SOURCES AND EFFECTS OF IONIZING RADIATION

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NOTE

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ANNEX F

DNA repair and mutagenesis

CONTENTS

INTRODUCTION

1. Risk estimates for the induction of human disease are obtained primarily from epidemiological studies. These studies can clearly distinguish radiation effects only at relatively high doses and dose rates. To gain information at low doses and low dose rates, which are more relevant to typical human radiation exposures, it is necessary to extrapolate the results of these studies. To be valid, this extrapolation requires a detailed understanding of the mechanisms by which radiation induces cancer and genetic disorders.

2. Several lines of evidence show that sites of radiation-induced cell lethality, mutation, and malignant change are situated within the nucleus and that DNA is the primary target. When DNA is damaged by radiation, enzymes within the cell nucleus attempt to repair that damage. The efficiency of the enzymatic repair processes determines the outcome: most commonly, the structure of DNA is repaired correctly and cellular functions return to normal. If the repair is unsuccessful, incomplete, or imprecise, the cell may die or may suffer alteration and loss of genetic information (seen as mutation and chromosomal aberration). These information changes determine heritable genetic defects and are thought to be important in the development of radiation-induced cancer. The more complete the knowledge of the ways in which human cells respond to damage and of the mechanisms underlying the formation of mutations and chromosomal aberrations, the more accurate will be the predictions of the oncogenic and hereditary effects of ionizing radiation.

3. DNA repair is itself controlled by a specific set of genes encoding the enzymes that catalyse cellular response to DNA damage. Loss of repair function, or alteration of the control of repair processes, can have very serious consequences for cells and individuals. It is anticipated that DNA repair plays a critical role in protecting normal individuals from radiation effects, including cancer. Clinical experience has revealed individuals who are both hypersensitive to radiation and cancer-prone; some of these individuals have recently been shown to have defects in genes involved in the response to DNA damage.

4. In recent years there have been significant advances in the molecular analysis of repair processes and the understanding of the mechanisms that induce genetic changes. Additionally, new methods have been developed to simplify the identification of the genes involved. As the details of damage-repair processes become clearer, it is seen that these processes have considerable overlap with other cellular control functions, such as those regulating the cell cycle and immune defences. In this Annex the Committee continues to review such developments in molecular radiobiology, as it began to do in Annex E, “Mechanisms of radiation carcinogenesis” of the UNSCEAR 1993 Report [U3], in order to improve the understanding of how radiation effects are manifested in cells and organisms.

I. DNA DAMAGE AND REPAIR

A. THE ROLE OF DNA REPAIR GENES IN CELL FUNCTION

5. The information needed to control cellular functions such as growth, division, and differentiation is carried by the genes. Genes, which are specific sequences of DNA, act mainly through the production of complementary messages (mRNA) that are translated into proteins. Proteins can have a structural role but commonly work as enzymes, each of which catalyses a particular metabolic reaction. Thus specific genes contain the code for (encode) specific cellular functions. The production of proteins can be timed so that they work at specific points in the development of a cell or organism, but protein function can also be controlled by post-translational modifications. These modifications are carried out by other proteins, so that a complex set of interactions is necessary to fine-tune cellular functions. Proteins involved in important aspects of cell metabolism (e.g. DNA replication) may also work in multi-protein complexes [A1]. There is some evidence also that some of the complexes are assembled into larger structures situated in defined regions of the nucleus (e.g. the nuclear matrix) [H2].

6. Loss or alteration of information in a specific gene may mean that none of that gene product (protein) is formed, or that the protein is less active, or that it is formed in an uncontrolled fashion (e.g. at the wrong time or in the wrong amount). While some minor genetic alterations may not affect protein activity or interactions, others may significantly disrupt cellular function. Since certain proteins work in a number of different processes or complexes, the loss or impairment of one type of protein can affect several different functions of the cell and organism (pleiotropic effect).

7. A very large number of genes, 60,000-70,000 [F14], are required to control the normal functions of mammalian cells and organisms. However, the genes form only a small part of the genome (the complete DNA sequence of an organism), the remainder of which largely consists of many copies of repetetive DNA sequence. The genes are linked in linear arrays interspersed by non-coding sequences, to form chromosomes located in the cell nucleus. Most genes are present in only two copies, each on a separate homologous chromosome, one inherited from the mother and one from the father. To monitor damage and to maintain the genes without significant alteration is a major concern for the cell. Repair

processes are common to all organisms from bacteria to humans and have evolved to correct errors made in replicating the genes and to restore damaged DNA. This fact has in recent years provided a useful tool for molecular geneticists in the analysis of repair processes; well characterized micro-organisms can serve as model systems to understand the structure and function of repair genes. The information gained in this way can sometimes also be used directly to isolate human genes of related function [L1]. While the structure and function of repair genes appears to be highly conserved from lower to higher organisms, the regulation of their activities may differ in different organisms.

8. The consequences of loss of repair capacity are seen in a number of human syndromes and mutant cell lines. These show hypersensitivity to environmental agents, and the human syndromes often have multiple symptoms, including cancer-proneness, neurological disorder, and immune dysfunction. Good progress has been made over the last few years in mapping and cloning the genes involved.

9. Ionizing radiation damages DNA and causes mutation and chromosomal changes in cells and organisms. Damage by radiation or radiomimetic agents also leads to cell transformation (a stage in cancer development) and cell death. In the light of current research, it is seen that the final response to radiation damage is determined not only by cellular repair processes but also by related cellular functions that optimize the opportunity for recovery from damage. For example, radiation damage may cause an arrest in the cell cycle; this is thought to be a damage-limitation step, allowing time for repair and reducing the consequences of a given dose [L22]. There is now some understanding of the way in which radiation alters cell cycle timing (Section II.B.2), although the roles of a number of enzymatic activities that are induced or repressed shortly after irradiation remain to be clarified (Section III.B).

10. The severity of DNA damage, or the context in which damage occurs (e.g. during DNA replication), will often dictate a repair strategy that places survival first and incurs genetic change. DNA replication may bypass sites of single-stranded DNA damage, inserting an incorrect base opposite the altered or lost base. Additionally, in attempting to repair damage to DNA, enzymes may not be able to restore the structure with fidelity. Thus, mutation and chromosomal rearrangement are not passive responses to damage; rather, they are a consequence of the interaction of cellular processes with damage. The types of genetic change that occur will depend on the types of initial DNA damage, from their potential to miscode at replication and from the probability that specific repair enzymes will act on given types of damage.

B. TYPES OF DAMAGE AND PATHWAYS OF REPAIR

11. DNA is a double-helical macromolecule consisting of four units: the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T) and cytosine (C). The bases are arranged in two linear arrays (or strands) held together by hydrogen bonds centrally and linked externally by covalent bonds to sugar-phosphate residues (the DNA "backbone"). The adenine base pairs naturally with thymine (A:T base pair), while guanine pairs with cytosine (G:C base pair), so that one DNA strand has the complementary sequence of the other. The sequence of the bases defines the genetic code; each gene has a unique sequence, although certain common sequences exist in control and structural DNA elements. Damage to DNA may affect any one of its components, but it is the loss or alteration of base sequence that has genetic consequences.

12. Ionizing radiation deposits energy in tracks of ionizations from moving charged particles within cells, and radiations of different quality may be arbitrarily divided into sparsely ionizing, or low linear energy transfer (low-LET), and densely ionizing (high-LET). Each track of low-LET radiations, such as x rays or gamma rays, consists of only a relatively small number of ionizations across an average-sized cell nucleus (e.g. a gamma-ray electron track crossing an 8 μm diameter nucleus gives an average of about 70 ionizations, equivalent to about 1 mGy absorbed dose, although individual tracks vary widely about this value because of track stochastics and varying path lengths through the nucleus). Each track of a high-LET radiation may consist of many thousands of ionizations and give a relatively high dose to the cell; for example, a 4 MeV alpha-particle track has, on average, about 23,000 ionizations (370 mGy) in an average-sized cell nucleus [G27, U3]. However, within the nucleus even low-LET radiations will give some small regions of relatively dense ionization over the dimensions of DNA structures, for example, where a low-energy secondary electron comes to rest within a cell.

13. Radiation tracks may deposit energy directly in DNA (direct effect) or may ionize other molecules closely associated with DNA, especially water, to form free radicals that can damage DNA (indirect effect). Within a cell the indirect effect occurs over very short distances, of the order of a few nanometres, because the diffusion distance of radicals is limited by their reactivity. Although it is difficult to measure accurately the different contributions made by the direct and indirect effects to DNA damage caused by low-LET radiation, evidence from radical scavengers introduced into cells suggests that about 35% is exclusively direct and 65% has an indirect (scavengeable) component [R21]. It has been argued that both direct and indirect effects cause similar early damage to DNA; this is because the ion radicals produced by direct ionization of DNA may react further to produce DNA radicals similar to those produced by water-radical attack on DNA [W43].

14. Ionization will frequently disrupt chemical bonding in cellular molecules such as DNA, but where the majority of ionizations occur as single isolated events (low-LET radiations), these disruptions will be readily repaired by cellular enzymes. However, the average density of ionization by high-LET radiations is such that several ionizations are likely to occur as the particle traverses a DNA double helix.

Therefore, much of the damage from high-LET radiations, as well as a minority of the DNA damage from low-LET radiations, will derive from localized clusters of ionizations that can severely disrupt the DNA structure [G27, W44]. While the extent of local clustering of ionizations in DNA from single tracks of low- and high-LET radiations will overlap, high-LET radiation tracks are more efficient at inducing larger clusters, and hence more complex damage. Also, high-LET radiations will induce some very large clusters of ionizations that do not occur with low-LET radiations; the resulting damage may be irrepairable but may also have unique cellular consequences (see paras. 192, 199, and 201) [G28]. Additionally, when a cell is damaged by high-LET radiation, each track will give large numbers of ionizations, so that the cell will receive a relatively high dose and there will be a greater probability of correlated damage within a single DNA molecule (or chromosome) or in separate chromosomes. As a consequence, the irradiation of a population of cells or a tissue with a "low dose" of high-LET radiation results in a few cells being hit with a relatively high dose (one track) rather than in each cell receiving a small dose. In contrast, low-LET radiation is more uniformly distributed over the cell population; at doses of low-LET radiation in excess of about 1 mGy (for an average-size cell nucleus of 8 μm diameter), each cell nucleus is likely to be traversed by more than one sparsely-ionizing track.

15. The interaction of ionizing radiation with DNA produces numerous types of damage; the chemical products of many of these have been identified and classified according to their structure [H4, S3]. These products differ according to which chemical bond is attacked, which base is modified, and the extent of the damage within a given segment of DNA. Table 1 lists some of the main damage products that can be measured following low-LET irradiation of DNA, with a rough estimate of their abundance. Attempts have also been made to predict the frequencies of different damage types from a knowledge of radiation track structure, with certain assumptions about the minimum energy deposition (number of ionizations) required. Interactions can be classified according to the probability they will cause a single-strand DNA alteration (e.g. a break in the backbone or base alteration) or alterations in both strands in close proximity in one DNA molecule (e.g. a double-strand break), or a more complex type of DNA damage (e.g. a double-strand break with adjacent damage). Good agreement has been obtained between these predictions and direct measurements of single-strand breaks, but there is less good agreement for other categories of damage [C47]. While complex forms of damage are difficult to quantify with current experimental techniques, the use of enzymes that cut DNA at sites of base damage suggests that irradiation of DNA in solution gives complex damage sites consisting mainly of closely-spaced base damage (measured as oxidised bases or abasic sites); double-strand breaks were associated with only 20% of the complex damage sites [S87]. It is expected that the occurrence of more complex types of damage will increase with increasing LET, and that this category of damage will be less repairable than the simpler forms of damage. Theoretical simulations have predicted that about 30% of DNA double-strand breaks from low-LET radiation are complex by virtue of additional breaks [N19] and that this proportion rises to more than 70%, and the degree of complexity increases, for high-LET particles [G29].

16. Some of the DNA damage caused by ionizing radiation is chemically similar to damage that occurs naturally in the cell: this "spontaneous" damage arises from the thermal instability of DNA as well as endogenous oxidative and enzymatic processes [L2, M40]. Several metabolic pathways generate oxidative radicals within the cell, and these radicals can attack DNA to give both DNA base damage and breakage, mostly as isolated events [B46]. The more complex types of damage caused by ionizing radiation may not occur spontaneously, since localized concentrations of endogenous radicals are less likely to be generated in the immediate vicinity of DNA. This theme is taken up in Annex G, "*Biological effects at low radiation doses*", which considers the cellular responses to low doses of radiation.

Table 1
Estimated yields of DNA damage in mammalian cells caused by low-LET radiation exposure
[L60, P31, W39]

Type of damage	*Yield (number of defects per cell Gy^{-1})*
Single-strand breaks	1 000
Base damage [a]	500
Double-strand breaks	40
DNA-protein cross-links	150

a Base excision enzyme-sensitive sites [P31] or antibody detection of thymine glycol [L60].

17. Measurement of the endogenous levels of DNA base damage has been difficult because of the artefactual production of damage during the preparation of the DNA for analysis (e.g. by gas chromatography/mass spectrometry) [C55]. This difficulty explains the presence in the literature of considerably inflated (by factors of at least 100) values for background levels of base damage. Interestingly, the recognition of damage by base excision repair enzymes (paragraph 22) has provided a less discordant method of measurement, although the specificity of the enzymes for different types of base damage is not precisely known. These enzymes cut the DNA at the site of base damage, to give a single-strand break that

can be measured accurately by a number of techniques. Using this method, measurement of an important form of oxidative damage, 7,8-dihydro-8-oxoguanine (generally known as 8-oxoguanine)), has given steady-state levels of 500-2000 per cell, depending on cell type [P30]. Using similar measurement methods, the level of 8-oxoguanine induced in cellular DNA by gamma rays is about 250 per cell per Gy [P31]. A newly developed ultrasensitive assay for another type of base damage in human cellular DNA, thymine glycol, couples antibody detection with capillary electrophoresis. This method showed a linear response for yield of thymine glycol with gamma-ray dose down to 0.05 Gy, giving a level of about 500 thymine glycols per cell per Gy against a background of 6 thymine glycols per cell [L60]. The difficulties experienced in measuring base damage accurately in cellular DNA and the relatively low levels now found for the commoner types of damage have also called into question the extent to which some previously identified forms of base damage occur in cells following irradiation.

18. The measurement of endogenous levels of other types of DNA damage, such as double-strand breaks, has involved similar technical difficulties. Many of the methods used to measure double-strand breaks in mammalian cells introduce this form of damage either inadvertently or deliberately as part of the methodology. This is because the mammalian genome is so large that it had to be reduced in size by random breakage first before useful measurements could be made. This problem has been overcome in part by the introduction of methods based on the gentle release of DNA from cells by their lysis in a gel matrix [C64, O3], but there is commonly still a background level of DNA breakage amounting to a few per cent of the total DNA. However, as documented in Section II.B, it is unlikely that mammalian cells have a high steady-state level of DNA double-strand breakage, since these breaks act as a signal for damage-recognition processes that can block the cell cycle or induce programmed cell death. It is possible that even one unrepaired double-strand break can trigger this cellular response (paragraph 101). It has also been found that one unrepaired double-strand break can cause lethality in irradiated yeast cells (paragraph 108). Thus, tolerance of this form of damage in cells is likely to be very low.

19. While the precise nature of the damage will influence repairability, it is possible to consider a few general categories of damage in order to describe their consequences. A simplified classification can be based on the ability of enzymes to use the complementary base structure of DNA to facilitate repair of the damage site. Thus, damage to single strands (base modifications, single-strand breaks) can be removed or modified, followed by resynthesis using the undamaged strand as a template. Where the damage affects both strands of a DNA molecule in close proximity (double-strand breaks, cross-links), it is more difficult to repair and requires different enzymatic pathways for its resolution. To resolve successfully more complex types of damage may require enzymes from more than one repair pathway. To illustrate the knowledge of the different repair pathways available to the cell, the following account (to paragraph 34) includes a discussion of the repair of damage caused by various DNA-damaging agents as well as ionizing radiation.

20. DNA repair enzymes can be characterized as cellular proteins acting directly on damaged DNA in an attempt to restore the correct DNA sequence and structure. These relatively specialized enzymes appear to undertake the initial stages of recognition and repair of specific forms of DNA damage. For example, DNA glycosylases catalyze the cleavage of base-sugar bonds in DNA, acting only on altered or damaged bases [W1]. Further, there are several different types of glycosylase that recognize chemically different forms of base damage. However, enzymes that carry out normal DNA metabolism are also part of the repair process for many different forms of damage. In the latter category there are, for example, enzymes involved in the synthesis of DNA strands (DNA polymerases) and enzymes involved in the joining of the DNA backbone (DNA ligases). Several different types of DNA polymerases and ligases have been identified; it is thought that they have different roles in normal DNA metabolism and that only some are active in DNA repair [L3, P1].

21. The simplest repair processes directly reverse the damage; for example, many organisms, but not mammals, possess an enzyme that directly photoreactivates the UV-induced dimerization of pyrimidine bases [S1]. Similarly, the enzyme O^6methylguanine-methyltransferase directly removes methyl groups induced in DNA by alkylating carcinogens [P13]. However, most damage types require the concerted action of a number of enzymes, forming a repair pathway. Several apparently discrete repair pathways have been identified, as described below and illustrated in Figure I.

22. Damage to individual bases in DNA may be corrected simply by removing the base, cleaning up the site, and resynthesis. In this process, termed the base-excision repair pathway, a DNA glycosylase removes the damaged base, a DNA endonuclease cuts the DNA backbone, the sugar-phosphate remnants are removed by a phosphodiesterase, and a polymerase fills in the gap using the opposite base as a template (Figure Ia) [L2, S49]. Even where a single base is damaged, therefore, several different enzymes are required to give correct repair. The latter part of this process may also be used to repair single-strand breaks in DNA. Radiation-induced DNA breaks are generally not rejoined by a simple ligation step, because sugar damage and, often, base loss occur at the site of a break. Base-excision repair is generally localized to the single DNA base and is very rapid [D16, S58]; however, in mammalian cells a minority of repair patches of up to 6 bases have been found, indicating a second “long-patch” pathway (see paragraph 75).

