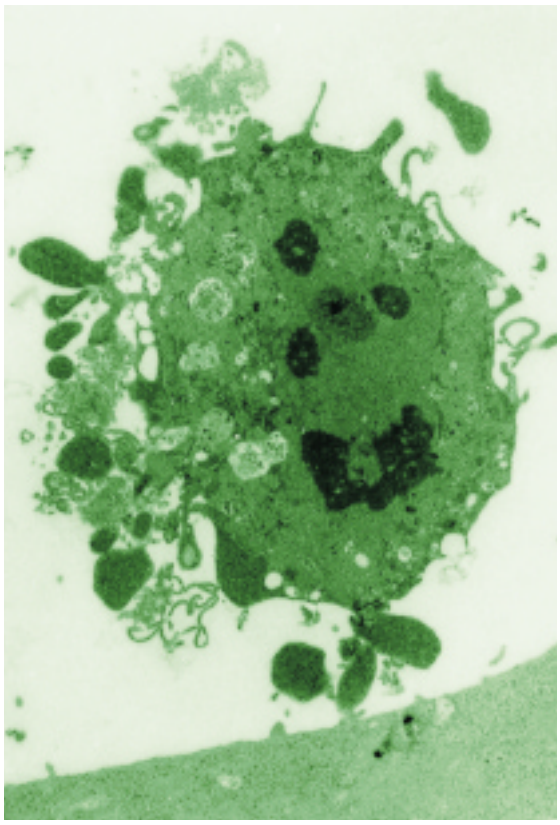
Apoptosis, a mechanism of programmed cell death, often occurs naturally, or can be induced by the action of genotoxic agents. For example, when a reptile moults, a new skin replaces the old one, the cells of which have died by apoptosis. Here: nuclei of rat cerebral cortex cells grown in culture after 1 Gy irradiation. Several cells (arrows) are undergoing apoptosis.

Two types of cell death

In adults the vast majority of **cells** are in a **quiescent** state. Even so, some die and are replaced by the process of cell division (box E, ***The cell division cycle: under control duplication***). This happens for example in organs such as the liver or kidney. Other organs such as the bone marrow, the small intestine, the testicle and the skin also display very high cell turn over. By contrast, the neurons of the brain do not divide, or only rarely, and some of them die during the life of the individual. But in all cases these cell deaths are isolated in the organ and apoptotic bodies are discreetly phagocytosed. The dead cells are captured, ingested and digested by neighboring cells or by specialized cells, the **macrophages**, without any inflammatory reaction occurring. This type of death is actively induced by the cell itself. This is why we use the term *programmed cell death* or **apoptosis**. The moulting of insects, batrachians and reptiles takes place by this mechanism. Before the concept of programmed cell death came to be employed, the term necrosis was used as a hold-all term. Today this term is reserved for cell death over a localized area due to disturbed tissue homeostasis(1), such as may result from ischemia(2), hyperthermia, a chemical injury or a physical shock. These two types of cell death, apoptosis and necrosis, differ in their tissue distribution and in the structural and morphological changes that take place in the cell (box D, ***The cell, the essential link***). During apoptosis the cell shrinks, but the **organelles** (**mitochondria, Golgi body** and **endoplasmic reticulum**) retain their normal appearance. In the **nucleus**, the **chromatin** condenses and then fragments into **nucleosomes**. Finally, the **cytoplasmic membrane** breaks up to form apoptotic bodies containing **cytoplasm** and organelles. In contrast, during necrosis cells and organelles swell and burst, causing an inflammatory reaction and forming a necrotic site.

The process of apoptosis plays a critical role during embryogenesis. It allows shaping of the new being by the removal of ancestral tissue (e.g., webbing between the fingers), and the correct installation of the nervous and immune systems. Apoptosis also occurs after the action of a **genotoxic** agent or in the course of an illness. In cancer, apoptosis seems to be reduced or suppressed to allow the cancer cells to survive and multiply.



