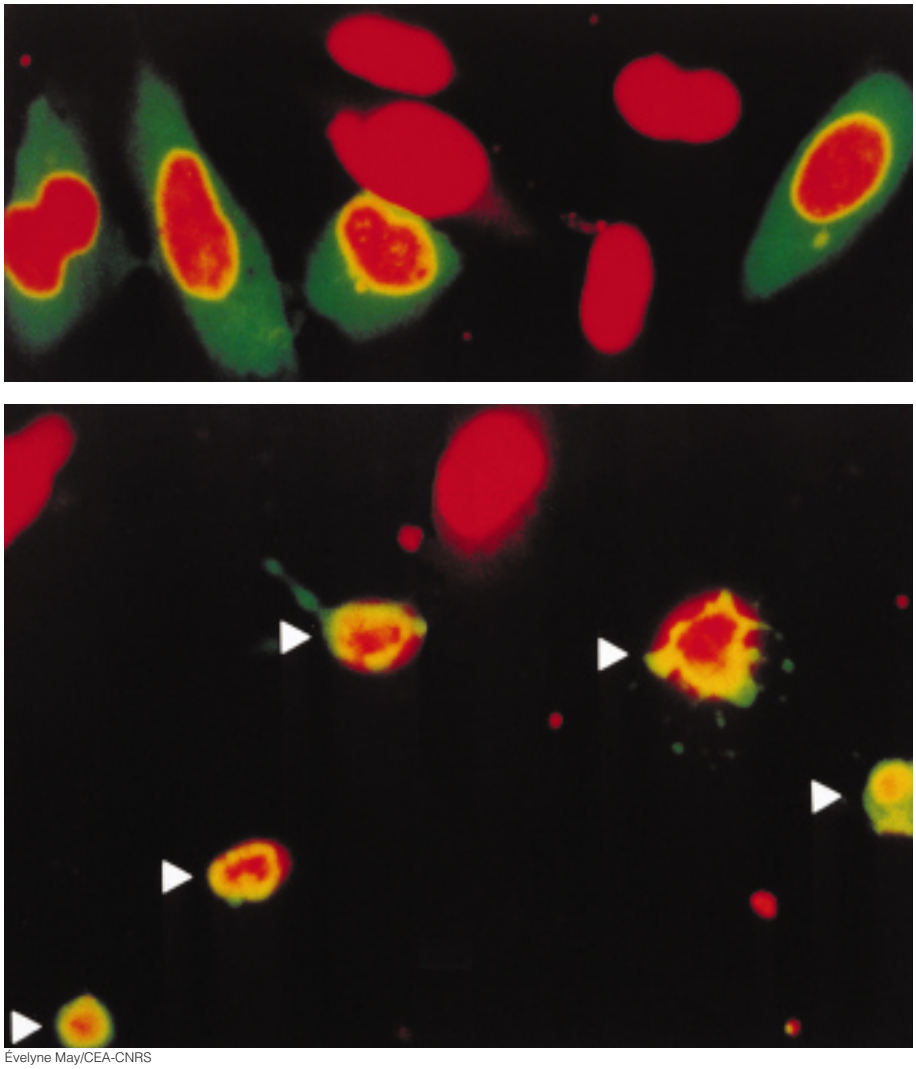
RADIATION-INDUCED GENES

The monitoring system of genome integrity following radiation exposure requires more than the recruitment of repair enzymes at the damaged sites and the subsequent repair of DNA lesions. It also generates an «alarm signal» that ultimately results in the activation of specific genes implicated in cell cycle arrest allowing time for repair to take place, and the elimination of cells that are too heavily damaged. Among the numerous genes involved in the cell's response to radiation damage, the target genes that are activated by the p53 protein in mammalian cells have been especially studied. The so-called guardian of the genome, the p53 protein plays a central role in the cell's response to ionizing radiation. This response has been less studied in plant cells. Identifying radiation-induced genes in plant cells appears to be an interesting approach which might hopefully benefit from lessons using mammalian cells. Together with the complete genome sequence of the «plant model» Arabidopsis thaliana, recent development of powerful techniques will allow a rapid expansion of the knowledge in this research field of plant biology.