23. Many DNA glycosylases are specific for the removal of one type of altered base from DNA; for example, uracil-DNA glycosylase removes only uracil and some oxidation products of uracil [F15]. However, there is some overlap in the specificity of some base-excision repair enzymes. An

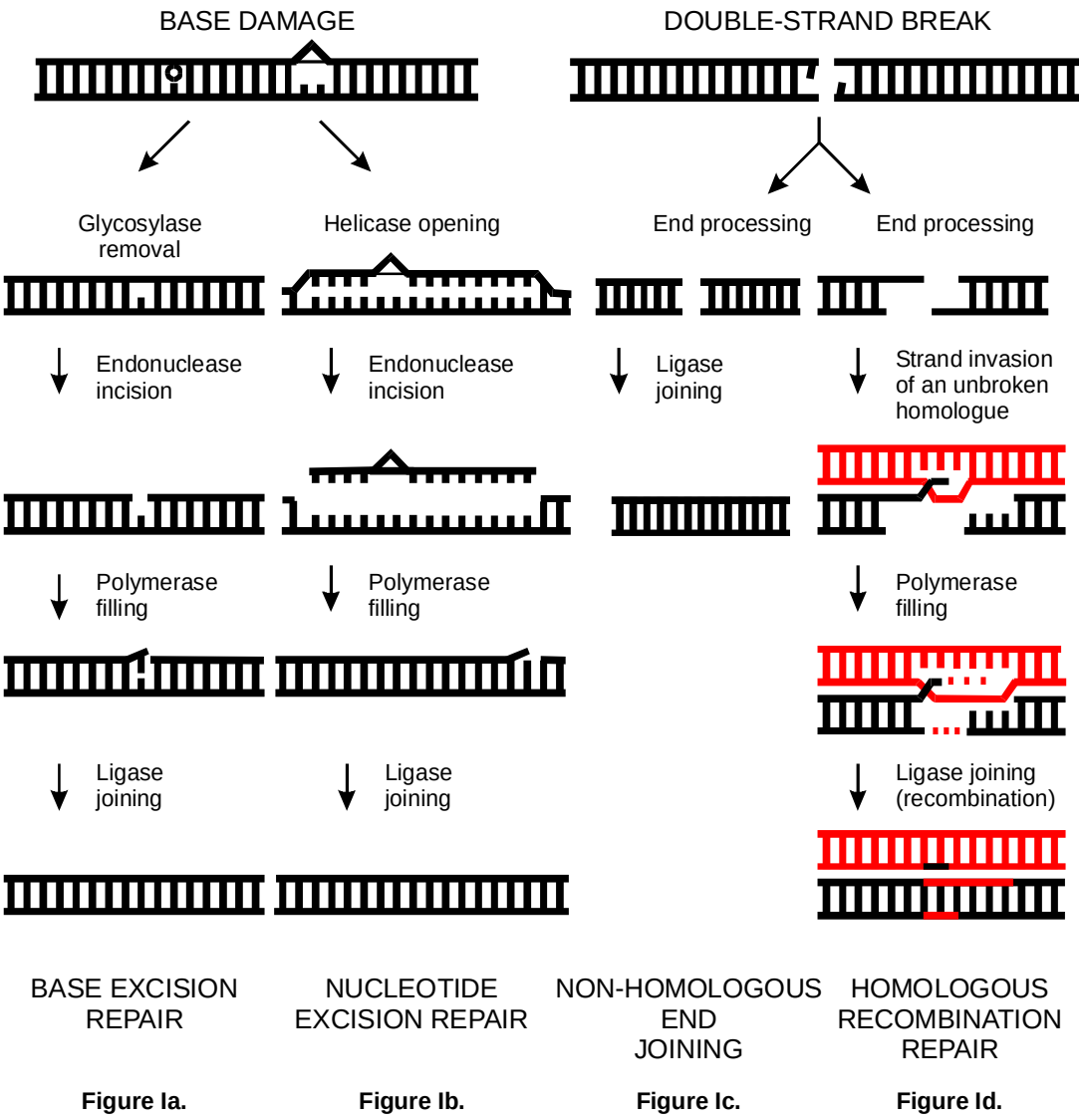

Figure I.
Mechanisms of DNA repair (simplified).

Figure Ia: Base damage is excised by a specific glycosylase: the DNA backbone is cut and the gap filled by a polymerase. The resulting gap is refilled.

Figure Ib: Bulky base damage is removed along with an oligonucleotide (of about 30 bases in human cells). Resynthesis takes place using the opposite strand as a template.

Figure Ic: A double-strand break is rejoined end-to-end.

Figure Id: A double-strand break is repaired with the help of a homologous undamaged molecule (shown in red). Strand invasion allows resynthesis on complementary sequence, followed by a resolution of the strands and rejoining.

example of this is seen in the response to ionizing radiation of bacterial cells defective for one or more nucleases involved in base-excision repair: while cells defective for either nuclease alone were not radiation sensitive, the loss of both nucleases made the cells extremely radiation sensitive [C60, Z10]. Some glycosylases have a very broad specificity for different types of base structure, such as 3-methyladenine-DNA glycosylase II, which acts on a variety of chemically-modified purines and pyrimidines, 5-methyloxidized thymines, and hypoxanthine. It has recently been found that 3-methyladenine-DNA glycosylase II will also remove natural bases from DNA, especially purines, at significant rates. This finding has led to the novel suggestion that the rate of excision of broad-specificity glycosylases is a function of the chemical bond stability in DNA. On this basis, damaged bases are excised more readily than natural bases because their chemical bonds are less stable [B57].

24. A specialized form of base-excision repair involves the removal of mismatched DNA bases that occur as errors of DNA replication or from the miscoding properties of damaged bases. For example, 8-oxoguanine is a common product of oxidative damage to guanine bases (paragraph 17); this product is highly mutagenic because of its ability to miscode

(polymerases incorporate adenine instead of cytosine opposite 8-oxoguanine, thus changing the DNA sequence). Cells have evolved three different methods to deal with the formation of 8-oxoguanine: one glycosylase can correct the mismatch by removing 8-oxoguanine from DNA, while a different glycosylase can remove the mismatched adenine after DNA replication [A27, T48]. Excision and resynthesis of the missing base occur as in the general base-excision repair process. Additionally, another enzyme can remove the precursor of 8-oxoguanine (8-hydroxy-GTP) from the nucleotide pool before it is incorporated into DNA [M51]. A number of other mismatches, arising most commonly in DNA replication and recombination, are corrected by a separate "long patch" pathway, known as the MutHLS pathway, that is similarly important in protecting cells from high frequencies of mutation (see paragraph 165) [K38].

25. Where a damaged base is close to another damaged base or to a single-strand break, which is the most simple form of clustered damage, repair may be compromised. Examination of model DNA substrates with base damage on opposite strands, separated by different numbers of bases, has shown that glycosylases do not repair both sites of damage when they are very close to one another (1-3 bases) [C56, C57, H49]. Further, attempted repair of both sites of base damage can result in a DNA double-strand break, because the DNA backbone is cut as part of the repair process.

26. In contrast to base-excision repair, nucleotide-excision repair removes a whole section of single-stranded DNA containing a site of damage, generally a bulky DNA adduct causing distortion of the double helix. These enzymes have to perform such functions as recognition of damage, cutting of the strand at a specified distance either side of the damage, and unwinding and removal of the strand (Figure Ib). As might be expected, at least 11 enzymes have already been identified as components of nucleotide-excision repair [W52], not including polymerase and ligase. The enzymes of nucleotide-excision repair are highly conserved from microbes to humans, and this feature has been used to assist in the isolation of the genes encoding these functions. Several of these genes have been found to be mutated in humans, giving rise to a series of disorders, including xeroderma pigmentosum and Cockayne's syndrome [H3]. Individuals inheriting mutated nucleotide-excision repair genes are generally sensitive to sunlight and chemical agents causing bulky damage in DNA, but a few individuals show cross-sensitivity to ionizing radiation [A2, R15]. Additionally, a small fraction of damage induced by gamma rays is not repaired in cells derived from individuals with xeroderma pigmentosum, which suggests that ionizing radiation induces some bulky damage (e.g. purine dimers) that cannot be removed by the base-excision repair pathway [S56]. Alternatively, a fraction of non-bulky base damage (8-oxoguanine, thymine glycol) may be removed by the nucleotide-excision repair system, particularly in long-lived cells such as neurons that sustain a great deal of endogenous oxidative damage [R22]. These possibilities may explain why severe cases of xeroderma pigmentosum also suffer progressive neurological degeneration.

27. A surprising recent discovery was that some nucleotide-excision repair enzymes are also involved in the normal process of gene expression (transcription). Thus, when genes are actively expressing, they require some of the same functions needed for repair, such as unwinding the DNA helix, and it seems that the same proteins are used. This finding explains the previously puzzling observation of a link between human disorders with sensitivity to sunlight and those with complex defects in gene expression (such as trichothiodystrophy [L70]).

28. Another important discovery about nucleotide-excision repair is that it operates at different rates in different parts of the genome [H1]. Thus, actively expressing genes are repaired much faster than the remainder of the genome. Much of the detail of this process has now been elucidated: it is thought that when damage occurs in a gene that is actively expressing, the proteins involved in this process (the RNA polymerase II transcription complex) stop working, and the stalled complex acts as a signal to the repair proteins to go to the damage site. This two-tier repair system has been found in organisms from bacteria to humans, and the protein mediating the signal to bring repair to the damage site has been identified in bacteria [S2]. The presence in the cell of a fast transcription-coupled repair process has also been found to have genetic consequences: only one of the DNA strands of the duplex is transcribed, and only this strand is repaired rapidly. It has been found in normal cells that most of the mutations induced by DNA damage are in the non-transcribed strand, presumably because lack of fast repair allows the damage to interact with other processes, causing mutations [M2]. In contrast, repair-defective cell lines show a completely altered mutation spectrum, with most mutations recovered in the transcribed strand. Much of this detail has been established using UV-light damage, but the repair of other types of DNA damage, including certain forms of base damage induced by ionizing radiation such as thymine glycols, is influenced by transcription-coupled processes [H1, C41]. Again, certain human sun-sensitive disorders have been found to lack either the fast repair path (Cockayne's syndrome) or the slower overall path of nucleotide-excision repair (xeroderma pigmentosum group C). Loss of the fast or slower pathways may also affect the clinical outcome of sun-sensitive disorders: Cockayne's syndrome does not result in cancer-proneness, while xeroderma pigmentosum patients are highly prone to skin cancers [M2].

29. More severe forms of damage require yet more resources for their correct repair. This is especially true of damage affecting both DNA strands simultaneously, since there is no undamaged strand to act as a template for repair. Severe damage may occur directly by a damaging agent causing complex DNA changes or may arise during the replication of unrepaired single-stranded DNA damage. It is likely that such severe damage will be repaired by recombination enzymes, which rejoin or replace damaged sequences through a variety of mechanisms. In general, there are two main types of recombination repair processes: homologous recombination and illegitimate recombination, although site-specific recombination processes also occur. The principles of recombination repair have been well

established in micro-organisms, and it has recently been found that similar processes occur in human cells.

30. Homologous recombination takes advantage of the sequence identity between certain regions of DNA to repair damage; such regions exist, for example, in the maternal and paternal copies of chromosomes and in the duplicated chromosome (sister chromatids) following DNA replication. The DNA sequence, from which information is derived to repair the damaged copy, must be identical over a considerable length ($\geq$200 base pairs). It is known in the budding yeast (*Saccharomyces cerevisiae*), for example, that homologous recombination is the main method for DNA double-strand break repair. Several of the *rad52* group of yeast mutants were isolated on the basis of their extreme sensitivity to ionizing radiation and have been shown to be defective in both DNA double-strand break repair and homologous recombination [G1]. In recombination, the broken 3' end of a DNA strand invades an unbroken double-stranded homologue, and resynthesis on this template re-forms the damaged strand (Figure Id). Separation of the joint product of this reaction requires the activity of enzymes cutting and rejoining the newly-synthesized DNA strands. Depending on which strands are cut and rejoined, this reaction may also result in crossing over (genetic exchange) of DNA strands.

31. Illegitimate recombination (including DNA end-joining processes) is a common mechanism for rejoining broken DNA sequences in mammalian cells (Figure Ic). When foreign DNA molecules are integrated into the genome [R1] or when the genomic breakpoints of deletions and rearrangements are analysed (Section IV.C), it is found that these genomic sites show little sequence homology. There appears to be more than one repair pathway involved, and terms such as non-homologous end joining and direct-repeat end joining are used to describe different pathways in this Annex (see Section II.B.1). It can be argued that illegitimate recombination is a mechanism for rapidly rejoining broken DNA ends without the need for the complex machinery of homologous recombination [R1]. It is also likely that because of the large amount of repetitive DNA sequence in mammalian cells, if the processes of homologous recombination were generally available in cells, there would be an intolerable level of reshuffling of the genome. While homologous recombination is thought to be a mechanism for repairing DNA with little error, illegitimate recombination is likely to cause alteration and/or loss of DNA sequence.

32. It is likely that both homologous and illegitimate recombination processes are able to repair severe damage in the genome. However, a surprising recent discovery in mammalian cells is that the some of the enzymes involved in repairing radiation-induced breakage of DNA also take part in a site-specific recombination process, V(D)J immune-system recombination. This process assembles functional immune genes from separate genomic regions, through somatic gene rearrangement, and is dealt with in more detail in Section II.B.1.

33. There is also evidence that cells have specific surveillance mechanisms for DNA damage and that these mechanisms interface with other aspects of cellular metabolism such as cell-cycle progression [M3]. Thus it is envisaged that when the genome, and perhaps other parts of the cell, sustains damage, a response mechanism is set up to maximize the chance of repairing the damage (or in some cases to commit the cell to a programmed death). The details of these mechanisms, as well as how the overall response is coordinated, are not yet clear.

34. More than 50 genes are already known to affect the repair of DNA damage in lower eukaryotic organisms such as yeasts [F1], but this figure includes genes involved in processes such as cell-cycle checkpoints (Section II.B.2). Additionally, new genes are being found continually, both in searches for homologues of existing repair genes and in genome mapping projects. In view of the numbers already discovered, the multiplicity of types of damage requiring repair, and the recent discoveries of complexity in repair pathways, it would not be surprising if the overall number in humans is a few hundred genes. Therefore, a significant fraction of the genome (paragraph 7) is devoted to maintaining the integrity of DNA. Since the damage to DNA from ionizing radiation is also very diverse, many of these genes will play a role in its repair.

C. SUMMARY

35. Ionizing radiation interacts with DNA to give many different types of damage. Radiation track structure considerations indicate that the complexity of the damage increases with linear energy transfer, and that this complexity may distinguish radiation damage from alterations occurring spontaneously and by other agents. Attempts to measure endogenous levels of damage have sufferred from high levels of artefacts, and despite improved methods there are still large margins of error in these estimates. At present, therefore, it is difficult to compare radiation-induced levels of damage with those occurring spontaneously, especially when damage complexity is taken into account. The importance of the relationship between spontaneous and induced levels of damage in the determination of low dose responses is taken up in Annex G, "*Biological effects at low radiation doses*".

36. A large number of genes have evolved in all organisms to repair DNA damage; the repair gene products operate in a co-ordinated fashion to form repair pathways that control restitution of specific types of damage. Repair pathways are further co-ordinated with other metabolic processes, such as cell cycle control, to optimize the prospects of successful repair.

37. It is likely that the simpler forms of DNA damage (single sites of base damage, single-strand breaks) arising endogenously and from exposure to ionizing radiation will be repaired rapidly and efficiently by base-excision repair processes, so these types of damage are not normally a

serious challenge to biological organisms. However, because of the relatively large amount of base damage and single-strand breaks induced (Table 1), if base-excision repair systems are compromised, the consequences would be very serious for the cell and the individual. DNA damage such as double-strand breaks represents a more difficult problem for cellular repair processes, but more than one recombination repair pathway has evolved to cope with this damage. Damage caused by large clusters of ionizations in or near DNA, giving more complex DNA alterations, may represent a special case for which separate repair pathways have to come together to effect repair, or where there is a consequent loss or alteration of the DNA sequence as a result of incorrect or inadequate repair.

II. REPAIR PROCESSES AND RADIOSENSITIVITY

A. RADIOSENSITIVITY IN MAMMALIAN CELLS AND HUMANS

1. The identification of radiosensitive cell lines and disorders

38. Individuals vary in their sensitivity to ionizing radiation. Highly radiosensitive individuals have been detected when they present for cancer therapy; these are seen as rare patients suffering severe normal tissue damage after standard therapy treatments. It has been possible to group some radiosensitive patients into defined disorders, such as ataxia-telangiectasia and the Nijmegen breakage syndrome, but others appear to be asymptomatic (that is, with none of the symptoms of known sensitivity disorders, but also discovered following treatment for cancer). Additionally there are individuals who show less extreme radiosensitivity, some of whom may be variants of known disorders such as ataxia-telangiectasia.

39. Ataxia-telangiectasia is the best described of radiosensitive disorders. It has a complex phenotype; cerebellar ataxia, neuromuscular degeneration, dilated ocular blood vessels (telangiectasia), immunodeficiency, chromosomal instability, and a substantially increased incidence of some cancers and neoplasms are common to ataxia-telangiectasia patients [B10]. Ataxia-telangiectasia is inherited primarily as an autosomal recessive trait, although it has been suggested that both radiosensitivity and cancer-proneness behave with some dominance. The disease is progressive, with most affected individuals surviving only to adolescence or early adulthood. Lymphocytic leukaemia and non-Hodgkin's lymphoma appear to be the commonest forms of cancer, but solid tumours in various organs are also associated with ataxia-telangiectasia [H9]. Estimates of the frequency of the disorder vary but suggest an average of about 1 per 100,000 [P8, S12, W10].

40. The radiosensitive phenotype of ataxia-telangiectasia is also readily demonstrated in cells cultured from patients, using cell survival and chromosome damage assays. For example in a survey comparing the survival of cells cultured from 42 normal individuals with those from 10 ataxia-telangiectasia individuals following x-irradiation, the ataxia-telangiectasia cells were, on average, 2.7 times more sensitive than the normal cells (see Figure II) [C8]. Compared to normal cells, an elevated frequency of chromosomal aberrations is found both spontaneously and after irradiation of ataxia-telangiectasia cells. Also, while irradiation of normal cells in the pre-synthesis (G_0) phase of the cell cycle yields only chromosome-type aberrations, both chromatid- and chromosome-type aberrations are found in ataxia-telangiectasia [T1]. A striking feature of ataxia-telangiectasia cells is their resistance to radiation-induced DNA synthesis delay: normal cells show a rapid inhibition of DNA synthesis after irradiation, while ataxia-telangiectasia cells have a delayed and/or much reduced inhibition [P4].

