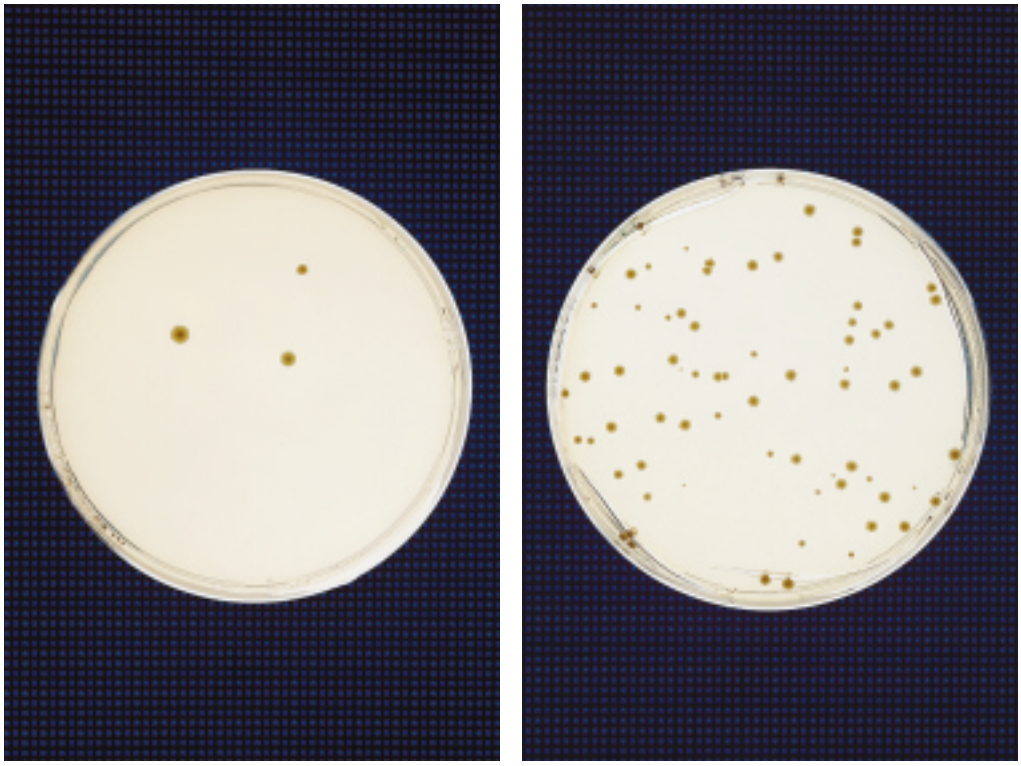
THE CARETAKERS OF THE GENOME

Repair of DNA lesions induced by ultraviolet-light and ionizing radiation

Every day the DNA contained in the nucleus of each of our cells suffers thousands of lesions caused by agents that are potentially able to modify the genetic information in it. These agents include ultraviolet light from the sun, and ionizing radiation, both natural and man-made. Yet this information remains astonishingly stable. Veritable «caretakers of the genome» repair DNA efficiently and faithfully, provided the cell is not overwhelmed by the initial injury. The importance of these repair mechanisms is such that any impairment in their function results in very severe syndromes. The failure of one of these genomic caretakers, the gene OGG1, seems to be implicated in the formation of cancer. This may offer a means of screening for predisposition to radio-induced cancer, or cancer caused by other genotoxic agents.



A. Gonin/CEA

***OGG1* gene inactivation experiment in baker's yeast. In each dish ten million cells have been spread on a nutrient medium containing an antibiotic that kills the cells. Dish A (left) is a reference yeast. Dish B (right) is a yeast in which the *OGG1* gene has been inactivated. Three colonies, each representing the lineage of one cell, have grown in dish A, indicating that three (mutant) cells were resistant to the antibiotic. Sixty-four colonies were counted in dish B, showing that the inactivation of the *OGG1* gene has resulted in a 21-fold increase in the mutation rate (64/3).**

Necessity and mechanisms of DNA repair

DNA suffers numerous unavoidable injuries inflicted by the intracellular medium or by the environment. These injuries result in the formation of several thousand lesions per day per **cell**. Reactive oxygen species produced by the cells during respiratory utilization of oxygen, and possibly also by the **radiolysis** of water, are liable to generate DNA lesions. Sunlight is partly composed of ultraviolet radiation, which adversely affects DNA either directly (UVB) or indirectly (UVA) after excitation of receptor molecules normally present in the cell. DNA is also highly sensitive to **ionizing radiation**, both naturally-occurring (terrestrial radioactivity and cosmic rays), and generated by human activity (nuclear medicine and, to a small degree, energy production).