Évelyne May/CEA-CNRS

Human cancer cells with mutated p53 protein are unable to undergo apoptosis (top). Introduction of wild-type p53 protein into tumor cells triggers the process of programmed death, apoptotic cells detected by fluorescence staining are indicated by arrows (bottom).

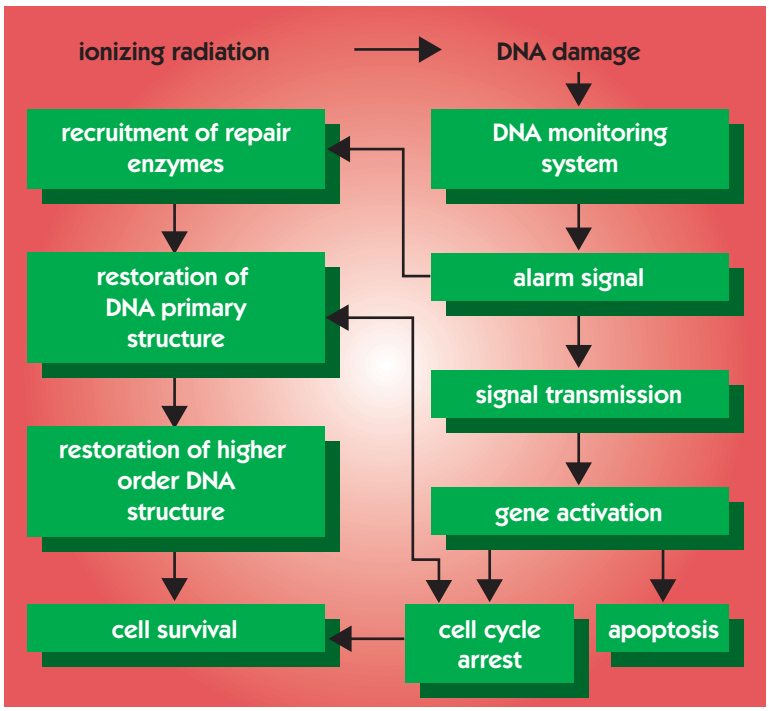


Figure 1. Sequence of cellular and molecular events following exposure to ionizing radiation in mammalian cells.

Genes are specifically activated

The **cell**'s response to radiation-induced **DNA** damage involves a series of molecular events that ultimately leads to the activation of specific **genes** (Figure 1). Radiation-induced DNA damage is repaired within the first minutes or at the very most within the first hours following exposure if the damage is severe. The activation of these highly specific genes results in the synthesis of **proteins** (box F, ***Amino acids, the chemical alphabet of proteins***). These proteins play a role in either the arrest of cell proliferation (see *Effects of radiation on the cell division cycle*), or **apoptosis** (see *Cell suicide*) according to the complexity of the lesions and the fidelity of repair (Figure 1). When the lesions are not too severe, they can be correctly repaired resulting in the restoration of the genetic information. Cell cycle arrest at precise points is presumed to allow the cell time to repair the DNA lesions. Then, the cell resumes the cycle. However, when the number of lesions is high and cannot be repaired, damaged cells are eliminated through the activation of genes involved in the process of apoptosis. This strategy developed by the irradiated cell, tissue or organism is thus to avoid the transmission of **mutations** to daughter cells, which may cause neoplastic transformation.

A very large number of genes are involved in the control of the cell response to radiation. The expression of these genes and their products can be regulated at different levels. The single aspect of gene activation at the level of **transcription** (production of messenger **RNA**) will be evoked here through the example of the target genes transactivated by the p53 protein. The importance of gene activation in the radiation response will also be illustrated by the recent advances in the field of radiation-induced genes in plant cells.