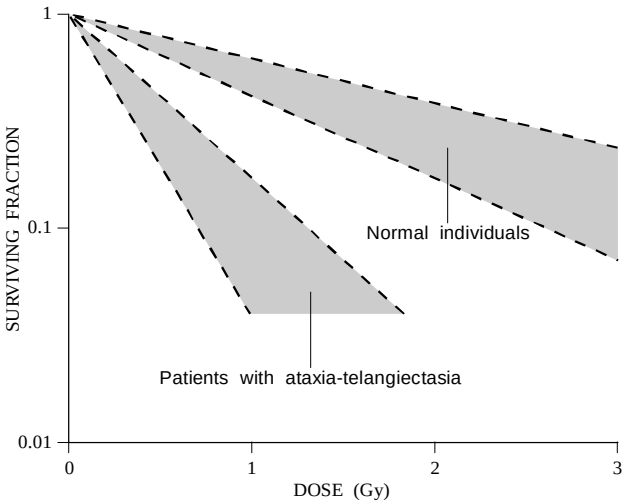

Figure II. Survival of human fibroblast cells after x-irradiation as measured by their colony-forming ability [C8]. *The range of D_0 (the dose required to kill 63% of the cells) is 0.3–0.6 Gy in 10 patients with ataxia-telangiectasia and 1–1.6 Gy in 42 normal individuals.*

41. Nijmegen breakage syndrome is a clinically separate radiosensitive disorder characterized by variable immune deficiencies, microcephaly, developmental delay, chromosomal instability, and cancer susceptibility [B4, S7, V7, W4]. Lymphoreticular cancers again seem to characterize this disorder [S8]. Nijmegen breakage syndrome patients show no ataxia or telangiectasia, but their cellular phenotype is very similar to that of ataxia-telangiectasia [A17, J1, N6, T2]. Other patients with similarities to ataxia-telangiectasia and Nijmegen breakage syndrome have been found and in some cases classified separately by genetic analysis ([C12, W5]; see also below). One case has been reported of combined ataxia-telangiectasia and Nijmegen breakage syndrome and called A-T_{FRESNO} [C18]. Also, there are a number of reports of families or individuals who show symptoms that partially overlap

ataxia-telangiectasia and Nijmegen breakage syndrome; an example would be a family with individuals showing either ataxia-telangiectasia or a disorder involving ataxia, microephaly, and congenital cataract [Z3].

42. To add to this complexity, a number of individuals with a variant form of ataxia-telangiectasia have been described; these patients may show a slower onset of symptoms and have intermediate levels of cellular radiosensitivity [C4, C7, F2, J2, T3, Y1, Z1].

43. In addition to individuals having multiple symptoms associated with radiosensitivity, some otherwise normal persons have been found to be highly radiosensitive. Woods et al. [W9] described an apparently normal 13-year-old girl who developed multiple complications following radiotherapy for Hodgkin's disease. Cells derived from a skin biopsy showed a highly sensitive radiation survival response, similar to that for ataxia-telangiectasia cells. Plowman et al. [P7] reported similar findings for a 14-year-old boy with acute lymphoblastic leukaemia; again, no ataxia-telangiectasia or Nijmegen breakage syndrome-like symptoms were present, but both whole-body and cellular radiosensitivity were as extreme as for ataxia-telangiectasia. This individual has now provided the first example of radiosensitivity in humans where a defined cellular repair defect (in the ligase IV enzyme) can be readily demonstrated; repair of radiation-induced DNA double-strand breaks and interphase chromosome damage are similarly impaired (see also paragraph 66) [B15].

44. There is some evidence for ionizing radiation sensitivity in several other cancer-prone disorders, although the published data do not always agree on the degree of sensitivity (see also Section III.A). Bloom's syndrome is a rare autosomal recessive disorder showing severe growth retardation, variable immune deficiencies, and abnormal spermatogenesis [G20]. The age of onset of cancer is considerably earlier than normal; about one third of surviving cancer patients with Bloom's syndrome develop multiple primary tumours with no consistent pattern of cancer type or location. Individuals with Bloom's syndrome develop a distinct facial rash from sunlight sensitivity, and their cells are not only hypersensitive to several different DNA-damaging agents but also show DNA replica ion abnormalities [L47]. Genetic instability is seen in high levels of spontaneously-occurring chromosomal aberrations and sister chromatid exchanges; chromosomal sensitivity to x rays has been found especially in cells in the G_2 phase of growth [A16, K30]. One case of Bloom's syndrome developed oesophageal stricture following standard radiotherapy treat-ent for lung cancer. This is very rarely seen in such treatment and is suggestive of hypersensitivity to radiation [K31].

45. Fanconi's anaemia is a cancer-prone disorder, most commonly presenting with acute myeloid leukaemia (15,000-fold increased risk), although solid tumours are also found. Bone marrow failure is a common diagnostic feature of this disorder, although symptoms may include congenital malformations, abnormal skin pigmentation, and skeletal and renal abnormalities [J15]. Fanconi's anaemia cells show high levels of chromosomal aberrations and are hypersensitive to DNA cross-linking agents (e.g. mitomycin C, diepoxybutane). Additionally, a high proportion of deletions has been reported in certain genes of Fanconi's anaemia cell lines, giving a higher frequency of mutation in assays measuring loss of heterozygosity (see paragraph 172) [S68]. There has been some dispute over the extent to which Fanconi's anaemia cells are sensitive to ionizing radiation; a lack of genetic classification in these experiments may account for some of the variability found (see paragraph 68). However, when they compared published data on the sensitivity of human fibroblasts, Deschavanne et al. [D7] concluded that Fanconi's anaemia was one of the few disorders for which sensitivity to radiation could be distinguished from that of normal cells.

46. The genes controlling radiosensitivity in humans will not be identified simply by analysing radiosensitive disorders. This is because mutations in many genes are deleterious to the extent that the development of a viable organism is inhibited. This point was illustrated by the creation of a “knockout” mouse for the UV-damage repair gene *ERCC1* (knockout meaning that both copies of the gene are inactivated). No human variant for the *ERCC1* gene has been found, and the knockout mouse dies before weaning, apparently as the result of a massive load of (unrepaired) damage [M7]. Therefore, to examine the full range of genes involved, it has been necessary to derive radiosensitive mutant lines from cells in culture. To this end, more than 50 mutant cell lines sensitive to various genotoxic agents have been identified; many of these show some degree of x-ray sensitivity and are being used to dissect repair pathways in mammalian cells [C16, H11]. These cell lines are especially useful for gene cloning, since this has often proved difficult to achieve using cells derived from human patients. It is possible at the present stage of knowledge to group these mutant cell lines into several categories based on their responses, and recently several of the genes involved have been mapped or cloned (Table 2). As radiosensitive cell lines have been developed in laboratories around the world, almost all have been found to represent defects in different genes. For this reason, and because ionizing radiation produces a diversity of DNA damage, it is anticipated that a large number of the human genes involved in determining radiation resistance remain undiscovered.

47. The discovery and analysis of the genetic basis of radiation sensitivity is also being pursued through other strategies, including the biochemical analysis of repair reactions and the purification of repair proteins, as well as the identification of human repair genes by homology to their counterparts in lower organisms (Section II.A.3). In many instances, the genes discovered by these routes are found to give rise to a high level of sensitivity when mutated but to affect only a small fraction of the human population or to be inconsistent with life. However, some genes affecting radiation sensitivity will probably have more subtle effects, either because the particular gene mutation only partially reduces gene product activity or because the gene is not vital for cellular response to radiation. Studies exploring the latter types of response, which may affect a much larger fraction of the human population, are described in Section III.A.

Table 2
Classification of radiosensitive disorders and cell lines

Type of defect	*Disorder / defective cell line(s)*	*Human gene designation* [a]	*Human gene location*	*Animal model phenotype* [b]
Probable DNA break repair defect and loss of cell-cyle control following damage	Ataxia telangiectasia Nijmegen breakage syndrome *irs 2*/V-series	*ATM* *NBS1* *XRCC8*	11q23 8q21 ?	AT-like [c] – –
DNA double-strand break repair defective and V(D)J recombination defective	*xrs* XR-1/M10 V3/*scid*/SX9 180BR	*XRCC5* *XRCC4* *XRCC7* *LIG4*	2q35 5q13 8p11–q11 13q33–q34	Immune deficiency [d] Embryonic lethal Immune deficiency [d] Embryonic lethal
Sensitivity to many different agents; some have DNA single-strand break repair defect and/or replication defect	46BR Bloom's syndrome EM9 irs1 irs1SF UV40	*L1G1* *BLM* *XRCC1* *XRCC2* *XRCC3* *XRCC9(FANCG)*	19q13 15q26 19q13 7q36 14q32 9p13	Viable (acute anaemia) Embryonic lethal Embryonic lethal Embryonic lethal – –
Radiosensitivity inferred from structural homology of genes to those known to be involved in response to radiation damage in lower organisms	– – – – – –	*ATR* *hRAD50* *hRAD51* *hRAD52* *hRAD54* *hMRE11*	3q22–23 5q23–31 15q 12p13.3 1p32 11q21	Embryonic lethal Embryonic lethal Embryonic lethal Viable Viable Embryonic lethal

a XRCC = x-ray cross complementing gene.
b Knockout mice except for *XRCC7;* (–) indicates no model yet available
c Symptoms similar to ataxia telangiectasia (see paragraph 62).
d Severe combined immune deficiency.

2. Mechanisms of enhanced sensitivity in human disorders

48. In addition to being radiosensitive, ataxia-telangiectasia cell lines show enhanced sensitivity to agents that have in common an ability to damage DNA molecules by producing highly reactive chemical radicals, causing both base damage and sugar damage, leading to breakage of DNA strands. Ataxia-telangiectasia cells have been found to be hyper-ensitive to a variety of chemicals that cause such DNA damage through radical action (bleomycin, neocarzinostatin, hydrogen peroxide, streptonigrin, phorbol ester [M5]). Also, inhibitors of DNA topoisomerases that can trap these enzymes during DNA-strand passage, leaving open breaks, are more effective at inducing chromosomal damage and cell killing in ataxia-telangiectasia cells than in normal cells [C1, H7, S11]. More recently, ataxia-telangiectasia cell lines have been found to be hypersensitive to restriction endonucleases; these enzymes produce only double-strand breaks in DNA by direct enzymatic cutting [C17, L56]. There is also evidence of modest chromosomal hypersensitivity in some ataxia-telangiectasia lines to agents such as UV light, especially when irradiated in extended G_1 phase, possibly because of the excessive production of breaks when DNA synthesis is attempted [E1, K4].

49. Experiments varying the time component either during or following irradiation have revealed the general nature of the defect in ataxia-telangiectasia cells. It was shown that normal cells held after irradiation in a non-growing state had some recovery (or sparing) from lethal effects, while ataxia-telangiectasia cell lines showed little or no sparing [C9, U15, W2]. More strikingly, irradiation at low dose rates, where the same dose was given over a period of days instead of minutes (factor of 500 difference in dose rates) showed a very large sparing effect on normal cells and little or no effect on ataxia-telangiectasia cells [C10]. These observations are consistent with an inability of ataxia-telangiectasia cells to recover from radiation damage, and they also show that the defect cannot be abrogated simply by allowing more time for damage restitution.

50. Lack of a sparing effect appears to be typical of a particular class of radiosensitive cell lines. Thus lines that are known to have a defect in the repair of DNA double-strand breaks, for example the xrs series and XR-1, also lack recovery under irradiation conditions in which their normal counterparts show a large sparing effect [S14, T7]. Similarly, yeast radiosensitive lines that are unable to rejoin double-strand breaks, because of a defect in recombination (*rad50, rad51, rad52)*, also lack sparing [R2, R3]. A substantial body of data in yeast supports the contention that the double-strand break is the DNA damage most likely to be lethal to cells, and that its repair is responsible for the recovery seen under sparing conditions [F3].

51. In contrast to these cellular studies implicating strand breakage as the type of DNA damage involved in the ataxia-telangiectasia defect, it has been difficult to prove that ataxia-telangiectasia cells have a break-repair defect at the molecular

level [M5]. Recently, irradiation at 37°C with low-dose-rate gamma rays has shown a small increase in DNA double-strand breaks following repair ("residual damage") in ataxia-telangiectasia cells relative to normal cells [B9, F10, F16]. However, cytogenetic studies have provided more satisfactory evidence for a break-repair defect: a significantly elevated fraction of unrestituted chromosomal breaks remain in ataxia-telangiectasia cells after irradiation [C11, T1]. Support for these findings has been obtained from the measurement of both DNA double-strand breaks and chromosomal breaks (using prematurely condensed chromosomes to allow rapid analysis) after gamma irradiation of normal and ataxia-telangiectasia lymphoblastoid cells at different phases of the cell cycle [P5]. A consistent decrease in the rapid component of repair was found in ataxia-telangiectasia relative to normal cells; this decrease was small and usually statistically non-significant for DNA double-strand breaks, but larger and significant for chromosomal breaks. Differences in amounts of residual chromosomal damage between normal and ataxia-telangiectasia cells give a close, but not exact, approximation to their relative survival levels after irradiation [C14, P5].

52. Hamster cell lines showing strong similarities to ataxia-telangiectasia have been isolated and their responses characterized (the irs2 line [J5] and the V-series [Z5]). These lines are hypersensitive to agents known to cause DNA breakage, have radioresistant DNA synthesis, and have no measurable biochemical defect in DNA break repair [T8, Z6]. It has also been shown that the radiation sparing effect is absent in irs2, while it is present in other lines that have similar radiosensitivity but do not show ataxia-telangiectasia-like characteristics (such as irs1 and irs3) [T30].

53. Overall, these studies strongly support the view that the increased sensitivity of ataxia-telangiectasia and related cell lines to agents such as ionizing radiation derives from an inability to recover from DNA breakage, leading to a higher level of residual chromosomal damage. However, the molecular mechanisms leading to radiosensitivity in this disorder are still not fully understood, despite considerable recent progress in defining the function of the ataxia-telangiectasia gene product (see Section II.A.3).

54. The functional defects in other cancer-prone disorders have also not been well characterized. Primary cells from Fanconi's anaemia patients have spontaneous delay and arrest in the G_2 phase of growth, as well as an increased frequency of chromosomal aberrations [J15] and recombination [T39]. G_2 delay and aberration frequency increases are corrected by lowering the oxygen tension during growth, leading to the suggestion that reduced detoxification of oxygen radicals may be responsible for the phenotype [C62, J17]. However, immortalized Fanconi's anaemia fibroblasts have lost this oxygen effect, showing that this factor is not a basic (or underlying) defect [S48]. In Bloom's syndrome, the high frequency of sister chromatid exchanges and specific types of chromosome aberrations suggested a defect in DNA repair and/or DNA replication.

3. Analysis of genes determining radiosensitivity

55. The classification of radiosensitive disorders and cell lines into genetic groups, followed by mapping and cloning of the affected genes, has dramatically increased knowledge of the molecular mechanisms of recovery from DNA damage. Once the affected gene has been cloned, its sequence may reveal the nature of the gene product (protein), because of similarities to known genes. Gene sequence data from at-risk groups will also allow deleterious mutations to be identified, and permit analysis of the role of these genes in disorders such as cancer. Manipulation and expression of the gene under defined experimental conditions allow specific functions to be studied in cells and in animals. Animal models of the human disorder can be created by replacing the normal pair of genes with defective copies (a knockout animal; see paragraph 46) and assessing the resulting phenotype.

56. Genetic classification of the ataxia-telangiectasia disrder initially indicated that several different genetic groups might exist [C3, J2], but mapping and cloning of a gene (*ATM*) found to be mutated in patients has cast doubt on this designation. The genetic mapping data, based on ataxia-telangiectasia family studies, mostly placed the affected gene into one chromosomal region, 11q23.1 [G2, Z2]. Positional cloning procedures in this region led to the identification of the *ATM* gene, which has homology to a gene family encoding PI-3 kinases [B31, S24, S25]. The PI-3 kinase family contains a number of large proteins involved in cell-cycle checkpoints, the regulation of chromosome-end length, and DNA break repair, including site-specific recombination (see Section II.B.1). It is therefore likely that *ATM* and other members of this family are involved in the detection of certain types of DNA alterations and may coordinate response by signalling these changes to other regulatory molecules in the cell [J8, K3, S25, T29].

57. Analysis of mutations in the *ATM* gene of ataxia-telangiectasia patients showed that the majority are compound heterozygotes (i.e. the mutations in the two gene copies derive from independent events) and that these commonly lead to an inactive, truncated protein [G19, M30]. However, individuals from 10 families in the United Kingdom with less severe symptoms (paragraph 42) all have the same mutation in one copy of *ATM* (a 137-bp insertion caused by a point mutation in a splice site) but differ in the mutation in the other gene copy. The less severe phenotype appears to arise from a low level of production of normal protein from the insertion-containing gene copy. Two more families with this less severe phenotype have mutations leading to the production of an altered but full-length protein, again suggesting that the severity of the symptoms in ataxia-telangiectasia is linked to the genotype of the individual [L41, M30]. However, it is possible that individuals with less severe symptoms have an increased risk of developing specific types of cancer [S78] (see also paragraph 139).

58. A further two families presenting with many of the symptoms of ataxia-telangiectasia (paragraph 39) but

without dilated ocular blood vessels failed to show mutations in the *ATM* gene. The cellular characteristics of family members, such as radiation sensitivity and DNA-synthesis delay (paragraph 40), were mostly intermediate between those of classical ataxia-telangiectasia patients and normal individuals. Examination of other genes implicated in the repair of DNA double-strand breaks revealed that the human homologue of the yeast *MRE11* gene (paragraph 74) was mutated in these families [S82].

59. A rare form of leukaemia, sporadic T-cell prolymphocytic leukaemia (T-PLL), shows a high frequency of *ATM* mutations; although some of the changes identified in the *ATM* gene were rearrangements, most mutations in T-PLL were single DNA base-pair changes in the kinase region of the *ATM* gene and did not lead to protein truncation [V5, Y8]. This finding has prompted the suggestion that the *ATM* gene acts as a tumour suppressor in cells that may develop T-PLL. There is no evidence for an involvement of the *ATM* gene in T-cell lymphoblastic leukaemias [L58], but in B-cell chronic lymphocytic leukaemia (B-CLL), 34%-40% of tumour samples showed about 50% reduction in levels of ATM protein [S70, S76]. *ATM* mutations were detected in the tumours of 6 out of 32 patients (18%); also 2 of these 6 patients had mutations in both tumour and normal cell DNA, indicating that they were carriers of an *ATM* gene defect (i.e, they inherited a mutation in one copy of the *ATM* gene; see paragraph 137 *et seq.*) [S76]. Similar results were obtained in a separate study [B62], and although they are based at present on small samples, the data suggest that the frequency of *ATM* mutations in the normal cells of patients developing B-CLL may be much higher than in the general population [B62, S76]. Patients with ATM deficiency also had significantly shorter survival times [S70].

60. Study of the ATM protein has shown that it has a nuclear location and is expressed in many different human tissues. Additionally, ATM protein does not increase in amount in response to cell irradiation, consistent with the idea that it is part of a DNA-damage-detection system rather than being regulated in response to DNA damage [L41]. However, the ATM protein does associate with DNA, and this interaction increases when the DNA is irradiated [S77]. The *ATM* gene has a complex structure with multiple transcription start sites, leading to messenger RNAs of different length and predicted secondary structure. This multiplicity of *ATM* transcripts may allow cells to modulate ATM protein levels in response to alterations in environmental signals or cellular metabolism [S40].

61. Initially it was also suggested that the Nijmegen breakage syndrome involves more than one genetic group [J1], but recent evidence shows that only one gene is involved [M41] and that this gene maps to chromosome 8q21 [M41, S59]. These data support clinical findings (paragraph 41) showing that the Nijmegen breakage syndrome is a separate radiosensitive disorder, distinct from ataxia-telangiectasia. The cloning of the gene (*NBS1*) mutated in this syndrome has confirmed this (paragraph 74), with the majority of patients carrying small deletions in this gene [M45, V6].