The mechanisms of formation and the nature of the lesions induced in DNA have been described in Part I. The fact that DNA is so readily damaged may seem surprising given the observed stability of the genetic information passed down from one generation to the next. Studies of **mutation** rate suggest an instability of less than one error per ten thousand million **nucleotides** copied, i.e., less than one error per generation in a human **genome**.

Excision and resynthesis

Evolution has solved this paradox by setting up efficient and accurate mechanisms to repair DNA. The presence of lesions induced in DNA by solar ultraviolet or ionizing radiation is a major problem for the cell, because it can lead either to the death of the cell, or an alteration of the genetic message (mutation). Rebuilding by *excision* and *resynthesis* is the main repair pathway in all living organisms. It consists in removing a damaged segment (excision) and recopying the initial information (resynthesis). This mechanism implies the existence of «scouts» that detect the DNA lesions and «caretakers» that ensure the removal of the damaged part and its exact reconstruction (Figure 1). These roles are played in the cell by the DNA repair **proteins**. These are enzymes able to act before DNA **replication** in the cell (box B, ***Replication of DNA: near-perfect fidelity***).

The DNA thus repaired by excision and resynthesis is exactly as it was before it was damaged. This is therefore a conservative mechanism, which tends to preserve the genetic information from one generation to the next. The existence and the efficacy of these molecular mechanisms explain how cellular DNA can preserve the integrity of the genetic message in time. It is important to note that the DNA repair mechanisms are very similar throughout all living organisms, from **bacteria**, the simplest ones, to humans. This similarity means that these mechanisms must have evolved very early on, and underlines how vital they are in a living world that uses DNA to store genetic information.

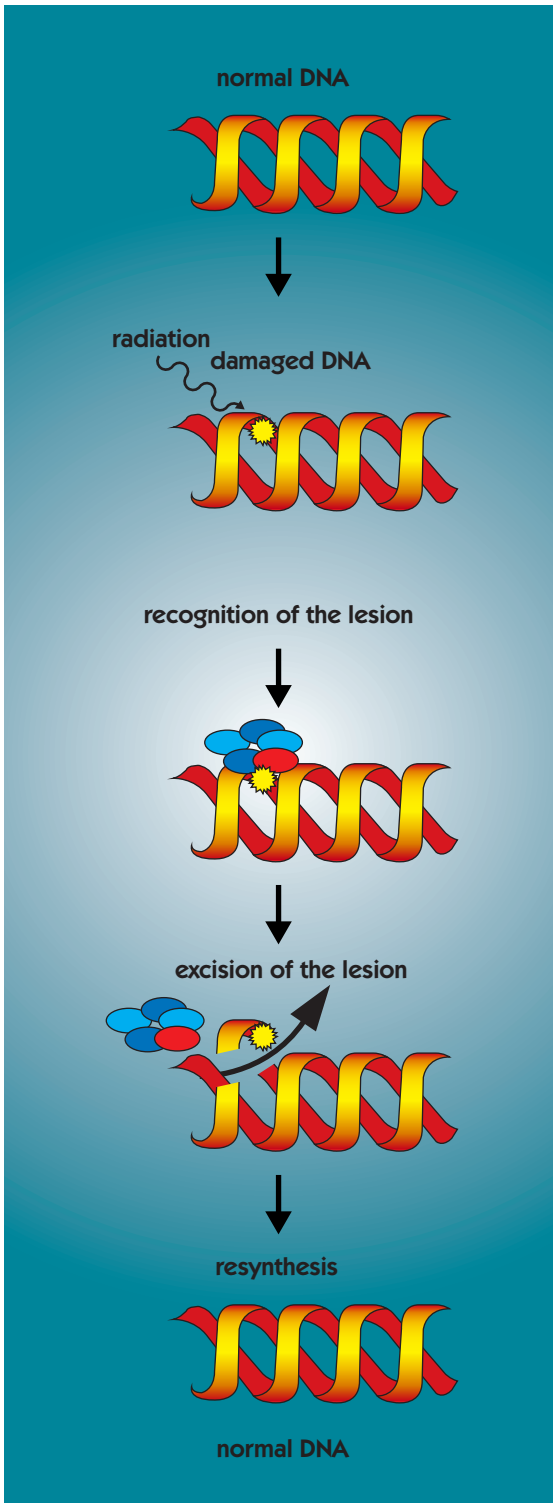


Figure 1. Repair of DNA by excision and resynthesis. The DNA repair enzymes «work» by excising, i.e., cutting out a segment containing a lesion, after prior recognition, from one of the DNA strands. To rebuild the removed fragment as it was before it was damaged, a sequence complementary to the one opposite it on the other DNA strand is synthesized.