Genes activated by p53 in mammalian cells

The p53 gene encodes a **transcription factor** which has been called a «caretaker» of the **genome**. Its key role in the cellular response to ionizing radiation has been extensively documented. DNA damage induces structural alterations of p53 and consequently its stabilization and its accumulation in the nucleus. By binding to specific gene regulatory sequences, p53 activates transcription. The main p53-induced genes in response to radiation are represented in Figure 2. Activation of these genes leads to the transitory arrest of cell cycle progression or cell death by apoptosis.

p53 and cell cycle progression

Several radiation-induced genes under the control of p53 participate in cell proliferation arrest at precise points of the cell cycle. Before detailing the mechanism involved, it has to be remembered that the correct succession of the different steps of the cell cycle is orchestrated by a complex control system which includes various families of proteins such as **cyclins**, along with their associated **kinases** (cdk) and their inhibitors. Each transition from one phase to another (box E, ***The cell division cycle: under control duplication***) is under the control of a specific cdk-cyclin complex. The importance of this control is illustrated by the observation that mutations of certain proteins of these complexes abrogate their function in the cell cycle machinery, e.g., the p16 protein involved in the complex required for the G1-S transition. As represented in Figure 2, the radiation-induced cell cycle arrest or delay can occur at different checkpoints: G1/S transition, S phase and G2/M transition.

The «p53-dependent» response involves the activation of the p21 gene. Following upon the binding of the p21 protein to the cdk-cyclin complex involved in the G1/S transition, cells are arrested in G1 phase. It is generally assumed that this delay would allow the cell time

Amino acids, the chemical alphabet of proteins

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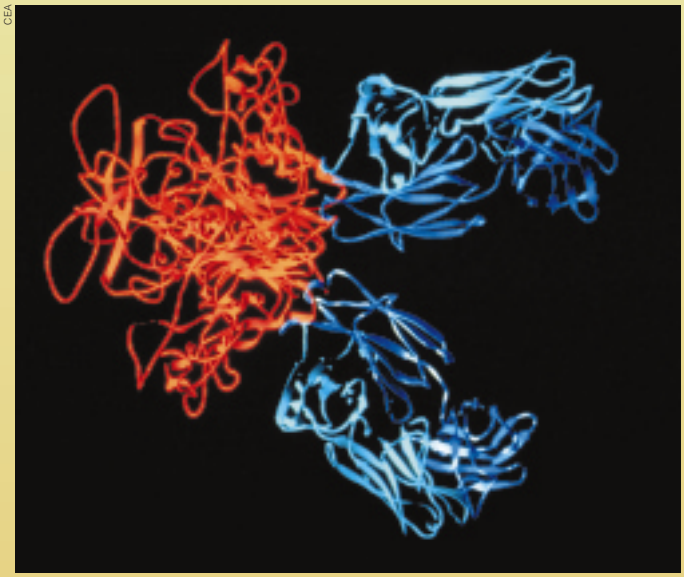
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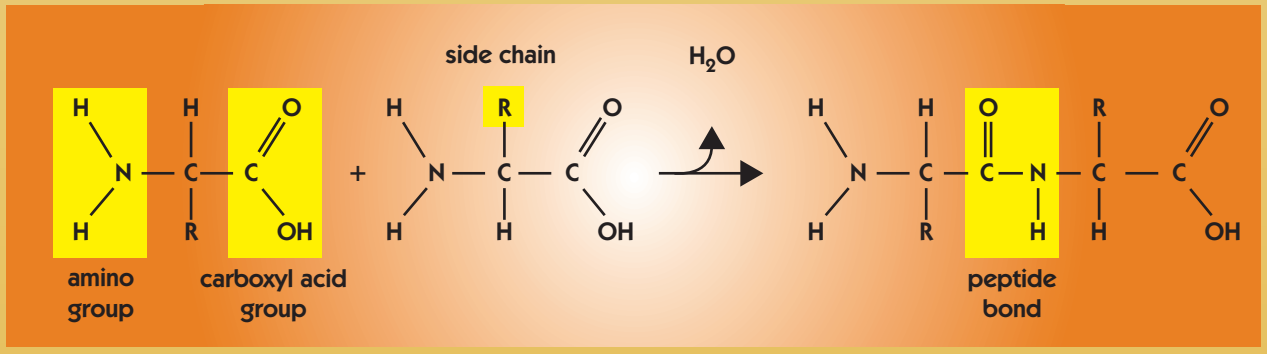
Representation of the structure of an immunoglobulin (antibody), an example of a protein with a very high molecular weight.