62. Knockout mice that lack a functional homologue of the ataxia-telangiectasia gene (*Atm*) have recently been bred; they show many of the symptoms of the human disorder, but also give further insights into the action of this gene [B33, E9, X3]. For example, the *Atm* knockout mice are viable but growth-retarded and infertile. They are also very sensitive to acute gamma radiation; at a whole-body dose of 4 Gy, about two thirds of the *Atm* knockout mice died after 5-7 days, while normal and heterozygous mice remained without morbidity after two months [B33]. Primary cells derived from the *Atm* knockout mice also show many of the characteristic features of cells from ataxia-telangiectasia patients [X4]. The cells grow poorly, are hypersensitive to gamma radiation, and fail to undergo arrest of the cell cycle following irradiation (see also paragraph 102). The *Atm* gene product was found to locate to homologous chromosomes as they associate (synapse) at meiosis in germ cells [K27]; loss of fertility in the knockout mice results from failure of meiosis due to abnormal synapsis and subsequent chromosome fragmentation [X3]. Immune defects occur in these mice, and the majority develop thymic lymphomas and die before four months. Based on the understanding to date of the defective response to DNA breakage in ataxia-telangiectasia, the meiotic failure and immune defects in these mice could both relate to an inability to respond to "programmed" DNA double-strand breaks (site-specific breaks that occur in the course of normal cellular processes). Such breaks are thought to be essential in meiosis, to drive the process of meiotic recombination, and they are required for V(D)J recombination in immune system development (see paragraph 82).

63. Unlike in the human disorder, no evidence was found of gross cerebellar degeneration in *Atm* knockout mice aged 1–4 months, but it is possible that the animals are dying too early for this symptom to be revealed [B33, X4]. However, behavioural tests indicated some impairment of cerebellar function [B33], and detailed studies of the brain in these animals showed that *Atm* deficiency can severely affect dopaminergic neurons in the central nervous system [E14]. There is an almost complete absence of radiation-induced apoptotic cell death (Section II.B.3) in the developing central nervous system of *Atm*-deficient mice, while the thymus shows normal levels of apoptosis after irradiation [H48]. Additionally, elevated levels of oxidative damage were recorded in mouse tissues affected by the loss of ATM, especially the cerebellum [B63].

64. In yeast cells, the closest sequence homologue to the *ATM* gene product is the Tel1 protein; mutations in the *TEL1* gene are associated with shortened chromosome ends (telomeres) and genetic instability (three- to fourfold increased levels of mitotic recombination and chromosome loss) [G22]. It has been known for many years that chromosomes in ataxia-telangiectasia cells show a relatively high incidence of telomere fusions, and recent studies have shown that preleukaemic cells from ataxia-telangiectasia patients have an increased rate of telomere loss [M29]. This loss may contribute to chromosomal instability [R40]. However, as yet the relationship between the telomere fusions and tumorigenesis in ataxia-telangiectasia is not clear.

Interestingly, loss of the *TEL1* gene does not lead to x- or gamma-ray sensitivity [G22, M31], but the combined loss of *TEL1* and another gene in the same family (*ESR1/MEC1*; see next paragraph) gives extreme sensitivity to gamma rays, suggesting that these two genes may functionally overlap in protecting the cell against radiation damage [M31]. In fission yeast, following prolonged growth, loss of these two genes led to circular chromosomes lacking telomeric sequences [N26].

65. A further member of the human PI-3 kinase family, related to *ATM*, has been found through homology searches [B32, C38]. The gene (named *ATR* or *FRP1*) encodes a protein that is most closely similar to the fission yeast *rad3* gene product, which is itself structurally and functionally related to the budding yeast *ESR1/MEC1* and the fruit fly *mei-41* gene products. Mutations in these genes render the yeast or flies sensitive to killing by both ionizing and UV radiation, and are known to play important roles in mitotic and meiotic cell-cycle controls. Expression of an inactive form of *ATR* at a high level in human fibroblasts increased radiation sensitivity by a factor of 2-3 and abrogated radiation-induced G_2 arrest (see paragraph 108). Additionally, cells with defective *ATR* develop abnormal nuclear morphologies, which may indicate further cell-cycle perturbations [C48, W50]. The *ATR* gene product is expressed at a high level in germinal tissue and localizes along unsynapsed meiotic chromosomes [K27], thereby playing a role that is complementary to that of the *ATM* gene product (paragraph 62). Thus in addition to their roles in mitotic cells, *ATM* and *ATR* gene products may be involved in the co-ordination of meiotic chromosome synapsis, perhaps by signalling breaks and monitoring repair synthesis to guard against genetic instability.

66. Some other genes affecting radiosensitivity in humans have also been mapped and cloned. Following the cloning of the human DNA ligase I gene, it was discovered that this was defective in a unique individual with growth retardation, immunological abnormalities, and cellular sensitivity to a variety of DNA-damaging agents, including radiation [B5]. The genes for other human DNA ligases have also been cloned using homology searches or protein purification [C31, W19], and subsequently another unique radiosensitive individual with impairment of double-strand break rejoining (described in paragraph 43) was found to be defective in DNA ligase IV [R38]. It has been established in yeast and mammalian cells that DNA ligase IV is specifically involved in the repair of DNA breaks by non-homologous end joining (Section II.B.1) [S64, T40, W46]. DNA ligase III has been implicated in the base-excision repair pathway (see paragraph 78).

67. Bloom's syndrome patients of different ethnic origins (Ashkenasi Jewish, French-Canadian, Mennonite, and Japanese) were found by cell fusion analysis to fall into one genetic group [W36]. The gene defective in Bloom's syndrome (named *BLM*) has been positionally cloned and shown to be similar to the bacterial RecQ helicase, a type of enzyme that opens up the DNA helix and is associated with genetic recombination in bacteria [E6, K39]. Knocking out the gene homologous to *BLM* in mice results in embryonic growth retardation and lethality, apparently because of a wave of increased apoptotic death [C61]. The yeast homologue of the *BLM* gene product interacts with DNA topoisomerase enzymes, known to be required for the resolution of interlocking DNA molecules following replication. In the absence of the *BLM* gene product, it is suggested that replicated DNA (in mitosis or meiosis) is entangled and may give rise to sister-chromatid exchange and chromosomal non-disjunction [W35]. Support for this idea comes from studies with the fission yeast, where the recQ helicase is required for the recovery from cell-cycle arrest during DNA replication following DNA damage; absence of the recQ helicase leads to an increase in the rate of genetic recombination [S60].

68. Cells derived from Fanconi's anaemia patients have been classified into eight genetic groups [J16, J19, S46], consistent with the heterogeneity of symptoms found (paragraph 45). To date, three of these genes have been mapped, *FANCA* to chromosome 16q24 [G21, P20], *FANCC* to 9q22 [S46], and *FANCD* to 3p22-26 [W37]. The *FANCA* and *FANCC* genes have now been cloned [F9, L43, S47]; these gene sequences predict proteins that are structurally different from each other and from other known proteins. Mutations of the *FANCA* gene were analysed in 97 patients from different ethnic groups with Fanconi's anaemia; the majority of mutations detected were either DNA base-pair alterations or small deletions and insertions scattered throughout the gene, with a smaller number of large deletions [L49]. The *FANCC* gene product is a cytoplasmic protein [Y7], but it interacts with the *FANCA* gene product, and the complex translocates to the nucleus [K40]. While this nuclear localization is consistent with a possible role in DNA repair, the precise function of these gene products remains unclear. *FANCC* also has binding sites for the p53 tumour suppressor protein (paragraph 100), and binding of p53 to the gene can regulate its expression [L46].

69. One of the more recently identified genetic groups of Fanconi's anaemia, FA-G, has been found to be caused by mutation of a previously-identified gene, *XRCC9* [D18]. This gene was identified by the complementation of a rodent cell line sensitive to a variety of DNA-damaging agents including a twofold enhanced sensitivity to x rays, and is potentially involved in post-replication repair of DNA [L59] (Table 2).

70. Human genes implicated in the repair of radiation-induced DNA damage have also been cloned by their structural homology to genes involved in specific repair pathways in lower organisms. For example, it is known that the main pathway for the repair of DNA double-strand breaks in lower organisms involves homologous recombination (paragraph 30); in budding yeast, the genes responsible are *RAD50*, *RAD51*, *RAD52*, *RAD53*, *RAD54*, *RAD55*, *RAD57*, *MRE11*, and *XRS2* (often called the *RAD52* group of genes). Mutations in these genes render the yeast cells defective in mitotic and/or meiotic

recombination and sensitive to ionizing radiation. At least some of the products of these genes act together in a multi-protein complex to effect recombination [H27, J14, U18]. In yeast, functional relationships have also been found between proteins of the PI-3 kinase family and some of the Rad52 group proteins. For example, the activity of the *RAD53* gene product, another protein kinase, is regulated by both *ESR1/MEC1* and *TEL1* gene products, suggesting that the Rad53 protein acts as a signal transducer in the DNA damage response pathway downstream of these ATM-like proteins [S43]. Sequence similarities have led to the cloning of human homologues of some of the yeast *RAD52* gene group: *hRAD50* [D16], *hRAD51* [S10, Y6], *hRAD52* [M10, S44], *hRAD54* [K28], and *hMRE11* [P22]. Preliminary evidence has been obtained from their abilities to partially correct the defects in radiosensitive yeast cell lines that some of these genes, or their mouse homologues, are involved in the repair of radiation damage [K28, M37]. Further, knocking out the *RAD54* gene in both the fruit fly [K41] and mouse [E12] confers radiation sensitivity and a defect in homologous recombination. There is also evidence for regulated expression of these recombination proteins through the cell cycle, with levels of RAD51 and RAD52 increasing during the S phase and peaking in G_2 in human and rodent cells [C5].

71. The human *RAD51* gene product has been shown to bind DNA and thereby underwind the double helix, an early step in the recombination process [B34]. The *RAD51* gene in yeast is not essential for cell survival, but recently the homologous gene has been knocked out in mice and surprisingly was found to give embryonic lethality [L42, T34]. Knockout embryos arrested early in development but progressed further if there was also a mutation in the gene encoding the tumour suppressor p53 (*TP53)*, possibly because of a reduction in programmed cell death (see paragraph 112). This may suggest that *RAD51* in mammals has functions in addition to recombination and repair, and in a separate study the human RAD51 protein has been shown to directly interact with p53 [S45]. Cells from the mouse *Rad51* knockout were hypersensitive to gamma rays, although this response has not been quantified, and show chromosome loss in mitotic cells [L42]. In mitotic cells, the mouse RAD51 protein is found to concentrate in multiple foci within the nucleus at the DNA-synthesis (S) phase of the cell cycle [T37]. In meiotic cells, RAD51 is found on synapsing chromosomes and disappears shortly after this stage [P23], but this pattern of localization of RAD51 is disrupted in *Atm*-deficient mice [B47].

72. The pattern of cellular localization found for the RAD51 protein has also been found for the product of a gene commonly mutated in familial breast and ovarian cancer patients, *BRCA1*; subsequently, RAD51 and BRCA1 proteins were shown to interact directly [S51]. *Brca1*-deficient mouse cells show a modest increase in sensitivity to ionizing radiation and have a reduced capacity to repair base damage in transcribing DNA (transcription-coupled repair, paragraph 28) [G34]. In human *BRCA1*-deficient cells, increased sensitivity to cell killing by gamma rays and reduced repair of DNA double-strand breaks can be partially corrected by introduction of the normal gene, while mutant *BRCA1* genes fail to restore these defects [S86]. A product of a second gene associated with familial breast cancer susceptibility, *BRCA2*, has similarly been shown to interact with RAD51 [M42, S61], as well as with p53 [M46]. *BRCA1* and *BRCA2* are highly expressed in rapidly proliferating cells, with expression highest at the start of S phase of the cell cycle [R23]. Analysis of mutations in the *BRCA* genes has shown that families with a high proportion of breast cancer tend to have mutations in different parts of the gene from families having a predisposition to ovarian cancer [G37, G38]. Despite their importance in cancer predisposition, the molecular function of the *BRCA* genes is unknown, and the finding of interaction with RAD51 provided the first clue that their role may be in DNA repair. As noted in paragraph 62, the ATM protein also has a specific pattern of localization to meiotic chromosomes, and loss of ATM can disrupt the localization pattern of RAD51. Thus, through their connections with *RAD51*, these data provide for the first time a mechanistic link between four different genes involved in cancer susceptibility (*ATM*, *TP53*, *BRCA1*, and *BRCA2*).

73. Mice have been bred with a knockout of the *Brca1* gene or the *Brca2* gene; like *Rad51* knockout mice, they were both found to arrest early in embryonic development [H40, L50, S61]. However, viable mice have been bred with a mutation in *Brca1* or *Brca2* that does not completely inactivate the gene. A conditional deletion of the *Brca1* gene in mice, confining the defect to the mammary glands, gave a low frequency of mammary tumour formation with long latency (see also paragraph 106) [X7]. Embryonic fibroblasts derived from *Brca1*-deficient mice proliferate poorly and show genetic instability [X8] and a reduction in the frequency of homologous recombination [M50]. About one third of the *Brca2*-deficient mice survived to adulthood but showed small size, poor tissue differentiation, absence of germ cells, and development of thymic lymphomas [C59]. Cells derived from viable *Brca2*-defective mice proliferate poorly, apparently as a result of spontaneously high levels of p53 and p21, causing cell-cycle arrest (see paragraph 100) [C59, P32]. These cells accumulate high levels of chromosomal aberrations and show enhanced sensitivity to DNA-damaging agents, including x rays [M48, P32]. The evidence linking the *BRCA* genes to DNA repair processes suggests that they may not function as tumour suppressors but are involved in the maintenance of genome integrity.

74. The yeast *RAD50, MRE11,* and *XRS2* gene products assemble into a multi-protein complex that is implicated in the processing of broken DNA, as well as in a number of other functions, including meiotic recombination and telomere maintenance [H46]. This multiplicity of roles for the complex may arise from its nuclease activities on both single- and double-stranded DNA ends that trim up the DNA in preparation for end-joining and homologous recombination processes. Human gene products with similar functions have been identified (paragraph 70); significantly, the gene mutated in the Nijmegen breakage syndrome *(NBS1*) has been found

to be the functional homologue of *XRS2* (paragraphs 41 and 61) [C54, M45, V6], and individuals with a variant form of ataxia-telangiectasia have mutations in the human homologue of *MRE11* (paragraphs 42 and 58) [S82]. The *NBS1* gene product contains motifs that are commonly present in proteins involved in cell-cycle regulation and DNA-damage response, suggesting that like ATM, this gene has a role in the signalling mechanism following damage to DNA by ionizing radiation. Study of the purified protein shows that DNA unwinding and nuclease activities of RAD50/MRE11 are promoted by the presence of NBS1 [P34]. The human RAD50 and MRE11 proteins co-localize in cell nuclei following ionizing radiation damage (but not after UV irradiation); their sites of localization were distinct from those of the RAD51 protein, consistent with their different roles in DNA repair [D17]. Co-localization of MRE11/RAD50 proteins was much reduced in ataxia-telangiectasia cells, suggesting that *ATM* gene signalling is important for the assembly of the break-rejoin complex [M28]. Additionally, the BRCA1 protein has been shown to colocalize and interact with RAD50, and radiation-induced sites of localization including MRE11, RAD50 and NBS1 proteins were much reduced in breast-cancer cells lacking BRCA1 [Z12].

75. Human genes encoding enzymes responsible for the repair of the numerous forms of base damage caused by DNA-damaging agents, including ionizing radiation, are also being discovered [L69, S49]. A number of key enzymes (DNA glycosylases, endonucleases) in the base-excision repair pathway have been isolated by biochemical purification, followed by protein sequencing and gene identification, or through their homologies to known enzymes in lower organisms. For example, the hOGG1 glycosylase [R41] removes 8-oxoguanine opposite a cytosine (paragraph 24), while the hNTH1 glycosylase [A28] removes oxidized pyrimidines such as thymine glycol (paragraph 17). The pathway of base-excision repair that results in the incorporation of a single nucleotide ("short-patch repair") has been reconstituted under cell-free conditions, using purified enzymes [K24]. Additionally, a second "long-patch repair" pathway has been identified, in which between two and six nucleotides are replaced following repair of a reduced or oxidized base-less site; this pathway also requires the structure-specific nuclease DNase IV (also known as FEN1) to remove the displaced nucleotides during repair [K42]. The reconstitution of base-excision repair of oxidized pyrimidines in DNA has revealed that hNTH1 glycosylase is strongly stimulated by one of the proteins involved in nucleotide-excision repair, XPG (xeroderma pigmentosum group G protein). The XPG protein binds to non-paired regions of DNA, and acts as a structure-specific nuclease where the unpaired region is greater than five base pairs, as will happen at sites of bulky damage. However, at sites of oxidative base damage it seems that the unpaired region is less than five base pairs, and instead of cutting DNA the XPG protein promotes the activity of the hNTH1 in removing the base damage [K46]. These findings may explain the extreme symptoms of some patients lacking XPG, including growth failure and neurological dysfunction, and the early death of mice carrying a knockout of the *Xpg* gene [H51] (while mice that are totally defective for nucleotide-excision repair such as those defective in *Xpa* are viable [N27]).

76. The main endonuclease involved in the repair of base-less sites (Figure Ia) in human cells, known variously as HAP1, APE, APEX, or Ref-1, was cloned in several laboratories following biochemical purification and was found to have a surprising additional function when compared with the bacterial enzyme. It stimulates the DNA-binding activity of transcription factors involved in signal transduction such as Fos, Jun, NFκB, and p53 by reduction/oxidation mechanisms [X5]. Signalling and repair responses to oxidative damage may therefore be coordinated through this one enzyme; this may be especially important during tissue proliferation, since *Ref-1* knockout mice die during embryonic development [X6]. Knockout mice have been produced for several other genes in the base-excision pathways, and a number of these have also proved to be embryonic-lethal, showing their importance for the normal functioning of cells and tissues [W45]. However, a knockout mouse for the broad-specificity glycosylase 3-methyladenine-DNA glycosylase II was recently shown to be viable [E13], possibly because of its mode of action (paragraph 23) and the likelihood that other more specialized glycosylases can substitute for it in removing damaged bases.

