# EFFECTS OF RADIATION ON THE CELL DIVISION CYCLE
Using yeasts as models

Ever since their earthly beginnings, even the simplest living organisms have suffered many kinds of DNA damage from various sources. They therefore soon developed systems that would respond to lesions of their DNA resulting from these insults, in particular those due to radiation. The cell can carry out a «tour of inspection» at various stages in its division cycle, slowing down or even blocking its cycle while repairs are done, and then resume its division. These mechanisms have remained remarkably similar throughout evolutionary time. The relatively straightforward study of these mechanisms in yeast serves as a model for the study of the corresponding human systems. Knowledge of the DNA damage surveillance pathways has important applications in cancer research.

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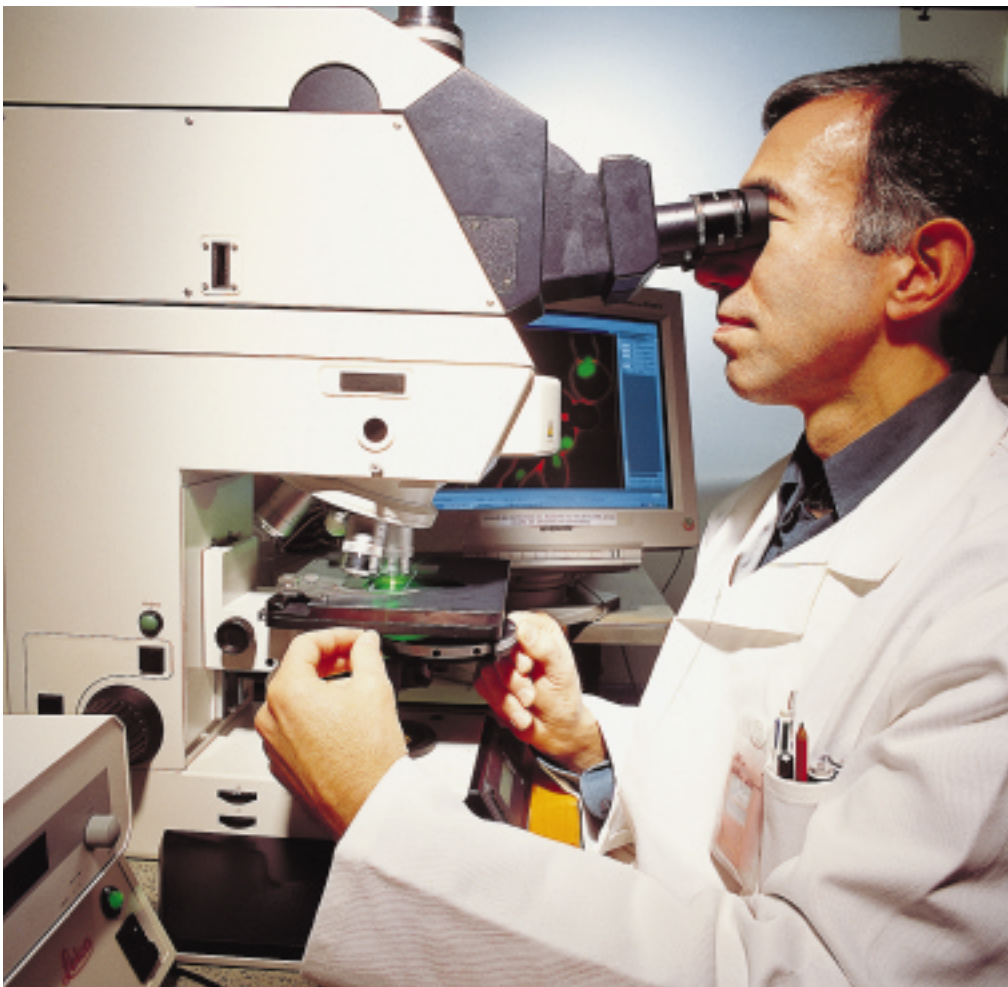
Localization by fluorescence microscopy of a RAD53-GFP hybrid protein expressed in yeast cells. The outlines of the cells appear in red on the screen of the image processing work station and the RAD53-GFP in the nucleus is in green.

The cell, the essential link

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The cell is the fundamental building block of all living organisms, and each one contains essentially all the information necessary for the construction of the entire organism. However, each type of cell expresses only a part of its genetic information corresponding to its «specialty» (in the case of a pancreatic cell, for instance, the part that enables it to make insulin).