The information stored by a **gene** (sequence of nucleotides in DNA) determines a very precise sequence of **amino acids** that corresponds to a specific **protein**. Proteins are the major macromolecules of a cell. Depending on their chemical properties, proteins can determine the structure and the shape of the cell, recognize foreign biological material, serve as energy transporters or act as **enzymes** which catalyze specific chemical reactions. The amino acids, twenty in number, have a similar general molecular structure: an **amino group** NH_2 and a **carboxyl group** COOH, both linked to a single carbon atom. Each of the twenty amino acids differ by the side chain R attached to the central carbon. Amino acids are joined together by a linkage called a **peptide bond** (diagramme). The number of amino acid of a protein varies from less than a hundred to several hundreds and can reach one thousand for the biggest proteins. A particular amino acid's position in the final protein is determined by a succession of three **bases**, called a **codon** present on the **messenger RNA**.

The synthesis of a protein from a **nucleotide** sequence of the DNA, called a coding region or gene involves two main steps involving different types of RNA molecules. First, the information carried by a gene is copied into a complementary sequence of nucleotides called the messenger RNA (**transcription**). This latter is subsequently exported to the **cytoplasm**. Secondly, the messenger is read in sets of three nucleotide bases, the codons which are recognized by specific **transfer RNA** molecules. A codon determines a unique amino acid to be added to the growing protein by the transfer RNA. The different molecules involved in this step of translation are kept tightly associated by **ribosomes**, a complex of proteins and **ribosomal RNA** molecules.



to repair DNA damage, preventing the fixation of mutations that would occur through replication of a damaged DNA template. Furthermore, p21 also controls the G2 block by inhibiting the kinase activity of the cdk complex regulating the G2/M transition. Other genes known as p53-induced genes also participate in cell cycle control in response to radiation, e.g. those coding cyclin G, the so-called 14-3-3 proteins and *GADD45* protein (for Growth-Arrest-DNA Damage, a group of genes induced by DNA-damaging agents). Indeed, by binding to the active p53, the 14-3-3 proteins increase its transcriptional activity. The *GADD45* protein blocks DNA replication (S phase) and may also contribute to damage repair.

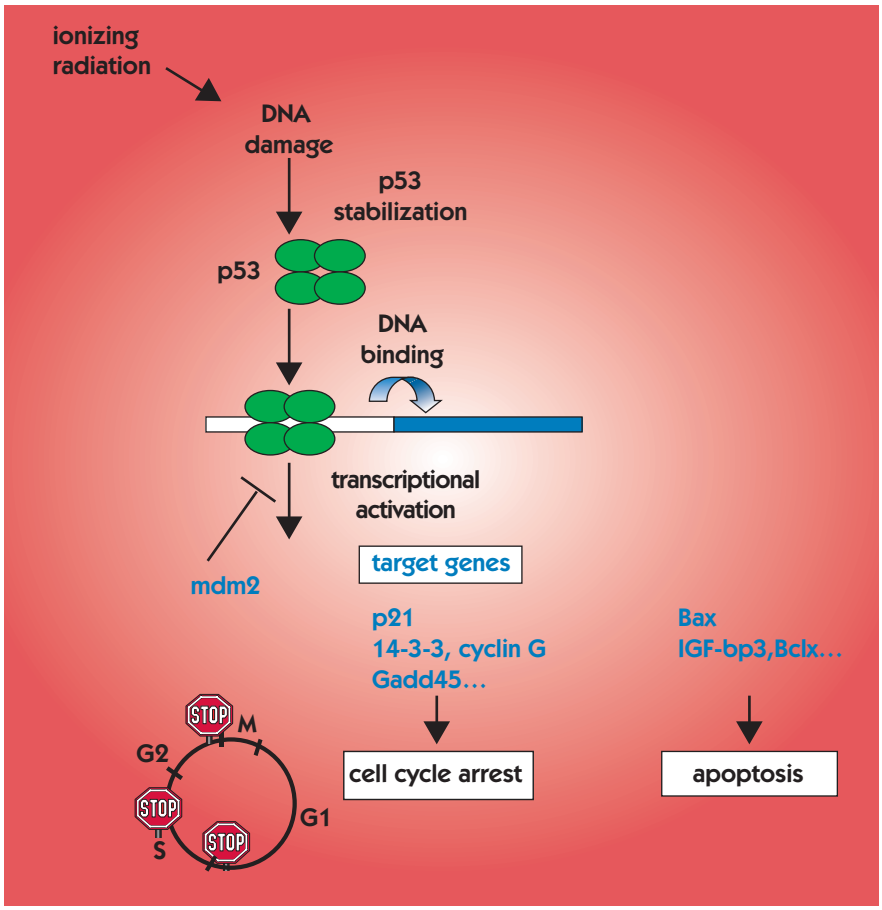
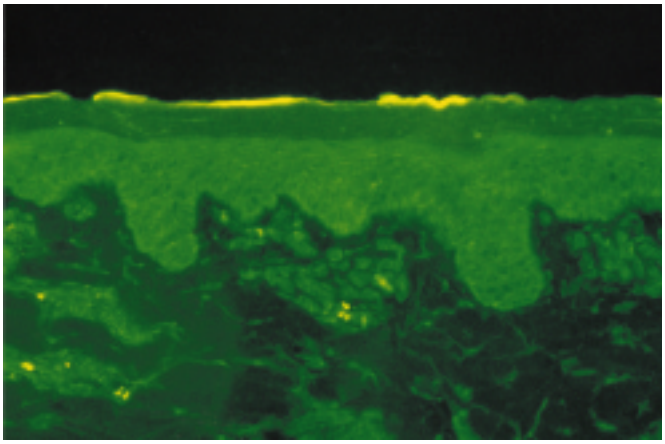