77. The abundant enzyme poly(ADP-ribose) polymerase, also known as PARP, is rapidly recruited to sites of DNA breakage following irradiation, where PARP transiently synthesizes long, branched chains of poly(ADP-ribose) on itself and other cellular proteins. These chains are degraded by another enzyme, poly(ADP-ribose) glycohydrolase, with a half-life of only a few minutes. The role of PARP in response to DNA damage, which has long been in dispute [C49], has been clarified from recent experiments in which the *Parp* gene was knocked out in mice [M9]. These mice are viable and fertile, although adult size is smaller than average and litter sizes are smaller than for normal mice. Following whole-body irradiation (8 Gy gamma rays), the *Parp* knockout mice died much more rapidly than normal mice from acute radiation toxicity to the small intestine; the survival half-time of these irradiated PARP-deficient mice was comparable to that of irradiated *Atm* knockout mice (paragraph 62). PARP-deficient cells or cells in which a mutated PARP is expressed [S63], show increases in chromosomal aberrations, sister-chromatid exchanges, and apoptosis following DNA damage. The average length of telomeres is significantly shorter in PARP-deficient mouse cells, leading to an increased frequency of chromosomal fusions and other aberrations [D21]. Recent biochemical data suggest that PARP has an important function as a molecular sensor of DNA breaks, especially single-strand breaks, and its absence reduces the efficiency of base-excision repair [O4]. In addition, it has been suggested that the synthesis of poly(ADP-ribose) chains causes negative-charge repulsion of damaged DNA strands, preventing accidental recombination between homologous sequences [S62].

78. To date, studies with radiosensitive mammalian lines have identified more than eight genetic groups [J3, T9, Z13]. The genes responsible have provisionally been named the *XRCC* (for *X-R*ay *C*ross-*C*omplementing) group, despite the fact that some of the cell lines are not primarily sensitive to x rays. The human gene corresponding to the first group, *XRCC1*, has been cloned and encodes a protein interacting with DNA ligase III [C2]. Further, the XRCC1 protein has been shown to associate with DNA polymerase β [C39, K29], which fills in the gaps created during the repair of damaged bases. It is suggested that XRCC1 may act as a scaffold protein in the final steps of base-excision repair (Figure Ia), supporting the activity of DNA polymerase β and DNA ligase III [K29]. The *XRCC2* gene [C66, L65, T35] and the *XRCC3* gene [L65]have been cloned recently, and both genes have structural homology to the yeast and human *RAD51* genes [T47]; it is likely, therefore that they are involved in repair of damage by homologous recombination. Other human genes recently cloned using mammalian cell lines (*XRCC4, XRCC5, XRCC7*) are involved in the repair of radiation-induced DNA double-strand breaks as well as in immune gene recombination, as detailed below (Section II.B.1). Current mapping data place all of the remaining genes on different chromosomes, and none maps to the location of characterized radiosensitive human syndromes (Table 2).

4. Summary

79. Several radiosensitive human disorders and examples of individual radiosensitivity have been identified in recent years. The sensitivity in these individuals is characterized by a greatly increased susceptibility to cancer, although this cancer is not necessarily radiation-induced. Many radiosensitive lines of cultured cells have also been established, and these have been useful for identifying the genes involved and for functional studies. In general, it has been found that enhanced sensitivity arises from an inability to recover from DNA damage, because of a reduction in damage detection and/or repair processes. Reduction of repair capacity commonly leads to a lack of low-dose-rate sparing and a higher level of genetic changes.

80. Considerable progress has recently been made in defining repair gene functions in human and other mammalian cells, and a summary of well-defined repair pathways for damage by ionizing radiation is shown in Figure III. A number of important genes have been cloned, including the gene *ATM*, which determines sensitivity in the ataxia-telangiectasia disorder. Studies with the *ATM* gene product and the production of mice defective for the gene suggest that it participates in the detection of DNA double-strand breaks and passes this information on to other important molecules regulating cellular response processes. Other recently discovered genes act directly in the repair of radiation damage; for example, the *RAD51* gene is vital for repair by homologous recombination. The *RAD51* gene product has also been found to interact with products of the breast-cancer susceptibility genes, *BRCA1* and *BRCA2*, suggesting an unsuspected role for these genes in damage recovery. In accordance with this finding, mice defective for the *BRCA* genes have symptoms very similar to *Rad51*-defective mice, and the *Brca2*-defective mice are radiation-sensitive. Additionally, some repair genes when knocked out in animals give embryonic lethality, showing that these genes have important roles in basic cellular processes influencing tissue development.

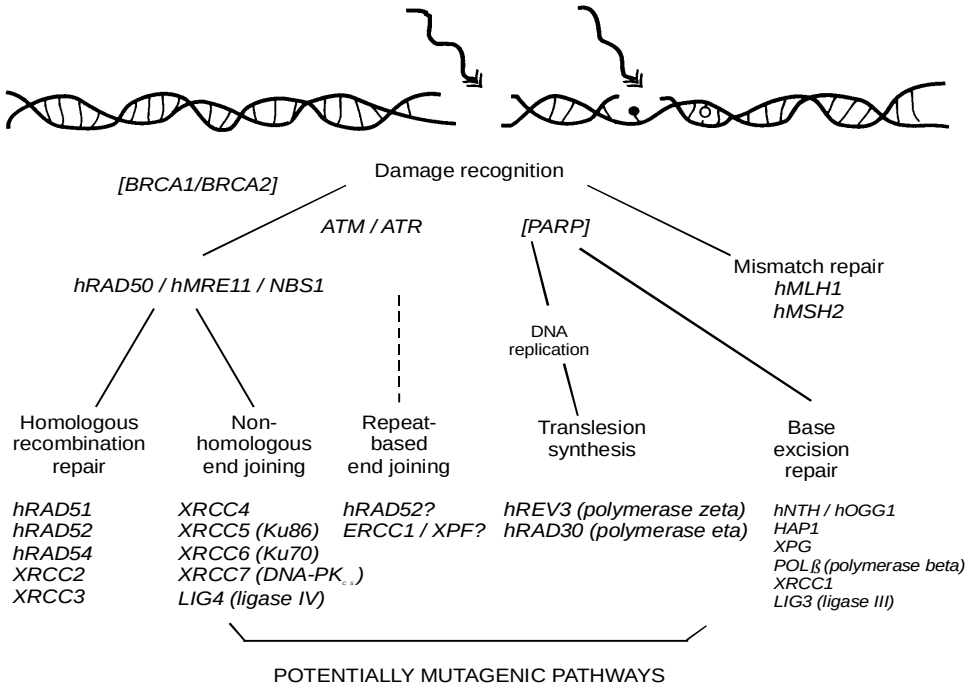

Figure III. Important genes in DNA repair pathways in human cells following damage by ionizing radiation.
Some of the important genes identified in each pathway are shown (the h prefix indicates a human homologue of a yeast gene); names of proteins are shown in brackets if different from the gene names. The BRCA genes and the PARP gene (square brackets) have speculative roles in recombination repair and base-excision repair, respectively. Pathways that can lead to mutation are indicated.

81. The conservation of repair genes from lower to higher organisms and progress in understanding the human genome suggest that further rapid progress will be made in the discovery and analysis of genes influencing radiation sensitivity. These genes will be important tools in understanding the extent of variation in radiation sensitivity in the human population and should help to identify individuals especially at risk.

B. RELATIONSHIP BETWEEN REPAIR AND OTHER CELL REGULATORY PROCESSES

1. Radiosensitivity and defective recombination in the immune system: non-homologous end joining of DNA double-strand breaks

82. It has been noted that radiosensitive disorders are often associated with some degree of immune deficiency. The recent discovery that the *scid* (severe *c*ombined *i*mmune-*d*eficient) mouse strain is radiosensitive has led to a reexamination of this relationship and to rapid progress in understanding at least one mechanism of DNA-break rejoining. In the immune system, functional immunoglobulin and T-cell receptor genes are assembled from separate genetic regions during lymphoid differentiation [A5]. The separate regions (termed "V" for variable; "D" for diversity, and "J" for joining) involved in this type of site-specific recombination are flanked by recognition (signal) sequences at which double-strand DNA breakage occurs prior to the rejoining of the regions. Mice homozygous for the *scid* mutation lack functional T-cells and B-cells because of a defect in V(D)J recombination [H8, L9], and about 15% of the mice develop lymphomas [C50]. It was shown that fibroblasts derived from homozygous *scid* mice are three to four times as sensitive as normal mice to x rays or gamma rays and have a reduced ability to repair DNA double-strand breaks [B8, H10]. Mice heterozygous for the *scid* mutation also show some increased sensitivity to gamma rays, compared to normal mice, and cultured bone marrow cells from *scid* heterozygotes are marginally more radiosensitive than normal cells [K45]. Detailed studies of repair capacity in *scid* cells show that the rate of repair of double-strand breaks is reduced by a factor of about 5, but if sufficient time is allowed (24 h) the final levels of repair are similar to those of normal cells [N20]. Subsequently, similar tests on several radiosensitive mammalian lines derived from cultured cells showed that those with large defects in double-strand break repair are also defective in V(D)J recombination, while radiosensitive lines, including ataxia-telangiectasia cells, with near-normal double-strand break repair are not V(D)J-recombination-deficient (Table 2) [H12, K25, P6, T4, T11].

83. At least four different gene products are common to V(D)J recombination and repair of radiation-induced DNA breaks; three of these products are defined by their respective rodent sensitive lines: xrs, scid/V3, and XR-1/M10 (Table 2). Recently there has been a breakthrough in identifying the genes and gene products complementing the defects in these lines, to define a repair pathway termed non-homologous end joining (paragraph 31). The xrs-complementing gene (also known as *XRCC5*) codes for a subunit of the Ku antigen (p86), a DNA end-binding protein discovered in normal human cells that reacted with sera from patients with certain autoimmune diseases [T5]. The *scid*/V3 protein (*XRCC7* gene product) has been found to be the catalytic subunit of a large DNA-dependent protein kinase, also known as p460, belonging to the PI-3 kinase family and related to the ataxia-telangiectasia gene product (see paragraph 56) [H17]. The Ku antigen consists of two subunits, p70 and p86, which interact to form a dimer; this dimer binds to broken ends of DNA, recruiting the p460 subunit and conferring kinase activity on the complex [G7]. The DNA-dependent protein kinase has been shown to phosphorylate a number of substrates *in vitro*, including the p53 tumour suppressor and RNA polymerase II [A4], but it is not clear whether these constitute important targets *in vivo*. The most recently cloned gene of this series, the human *XRCC4* gene complementing the XR-1 line, encodes a protein unrelated to any other yet described [L30]. Functional studies of the XRCC4 protein show that it is a nuclear phosphoprotein that is a substrate *in vitro* for the DNA-dependent protein kinase and that it associates with the recently discovered DNA ligase IV [C51, G30]. This discovery suggests that XRCC4 acts as a go-between in the assembly of a DNA-break repair complex in which the final step is mediated DNA ligase IV (paragraph 66). Experiments using cell extracts show that the rejoining of breaks by mammalian DNA ligases is stimulated by purified Ku86 protein, especially when the break ends cohere poorly [R24]. This observation suggests that Ku86 may function to stimulate ligation, perhaps through its ability to bridge the gap between broken ends. However, the precise mode of action of these proteins in promoting the repair of DNA double-strand breaks (and presumably in initiating a coordinated response through kinase action on relevant proteins) is presently under intensive study.

84. The different subunits of the DNA-dependent protein kinase are not induced following x irradiation [J22]. However, the gene encoding Ku86 in primates (but not in rodents) can be expressed in a form that has been found to respond to radiation damage. In this form the gene gives rise to a related protein, termed KARP-1, which includes a p53 binding site [M43], and the RNA transcript and protein were increased by a factor of up to 6 at 90 min following x-ray irradiation. Interestingly, this induction did not occur in cells defective in either p53 or ATM, suggesting that at least some of the non-homologous end joining is activated through interactions with p53 and ATM signalling processes [M44].

85. Sequencing the entire gene encoding the p460 kinase from *scid* cells showed that a single DNA base alteration leads to a premature stop codon in the highly conserved terminal region of the gene [A20], as other data had predicted [B37, D11]. This mutation gives a protein truncated by 83 amino acids in *scid* cells, leading to a partial abolition of kinase activity. Mutations in this gene have subsequently been

found in other radiation-sensitive lines of cultured cells; for example, the SX9 line (Table 2) has a mutation giving rise to a single amino-acid substitution at a position before that of the *scid* mutation, and leads to a more severe phenotype than the *scid* mutation [F25]. The partial loss of activity in *scid* [A19, B38, C52, P26] may explain another surprising phenomenon, whereby sublethal irradiation of *scid* mice can transiently rescue V(D)J recombination [D12]. While the mechanism of this rescue is still unknown, it suggests that p460 in *scid* is not completely inactive.

86. The importance of the catalytic subunit of DNA-dependent protein kinase in the development of T-cells and B-cells and in protecting animals against cancer has been illustrated by the chance finding of transgenic mice in which the *Xrcc7* gene was knocked out [J20]. These mice, named *slip*, showed a lack of mature lymphocytes, but most remarkably all animals died within 5-6 months from thymic lymphoblastic lymphomas. No tumours of B-cell or myeloid linkage were found, suggesting that *Xrcc7* acts as a tumour suppressor of the T-cell lineage. Treatment of *scid* mice with gamma rays (1 Gy) at 24-48 h after birth led to 86% developing T-cell lymphoma with very short latency, with no other tumour type observed [G35]. Further, mice having both the *scid* defect and a knockout of the *Parp* gene (paragraph 77) also develop very high levels of T-cell lymphoma, suggesting that the carcinogenic effects of a partial defect in double-strand break rejoining is exacerbated by a reduction in repair capacity for other types of DNA damage [M6].

87. The assessment of radiation sensitivity of different tissues has shown that epithelial cells of the intestine and kidney in *scid* mice show a much greater radiosensitization than bone marrow cells from the same animals, compared to the relative sensitivity of these cells in normal animals [H28]. However, the additional sensitivity of *scid* epithelial cells effectively made these cells similar in overall sensitivity to *scid* marrow cells (e.g. at a dose of 2 Gy), since normal epithelial cells are more radioresistant than normal marrow cells. Irradiation of epithelial cells at low dose rate (16 mGy min^{-1}) altered the survival of *scid* cells much less than it did for normal cells [H28], as expected for this type of radiosensitive cell line (see paragraphs 49–50).

88. In addition to the *scid* mouse as a model of defective DNA double-strand break repair, knockout mice for the gene encoding Ku86 have been created [N21, Z9]. These mice weigh 40%-60% less than normal mice, and further weight loss occurred when newborn mice were gamma-irradiated at whole-body doses in excess of 0.25 Gy. Survival of Ku86-deficient mice irradiated at 2-4 months old with gamma rays was also compromised; doses of 3-4 Gy caused 50% mortality in two weeks. Ku86-deficient mice given 2 Gy gamma rays survived for up to 12 weeks but unlike normal mice showed severe hair loss within one month post-irradiation. Examination of tissues at four days post-irradiation showed severe injury to the GI tract at much lower doses than for the normal mice, along with atrophy of lymphoid organs [N21]. In these mice it was found that T- and B-cell development was arrested at an early stage, as in *scid* mice. The Ku70 subunit has been knocked out in mouse embryonic stem cells, and these cells are sensitive to gamma rays. As might be expected, Ku70-deficient cells are defective in DNA end-binding activity and in V(D)J recombination [G31]. Ku70-deficient mice have similar growth reduction and radiation sensitivity to Ku86-defective mice, but they also develop a high frequency of spontaneous thymic and disseminated T-cell lymphomas [L57].

89. Mice lacking DNA ligase IV do not survive beyond the late embryonic stage of development; ligase IV-deficient embryonic fibroblasts are hypersensitive to gamma rays but not to ultraviolet light and are defective in V(D)J recombination [F20]. Very similar defects have been found in mice and murine fibroblasts lacking the *Xrcc4* gene. Further, lethality in XRCC4- and ligase IV-deficient mouse embryos is associated with severe disruption of the development of the nervous system due to extensive apoptotic cell death [G33]. It is presumed that the enhanced sensitivity of neuronal cells relates to an inability to repair DNA double-strand breaks, although it is not known whether these breaks result from normal metabolism or a specific neuronal process (e.g. a recombination process) required for neuronal function. It is of considerable interest that a single person defective in DNA ligase IV has been identified (paragraphs 43 and 66); this person showed extreme sensitivity to radiation and developed acute lymphoblastic leukaemia at age 14, but was not severely impaired otherwise. Analysis of the ligase IV gene and protein in cells derived from this person showed that ligase function was not completely defective, presumably explaining their relatively normal developmental progress [R38]. This example of repair deficiency is important in revealing that mutations leading to partial activity of the gene product may be permissive for growth and development, but may have undesirable consequences including the possibility of cancer formation.

90. The search for human mutations in the *XRCC4, 5, 6,* and *7* genes among patients known to be compromised in immune functions is beginning to yield some candidates [C28, H26].

91. There is a difference between the “programmed” double-strand breaks generated in the V(D)J recombination process and those caused by radiation damage. The breaks at V(D)J sites have 5'-phosphorylated blunt ends and can be rejoined by a DNA ligase without further processing [S6], while radiation-induced breaks are often not directly ligatable because of extensive damage to the sugars and bases at the break sites (see Section I.B). Since, in the cell, the non-homologous end joining pathway is involved in repairing both types of break, it may be suggested that the exact structure (and possibly complexity) of the breaks does not influence their recognition by the proteins that initiate the rejoining process. However, it seems likely that the context in which the break occurs will influence its repair; in yeast cells, three proteins involved in modulating chromatin structure (Sir2, Sir3, and Sir4) interact with the Ku70 protein and have a role in DNA break rejoining

[T41]. A defect in any of the three *SIR* genes led to increased sensitivity to gamma radiation, providing the homologous recombination pathway was inactive. While the changes in chromatin structure brought about by the Sir proteins are not well understood, it is thought that they may make the broken DNA inaccessible to other DNA-modifying enzymes such as nucleases and thereby protect the damaged DNA from loss. It has also been found that the V(D)J recombination process is restricted to the G_1 phase of the cell cycle [S6]. It is likely that this rejoining process is also cell-cycle-controlled in the repair of radiation-induced double-strand breaks [L51]. Thus, the XR-1 line is highly sensitive and defective in radiation-induced double-strand break repair in G_1 but has nearly normal sensitivity and break repair in late S phase [G5]. One implication of this analysis is that there must be at least two major pathways for the repair of DNA double-strand breaks: the non-homologous end-joining pathway active in G_1 and at least one other pathway operating in other stages of the cell cycle.

92. As noted in paragraph 70, there is evidence suggesting that the homologous recombination repair pathway is active in late S/G_2 stages of the cell cycle; additionally, chick cells defective in the *RAD54* gene were found to be more sensitive than normal cells in this part of the cycle. Chick cell lines were generated with defects in both the homologous recombination repair pathway and the non-homologous end-joining pathway (RAD54/Ku70-defective), and these showed increased radiation sensitivity and higher levels of chromosomal aberrations than either single defect [T42].