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Electron microscope image of a human epithelial cancer cell into which has been introduced a molecule of DNA to induce the expression of protein p53 in the terminal phase of apoptosis. The cytoplasmic membrane has broken up causing the formation of apoptotic bodies containing cytoplasm and organelles.

The central role of protein p53

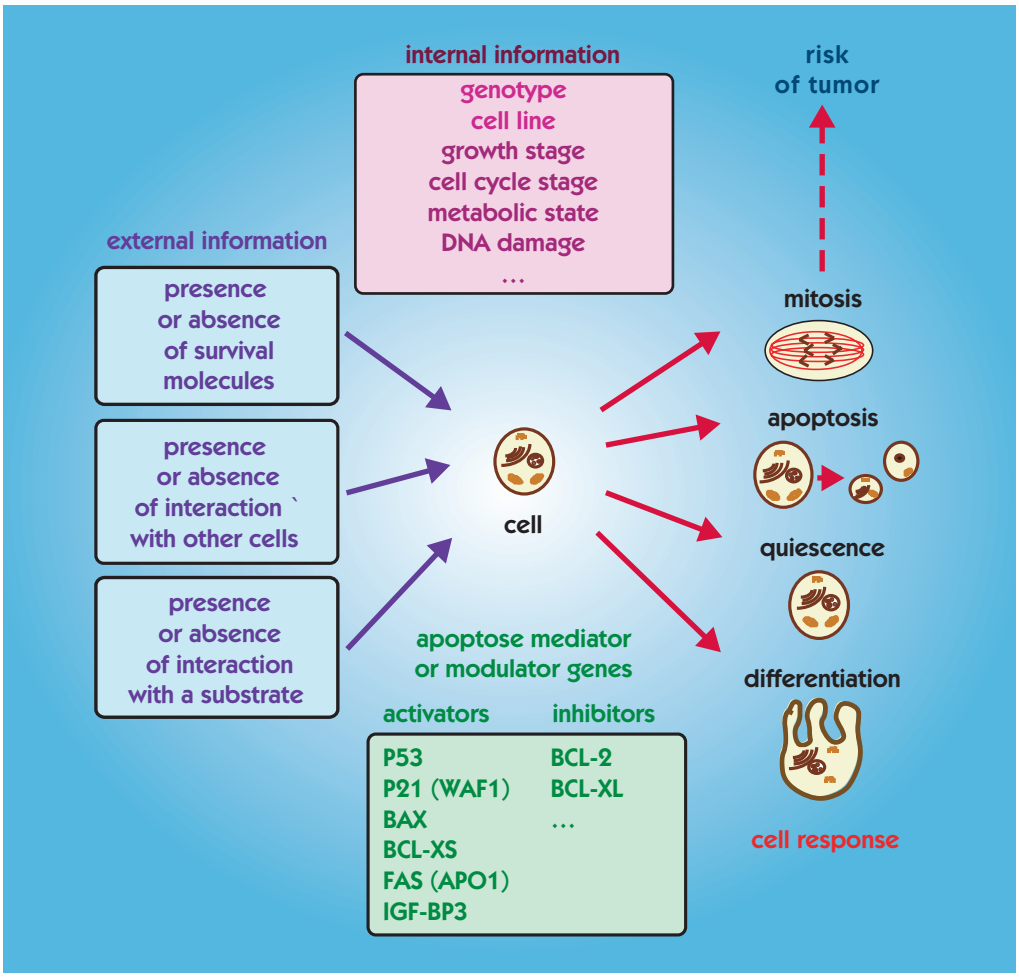
DNA is the principal target for the action of **ionizing radiations**, which induce single- and double-**strand** breaks. However, ultraviolet rays and various chemicals, including some that can splice into the DNA molecule, also cause damage to DNA. It seems that all such damage can be recognized directly by the C-terminal(3) part of **protein** p53 or indirectly by protein ATM (see previously). This induces stabilization of protein p53, which in turn activates the **expression** of several other **genes** including P21 (WAF1-Cip-1), GADD45, BAX, MDM2, etc.