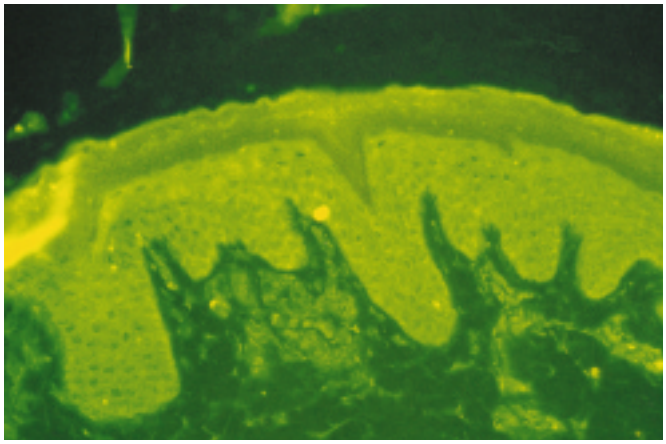
Figure 2. p53-induced genes in response to ionizing radiation in mammalian cells.

p53 and apoptosis

The p53 protein mediates apoptosis not only when excessive damage persists but also in other situations such as the activation of **oncogenes**. This is probably one of the reasons why tumor cells are frequently characterized by loss of functional p53. This defect would confer a selective advantage through the failure to undergo apoptosis (see *Cell suicide*). Mutations of this so-called **tumor suppressor** gene are found in more than 50% of cancers.

Once the damage is repaired, the «p53 signalling» has to be switched off, i.e., there is a negative feedback control of p53-inducible genes so that cells can resume the cell cycle. Indeed, there is a self-regulating mechanism of the p53 response. It implies the *MDM2* gene, another p53-inducible gene; by binding to p53, the mdm2 protein inhibits its transcriptional activity. This mechanism requires a third participant, the DNA-dependent protein kinase (DNA-PK), which plays a major role in signal transduction and repair of DNA double-





Immunofluorescence staining of TGF-β1 (Transforming Growth Factor-β1) in the irradiated skin of a pig. TGF-β1 staining (green color) is low in non-irradiated skin (left) whereas intense immunostaining is observed in irradiated skin (32 grays) at six hours after exposure (right).

M. Martin-LREG/CEA

strand breaks.

This enzyme phosphorylates the mdm2 protein, which becomes inactive. Once DNA is repaired, DNA-PK activity is switched off so that mdm2 can associate with p53 leading to p53 degradation.