93. Unlike mammalian cells, in lower eukaryotic organisms such as the budding yeast, repair of DNA double-strand breaks is mainly effected through homologous recombination processes. However, proteins similar to the mammalian Ku70/86 have recently been found in yeast, and these form a dimeric complex binding directly to DNA ends. DNA end-joining in yeast was shown to be impaired when the genes encoding the yeast Ku proteins are mutated, and this process is distinct from the rejoining mediated by homologous recombination. However, it is possible that some activities are shared by both homologous and illegitimate recombination pathways, since it was found that the *RAD50* gene product (paragraph 70) interacts with the yeast Ku proteins [M32]. Mutation of the gene encoding the smaller Ku subunit (*HDF1*) has also been found to lead to shortened telomeres, similarly to *TEL1* mutations (paragraph 64), suggesting that in addition to their end-joining role these proteins may help protect yeast chromosome ends [P21]. Intriguingly, both the Ku and Sir proteins are located at telomeres in undamaged yeast cells, but following the induction of DNA double-strand breaks they relocate to break sites in the genome [M54]. Loss of *HDF1* gene function alone does not, however, lead to radiation sensitivity or a measurable defect in DNA double-strand break repair in yeast, while the combined loss of *HDF1* and homologous-recombination gene functions leads to extreme sensitivity [B35, S50]. Additionally, while the loss of homologous recombination repair in yeast leads to an elevated frequency of chromosomal aberrations, the combined loss of these two pathways gives a reduction in chromosomal aberration frequency [F24]. This observation has been considered as evidence for the involvement of non-homologous end joining mechanisms in chromosomal aberration formation.

94. While ataxia-telangiectasia cells are not defective in V(D)J recombination, a recurrent feature of the disorder is the appearance of T-cell clones with characteristic chromosomal rearrangements at sites of immunoglobulin and T-cell receptor genes [A7, B2, R4]. Elevated levels of recombination of T-cell receptor genes have been described in lymphocytes from ataxia-telangiectasia patients relative to those from normal individuals [L10]. ATM-deficient mice develop thymic lymphomas and die by 5 months (paragraph 62), but if the gene that normally causes programmed double-strand breaks leading to V(D)J recombination is inactivated, no lymphomas develop [L66]. This striking observation suggests that ATM normally suppresses aberrant recombination events.

95. The rejoining of DNA double-strand breaks, produced enzymatically, has also been examined using small DNA molecules transfected into mammalian cells or exposed to cell extracts. These studies have shown that almost any sort of break end (with flush ends, or with complementary sequence at the ends, or with mismatched ends) can be rejoined by cellular enzymes [N7, P29, R1]. However, even with complementary ends, a fraction of the breaks are rejoined with a loss of sequence around the break sites. This mis-rejoining process was found to occur by a non-conservative recombination mechanism [T10] that appears to differ from the non-homologous end-joining pathway. The mechanism entails deletion of DNA bases between short (2-6 bp) direct repeat sequences, such that one of the repeats is also lost (Figure IV; see also paragraph 182). Occasionally the mis-rejoining can be more complex, with an insertion of DNA at the deletion site. Using substrates that attempt to model more closely DNA double-strand breaks produced by radiation damage, where the sequences at the break-ends are not complementary, breaks were shown to be rejoined by either this repeat-driven mechanism or by a process of blunting the ends before rejoining [M33, R18]. The repeat-driven process of mis-rejoining has been shown to be independent of the Ku proteins [M33]. Recent data with yeast cells reveal that this mis-rejoining mechanism prevails where both the homologous recombination and non-homologous end-joining pathways are knocked out [B35, B36]. This error-prone mechanism constitutes, therefore, a third pathway for the rejoining of double-strand breaks common to many organisms. Interestingly, in extracts from two ataxia-telangiectasia cell lines, the frequency of mis-rejoining by this mechanism was about 20 times higher than in extracts from normal cell lines [G3, N7]. The genetic basis of this short repeat-driven process is unknown, although in principle it may require similar enzymes to those that recombine adjacent homologous sequences within a chromosome. Adjacent sequences with homology of several hundred base pairs recombine in a process termed single-strand annealing in both mammalian cells and yeast; in budding yeast some of these recombination

events are *RAD52*-dependent, but also require the products of the *RAD1* and *RAD10* genes [O5]. *RAD1* and *RAD10* combine to give a single-stranded endonuclease required to snip off the overhanging DNA strands generated by the recombination of adjacent repeats. In mammalian cells, the homologues of *RAD1* and *RAD10* are the nucleotide-excision repair genes *ERCC1* and *XPF*, respectively [W52], and there is evidence that the *ERCC1* gene is involved in recombination-dependent deletion formation in mammalian cells [S85].

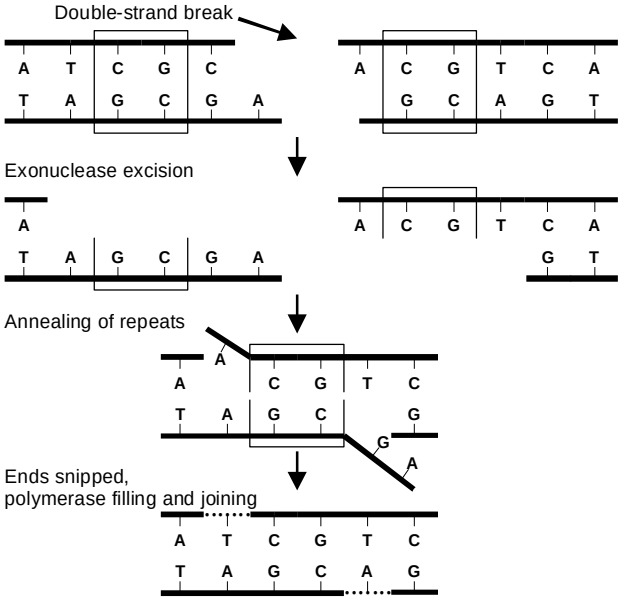

Figure IV. Direct-repeat end joining model.
Direct repeats (shown in boxes) are identified on either side of the initial double-strand break. Exonuclease removes opposite strand beyond repeats. Repeats are annealed, leaving extended tails (snip ends), which are removed. Strands are infilled by polymerase and ligated to give the deleted form with only one copy of the repeat.

96. Ataxia-telangiectasia cell lines were found to have elevated rates (by a factor of 30-200) of intrachromosomal recombination in an introduced DNA construct [M8]. These findings suggest that ataxia-telangiectasia cells have a general disturbance in recombination processes, and that the cell extract studies highlight those events leading to sequence loss and rearrangement (see Section IV.B). It seems likely that an elevated level of non-conservative recombination would also lead to an increase in mutation frequency, provided that the mutation system (i.e. target genomic region) allows detection of relatively large deletions and rearrangements. Evidence of an increased spontaneous frequency of mutations in ataxia-telangiectasia relative to normal persons has been found from direct analysis of their blood cells. Measurements made in ataxia-telangiectasia erythrocytes (loss of heterozygosity in the GPA system, paragraph 213) show loss mutations were elevated, on average, by a factor of 10, while ataxia-telangiectasia lymphocytes (*HPRT* gene mutation) show an average elevation of about 4 [C15]. In each system, however, there was a wide range of frequencies, from nearly normal to more than 100-fold higher than normal. These results are also supported by studies of transfection of small target genes carrying breaks at specific sites (by endonuclease cutting) on shuttle vectors. The proportion of correctly rejoined target genes was lower in ataxia-telangiectasia than normal cell lines [C13, R5, T6]. It is unclear whether this recombination defect is directly related to radiosensitivity in ataxia-telangiectasia, but disturbance of recombination would tend to give genetic instability and ultimately may lead to cancer-proneness.

2. Radiosensitivity and the cell cycle

97. Mammalian cells vary in their radiosensitivity through the cell cycle, generally showing greatest resistance during the period of DNA synthesis (S phase) and least resistance during G_2/mitosis and late G_1 (where a long G_1 exists) [S37]. To account for this variation in response, different hypotheses were advanced, such as fluctuation in radioprotective substances in the cell and/or duplication of the genetic material in S phase [S38, T27]. However, a number of studies indicate that the fluctuation in response correlates to the ability of cells to repair radiation damage when irradiated in certain cell-cycle phases [I3, W25]. Additionally, it has more recently been found that certain DNA repair-defective cell lines have altered cell-cycle responses (see paragraph 91). These results suggest that the opportunity for repair of damage in relation to DNA synthesis or segregation is an important determinant of response (see paragraph 9). Accordingly, the ability of the cell to optimize repair and to avoid the interaction of damage with other cellular processes by modulating the cell cycle after irradiation may be crucial to recovery and genome stability.

98. It is now well established that some radiosensitive (*rad*) cell lines in yeasts are defective primarily in regulation of the cell cycle. The paradigm for this type of defect is the *rad9* line of budding yeast. Although identified many years ago by sensitivity to x rays and UV light, it was discovered more recently to be defective for a regulatory function (termed a checkpoint) in the G_2 phase of the cell cycle. The loss of this checkpoint allows *rad9* cells carrying DNA damage to proceed into mitosis and die. The normal *RAD9* gene product appears to respond only to damage, since *rad9* mutant cells have normal division kinetics when unirradiated, although they do show spontaneous chromosomal instability [W6, W7]. Irradiated *rad9* cells have been shown to be defective also in extended-G_1 arrest, indicating that the normal *RAD9* gene product operates at more than one transition point in the cell cycle [S9].

99. Numerous other checkpoint genes have now been identified, especially in lower eukaryotes such as yeasts, and homologues of many of these regulatory genes are present in human cells [C42]. It is clear, therefore, that all cells regulate their cycling in relation to a variety of signals to make sure that a proper progression of phases occurs (e.g. that mitosis does not occur until the S phase is finished) but also to ensure that the genome is intact before starting a new phase. Certain gene products, for example, the tumour suppressor p53, appear to be central to this monitoring process in mammalian cells [A21, C43], and the detailed workings of this regulatory network are currently the subject of intense research.

100. It has been known for many years that x irradiation of mammalian cells in extended-G_1 phase (slowly proliferating primary cultures or cells grown until they run out of nutrients ["plateau phase"]) induces a dose-dependent G_1 arrest [L7, N1]. This arrest is evident at doses as low as 100 mGy, but the duration of the arrest is reduced if the G_1 cells are allowed time to recover before restarting the cycle. Kastan et al. [K2] observed increases in the levels of the tumour suppressor protein p53 correlated to a transient G_1 arrest after relatively low doses (500 mGy) of gamma radiation in normal human haematopoietic cells. Cells that were mutant for p53 showed no G_1 arrest but retained the G_2 arrest found typically after irradiation (but see also paragraph 109). The apparent increase in p53 derives from a prolonged half-life of the protein (the protein half-life is normally only about 30 minutes). The link between p53 and G_1 arrest has been generalized for other cell types and for a variety of DNA-damaging agents [F4]. A likely role for p53, given its function as a transcriptional activator, is to activate the genes involved in negative growth control. Radiation-induced G_1 arrest was found to correlate to inhibition of at least two members of the compound family of proteins that controls cell-cycle progression (the cyclin/cdk proteins); this inhibition was dependent on p53 and was mediated by the p53-inducible kinase inhibitor p21 [D4].

101. The signal for p53-dependent G_1 arrest is likely to be DNA breakage; agents causing DNA double-strand breakage (ionizing radiation, bleomycin, DNA topoisomerase inhibitors) are very effective inducers, while UV light appears to cause p53 induction through the processing of base damage into breaks. The introduction of restriction endonucleases into cells has shown that DNA double-strand breakage alone is sufficient for this response [L14, N5]. Microinjection of broken DNA molecules into cell nuclei has suggested that very few double-strand breaks may be required for arrest, and only one or two may be sufficient [H29].

102. Ataxia-telangiectasia cells lack DNA-synthesis arrest (Section II.A.1) and G_1 arrest, and the DNA-damaging agents that strongly induce p53 are also the agents to which ataxia-telangiectasia cells are sensitive [L14, N5]. It has further been shown that p53 induction is reduced and/or delayed in irradiated ataxia-telangiectasia cells and suggested that the ataxia-telangiectasia gene product (ATM) is part of a signalling mechanism that induces p53 [K3, K6, L14]. A similar alteration of p53 response has been found after irradiation of cells derived from patients with Nijmegen breakage syndrome [J21]. ATM has been shown to physically associate with p53; it is responsible for a rapid phosphorylation of a specific residue at the N-terminal end of p53; this phosphorylation occurs within minutes of treatment of cells with ionizing radiation or radiomimetic drugs but not after UV light treatment [B59, C58, K43, S73]. These processes contribute to its increased half-life following irradiation and also lead to the association of p53 with other proteins involved in damage-signalling pathways. In mouse fibroblasts deficient in both ATM and p53 proteins (or ATM and p21), the loss of the G_1/S cell-cycle checkpoint following irradiation is no worse than for either single defect. This again suggests that ATM and p53 operate in a common pathway of cell-cycle control in response to DNA damage. However, it seems that ATM may regulate only some aspects of p53-dependent responses; for example, the ATM defect leads to premature senescence in mouse fibroblasts, and in this case the combined deficiency of ATM and p53 [W47] or ATM and p21 [W49] alleviates the senescence.

103. The ATM protein has also been shown to interact directly with the proto-oncogene c-Abl [B48, S65], a protein kinase that itself interacts with other important cell-cycle regulators such as the retinoblastoma protein and with RNA polymerase II. When cells are irradiated, c-Abl is activated in normal cells, but this activation is absent in ataxia-telangiectasia cells. Additionally, cyclin-dependent kinases are resistant to inhibition by radiation in ataxia-telangiectasia cells, and this appears to be due to insufficient induction of p21 [B30]. A kinase named CHK1 that links DNA repair with cell-cycle checkpoints in yeast, has recently been found to be conserved in humans and mice and is dependent on ATM for its activity in meiosis. Like ATM (paragraph 62), CHK1 protein localizes along synapsed meiotic chromosomes, probably at the time that they are repairing DNA breaks. It is speculated that CHK1 acts to coordinate signals from both ATM and ATR (paragraph 65) to ensure the correct progression of meiosis [F17]. The activity of a second kinase, CHK2, which is homologous to the yeast RAD53 protein (paragraph 70), has also been shown to be ATM-dependent; this kinase is involved in the negative regulation of the cell cycle [M23]. It seems likely, therefore, that the ATM protein is involved in multiple signal-transduction pathways, by virtue of its interaction with a number of important regulatory proteins.

104. Alterations in the response of cell-cycle regulators in ataxia-telangiectasia cells may be involved in some aspects of the disorder but are unlikely to be responsible for the radiosensitivity of ataxia-telangiectasia. For example, p53-deficient cells have a tendency to be radioresistant rather than radiosensitive [L6], and SV40-transformed ataxia-telangiectasia cell lines in which p53 function is ablated still have the characteristic radiation sensitivity. It is more likely that any compromise of p53 response will affect cancer-proneness; both human patients (Li-Fraumeni syndrome) and *Tp53*-knockout mice show elevated levels of cancer [D3, M4]. Loss of one ($Tp53^{+/-}$) or both ($Tp53^{-/-}$) gene copies in mice also has significant effects on sensitivity to radiation-induced cancers, seen as a much reduced time for tumour onset. When $Tp53^{+/-}$ mice were irradiated with gamma rays to a dose of 4 Gy at 7-12 weeks, the tumour onset time was reduced from 70 to 40 weeks, while $Tp53^{-/-}$ mice showed a decreased latency only if irradiated when newborn (because at later times their frequency of spontaneous tumour development was already extremely high, masking the effect of irradiation). It is of interest that p53-deficient mice show a high incidence of thymic lymphomas, similar to ATM- and BRCA2-deficient mice (paragraphs 62 and 73), although it should be remembered that this result may be influenced by the fact that all of these mice were produced in the same genetic

background. In the *Tp53*$^{+/-}$ mice, the normal *Tp53* allele was commonly lost in tumour cells; it was notable that in radiation-induced tumours there was both a high rate of loss of the normal allele and a duplication of the mutant allele [K11].

105. Inactivation of mitotic checkpoint genes has been found in tumour cells derived from both BRCA1-deficient and BRCA2-deficient mice (paragraph 73), suggesting that these defects cooperate in cancer progression. Conditional deletion of the *Brca1* gene led to a low frequency and long latency for mammary tumours, but *Tp53*$^{+/-}$ mice carrying this *Brca1* mutation showed a significant acceleration in mammary tumour formation [X7]. It may be significant that the phosphorylation of BRCA1 in response to DNA damage is mediated by ATM [C67], in view of the potential involvement of ATM in breast cancer (paragraph 137 *et seq.*). In BRCA2-deficient cells, mutations in *Tp53* or genes controlling the mitotic checkpoint were implicated in promoting cellular transformation and the development of lymphomas [L64]. Centrosomes, which control the movement of chromosomes during mitosis, are abnormal in both BRCA1- and BRCA-2-deficient cells, leading to unequal chromosome segregation [T46, X8].

106. Mice that lack DNA-dependent kinase (*scid* phenotype, paragraph 82) accumulate high levels of p53 because of the presence of "natural" DNA double-strand breaks from unrepaired V(D)J recombination intermediates but show a typical G_1 arrest following irradiation. These mice develop thymic lymphoma with low incidence (15%) and long latency. The importance of p53 expression in protecting the animals from cancer was shown in mice that lack both DNA-dependent kinase and p53; these mice show prolonged survival of lymphocyte precursors and onset of lymphoma/leukaemia by 7-12 weeks of age (mice defective in p53 alone develop lymphoma to 50% incidence at 16-20 weeks). Cell lines derived from these double knockout mice were also about 10 times more resistant to gamma radiation than lines from *scid* mice. It was suggested that p53 may normally limit the survival of cells with broken DNA molecules and therefore that p53 loss promotes genetic instability [G23, N14].

107. Many of these p53-related responses may be associated with other functions of p53, in particular its role in radiation-induced apoptosis (programmed cell death, Section II.B.3). It is clear that DNA- break-inducing agents, including ionizing radiation, kill some cell types through a p53-dependent apoptotic pathway, although this pathway is not involved in the induction of apoptosis by other stimuli [C6, L13]. Alterations in the control of apoptosis may be linked to carcinogenesis, where a loss of p53 function may lead to the survival of precancerous cells, as has been shown in the induction of skin cancer by UV light [Z4].

108. It has also been known for many years that irradiated mammalian cells may arrest in G_2. The G_2 checkpoint is also controlled by specific cyclin/cdk proteins, and gamma radiation rapidly inhibits the kinase component ($p34^{cdc2}$) at doses resulting in G_2 arrest [L12]. Conversely, $p34^{cdc2}$ activation accompanies release of a radiation-induced G_2 block by drugs such as caffeine [H14]. In cultured cells, caffeine-induced release of the arrest decreases survival [B49] and increases the proportion of cells dying from apoptosis [B40]. However, it has also been found that caffeine treatment leads to an increased frequency of chromosomal aberrations [L40, T26], although the link between these two effects has been questioned [H18]. These observations suggest that the arrest promotes survival and may reduce the probability of genetic alterations. This view is supported by experiments with arrest-defective *rad9* yeast cells, where the imposition of an artificial G_2 delay promotes survival [W6]. However, in some radiosensitive mammalian cell lines, including ataxia-telangiectasia, enhanced delay in G_2 is commonly linked to enhanced sensitivity [B3, B6]. It seems likely that because of repair defects these cells never progress further in the cycle and die (that is, the cell-cycle arrest is irreversible, because the damage signal is not reduced). The signal for this response could be unrepaired DNA breaks; recent experiments in recombination-deficient yeast carrying a single unrepaired double-strand break in an inessential DNA molecule show that this can be lethal to a majority of cells [B7].