In higher **eukaryotes**[1], which include mammals and therefore human beings, the cell is composed of the following main elements:

● The **nucleus**, which contains the genetic information of the cell (the **genes**) in the form of DNA (organized when appropriate in **chromosomes**). It is in the nucleus that **RNA** is transcribed, which ensures that the information inscribed in the DNA can be translated into the synthesis of proteins (boxes A, B and C, *DNA mole-*
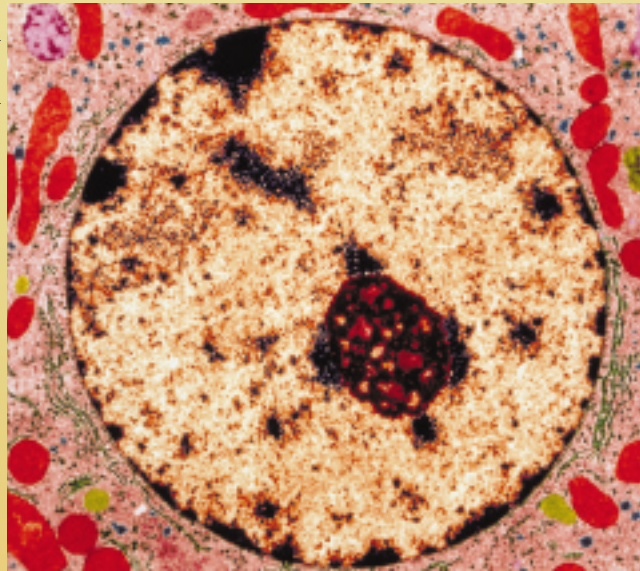
cule, heredity vector, Replication of DNA: near-perfect fidelity, and *Chromosomes, material supports for genes*).

View of a hepatocyte (liver cell) showing in particular the nucleus, a nucleolus and mitochondria (red), and the rough endoplasmic reticulum (green).

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(1) Living organisms composed of one or many cells that possess a distinct nucleus and cytoplasm. The eukaryotic lineage includes all form of life except for bacteria and some lower algae, which belong to the **prokaryotic** lineage, and viruses.

● The **cytoplasm**, composed of various elements bathed in a transparent substance that contains filaments and **microtubules** that make up the skeleton of the cell. This **cytoskeleton**, made up of protein fibers, gives the cell its shape and allows it to move.
● Microstructures with specific functions, called **cell organelles**, embedded in the cytoplasm. One of them, the **Golgi apparatus**, concentrates, sorts, conditions and excretes cellular protein products (**lipoproteins, enzymes,**

hormones). The **mitochondria**, which possess their own DNA, are the cell's power plants. They combine oxygen with fuel molecules to make **ATP** (adenosine triphosphate), an energy transporting molecule involved in a great number of the steps in cell metabolism. The **endoplasmic reticulum** is a tubular membrane network that transports proteins synthesized in the **ribosomes**, which are large particles made of RNA and proteins. The **lysosomes** are the cell's «stomach»; they use enzymes to destroy substances from outside or belonging to the cell itself. The **peroxyzomes** are specialized in the decomposition of hydrogen peroxide.
● The **cytoplasmic membrane** contains all the cytoplasm and the nucleus. It bounds the cell and isolates it while at the same time allowing exchange with the external medium by its permeability to water and certain substances dissolved in it, and by the presence of proteins that serve as «pumps» or «channels» for the transfer of certain molecules.

From micro-organisms to man

Radiation, whether non-ionizing, like ultraviolet light (UV) or **ionizing**, has been a natural cause of damage to the **DNA** of living organisms ever since the beginning of biological evolution. The damage caused to DNA by these physical agents ranges widely in nature (see *Radiation-induced damage to nucleic acids*), and includes modifications to bases, single-strand breaks, and double-strand breaks.

The **cells** must mend these lesions to prevent loss of the genetic information necessary for their survival. Both **prokaryotes** (cells without a nucleus) and **eukaryotes** (cells with a nucleus) possess highly elaborate systems to respond to DNA lesions. The first such system, the SOS system, was described in the **bacterium** *Escherichia coli* (a prokaryote) and it remains the best understood of the DNA damage surveillance systems. Its two main elements are LexA, a **transcriptional repressor** for **genes** induced in the event of DNA

damage, and RecA, an **enzyme** (co-protease) that becomes active if DNA is damaged and inactivates LexA, allowing the **transcription** of the genes it controls.

Analogous systems have been discovered in eukaryotic cells. Some of their features have been conserved extremely faithfully during evolution and are found today in both micro-organisms and man. Simple eukaryotes like the **yeasts** *Saccharomyces cerevisiae* (*S. cerevisiae*) or *Schizosaccharomyces pombe* (*S. pombe*) lend themselves much more readily to genetic and biochemical analysis than more highly organized ones such as mammals. Hence, yeasts are used as models for the study of the response of eukaryotic cells to DNA lesions. In what follows we focus on the response system of *S. cerevisiae*, referring to *S. pombe* and to mammals when these display marked differences from it.