DNA repair and genetic diseases

The importance of the mechanisms of DNA repair in humans is demonstrated by the study of various genetic diseases such as *xeroderma pigmentosum*, *Cockayne's syndrome*, and *ataxia telengiectasia*. These are rare **recessive autosomal** diseases, characterized clinically by three types of serious disorder: the early appearance of cancer, mental retardation, and retarded physical development. They are extremely severe insofar as those affected mostly die before they reach adulthood. Two of these diseases, *xeroderma pigmentosum* and *Cockayne's syndrome* are the direct consequence of a deficiency in the excision-resynthesis repair pathway for DNA lesions induced by UVB rays in sunlight and by numerous cancer-causing chemical agents. The early development of cancer in patients with *xeroderma pigmentosum* results from mutations caused by the accumulation of unrepaired lesions in DNA. These mutations affect the functioning of certain **genes** and lend the **mutant** cells properties of abnormal proliferation. This uncontrolled proliferation can sometimes give rise to a malignant tumor (box 1). In patients with *ataxia telengiectasia* cells are hypersensitive to ionizing radiation. The molecular defect responsible for this disorder has been identified as mutations in the ATM gene. This gene does not code directly for a DNA repair protein, but is involved in a complex pathway that enhances the efficacy of the mechanisms of repair of DNA lesions caused by ionizing radiation. The importance of the DNA repair mechanisms has also been demonstrated in mice in which certain genes can be inactivated experimentally. Recent studies have shown that inactivation of *HAP1* and *POLβ* genes involved in the excision-resynthesis repair pathway causes early death of the embryo in their host. Importantly, these genes have equivalents in human cells.

These different observations confirm that DNA repair is necessary for the survival of organisms, in particular mammals. Cancer predisposition in persons carrying mutated DNA repair genes also suggests that the repair mechanisms play