Many experimental models provide evidence for a link between loss or deregulation of p53 function and radiosensitivity, chromosomal instability and appearance of cancers (see *Chromosomic instability*). However, it must be added that the mechanisms of growth arrest and apoptosis depend not only on p53 transcriptional activity but also on other processes involving a wide variety of genes. This illustrates the great complexity of the multiple and often redundant control mechanisms that mediate the response to radiation-induced damage. It must also be emphasized that the tight regulation and cooperation among these genetically controlled molecular pathways are crucially important for cell survival and genome stability.

Two representative genes in plants

The response to ionizing radiation is less well described in plants than in animals. No **transcription factors** of the p53 type activated by damaged DNA or genes expressed under the control of such a protein are known in plants. The Laboratory of Plant Radiobiology at CEA/Cadarache has accordingly been studying this response in *Arabidopsis thaliana*, among other plants, with the aim of identifying potentially associated radiation-induced functions for the detection and repair of DNA damage and cell division cycle regulation. *Arabidopsis* was chosen as a study model because it displays a number of features that facilitate laboratory molecular approaches (simple genome, rapid growth in culture, large number of seeds), and also because the tools for the functional analysis of the genome exist. Two radiation-induced genes in *Arabidopsis* are representative examples. The first, PARP-1 codes for a protein already identified in animals. The second, AtGR1, codes for a new protein with an unknown function, which has so far only been found in plants.

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***Arabidopsis thaliana*, a plant used as a model by scientists for genetic studies in plants.**

The PARP-1 gene, a survival factor

In animals the protein poly (ADP-ribose) polymerase 1 (PARP-1) is a protein of the nucleus that detects and binds to DNA breaks. After binding PARP switches from an inactive to an active state in which it synthesizes poly (ADP-ribose) polymers. The transfer of these to acceptor proteins turns the DNA damage into an alarm signal that activates either the repair of the DNA, or else programmed cell death if the cells are too badly damaged to be repaired. Hence researchers have assigned a role as a survival factor to PARP-1 protein, as it determines the fate of a cell after genotoxic stress. The PARP-1 protein in *Arabidopsis* is very closely similar to animal forms in terms of structure and activity. The different structural domains that compose this protein (DNA linkage domain, nuclear localization signal, associated protein interaction domain, catalytic domain) are conserved from plant through to man, just as the specific recognition of DNA breaks and the synthesis of polymers dependent on DNA damage. However, research results at CEA/Cadarache point to different mechanisms for the control of the activity of PARP in animals and plants. Whereas in animals PARP activity is regulated by the activation of the protein after binding to damaged DNA, in *Arabidopsis* ionizing radiation very

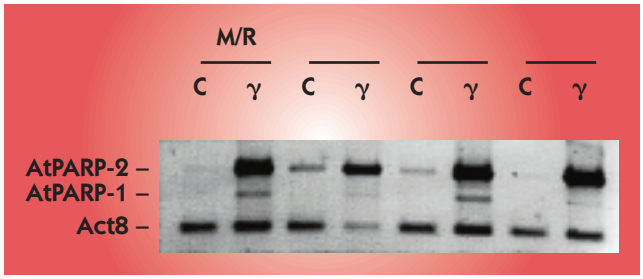

Figure 3. Demonstration of the accumulation of PARP-1 and PARP-2 messenger RNA in different tissues of Arabidopsis thaliana after irradiation with a cobalt-60 source. C corresponds to a non-stressed plant, γ to a plant treated with gamma rays. Act8 is the actin gene, used as control, because its expression, roughly consistent in all types of tissue, is not modified by ionizing radiation. (M/R is the cell fraction corresponding to the apical meristem and roots).

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Fusion of radiation-induced promotor AtGR1 with reporter gene GUS shows a close association between the level of the AtGR1 gene expression and the mitotic activity of the cells that compose the growing flower of a transgenic variety of Arabidopsis. The immature buds are deep blue; they then show preferential coloration of the germ cells, and at maturity a very weak coloration of the ovules.

strongly induces the level of expression of the PARP-1 gene, which justifies its classification among the radio-inducible genes. This presupposes some other mode of DNA damage recognition in plants. Also, the radiation-induced biosynthesis of the PARP-1 protein in *Arabidopsis* is preferentially detected in tissues composed of non-differentiated and (or) dividing cells, whereas the PARP-1 mRNA, which forms the template for the protein synthesis, accumulates in all the tissues analysed (Figure 3). Given that the protein synthesis is costly in energy for a cell, the selective accumulation of protein PARP-1 in certain types of cell suggests that, just as in animals, the plant PARP-1 activity is associated with keeping the genome intact during DNA **replication**. This does not concern the tissues composed of cells that have completed their differentiation process and are no longer dividing.