The cellular response to DNA lesions

Two main cellular responses have been characterized when DNA is damaged: **activation of the transcription** of genes coding for proteins involved in DNA repair, and temporary blockage of the cell division cycle (box E, *The cell division cycle: under control duplication*). In animal cells there exists another response, apoptosis, or programmed cell death (see *Cell suicide*).

The treatment of DNA lesions involves several repair mechanisms that proceed by excision of **nucleotides**, by recombination, by photoreactivation or by excision of bases (see *The caretakers of the genome*). When lesions appear, the transcription of genes coding for certain DNA repair enzymes is activated. In addition, damaged DNA activates pathways that inhibit cell division at precisely defined stages of the cell division cycle, namely in the G1, S or G2/M phases (Figure 1), according to where the cells are in their cycle when the damage is done. The mechanisms that allow these different halts in the division process have common features, in particular protein sensors that locate damaged DNA, but they differ in the targets of inhibition within the cell divi-

sion machinery. The most fully characterized response for yeast cell division is blockage at G2/M.

Inhibition of cell division plays a very important role in the resistance of cells to **genotoxic** stress. It stops the **replication** of DNA and the **segregation** of **chromosomes** (box B, *Replication of DNA: near-perfect fidelity*) until the lesions are completely repaired. The repair processes can thus operate for several hours until completion, without being interrupted by **mitosis**. Once the damage is fully repaired, the cells can resume their division cycle without any impairment of their viability (Figure 2B). By contrast, **mutant** cells that are unable to block their cycle if their DNA is damaged are extremely sensitive to radiation because they undergo mitosis with chromosome fragments that are imperfectly segregated during cell division (Figure 2D).
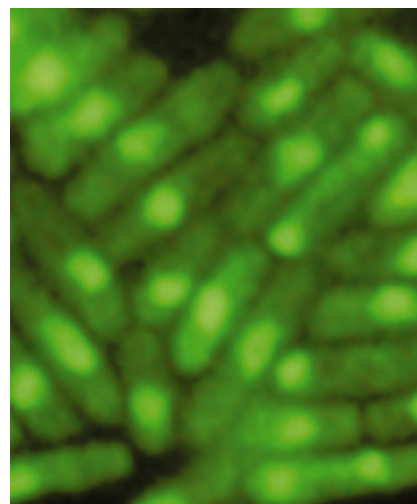

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Cells of Schizosaccharomyces pombe during division. This yeast, together with Saccharomyces cerevisiae, is one of the organisms that lend themselves most readily to genetic and biochemical analysis, making them suitable models for the study of the response of eukaryotic cells to DNA lesions.

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The cell division cycle: under control duplication

The life of a cell comprises several distinct phases, the most important of which is **mitosis**, the process of cell division. These phases are conventionally represented by a circle, each «revolution» corresponding to the appearance of two identical daughter cells derived from the division of the mother cell (diagram).

and replication of DNA, called S for synthesis, which culminates after a variable time (a few minutes to a few hours) in the **duplication** of the DNA content of the cell, which goes from n to $2\,n$. After the next step, called post-synthesis (G2) during which the cell makes sure the replication of its DNA is completed, it can begin mitosis (M), a process that
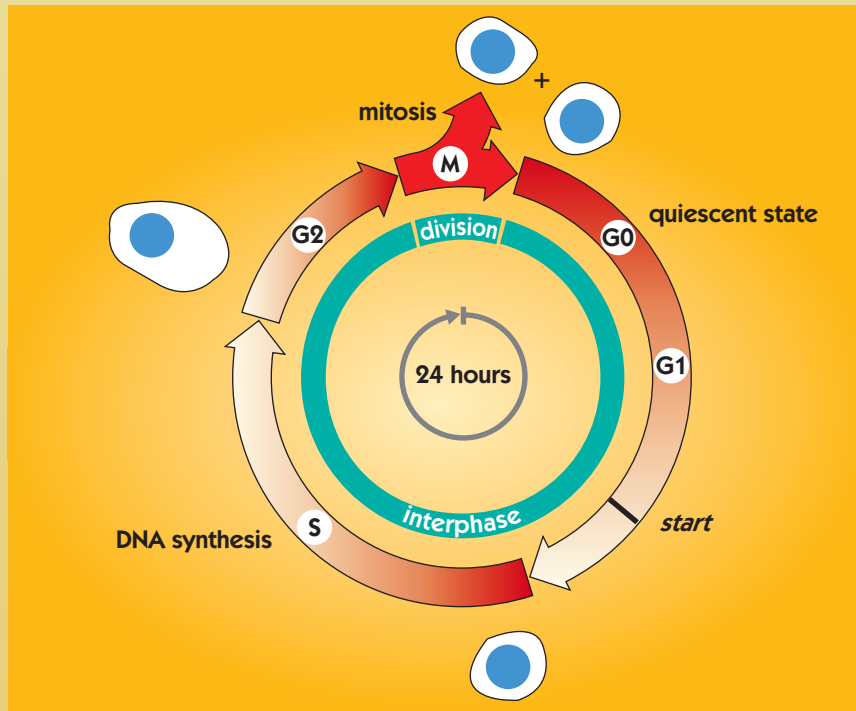