DNA repair and cancer formation

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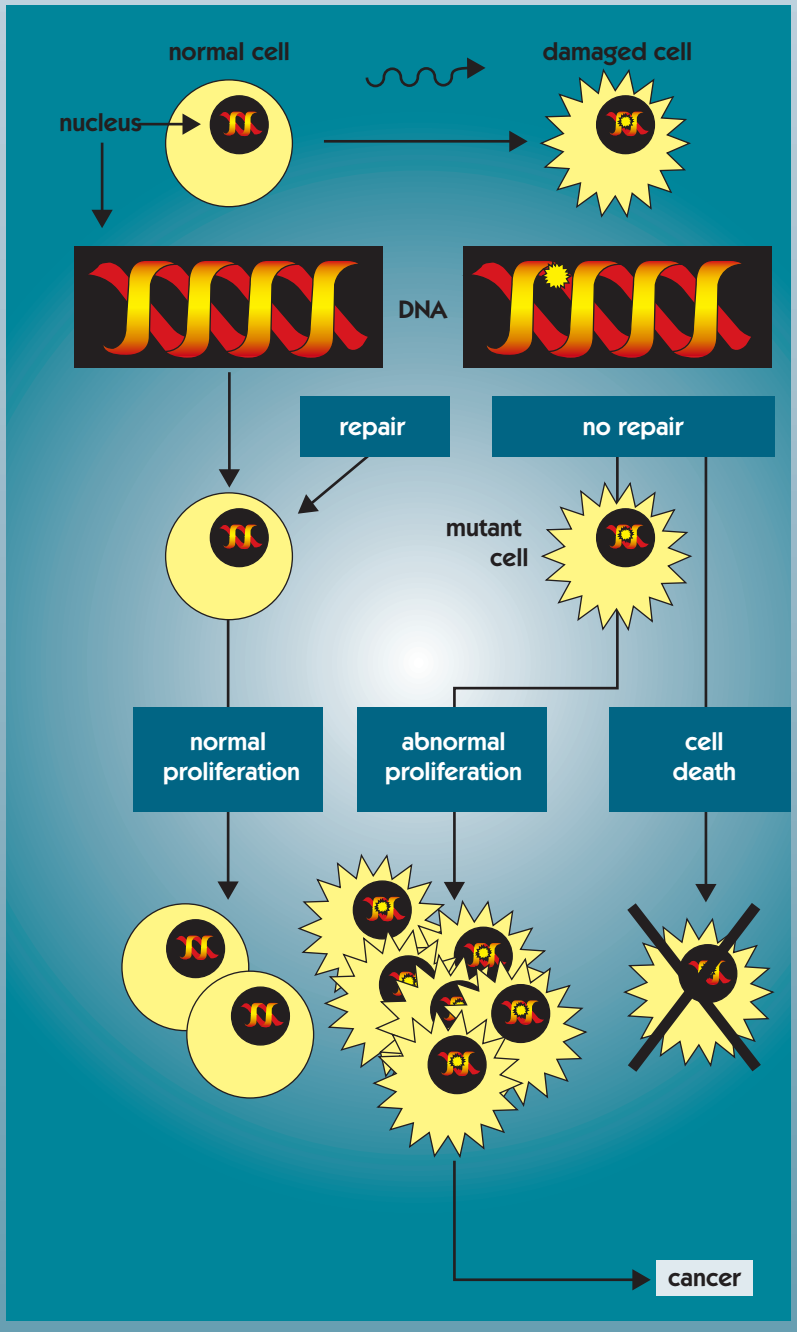
A cell containing damaged DNA can have two possible fates. In most cases the DNA undergoes full repair and the genetic program can run normally. When repair is not carried out or is incomplete the cell dies or mutates, depending on the nature of the lesions. Mutations in the genes that govern the cell division cycle (box E, ***The cell division cycle: under control duplication***) will lend the cells the property of proliferating in an uncontrolled manner, ending in cancer. This diagram illustrates how DNA repair helps prevent the occurrence of cancer in humans by removing the lesions induced by radiation or chemical agents. This preventive action against alterations in the genetic message underpins the concept of «caretaker of the genome».