The AtGR1 gene, a cell division controller?

The AtGR1 gene (*Arabidopsis thaliana Gamma Responsive 1*) has been identified by one of our research groups. This gene is expressed at a basal level in mitotically dividing cells[1] and at a very high level in cells in their endoreduplication phase. Endoreduplication is characterized by the successive duplication of **genomic** DNA in the absence of chromosome separation and **cytokinesis**. Such a situation is close to a blockage of cell division just before **mitosis**. Ionizing radiation induces a massive accumulation of the **transcript**, followed by that of the protein, which is particularly evident in the **germ** cells (ovules, pollen), which are never enga-

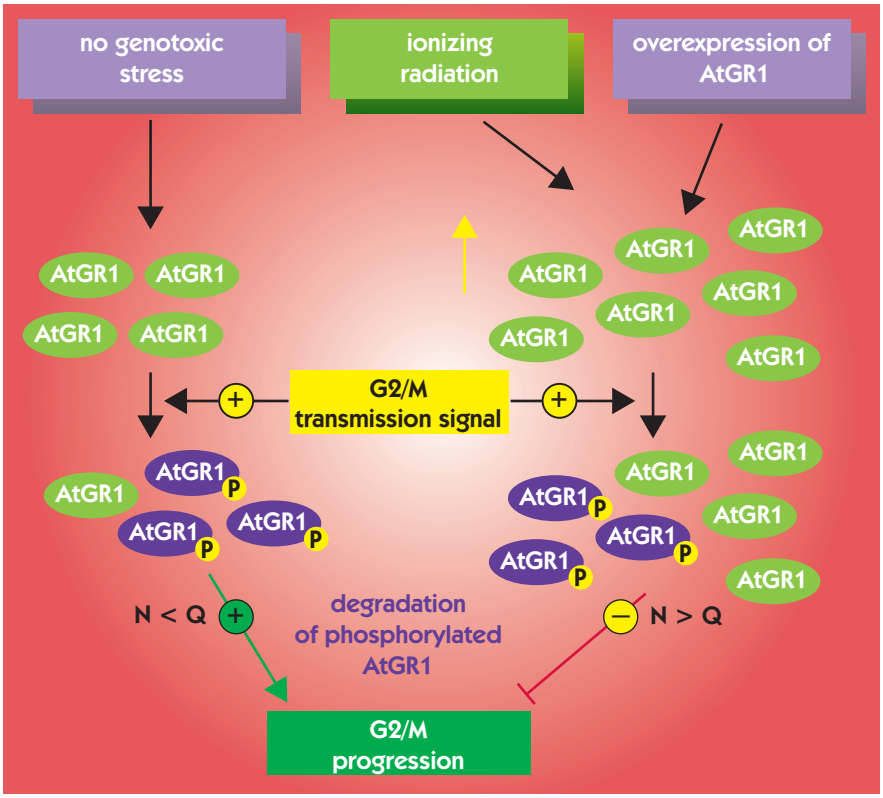
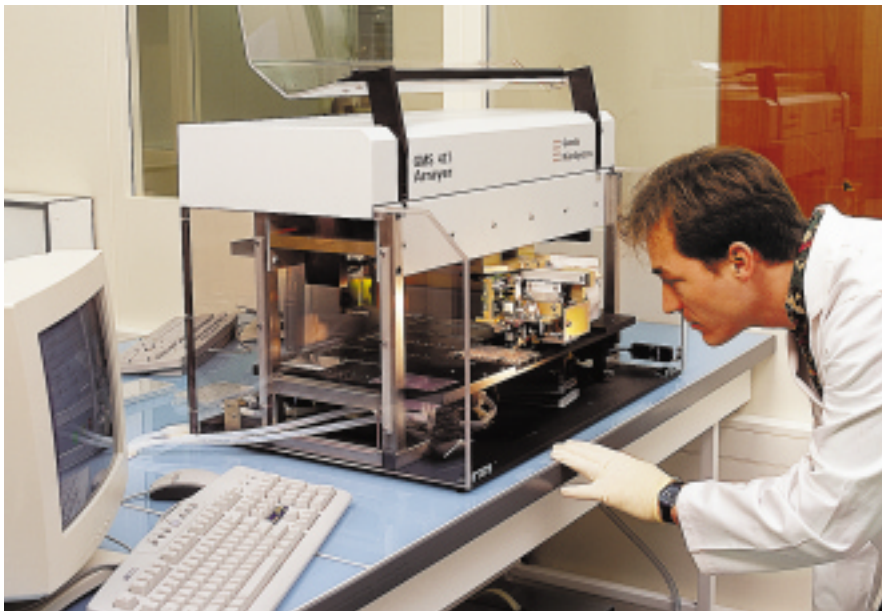