Protein p21 complexes with **cyclines** and their associated **kinases** to arrest the cell cycle at the G1/S transition (see *Radiation-induced genes*). This arrest in the cell cycle allows the cell to repair damage before entering the DNA duplication phase (S phase). If repair is impossible, incomplete or inaccurate then protein p53 directs the cell to undergo apoptosis by activating GADD45, FAS, IGF-BP3 (Insulin Growth Factor Binding Protein) and the **proto-oncogene** BAX.

The quantity of p53 present in the cell is controlled by the protein itself, which activates the expression of gene MDM2. The formation in the nucleus of a complex between proteins p53 and mdm2 causes the transport and specific degradation of p53 in the cytoplasm. This mechanism allows the cell division cycle to resume once the repair is completed.

(1) Mechanism that maintains or restores various physiological constants in an organism, in spite of variations in the environment.
(2) Insufficient blood circulation in an organ or a tissue.
(3) Proteins, which are long polymers of **amino acids** associated by **peptide linkages**, possess an **amine**, or N-terminal end ($-NH_3^+$) and a **carboxylic**, or C-terminal end ($-COO^-$).

Figure 1. The different responses of the cell according to the external or internal information it receives.



The fate of cell for not dividing (G1 arrest) or for apoptosis depends on many factors, especially intercellular contacts, and the presence or absence of survival molecules(4) including certain **metabolites**, growth factors, agents of the immune system (interleukins), and **hormones**. The nature of the response depends closely on the cell type. In cells growing in culture the same **dose** of radiation only blocks the cell cycle in **fibroblasts**, the cells of conjunctival tissue, yet induces apoptosis in thymocytes, the cells of the thymus(5). All the factors that regulate growth arrest or apoptosis can combine in various ways to give a range of conditions that will determine the fate of the cell (Figure 1).

«Organised» suicide

After irradiation the cells of the outer granular layer of the cerebellum in young rats die in large numbers in the first six to nine hours. An injection of actinomycin D (an **RNA** synthesis inhibitor) or of cycloheximide (protein synthesis inhibitor) temporarily halts this apoptotic wave. The activation of certain genes in the cell is thus necessary for apoptosis to occur. A study of the **mutations** present in a small parasitic worm, *Cœnorhabditis elegans*, revealed two pro-apoptotic genes, CED-3 and CED-4, and an anti-apoptotic gene, CED-9. In mammals the equivalent genes are respectively ICE, APAF-1 and BCL-2. The protein ICE (Interleukin 1 Converting Enzyme) belongs to the caspase(6) family which activate each other in a cascade culminating in the activation of an endonuclease(7) and many other **enzymes** mobilized to modify the **cytoskeleton** and proteins of the membrane surface in preparation for phagocytosis. Protein apaf-1 regulates caspase activity. The bcl-2 family is composed of two subfamilies, one including bcl-2 and bcl-xl that favors survival, the other including bax, bak, bad and bcl-xs that favors cell death.

The presence or absence of **survival factors** derived from the cell environment, plus the state of the **genome**, which mediates the role of protein p53, will stimulate or slow down the process of apoptosis. The mechanism of action of protein bax is to bind to the survival protein bcl-2. They form dimers and neutralize each other. An increase in either one of these proteins produces bcl-2-bcl-2 or bax-bax dimers, thereby favoring survival or cell death. The overexpression of bcl-2 neutralizes the apaf-1-caspase complex and blocks apoptosis. Conversely an excess of bax favors the permeabilization of the **mitochondrial** membrane and the formation of a cytochrome C-apaf-1-caspase com-

(4) Molecules that trigger intracellular reactions that favor survival.
(5) Glandular organ situated in front of the trachea, particularly well developed in children and young animals, which supports immunological responses.
(6) Enzymes containing a molecule of Cysteine and an ASPartate proteASE activity.
(7) Enzyme that breaks down DNA except at its ends.

plex, which triggers the program that executes apoptosis (Figure 2).