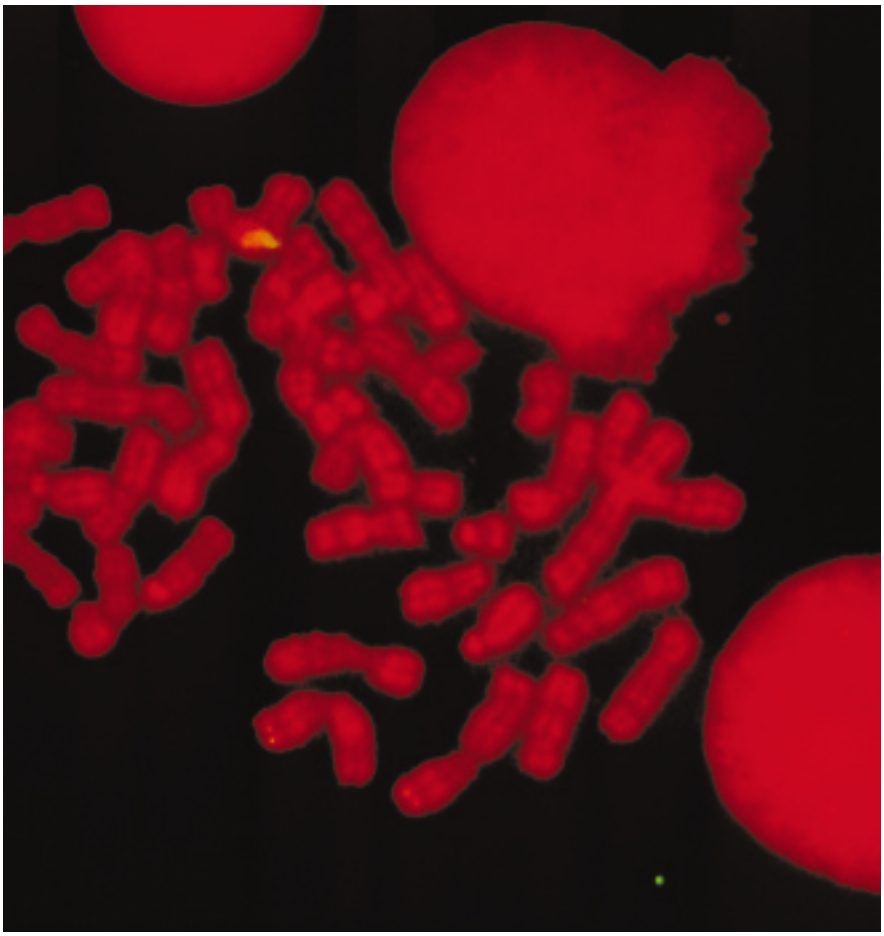


an essential role in the prevention of the process of **carcinogenesis**. In patients with xeroderma pigmentosum the time by which tumors appear is shortened by several decades compared with the normal population. This suggests the term «caretakers of the genome» to describe the DNA repair proteins that ensure the stability of the genetic information and thus delay the appearance of cancer in humans (box 1).

The *OGG1* gene, a caretaker of the genome

The genes directly involved in DNA repair in mammals may be a few dozen in number. For excision-resynthesis the diversity resides mainly in the initial lesion recognition step. It is noteworthy that despite the extraordinarily wide variety of lesions generated in DNA the cell uses only a small number of repair proteins. In fact most of them are multi-role, and recognize several types of lesion.

The research conducted by a joint CEA-CNRS (French Atomic Energy Commission-National Scientific Research Center) team focuses on the repair and the biological impact of a radio-induced DNA lesion that is repaired by excision and resynthesis, 8-oxo-7,8-dihydroguanine (8-oxoGua). This modification of DNA causes mutations that ultimately lead to the replacement of a **guanine-cytosine** pair by a **thymine-adenine** pair. This research team has isolated the *OGG1* gene that codes for the protein that recognizes and excises this lesion in baker's **yeast**, *Saccharomyces cerevisiae*. The inactivation of the *OGG1* gene in this yeast causes an increase in the genetic instability characterized by a spontaneous mutation rate 20 to 100 times higher than that observed in a baker's yeast possessing this DNA repair function. This instability is seen, for example, in the appearance within a population of an elevated number mutant cells that are resistant to an antibiotic.



CEA-CNRS

The four little fluorescent spots indicate the location of the human OGG1 gene at the end of the short arm of chromosome 3 (p region). This location is potentially important insofar as rearrangements of this region are frequent in many human tumors, chiefly of the lung and kidney. This result was obtained in collaboration with Dr Chantal Desmaze (Radiobiology and Oncology Laboratory, CEA/Fontenay-aux-Roses).

From yeast to humans

Of course, an essential step remained; extending this research to humans. This advance was achieved by the same group in 1997, when it succeeded in **cloning** the *OGG1* gene in humans and mice. The *OGG1* gene is **expressed** in all the human tissues analyzed, which is consistent with the hypothesis that its prime function is to eliminate lesions resulting from the respiratory **metabolism** of the cell. The *OGG1* gene is located on the short arm of **chromosome** 3 in humans. The subcellular localization of human Ogg1 protein shows an accumulation in cell nuclei, where DNA is found. The aim of this research is to elucidate the biological role of the *OGG1* gene by means of studies in humans and mice.

The *OGG1* gene is thus potentially one of the caretakers of the genome, which by ensuring the elimination of

Altogether about two meters long, placed end to end, the molecules of **DNA** present in the nucleus of each human cell carry all the body's genetic information in the form of tens of thousands of **genes**, which make up its **genome**, which comprises several thousand million pairs of **nucleotides**. The genes are not separately visible entities, but rather sets of information, a program of instructions. On the other hand, **chromosomes**, which support the genes, are perfectly distinct elements, at least from the time they appear during the first phases of cell division to when they finally disappear when this process ends (box E, ***The cell division cycle: under control duplication***). Each molecule of gene-bearing DNA takes on a very different appearance depending on when it is observed in the cell division cycle. Before the cell division it appears as a loose structure dispersed in the nucleus. It is only when the division is about to take place that, with the aid of associated molecules, it winds to form a compact and ordered structure, the chromosomes. The complex formed by these specialized molecules (the **histones**, along with other chromosomal proteins) and cell DNA is called chro-

V. Estiot/BSIP

Human karyotypes comprising 46 chromosomes: 22 pairs plus one pair of sex chromosomes,

Chromosomes, material supports for genes

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matin. The association of histones with DNA produces **nucleosomes**, elementary units of chromatin.