Cell division cycle of a standard eukaryotic cell (from Biologie moléculaire de la cellule, Flammarion ed.).

The typical cell division cycle takes about twenty-four hours in higher **eukaryotes**. The life of the cell is «paced» by phases of mitosis separated by a longer phase called the **interphase**. This interphase starts with a stage during which the cell carries out its «ordinary» functions without replication of **DNA**. It makes **proteins** and substances necessary for its growth or for its assigned work using chemical energy that it can store temporarily. The interphase starts with a presynthesis phase called G1, at the end of which the actual division cycle begins (Start). In the interphase the cell can enter a **quiescent** phase (G0) where it can remain for days or even years before resuming its division cycle. Soon after the Start point begins the crucial phase of synthesis

breaks down into five steps:
● The **prophase**, marked by the condensation of the chromatin dispersed in the nucleus into $2\,n$ separate **chromosomes**. Each chromosome is made up of two sister **chromatids** connected at a point called the **centromere**. The **mitotic spindle**, a bipolar structure made up of microtubules and associated proteins, begins to form.
● The **prometaphase**, which includes in particular the break-up of the nuclear envelope.
● The **metaphase**, characterized by the assembly of the chromosomes in a zone, the «equatorial plate», equidistant from the two poles of the mitotic spindle.
● The **anaphase**, in which the two chromosome sets separate (**segregation**)

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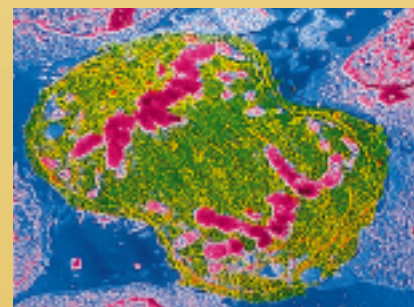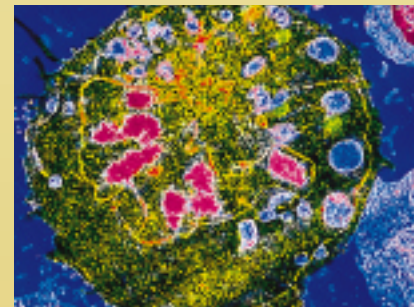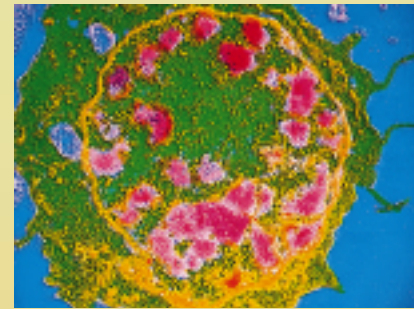
The successive phases in the mitosis of a human cell (lymphocyte) observed by transmission electron microscopy: prophase, prometaphase, metaphase, anaphase, telophase.

in a few minutes after cleavage of the centromeres, enabling each chromatid to move toward one of the poles.

● The **telophase**, marked by the individualization of the two daughter cell nuclei, a nuclear boundary membrane forming around the individual chromosomes, which decondense. The telophase is followed by **cytodieresis** (or **cytokinesis**), which ends in complete separation of the two daughter cells.