Natural and radio-induced apoptosis

Natural apoptosis is very important in the embryo and fetus at very precise stages in their growth. It intervenes for example to eliminate neurones that have not reached their target site which secrete survival factors, and so improves the quality of the inter-neuronal connections. Are these phases of natural apoptosis concomitant with phases of radio-sensitivity? In the rat the natural apoptosis of the neurones is intense in the days following birth, whereas the phase of high sensitivity of the cells to radio-induced apoptosis occurs at two-thirds of the gestation period.

The period of natural apoptosis corresponds to the phase of synaptogenesis (during which synapses, the connections between neurones, form), whereas the period of radio-induced apoptosis occurs during the multiplication phase of the neuroblasts (immature neurones). In the adult, most neurones do not divide and are not very sensitive to irradiation. Thus there are radio-sensitivity windows during embryo and fetal growth that are characteristic of each tissue. Natural apoptosis is independent of P53, because it occurs in mice whose gene is invalidated, i.e., mice that do not express p53. By contrast, cells irradiated in their multiplication phase undergo cycle arrest at G1 and p53-dependent apoptosis.

In the adult intestine, irradiation kills many cells in the small intestine, but few in the colon. This is because the absence of protein bcl-2 in the small intestine favors apoptosis, whereas its normal expression in the colon restrains it. The other rapidly-renewed tissues in adults undergo radio-induced apoptosis probably involving protein p53. In the same manner, chemotherapy causes generalized apoptosis in these tissues, but, fortunately, especially in **tumors**.

Protein p53 is implicated not just in cell cycle arrest and apoptosis. It is strongly expressed during the **meiotic prophase** of the spermatocytes, the male **germ** cells, when the spermatids are formed. These are **haploid** cells, which possess only half the number of **chromosomes** for the species, suggesting a role in the recombination and structural changes in chromatin.