To form chromosomes long pieces of DNA are coiled in different successive windings (diagram opposite), and not only at the scale of the double helix. The helix first winds around a «bead» (7 to 10 nm in diameter, one nanometer = 10^{-9} m) made of an assembly of eight histone molecules (1). The «string of beads» thereby formed coils back on itself to make a rope 30 nm in diameter (2). The rope then forms chromatin loops (3). Six of these loops make up a rosette (4). These rosettes are condensed to make a 300 nm rope, itself wound to make a fat coil (5) during the metaphase; the total length of a human chromosome is 1,000 to 10,000 nm. The **karyotype**, which corresponds to the full set of chromosomes of a cell in metaphase, comprises, for the human species, 46 chromosomes (22 pairs of homologous chromosomes or **autosomes**, and one pair of sex chromosomes, XX in women, and XY in men).

BSIP

Genes are located on the transverse stripes characteristic of a given chromosome.

The DNA molecule of a chromosome contains three nucleotide sequences that are vital for its replication: «**origins of replication**», a **centromere** and **telomeres**. To replicate, a DNA molecule needs a special sequence that marks the starting point. The centromere serves to keep together the two duplicated chromosome copies, called chromatids, and fix them to the mitotic spindle during mitosis, so that one copy is distributed to each new cell. The third sequence element, located at the two extreme ends of the chromosome, is the telomere. This prevents the DNA chain from shrinking at every replication. An enzyme, **telomerase**, periodically lengthens this sequence to make up for the few lost DNA telomere nucleotides, so that the chromosome is fully replicated.

Various staining techniques can be used to visualize the characteristic cross bands of each chromosome. The G bands indicate regions with DNA rich in adenine-thymine nucleotide pairs, R bands regions rich in guanine-cytosine nucleotide pairs. C bands mark the centromeres, among other areas. The genes are located on the G and R bands. The **alleles** of a particular gene take up the same position, or **locus**, on two homologous chromosomes, one paternal, the other maternal. If the two alleles are identical, then the individual is said to be **homozygous** for that type of gene; if not, it is said to be **heterozygous**. The two alleles have the same function, such as the synthesis of a protein, for example. The length of a gene ranges from a few tens to a few thousands of nucleotide pairs according to the complexity of the protein it stands for. The **expression of a gene** requires the presence of either a **dominant** allele on just one of the two homologous chromosomes, or a **recessive** allele on both of them.

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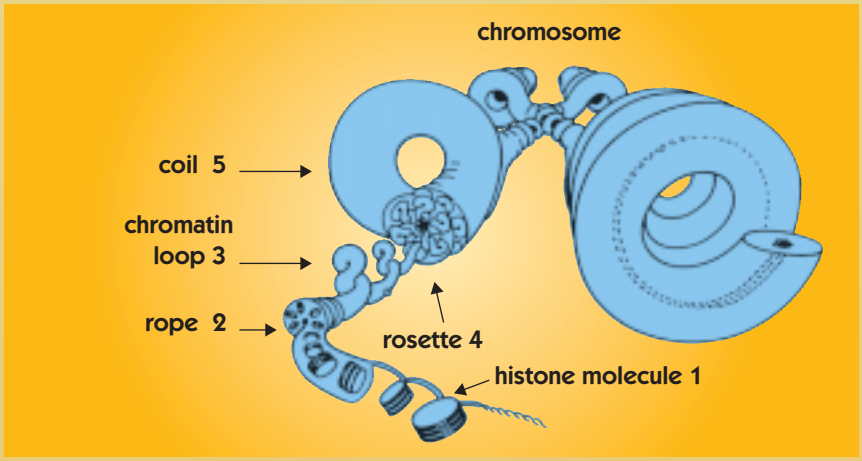

X and Y in men (green), X and X in women (red).