A particular mode of cell division is involved in the genesis of the sex cells, or **gametes** (ova or spermatozoa) each of which, on fecundation, supplies one half (*n*) of the new chromosomes. Called **meiosis**, this process produces, by two divisions instead of one after the **replication** of DNA, a halving (2 *n* to *n*) of the number of chromosomes in each daughter cell. The gametes, which possess only half the number of chromosomes characteristic of the species, are for that reason termed **haploid**, as

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Ovocytes of a marine mollusc preparing for meiosis.

opposed to **diploid**, which designates a cell possessing two sets of homologous chromosomes. The **somatic** cells, which make up the organism, are diploid, and divide by mitosis to produce two new cells that are also diploid. Only the **germ** cells undergo meiosis, generating four haploid cells, the gametes.
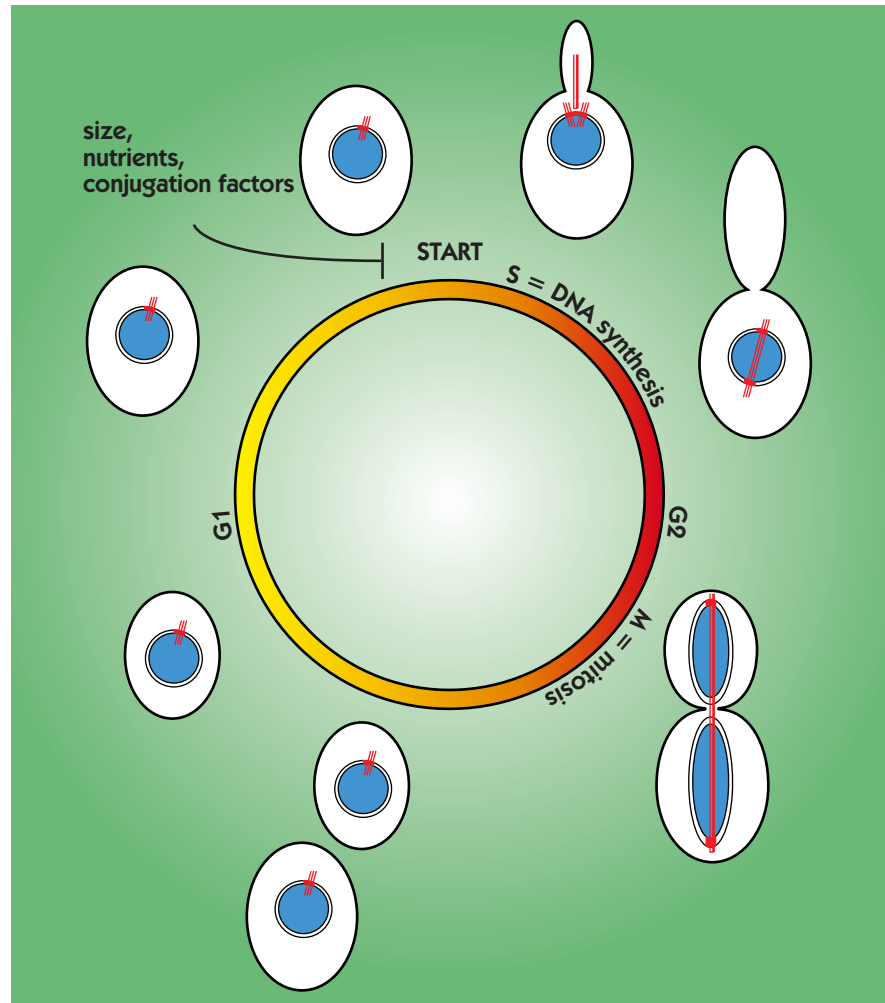

Division cycle control pathways

The mobilization of responses to DNA damage depends on cell-cycle **control pathways**, or «checkpoints» (Figure 2D). These allow a pause in the cycle for the time required so that the cells can repair their DNA before chromosome segregation occurs. In general, cell division control pathways are mechanisms that subordinate the execution of a cellular event to the prior completion of another event. There are checkpoints that require prior replication of the DNA and total absence of damage before any segregation of the chromosomes can take place, and others that will suspend the separation of the sister **chromatids** unless the **mitotic spindle** is correctly organized. As we will now see, the molecular mechanisms of the checkpoints that act when DNA is damaged are complex.

Figure 1. The cell division cycle of S. cerevisiae. The cells pass the Start point when certain conditions are met. This irreversible entry into the cell division cycle triggers three types of event: the replication of chromosomes, the duplication of the microtubule organizing center (red squares in the nuclear membrane) for the assembly of the mitotic spindle (red lines), and the formation of the bud. When DNA synthesis is finished the cells move on to the G2 phase, and then begin mitosis, the two daughter cells entering G1 phase. Damage to DNA causes the blockage of the cells in G1, S or G2/M phases.

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Figure 2. Response to irradiation of wild cells (B) and cells mutated in genes involved in DNA repair (C) or in checkpoints (D). After irradiation the wild-type cells block their division cycle until the damage is repaired. The cells with mutations in their repair genes detect the lesions and halt their division. However, being unable to repair the lesions they die. The cells with mutations in their checkpoint genes do not stop their division and segregate their chromosomes before they have time to repair their DNA. The damage, now permanent, kills the cell after a few divisions.