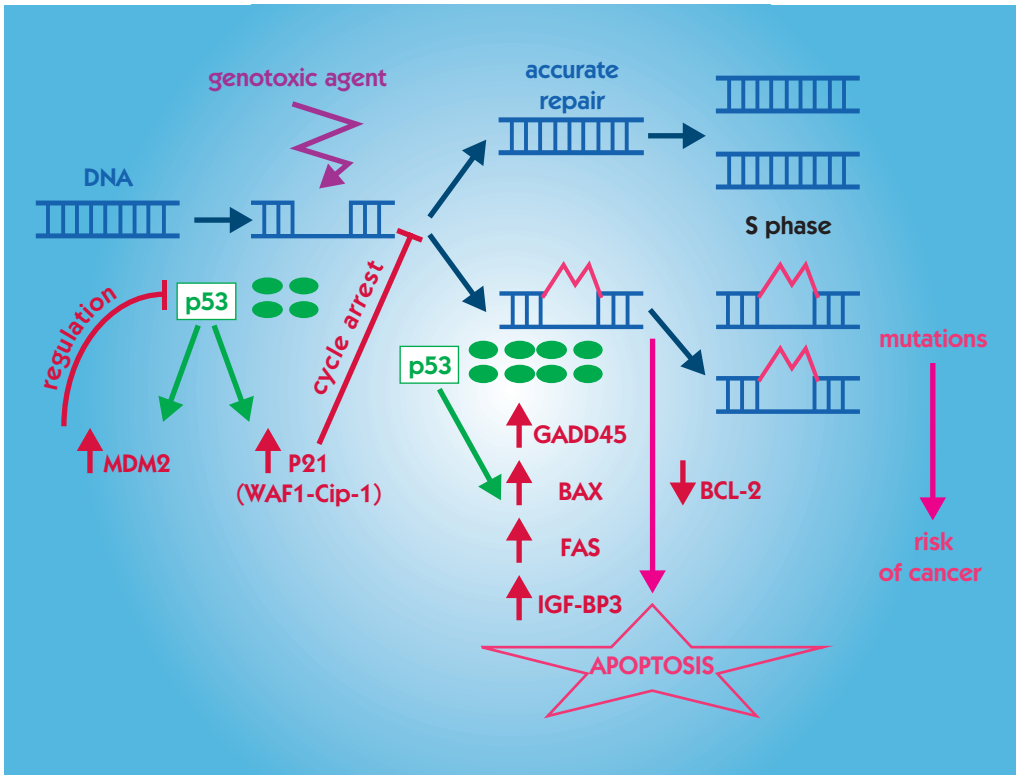
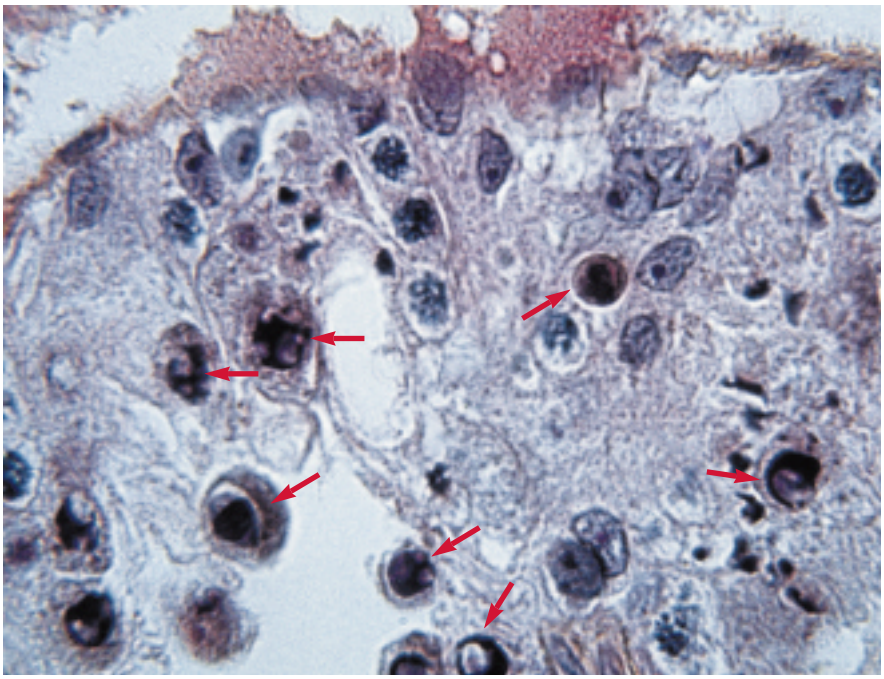


Figure 2. The mechanisms of cell cycle blockage and apoptosis induced by protein p53.



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Adult rat testis cells after irradiation at fetal age. Numerous cells undergoing apoptosis among the spermatocytes are arrowed.

Hope for cancer therapy

In a tumor, the increase in the number of cancer cells is the result of an uncontrolled proliferation with overexpression of particular proto-oncogenes, plus a reduced level of apoptosis or none at all. Thus the overexpression of the anti-apoptotic bcl-2 in certain tumors enables the cancerous cells to survive. After exposure to a genotoxic agent most of the cells containing damage that is not repaired or only partially repaired are eliminated by apoptosis under the control of protein p53. The mutation of the P53 gene, favoring the survival of cells bearing anomalies, is an important step in tumor growth.

The vital role of protein p53 in tumor growth is demonstrated by the fact that mice with two knock out **alleles** of the P53 gene rapidly die of various tumors in the first six months of their life. Hence the reduction or inhibition of cell suicide is a key step in cancer formation. Accordingly, much is expected of current research into treatments that favor radio- or chemo-induced apoptosis of tumor cells.

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