Finding *OGG1* mutations in human tumors

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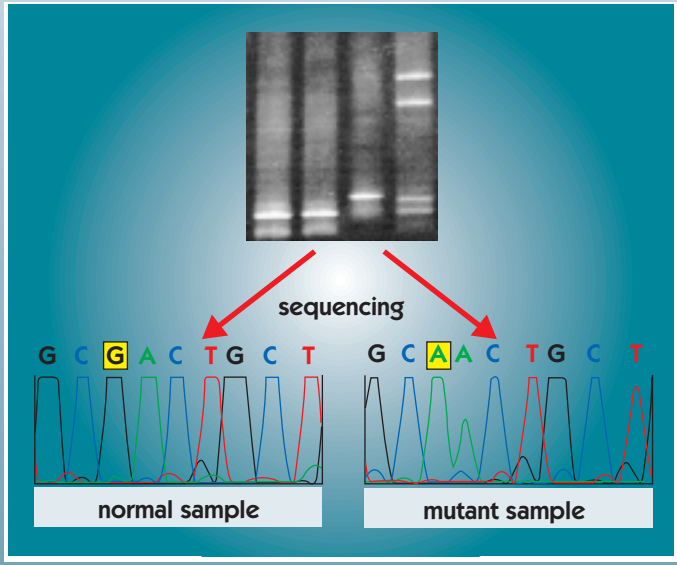
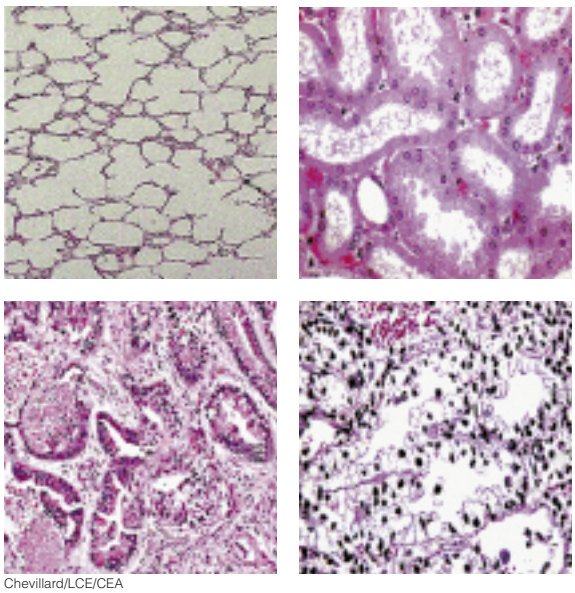
What is the connection between certain human cancers and the inactivation of the *OGG1* gene? Studying the quality of the gene in the tumor cells may help find the answer. To look for the presence of mutations in the gene, genetic material was extracted from tumors and from healthy tissue in the same patient. After specifically **amplifying** the *OGG1* gene, the DNA, which is electrically charged in its natural state, is placed in an electrophoresis field, where it migrates, appearing as white bands (Figure). At a particular time, the DNA of a mutant sample (the two columns on the right) does not migrate like normal DNA (the two columns on the left). This proves that the *OGG1* gene has mutated.



This mutation then has to be characterized; it lies in a modification of the base order (adenine, thymine, guanine and cytosine) that constitutes the genetic information contained in the DNA, coded using four «letters» (box A, **DNA molecule, heredity vector**). This code can be read using the technique of **sequencing**. It has been found that the guanine (G) in third position in the normal sample is replaced by an adenine (A) in the mutant sample. This modification is enough to account for the non-function of the Ogg1 protein.

mutagenic lesions of DNA, stave off the development of certain human cancers. Consequently the loss of this gene may favor the formation of mutations and so hasten cancer growth in target organs.

How can this hypothesis be tested in humans? If cancer is associated with the loss of the *OGG1* function, alterations to this gene should be found in the DNA of tumor cells. Accordingly, researchers have undertaken the analysis of the «quality» of the *OGG1* gene in human tumors of the kidney and lung (box 2). The results indicate that in kidney cancer about 5% of the tumors analyzed display a mutation in the *OGG1* gene. These still preliminary findings thus suggest a correlation between certain cancers of the kidney and the inactivation of the *OGG1* gene.



Sections of a normal lung (top) and a lung tumor (bottom). Sections of a normal kidney (top) and a kidney tumor (bottom).

Chevillard/LCE/CEA

Will screening identify populations at risk?

In the long term the status of the *OGG1* gene could be studied to detect possible at-risk populations presenting a predisposition to radio-induced can-

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Automatic DNA sequencer. The sample gel is loaded into the sequencer using a syringe. Each color on the screen corresponds to a DNA base after analysis of the sequence.

cer, or tumors attributable to **genotoxic** agents that work by oxidation of DNA. More generally, the detection of mutations in repair genes may help define those categories of persons who ought to be exposed as little as possible to certain agents present in natural, occupational or health environments.

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