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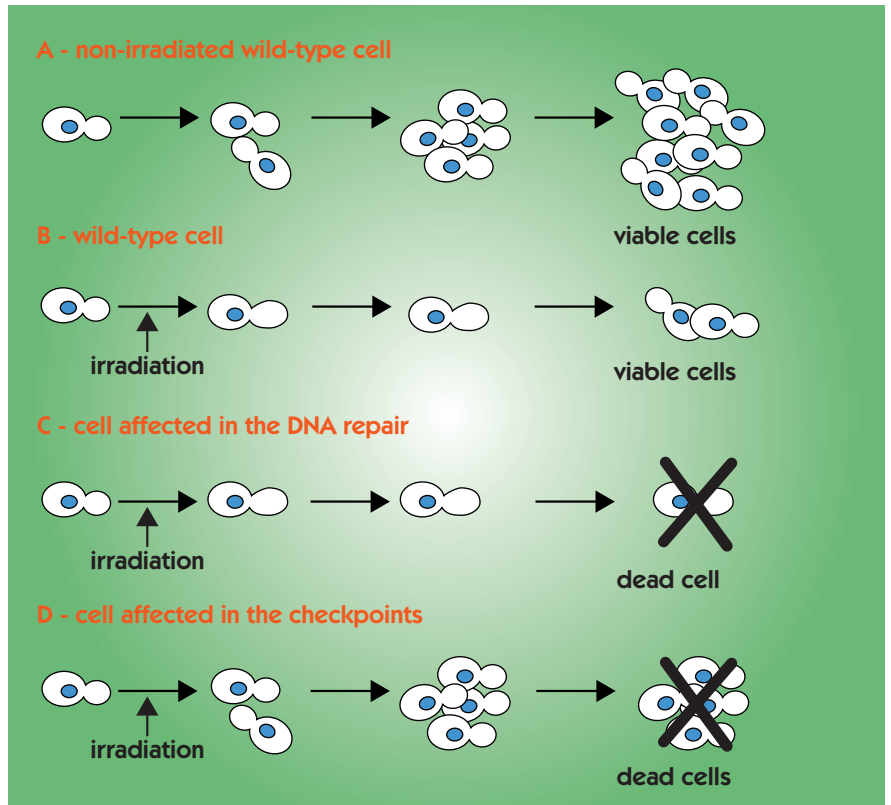
Molecular mechanisms of the checkpoint control pathways

The genes involved in the checkpoints have been identified by screening for mutants that are hypersensitive to genotoxic stress. Genetic and biochemical analysis can then indicate the order in which the gene products are involved, so that the molecular mechanisms are now known with some precision. The control pathway that causes the G2/M block in *S. cerevisiae* in response to DNA damage is one of the best characterized to date. Like any signal transmission pathway, this checkpoint comprises sensor elements, transmitter elements, and effector elements[1] (Figure 3).

The sensors give the signal

DNA lesions promptly undergo various enzymatic modifications, so that it is difficult to know whether the lesions are themselves the signal received by the checkpoint, or whether this signal is composed by lesions modified by repair complexes. Some data suggests that just as in bacterial systems, the presence of single-strand DNA is the trigger. Even

so, the products of four genes, *RAD9, RAD17, RAD24* and *MEC3* have properties compatible with the functions of sensors or lesion modifiers. In particular, the structure of Rad17 is close to that of the exonucleases[2] and Rad24 is homologous to a replication factor that binds to the DNA of the **replication fork**. These four genes form two groups: *RAD9* on the one hand, and *RAD17, RAD24* and *MEC3* on the other hand, act in parallel and additively to trigger the checkpoint.

The transmitter elements pass on the message

Two genes are essential for the transmission of the signal: these are *RAD53* and *MEC1*. All the responses to damaged DNA, both transcriptional and cellular, depend on the functional properties of their gene products. Mec1, although it belongs structurally to the «superfamily» of the lipid **kinases**, is probably a protein kinase. The *TEL1* gene, required for the maintenance of **telomere** lengths, is a homolog of *MEC1* in the genome of *S. cerevisiae*. Its deletion confers no hypersensitivity to genotoxic stress in a **wild**-type strain, but increases the sensitivity of a strain already mutant

for the *MEC1* gene. Tel1 thus seems to have a function that is more specific to the telomeres, but subsidiary for the checkpoints. Several functional homologs of *MEC1* and *TEL1* are known in other eukaryotes: the Rad3 gene of *S. pombe* and the human genes ATM and ATR, for example. ATM is the gene whose mutation is responsible for the syndrome called *ataxia telangiectasia*. The cells with this gene mutation are hypersensitive to ionizing radiation, because they are unable to block their division cycle after radiation damage, like the *mec1* mutants of *S. cerevisiae*. Patients with this syndrome also present an elevated incidence of cancer. ATR (ATM-related) is a gene that displays homologies with ATM. It plays a role in **meiotic** recombination, but what function it may have in response to DNA