Figure 4. Model for the action of protein AtGR1. In the absence of genotoxic stress the quantity N of non-degraded protein (AtGR1) drops below a critical threshold Q equal to the quantity of protein degraded by phosphorylation ($AtGR1_P$) and the cell can progress from phase G2 of the cell division cycle toward mitosis. Under stress the temporary accumulation of protein AtGR1 correspondingly delays the onset of mitosis.



Ph. Pons/CEA

Automated preparation of DNA chips being tested by the CEA Department of Functional Genomics at Evry. The chips thus obtained can simultaneously analyze up to 15,552 genes, decisively speeding up research in radiobiology, in both animals and plants.

ged in an **endoreduplication** cycle. This suggests a functional link between the presence of damaged DNA and the need to temporarily stop the cell division cycle before mitosis, which is achieved at least in part by an elevation of the intracellular protein AtGR1 content. This hypothesis is supported by measurements of the quantity of protein AtGR1 during the division cycle. Its concentration drops at the onset of mitosis, suggesting that its momentary disappearance acts as a positive signal for the progression of the cell division cycle toward that phase. The radiation-induced biosynthesis of AtGR1 constitutes a temporary blockage of progression to mitosis. Results obtained with **transgenic** plants that overexpress this protein support this hypothesis: these plants are sterile owing to a random distribution of genetic material during **meiosis**. This observation indicates a definitive blockage of cell division due to the deregulation of the quantity of AtGR1 in the cell. The biochemical activity of protein AtGR1 is still unknown, but the Laboratory of Plant Radiobiology has proposed a model of its action (Figure 4). Its role may be that of cell cycle controller at the G2/M checkpoint after radiation stress. DNA damaged by ionizing radiation induces the accumulation of protein AtGR1, which temporarily delays progression to mitosis long enough for the DNA damage to be put right.

Plants catching up with animals

The extraordinary complexity of the radiation-induced functions cannot be summarized here. Radiation-induced functions independent of p53 would have to be discussed. Likewise the role of other **transcription activators** such as BRCA1 and NFκB - less thoroughly studied - in the «concertation» of the response to ionizing radiation. The availability of powerful new techniques that allow global evaluation of changes in the transcriptome and proteome in a particular type of cell, and their application in the field of radiobiology will very soon help completely decipher the functions induced by DNA damage(2). Among such techniques are DNA chips (see box in *Radio-induced genetic risk estimated*) and SAGE (*Serial Analysis of Gene Expression*), both developed at CEA. These global approaches will prove highly useful in plant radiobiology, and should help it shorten the lead taken by radiobiology in animals.

(1) Root points, apical meristem (small central part of the rosette containing embryonic cells from which derive the leaves, scape and flower bud), leaf primordia and inflorescences.
(2) Various tools allow the level of gene expression to be characterized according to cell type, environment, stress, etc. The **transcriptome** is the part of the **genome** whose expression takes place in a given cell type under specific experimental conditions. The **transcripts** are the messenger **RNA** molecules. The **proteome** defines the qualitative and quantitative features of **proteins** present under these conditions.

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