109. Initially there was little evidence of a role for the p53 protein in G_2 arrest (paragraph 100), but more recent data with p53-deficient mice and cells have shown that p53 is required for correct control of entry into mitosis following DNA damage [B60, S74, S75]. Similarly, cells established from patients with germ-line *TP53* mutations (Li-Fraumeni syndrome) have a consistent defect in G_2 response [G36].

3. Apoptosis: an alternative to repair?

110. Early studies of irradiated mammalian cells recognized that there are different forms of cell death [O1]. Attempts to correlate cell death with the formation of chromosomal aberrations concluded that a major cause of death in fibroblastic cells is genetic damage resulting in the loss of chromosome fragments [B22, C26, D8]. The most conclusive experiments were those of Revell and co-workers, showing a close correlation between loss of chromosome fragments, observed as micronuclei, and cell death in individual diploid Syrian hamster BHK21 cells [J13]. It is envisaged in these cells that genetic imbalance, perhaps due to the loss of specific essential genes, results in death once the pre-irradiation levels of their gene products have decayed. However, cells from a number of different developmental lineages are known to undergo rapid cell death, often termed interphase death, after irradiation [H16, P18, Y4].

111. It is now recognized that the balance between cell proliferation and cell death is crucial to the correct development of organisms, and that cell deaths in many tissue lineages are programmed by a genetically-controlled process known as apoptosis. This process was first described as a characteristic breakdown of cellular structures, including DNA degradation [K14, W33]. Apoptosis is initiated through specific cell surface receptors in response to external developmental signals but

may also be induced by DNA damage. Radiation-induced interphase death is considered to be an example of apoptosis [A3, U14, Y3], although the occurrence of apoptosis has more recently been reported in cell lines dying at various times after irradiation [R8, Y5].

112. Initiation of rapid apoptosis in normal cells by radiation appears to be dependent on the induction of the p53 protein [C6, L13], although delayed apoptosis may occur in cell lines deficient in wild-type p53 protein [B27, H37]. While p53-dependent apoptosis does not involve the same receptors as developmentally-regulated apoptosis, thereafter the molecular pathways converge to a common cell death programme. The mechanism of signalling by p53 is not well understood, although p53 can bind DNA at specific sites ("response elements"), and this binding is strongly stimulated by DNA ends, short regions of single-stranded DNA, and short mismatched DNA segments [J9, L28]. The importance of this DNA binding is demonstrated by the fact that the vast majority of *TP53* mutations identified in tumour cells occur in the part of the gene specifying DNA-binding activity [H25]. Relatively stable binding to DNA (half-life >2 h) can allow a variety of subsidiary processes to occur, such as the activation of specific genes. Because p53 has the properties of a transcriptional activator, it may initiate apoptosis by switching on a specific set of genes. Alternatively there is evidence that in some situations p53 may act in the opposite way, repressing genes concerned with cell survival [C23, C32].

113. A second pathway of radiation-induced apoptosis is proposed to work through a membrane-associated signalling system responding to a variety of extracellular stresses. Sphingomyelin and possibly other sphingolipids at the membrane respond to stress by rapidly releasing ceramide, which accumulates and activates protein kinases to initiate cell-cycle arrest and apoptosis. It has been suggested that ceramide acts as a "biostat", measuring and initiating response to cell stresses in the same way as a thermostat measures and regulates temperature [H34]. Radiation can activate components of the ceramide pathway in isolated membranes, suggesting that DNA damage is not required [H35]; however, this suggestion has been challenged [R39], and recently it has been found that another function of the *ATM* gene is to modulate ceramide synthesis following radiation-induced DNA damage [L67]. Lymphoblasts from patients with the Niemann-Pick disorder have an inherited deficiency in acid sphingomyelinase, an enzyme hydrolysing sphingomyelin to give ceramide, and fail to show ceramide accumulation and apoptosis following irradiation. Comparison of mice with a knockout in the gene for acid sphingomyelinase with *Tp53*-knockout mice shows that the initial stages of the sphingomyelin-dependent apoptotic pathway are distinct from those of p53-mediated apoptosis. These two pathways may be prevalent in different tissues; sphingomyelin-dependent apoptosis prevails in the endothelium of the lung and heart and the mesothelium of the pleura and pericardium, while thymic apoptosis is especially dependent on p53. The acid-sphingomyelinase-deficient mice develop normally, showing also that this apoptotic pathway is distinct from developmentally-regulated apoptosis [S55]. In tumour cell lines where *TP53* is mutated, it has also been shown that resistance to radiation correlates to loss of ceramide accumulation [M1].

114. Whether or not the cell goes into apoptosis in response to a given radiation dose is thought to depend on the availability of other proteins that promote or inhibit further steps in the programme. The details of these steps are still being worked out, but it is clear that a diversity of factors influences apoptosis. For example, cells may become susceptible to radiation-induced apoptosis through functional loss of the retinoblastoma (Rb) protein, a cell-cycle regulator, by either mutation of the *RB* gene or the expression of viral oncoproteins that inactivate Rb [S42]. Conversely, the gene product of the *BCL-2* oncogene, first identified at a site of chromosome translocation in B-cell lymphomas, can block radiation-induced apoptosis [R10]. It has been found that BCL-2 and related proteins localize to cellular membranes, especially of mitochondria, where permeability transitions are important in regulating the apoptotic process [G25]. The competitive formation of protein complexes by members of the BCL-2 family is suggested to control susceptibility to apoptosis; some of these proteins are antagonists, like BCL-2, while others are agonists, and the relative proportions of these proteins determine whether a cell will respond to an apoptotic signal [K5, K19, W34].

115. The extent of apoptosis for a given radiation dose does not appear to be affected by changes in the dose rate or by dose fractionation [H38, L26, L32], although there has been a recent report of a reduction in apoptosis at very low dose rates from gamma rays (<1.5 mGy min^{-1}) [B66]. There is an effect of cell-cycle stage on the extent of apoptosis; in contrast to the "classical" pattern of resistance (Section II.B.2), mammalian cells appear to be resistant in G_1 and sensitive in S phase [L26, L33]. In cells that are relatively resistant to apoptosis, the oxygen enhancement ratio was found to be similar for apoptosis and for clonogenic cell survival [H36]. The relative biological effectiveness (RBE) of fast neutrons for apoptosis in thymocytes was found to be 1 [W16], in accordance with earlier measurements of interphase death in thymocytes and lymphocytes [G15, H19]. However, for intestinal crypt cells of mice, where there is a highly sensitive subpopulation of cells in the stem-cell zone, the RBE for apoptosis was 4 for 14.7 MeV neutrons and 2.7 for 600 MeV neutrons [H38, H39]. It is not known why the RBE varies for different tissues; both thymocytes and intestinal cells show p53-dependent apoptosis [L13, M39], but it is possible that their relative abilities to repair radiation damage differ (see paragraph 87). The proportion of cells dying from apoptosis, relative to other forms of death, was shown to be constant at different doses (range 2-6 Gy) in cells where the main cause of death was apoptosis [L26]. This result does not suggest, at least for doses in excess of 2 Gy, that cells switch on the apoptotic pathway because their repair systems are unable to cope with the level of damage. In human lymphocytes, significant induction of apoptosis by gamma rays could be measured at doses as low as 50 mGy, and some evidence was found that individuals vary in their apoptotic response [B41].

116. It has been suggested that alterations in the p53-dependent apoptotic pathway play an important part in the sensitivity of ataxia-telangiectasia [M38] and Fanconi's anaemia [R20, R34], but this view has not been supported by other studies [B51, D15, E15, K35, T43]. It has been found that p53-mediated apoptosis is normal in irradiated *Atm* knockout mice [B50], but that *ATM* is involved in the regulation of the ceramide-dependent pathway [L67]. Further study of apoptotic responses, especially p53-independent pathways, are required to clarify these responses, preferably in primary cells from these disorders.

4. Summary

117. A link between radiation sensitivity and immune system defects has been revealed from studies of DNA repair genes; the same gene products are used to assemble functional immune genes and to repair radiation-induced DNA breaks by non-homologous end joining. Cells and animals defective in genes in this pathway are extremely sensitive to ionizing radiation and lack low-dose-rate sparing. While non-homologous end joining seems to be the main mechanism in mammalian cells for DNA double-strand break rejoining, at least two other pathways exist, and in lower organisms double-strand breaks are mostly repaired by the homologous recombination pathway.

118. An important component of the non-homologous end-joining pathway, the *XRCC7* gene encoding the catalytic subunit of DNA-dependent kinase, is related in structure to the ataxia-telangiectasia gene product and has been shown to behave as a tumour suppressor. The mechanism of rejoining by this pathway and the relationship of the components of the pathway to other regulatory processes (such as cell-cycle control) are at present the subject of intense research activity.

119. It is clear that the fidelity of rejoining by the different repair pathways is an important determinant of the mutagenic consequences of DNA damage (see also Figure III). Homologous recombination repair is likely to be the only error-free pathway. However, since even this pathway depends on copying information from another DNA strand, the fidelity must relate to the quality of both the information copied and the enzyme (polymerase) used for the copying. Pathways of double-strand break repair based on illegitimate recombination (non-homologous end joining, repeat-driven rejoining) will commonly be error-prone, although in principle the non-homologous end joining pathway can be error-free where the nature of the damage allows this (e.g. repair of a double-strand break formed by the overlap of two single-strand breaks sufficiently separated to allow for templated repair). Repeat-driven rejoining leads to deletion formation (mis-rejoining), and may act as a back-up for damage that is not (or cannot be) repaired by the other pathways. It is still not clear which factors determine the likelihood that a specific damage site will be repaired by a particular pathway [T44]. The complexity of damage may influence this question (Section I.B), as well as the possibility that radiation damage induces the activity of specific repair enzymes or pathways (Section III.B). In future work it will be important to establish the relative efficiencies of these different pathways at different doses and dose rates and in different cell types, so that mutagenic consequences can be predicted. The potential consequences of error-prone repair pathways at low radiation doses are explored further in Annex G, "*Biological effects at low radiation doses*".

120. When growing cells are irradiated, they arrest their cell-cycle processes, apparently to allow time for repair to be completed satisfactorily. This arrest is part of a checkpoint function that monitors the physical state of DNA at different stages of the cycle. Cells that have lost a checkpoint may be as radiation sensitive as cells that have lost DNA repair capability. Many genes are involved in controlling the cell cycle and determining checkpoints. In mammalian cells, the p53 protein is important in response to DNA breakage and controls both arrest in the G_1 phase and one pathway of programmed cell death (apoptosis). Animals and humans (Li-Fraumeni syndrome) deficient in p53 show elevated levels of cancer; irradiation of p53-deficient mice has a marked effect on the latency period for tumour formation and gives a high incidence of thymic lymphomas. The ataxia-telangiectasia disorder has defects in cell-cycle checkpoint functions, and there is evidence suggesting that ATM and p53 operate in a common pathway of cell-cycle control in response to DNA damage.

121. Apoptotis (programmed cell death) is also induced by ionizing radiation, both through the p53-dependent pathway and a membrane-associated signalling pathway. The relative importance of these pathways varies in different tissues; also, the importance of apoptosis as a mechanism of cell death in response to radiation varies with the cell type and developmental stage. The relationship between apoptotic death and radiation injury differs from that for genetic death (loss of essential genes through damage to the genome). Changes in dose rate or dose fractionation do not appear to affect apoptotic responses, and the response of cell-cycle stage is the reverse of the pattern found for genetic death. While it is attractive to consider that in the face of excessive damage, radiation-induced apoptosis is an alternative to DNA repair, the evidence for this possibility is not convincing. Loss of ability to respond to apoptotic stimuli will allow the accumulation of cells that may carry genetic damage and can therefore be a cancer-promoting event [W8]. This phenomenon is seen, for example, in *Tp53*-knockout mice (paragraph 104) and in mice overexpressing the BCL-2 protein [M21]. In this respect, the correct functioning of apoptotic pathways can be viewed as a complementary mechanism to the repair of DNA damage, removing damaged cells from the population.

III. HUMAN RADIATION RESPONSES

A. CONTRIBUTION OF MUTANT GENES TO HUMAN RADIOSENSITIVITY

122. The term radiosensitivity is used to indicate an abnormally increased response to ionizing radiation of both the whole body (see Section II.A.1) and cells derived from body tissues. Further, in cells the radiation response may be measured in different ways. For example, the ability of cells to grow and form clones (cell survival) following irradiation is commonly used as a measure of sensitivity, but more sensitive assays based on chromosome damage may also be used. In the comparative measurement of cell survival, the terms D_0 (mean lethal dose calculated as the inverse of the slope for exponential survival curves represented semi-logarithmically), D_{bar} (mean lethal dose calculated as the area under the survival curve in linear representation [K34]), or D_{10} (dose to give 10% survival) are commonly used.

123. The range of sensitivity of cells from supposed normal individuals has been measured in survival experiments following multiple doses of acute low-LET radiation. In two major studies with human diploid fibro-blasts, the sensitivity to dose was found to vary by a factor of about 2: D_0 range = 0.98-1.6 Gy (mean = 1.22±0.17 Gy) in 34 cell lines tested [C8] and D_0 range = 0.89-1.75 Gy (mean = 1.23 ± 0.23 Gy) in 24 cell lines [L8]. The degree of interexperimental variation for a given cell line was generally small ($\leq$20%), although in one of these studies cell lines derived from a mother and daughter showed survival curves that could also vary by a dose factor of about 2 on repeated testing [L8]. However, it was concluded that neither cell culture conditions (including cloning efficiency and population doublings) nor age and sex of the donor were correlated to the observed differences in radiosensitivity [C8, L8]. This suggests that unknown genetic factors that affect radiosensitivity vary in the cells of normal individuals.

124. More recently it has also been possible to measure cell survival with peripheral blood T-lymphocytes, irradiated as resting (G_0 phase) cells, to assess the range of radiosensitivity in humans. G_0 lymphocytes tend to have a more curvilinear response to dose than cycling fibroblasts and, on average, a higher survival at low doses than fibroblasts. Early-passage fibroblast survival data generally show a good fit to a simple exponential, while the G_0 lymphocyte data are better fitted with models including a dose-squared term, such as the quadratic ($-\ln S = \alpha D + \beta D^2$). Using D_0 as a measure, the range of sensitivity to dose of lymphocytes from different individuals can also vary by a factor of about 2 [E7]; however, given the shape of the survival curves, D_0 is difficult to measure accurately, and the range is generally much less than 2 using measures such as D_{bar} or D_{10} [E7, G17, N4].

125. No correlation was found between the measured sensitivities of fibroblasts and lymphocytes from the same individual [G17, K23]. A similar lack of correlation was found between lymphocyte and fibroblast responses of pretreatment cancer patients, whether the fibroblasts were cycling or in the plateau phase, suggesting that these differences in response were not cell-cycle-dependent [G11]. The differences between fibroblasts and lymphocytes may reflect several other possibilities: modes of death may differ, cell-type-specific factors may affect the expression of genes that modify radiation response (especially after growth in culture), and/or there may be unknown variables in assay conditions. In support of the last possibility, Nakamura et al. [N4], using donors from one ethnic group, found a similar (relatively small) variation in the mean x-ray dose to kill 28 samples of T-lymphocytes from one individual (D_{10} = 3.66±0.21 Gy), very similar to results with samples from 31 different individuals (D_{10} = 3.59±0.18 Gy). Elyan et al. [E7] reported similar data with donors from three ethnic groups, although with a greater overall range of variability. In support of differences in cell-type-specific factors, other cell types such as keratinocytes also show survival that is significantly different from that of fibroblasts taken from the same individuals [S39].

126. The use of experimental conditions promoting recovery from irradiation, such as low-dose-rate irradiation, will, it is suggested, expand the range of sensitivities to dose shown by normal human cells. The exposure of non-growing fibroblasts from 14 different normal individuals to tritiated water (dose rate = 8.5-100 mGy h^{-1}) expanded the range of sensitivity to dose by a factor of more than 3 [L35]. Similarly, Elyan et al. [E7] found that low-dose-rate irradiation (9.8 mGy min^{-1}) of human G_0 lymphocytes from 19 individuals expanded the range of sensitivity to dose by a factor of about 4. When a higher dose rate (28.5 mGy min^{-1}) was used, however, there was little difference between the range of sensitivities measured for fibroblasts and lymphocytes and the range with a dose rate of 4.55 Gy min^{-1} [G11]. The latter result probably reflects incomplete recovery during the low-dose-rate irradiation period [E7, G11].

127. Other assays of cellular sensitivity have been developed based on the measurement of responses such as the growth of cells after single doses of radiation. A "growback" assay following gamma irradiation of lymphoblastoid cells, developed by Gentner and Morrison [G4], detected a wide range of sensitivity in the normal (asymptomatic) population. Of 270 lymphoblastoid lines tested, about 5% of normal lines showed hypersensitivity after acute irradiation, as measured by their overlap with the responses of ataxia-telangiectasia homozygotes, while on the same criterion about 12% were hypersensitive when assayed using chronic irradiation [G4, G13]. However, a wider range of sensitivity was detected using chronic irradiation, and rigorous statistical tests found that the proportion of lines showing a significantly hypersensitive response ($p = 0.05$) was 5%-6% under both irradiation conditions [G14].

128. There have been claims that a number of disorders with an increased incidence of cancer are hypersensitive to x rays, including retinoblastoma, basal-cell naevus syndrome, Gardner's syndrome, and Down's syndrome [A6, M2, W3]. The main method of determining sensitivity differences has been cell survival assays with cultured fibroblasts (as shown in Figure II), in which small numbers of cell cultures derived from normal individuals are compared with those of one or more derived from the patient(s). Unfortunately, such studies have not always given consistent sensitivity differences for the same disorder tested in different laboratories. In part this may be due to the large range of sensitivity found among normal individuals; if the normal lines used in a given laboratory are in the higher part of the survival range, a disorder showing relatively low survival may be classified as sensitive even though it falls within the overall normal range. However, it has been concluded from a statistical analysis of many published survival curves of human fibroblasts that the radiosensitivity of cells from some disorders, calculated as D_{bar}, can be discriminated from that of normal cells [D7]. The disorders include ataxia-telangiectasia homozygotes and heterozygotes, Cockayne's syndrome, Gardner's syndrome, Fanconi's anaemia, and 5-oxoprolinuria homozygotes and heterozygotes.