(1) In what follows the genes of *S. cerevisiae* are written in upper case italics (*RAD53*), the mutants in lower case italics (*rad53*) and the corresponding proteins in roman type with a capital initial (Rad53). This convention differs from those used for other organisms, e.g., *S. pombe*.
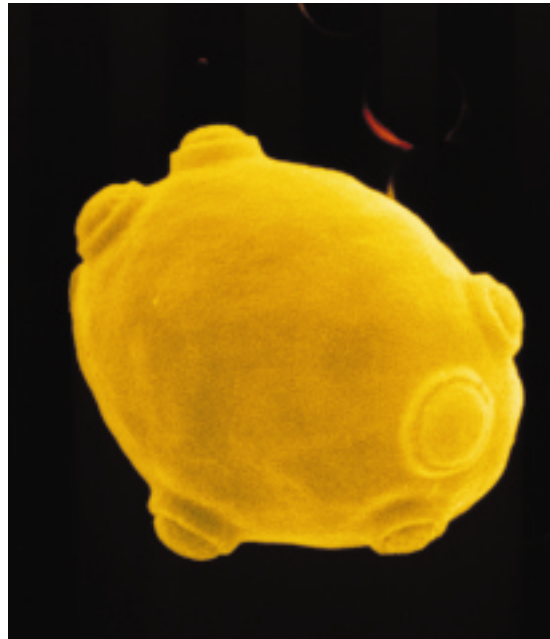(2) Enzyme that degrades DNA from its ends inwards.

damage is not yet clear. *RAD53* codes for a protein kinase, and its human homolog, hCHK2, has recently been shown to be implicated in the Li-Fraumeni multiple cancer syndrome. Rad53 is a protein kinase activated by **phosphorylation** when DNA is damaged. This phosphorylation depends on Rad9 and Mec1, indicating that Rad53 acts downstream of these **proteins**.

However, *rad53* mutants are much less sensitive to irradiation than *mec1* mutants, suggesting that Mec1 acts on other proteins independently of Rad53.

The effectors block the cycle

Finally, the effectors responsible for halting the division cycle in the event of DNA damage are not definitely identified in *S. cerevisiae*. A probable target is Pds1, a protein involved in the association of the sister chromatids during mitosis, and whose gene mutation abolishes the G2/M block after gamma ray irradiation. By contrast, in *S. pombe* and in mammals, the blockage of the cell division cycle at G2 in response to DNA damage is via the inhibition of the cycline-dependent kinase (Cdk) governing the onset of mitosis[3]. The phosphatase Cdc25, a protein that hydrolyzes phosphate, is necessary for the activation of the Cdk Cdc2. After irradiation, the Rad3 kinase of *S. pombe* activates

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Chk1 kinase by phosphorylation, which in turn phosphorylates the Cdc25 phosphatase. This phosphorylation brings about the sequestration of Cdc25 by proteins of type 14-3-3, which prevents the activation of Cdc2. The same mechanism is also found in mice and in humans.

The response of eukaryotic cells to DNA lesions shares many similarities in simple eukaryotes such as yeasts and in higher eukaryotes. The table above gives a list of homologous genes in *S. cerevisiae, S. pombe and Homo sapiens*, whose products function in the DNA integrity checkpoints. There are few

Saccharomyces cerevisiae viewed by scanning electron microscopy. The checkpoint pathway that causes the blockage of the division cycle of this yeast in response to DNA damage is one of the best known.

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Identification of the **Rad53** *protein after acrylamide gel electrophoresis. In the absence of damage to DNA the protein migrates in a single band. If lesions are caused by UV radiation then* **Rad53** *is phosphorylated, which modifies its electrophoretic mobility and causes several additional bands to appear. (A: phosphorylated* **Rad53**, *B: non-phosphorylated* **Rad53***).*
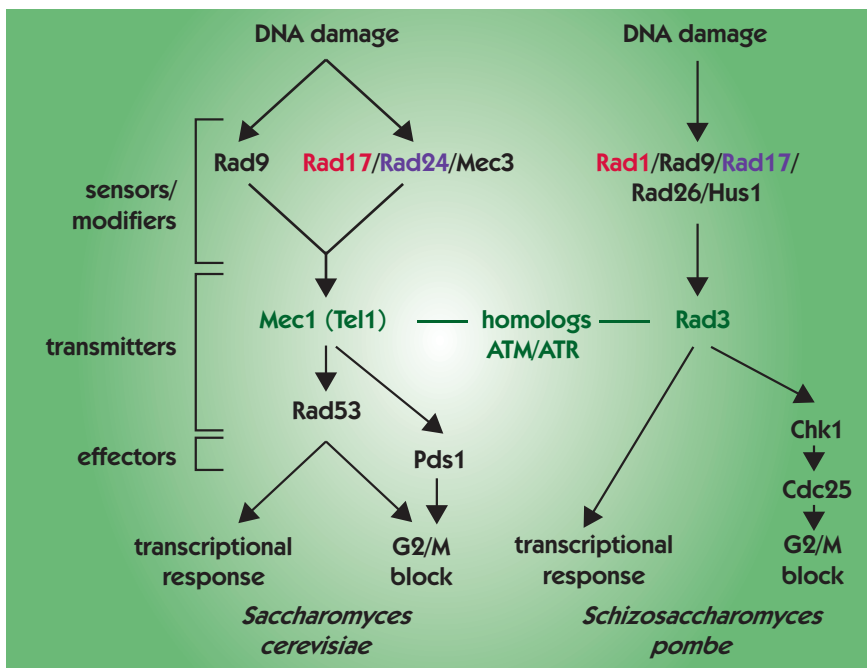
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*Figure 3. Response pathways to DNA damage in S. cerevisiae (G2/M) and in S. pombe (G2). The elements of the homologous gene pairs RAD17/*Rad1, *RAD24/*Rad17, *MEC1(TEL1)/*Rad3 *are indicated by the same colors.*

function	S. cerevisiae	S. pombe	Homo sapiens	biochemical characteristics	
sensors/ modifiers	RAD9 RAD17 RAD24	Rhp9 Rad1 Rad17 Hus1 Rad9	EST(a) EST EST hRAD9	BRCT(b) domains probable nuclease(c) homology with replication factor(d) C ? ?	(a) EST stands for Expressed Sequence Tag (fragment of a gene expressed in humans). (b) The BRCT domains are similar to the C-terminal domain (carboxylic -COO⁻ end) of human protein BRCA1 whose mutation is implicated in certain breast cancers. (c) Nucleases cleave DNA chains. (d) Replication factors are involved along with polymerases synthesizing DNA. (e) Protein kinases transfer phosphate groups onto amino acids of certain target proteins, which regulates their activity. (f) 14-3-3 type proteins recognize and bind certain phosphorylated protein motifs.
transmitters	MEC1 TEL1 RAD53	Rad3 Rad3 Cds1	ATR ATM hCHK2	protein kinase(e) protein kinase protein kinase	
effectors	CHK1 BMH1/2	Chk1 Rad24/25	hCHK1 gene of type 14-3-3	kinase of Cdc25 protein type 14-3-3(f)	

Table. Some homologous genes implicated in the checkpoints in S. cerevisiae, S. pombe and Homo sapiens (the genes of S. pombe and H. sapiens are written according to current typographical conventions).

checkpoint genes in *S. cerevisiae* and *S. pombe* that do not possess a human homolog, which justifies using these yeasts as model systems, even though it is clear that certain pathways such as apoptosis are specific to animal cells, and so cannot properly be studied in these organisms.

whose sensitivity to some genotoxins is comparable to that of certain cancer cell lines. As yeasts are easier to handle than **cell lines** of higher eukaryotes, they can serve as single-cell «guinea pigs» in the search for such drugs. ●

From control pathways to anti-cancer agents

Besides the scientific interest of knowing how eukaryotic cells respond to radiation, the study of checkpoints in yeast can lead to important therapeutic applications, in particular in the fight against cancer. Cancer cells by their very nature contain defects in their cell division control. The inactivation of checkpoint pathways allows the rapid accumulation of the genetic modifications necessary for the formation of tumors and may be one of the early events in cancer development. This characteristic is also the great weakness of cancer cells, because the absence of checkpoints makes them more sensitive than healthy cells to genotoxic treatments under certain conditions. These checkpoint defects are due to mutations in genes that often have homologs in *S. cerevisiae*. Some American laboratories are currently developing anti-cancer drug screening programs using repair or checkpoint mutants of *S. cerevisiae*,

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(3) The two key components of the cell division cycle control system are cycline-dependent kinase and cycline, without which the former is inactive. The complex formed acts as a protein kinase to trigger events of the cell division cycle.