129. A number of studies had previously suggested that cells from individuals heterozygous for the disorder ataxia-telangiectasia (carriers with one mutated gene, such as the parents of those showing the full symptoms) are more sensitive than normal to the lethal effects of irradiation [A6, A14, C33, K17, P11]. Additionally, ataxia-telangiectasia heterozygotes show an average level of dose-rate sparing that is intermediate between that of lines from ataxia-telangiectasia homozygotes (paragraph 49) and normal persons [P12, W18, W21]. However, even under low-dose-rate conditions, where a number of carriers have been tested, it is evident that their sensitivity range overlaps that of the most sensitive normal individuals [L35, W18]. Survival assays on cells derived from human sources cannot therefore unequivocally distinguish carriers of a mutated AT gene from normal individuals. Similarly, it has been found that protracted radiation exposure will not readily distinguish other disorders suggested to have minor degrees of hypersensitivity [L35]. Inbred mouse strains may show less variation in response from individual to individual, and mice heterozygous for a defective *Atm* gene were found to show a significant reduction in lifespan following irradiation (4 Gy at 2-4 months of age), as well as premature greying of coat colour, compared to normal littermates [B61].

130. The finding of a range of sensitivities to radiation among supposedly normal persons implies that in addition to those with increased sensitivity, there are individuals with greater radiation resistance than average. There is little evidence, however, for specific disorders associated with radiation resistance. It has been noted that cell lines derived from mice deficient in the p53 tumour suppressor are more resistant to radiation than normal (paragraph 104), although this response may be cell-type-dependent [B43]. Similar studies on fibroblasts derived from patients with the Li-Fraumeni syndrome, defective in p53, highlighted a family carrying a germ-line *TP53* gene mutation in codon 245. Cells from family members showed a radioresistance (D_0 value) that was increased by a factor of 1.2 [B44], compared to an approximately 1.5-fold increase for the p53-deficient mice [L6].

131. There is evidence from cell survival assays that radiation sensitivity at the cellular level may correlate with tissue response of cancer therapy patients. In a study of 811 patients treated with radiotherapy for breast cancer, five showed severe skin responses. The survival of fibroblast cell lines derived from these five patients was compared to six lines derived from women with normal radiotherapy responses (also from the same group of 811 patients). The sensitivity of the severe-response patients (D_0 = 0.97±0.11 Gy) was significantly higher than that of the normal group (D_0 = 1.16±0.08 Gy; see paragraph 123) [L39]. In a prospective study of 21 head-and-neck cancer patients it was found that the sensitivity of their cultured fibroblasts, but not their lymphocytes, correlated with whole-body late effects [G12]. Similarly, a study of fibroblasts derived from 10 radiotherapy patients showed a correlation between cellular sensitivity and late tissue reactions [B42]. The survival of lymphocytes from breast cancer patients suffering severe reactions to radiotherapy has also been studied in conjunction with ataxia-telangiectasia heterozygotes and homozygotes [W21]. Using high-dose-rate irradiation (1.55 Gy min^{-1}), the survival of lymphocytes from severe-response patients could not be distinguished from that of normal-response patients (or other normal controls), but with low-dose-rate irradiation (9.8 mGy min^{-1}) the cell survival of severe-response patients and ataxia-telangiectasia heterozygotes was similar and significantly different from that of normal individuals. Using the growback assay (paragraph 127), lymphoblastoid cell lines derived from newly presented, non-selected cancer patients showed a similar range of radiosensitivities to the normal lines, while patients already known to show some adverse reaction to radiotherapy showed a higher proportion with extreme sensitivity [G14].

132. Assays based on chromosomal damage have commonly shown a higher degree of sensitivity in the detection of differences between individuals. For example, the genetic disorders ataxia-telangiectasia, Bloom's syndrome, and Fanconi's anaemia were described as “chromosomal breakage syndromes” in early studies, showing significantly increased frequencies of chromosomal breaks and exchanges after irradiation [H24]. Chromosomal responses after low-dose-rate irradiation have also been used to examine the sensitivity of ataxia-telangiectasia heterozygotes [W13] and breast cancer patients who react severely to radiotherapy [J12]. Lymphocytes from 5 out of 16 patients (31%) sustaining severe reactions to radiotherapy had dicentric yields outside the control range [J12].

133. An assay based specifically on the chromosomal sensitivity of lymphocytes to irradiation in G_2 has been successful in detecting significant differences between

individuals carrying various cancer-prone disorders. The work of Sanford et al. is based on the measurement of chromatid breaks and gaps a short time after irradiation of lymphocyte cultures; this assay detected enhanced sensitivity in fibroblasts from a number of disorders, including ataxia-telangiectasia [T25], Fanconi's anaemia, Bloom's syndrome, Gardner's syndrome, basal-cell naevus syndrome, xeroderma pigmentosum, and familial polyposis [P2, S5]. In this G_2 assay, cells from cancer patients and tumour cells showed an even higher frequency of chromatid damage [H21, P3]. This assay has some technical difficulties; extensive tests in another laboratory using identical conditions failed to reproduce many of the results, which led to a modified assay that appears to be more encouraging [S28].

134. The modified G_2 chromosomal assay devised by Scott et al. [S27] has been used to assay the sensitivity of lymphocytes from 74 normal individuals, 28 ataxia-telangiectasia heterozygotes, and 50 breast cancer patients. The ataxia-telangiectasia heterozygotes were clearly distinguished in sensitivity from the majority of normal individuals, but about 9% of the normal individuals showed hypersensitivity. Human fibroblast cultures show a similar result, with four ataxia-telangiectasia heterozygotes distinguished from seven normal individuals [M47]. The range of sensitivities of lymphocytes from breast cancer patients was much greater than the range from normal individuals (42% overlapped the ataxia-telangiectasia heterozygote range). It was proposed that in addition to those mutated genes already identified with familial breast cancer, a number of other genes of lower penetrance may also predispose to breast cancer. It was suggested that these genes may be involved in the processing of DNA damage [H21, S27]. Family studies showed that the lymphocytes of first-degree relatives of sensitive individuals were significantly more sensitive to x rays than expectation; segregation analysis of family members (95 individuals in 20 families) suggested that a single gene accounts for most of the variability, but that a second rarer gene with an additive effect on radiosensitivity may also be present [R36].

135. It has also been found that a subset of patients with common variable immune deficiency (CVID) showed enhanced sensitivity to G_2 chromosomal damage [V1]. Among disorders with primary immune deficiency, CVID and ataxia-telangiectasia have the highest reported incidence of tumours, and both disorders show similar types of tumours, suggesting a common risk factor. This finding may relate to the link noted above (Section II.B.1) between immune system dysfunction and radiosensitivity. However, it is clear that the G_2 chromatid assay will detect enhanced levels of damage in cells showing a normal response in survival assays (e.g. xeroderma pigmentosum cells), and it may be argued that it is primarily a means of detecting genetic instability.

136. Another method based on alterations in the sedimentation of human lymphocyte nuclei is claimed to detect particularly sensitive individuals [S4]. When the repair of damage is measured by return to normal sedimentation properties after irradiation, it was possible to detect individuals sensitive to radiotherapy and patients with autoimmune disease [H5]. Further, patients showing post-therapy complications were found to be more sensitive by this sedimentation assay than those without complications [D2].

137. It has been suggested that individuals heterozygous for the ataxia-telangiectasia defect are also at increased risk of cancer. Swift et al. [S13] compared prospectively the incidence of cancer in 1,599 relatives of ataxia-telangiectasia patients in 161 families in the United States and found an increased relative risk of 3.8 in men and 3.5 in women for all cancers, and an increased relative risk of 5.1 for breast cancer in women. If borne out, this predisposition would contribute significantly to the cancer incidence in the general population: heterozygotes are much more frequent than ataxia-telangiectasia homozygotes (both genes mutant) and from these data could constitute as much as 5%-8% of all adults with cancer [S12]. However, commentary following the publication of the work of Swift et al. [S13] questioned two aspects of their findings. First, bias in the control group was suggested [B24, K22, W14], although this was contested by Swift et al. [S41]. Second, against a background radiation level of 1 mGy a^{-1}, it was considered surprising that an increase in breast cancer could be detected following diagnostic x-ray procedures of $\leq$10 mGy [B24, L25]. Differences in dose rate might influence the effectiveness of these two sources, and in other studies an acute dose as low as 16 mGy gave a significant increase in breast cancer in normal women aged 5-9 years at exposure [M26]. However, it is clear that a relatively large difference in sensitivity of the ataxia-telangiectasia heterozygotes to the acute diagnostic procedures would be required to attribute the observed cancer incidence to deleterious effects of one mutated copy of the ataxia-telangiectasia gene.

138. Genotyping of 99 ataxia-telangiectasia families using markers tightly linked to the *ATM* gene showed that 25 of 33 women with breast cancer were heterozygous compared with an expectation of 15 of 33 (relative risk = 3.8; 95% CI: 1.1–7.6) [A18]. Two smaller European studies of cancer incidence among ataxia-telangiectasia heterozygotes tend to support the data from the United States, giving an overall relative risk for breast cancer of 3.9 (CI: 2.1–7.2) [E4]. The average relative risk for other cancers was lower at 1.9, with the European studies showing no statistically significant increase over controls. Using these combined data, the proportion of breast cancer cases due to ataxia-telangiectasia carriers would be about 4%. This small proportion is consistent with an inability in two studies [C36, W31] to detect linkage to AT gene markers in breast cancer families (i.e. on the basis of the risk estimates and gene frequency, most cases of familial breast cancer will be caused by other genes) [E4].

139. The identification of the gene mutated in ataxia-telangiectasia (*ATM,* paragraph 56) has led to further attempts to correlate this gene defect with breast cancer incidence or with severity of response to radiotherapy. In a

study of *ATM* mutations in 38 sporadic breast cancers, only rare polymorphisms were detected, none of which would lead to truncation of the gene (paragraph 57), giving no evidence for an increased proportion of ataxia-telangiectasia heterozygotes in breast cancer patients [V8]. The same workers also looked at 88 unrelated primary breast cancer cases from families previously associated with cancer susceptibility and found three *ATM* mutations (3.4%); this number was considered to be higher than expected by chance, but these mutations did not necessarily segregate with cancer incidence in the families [V9]. Screening a further 100 breast cancer cases from families with a history of breast cancer, leukaemia, and lymphoma revealed only one mutation, consistent with minimal involvement of the *ATM* gene [C65]. Fitzgerald et al. [F21] detected heterozygous mutations in 2 of 202 (1%) healthy women with no history of cancer, compared to 2 of 410 (0.5%) patients with early-onset breast cancer, consistent with a lack of association. Similarly, in a study of 18 families associated with a high incidence of breast and gastric cancers, only one *ATM* mutation was found, and this did not cosegregate with the gastric cancer in the family [B58]. In a study of 41 breast cancer patients showing marked normal tissue response to radiotherapy, one was found with a heterozygous mutation in the *ATM* gene (2.4%), compared with none in a comparable number of control patients [S69]. The conclusion that mutation of *ATM* is not a major cause of radiotherapy complications was supported by studies of smaller numbers (15-16) of breast cancer patients showing severe normal tissue damage following radiotherapy, in whom no *ATM* mutations typical of those in heterozygotes were found [A22, R31]. In contrast, a relatively high frequency of *ATM* mutations has been found for a group of prostate cancer patients (3 of 17, 17.5%) with severe late responses to radiotherapy [H47]. The majority of these studies suggest that heterozygosity for the *ATM* gene is not an important cause of breast cancer susceptibility or severe response to radiotherapy, but most do not have sufficient numbers of patients to exclude completely a role for ataxia-telangiectasia heterozygotes.

140. The association of certain forms of lymphocytic leukaemia with mutations in the *ATM* gene has already been noted (paragraph 59). Individuals with less severe mutations in the *ATM* gene, allowing some expression of ATM protein (paragraph 57), may also be at greater risk of developing breast cancer [S78]. It has also been observed that loss of heterozygosity (Section IV.B) at chromosome 11q23 is a frequent occurrence in breast carcinomas [C27, H15]. At least two regions of 11q23 are commonly deleted, and one of these includes the *ATM* gene. Other common human malignancies such as lung, cervical, colon, and ovarian carcinomas, as well as neuroblastoma and melanoma, also show an association with loss of heterozygosity at 11q23 and may include the *ATM* gene region [R9]. In a recent study, 40% loss of heterozygosity was found for markers of *ATM* in cases of sporadic invasive ductal breast carcinoma; in the same tumours, markers for the *BRCA1* and *BRCA2* genes showed 31% and 23% loss of heterozygosity, respectively. Loss of heterozygosity of *ATM* correlated with higher grade tumours and a younger age at diagnosis [R32].

141. It is clearly important to detect individuals in the human population who are hypersensitive to radiation and to understand the connection between radiosensitivity and cancer-proneness. While there are a number of indicators of this hypersensitivity, it has been difficult on the basis of cell survival assays to distinguish normal individuals from those carrying mutant genes giving intermediate levels of sensitivity, when the full sensitivity range of normal cells is taken into account (paragraph 128). In the case of ataxia-telangiectasia heterozygotes, newer assays based on G_2 chromosomal sensitivity (paragraphs133–134), DNA damage levels measured by the Comet assay [D22], enhanced arrest in G_1 [N2, N3], or enhanced arrest in G_2 [H6, L4, L5] all show more promise, but it is still not clear whether any of the assays will detect these heterozygotes exclusively. The discovery of the *ATM* gene (paragraph 56) has made the task of detecting heterozygotes simpler, but because of the large size of this gene it is still a difficult task to screen large numbers of individuals for defects (see paragraph 139). Since many other genes are known to influence cellular radiosensitivity [Z13], it is likely that the molecular cloning of these genes will have a substantial impact on the ability to determine the importance of genetic predisposition to human radiation risk. Indeed, preliminary studies on nine recently-cloned repair genes including *XRCC1* and *XRCC3* (paragraph 78) showed that relatively common alterations in gene sequence exist in normal individuals. An average of 14 percent polymorphic sites yielding protein sequence variations was found in samples of 12-36 individuals, including some individuals who were homozygous for the variant site [M53, S84]. While the functional significance of these variations has yet to be established, their potential to reduce DNA repair capacity may influence individual response to radiation sensitivity and cancer susceptibility.

B. INFLUENCE OF REPAIR ON RADIATION RESPONSES

142. It is known that DNA repair processes influence the sensitivity of mammalian cells and organisms to radiation. If recovery from radiation damage is compromised, as in cell lines defective in double-strand break repair, the slope of the cellular survival curve is increased. Also, while normal cells show a “shouldered” curve, the repair-defective cells commonly show a loss of this feature. In simplistic terms, it is envisaged that repair processes in normal cells increase the chances of survival, but that as an acute dose increases, the amount of damage temporarily saturates the repair capacity of the cell [G6]. There is very good evidence in yeast cells that these aspects of survival are associated primarily with the repair of DNA double-strand breaks [F3]. It has been possible to make this correlation in yeast, because DNA double-strand breaks can be measured accurately, and mutant cells that are temperature-sensitive for double-strand break repair are available. Thus, as the post-irradiation temperature is altered, it is possible to see a concomitant alteration in double-strand break repair and survival. Unfortunately, it

has proved technically difficult to establish similar quantitative correlations in mammalian cells, which have a much larger genome than yeast.

143. Operationally defined measures of cellular recovery, termed potentially-lethal damage repair or sublethal damage repair, have been used for some years in cellular "repair" studies. Potentially-lethal damage repair is measured by the recovery found when cells are held in a non-proliferative state after irradiation, before respreading into fresh growth medium. Sublethal damage repair defines the recovery seen when survival is measured after splitting the dose over a suitable interval. While it has not been clear whether potentially-lethal damage repair and sublethal damage repair measure the same underlying repair processes, it is notable that both are absent in yeast single-gene mutants lacking DNA double-strand break repair (*rad52* series) [R2, R3]. It has also been argued from kinetic data that double-strand break repair is responsible for potentially-lethal damage repair in yeast [F3]. Similarly, with low-dose-rate irradiation, there is good evidence in yeast cells that DNA double-strand break repair is responsible for the dose sparing observed [F3]. Mammalian cell lines with defective double-strand break repair as well as ataxia-telangiectasia cells have also been shown to lack potentially-lethal damage repair (xrs, XR-1, ataxia-telangiectasia) or low-dose-rate sparing (xrs, ataxia-telangiectasia, irs2) (see Section II.A.2) [T30]. A correlation has recently been found for human normal and ataxia-telangiectasia cells, irradiated at high or low dose rates, between the amount of DNA double-strand breaks remaining after repair and radiosensitivity (measured as cell survival) [F18].

144. Mammalian cells irradiated with densely ionizing radiation, such as alpha particles, show both a more sensitive response (increased slope of survival curves) and, commonly, the loss of the curve shoulder region. Thus, characteristic increases in RBE are found as the density of ionization (LET) increases. This may well arise from the LET-dependence of damage complexity [A23, G28, G39] (see Section I.B), and refined measurement of DNA breakage following both x irradiation and high-LET particles has shown that there is a comparative excess of small fragments for the high-LET radiations [L53, R26]. However, several studies of mammalian cells using conventional biochemical techniques show that there is little or no difference in the numbers of DNA double-strand breaks induced by low- and high-LET radiations (RBE = ~1) but that fewer of the breaks induced by high-LET radiations are repaired [B52, C53, J4, L52, P27, P28, P37, R27]. Using these methods, a good correlation has been found between the relative number of non-rejoined DNA breaks and the RBE for mammalian cell inactivation over a wide range of LET [G32, R25]. Plasmid DNA, irradiated to give the same amount of damage by gamma rays or by alpha particles, showed much less repair of the alpha-particle damage when exposed to mammalian cell extracts [H41]. It is suggested that damage induced by high-LET radiations is more complex, because of the increased local clustering of ionizations, and therefore less repairable than low-LET radiation damage (see Section I.B). This finding is supported by measurement of the rejoining kinetics of large fragments of cellular DNA, where rejoining was generally slower after irradiation with high-LET particles than with x rays [L62]. Additionally, using this method the proportion of mis-rejoined fragments increased with x-ray dose (10%-50% over the dose range 5-80 Gy), while similar experiments with high-LET radiation gave a constant 50% of fragments mis-rejoined at all doses tested [L72, K47].

145. In radiosensitive cells, specifically ataxia-telangiectasia cells [C10] and the xrs series [T7], the increase in RBE with LET is very much reduced. This result would be expected if the damage caused by densely ionizing radiation is intrinsically more difficult for normal cells to repair than the damage caused by sparsely ionizing radiation. On this basis, radiosensitive cells show little alteration in effectiveness as the LET increases, because they are already inefficient at repairing low-LET radiation damage. This analysis suggests that the RBE-LET relationship is largely caused by the normal cell's ability to repair low-LET radiation damage.

146. Extensive studies in bacteria have shown that some repair processes are inducible by treatment with DNA-damaging agents [W11]. That is, DNA damage causes an increase in the expression or activity of repair enzymes, mitigating the effects of the damage. This process is part of a much wider series of so-called stress responses by which cells adapt to their environment. Knowledge of such inducible processes in mammalian cells is fragmentary, and in some cases the data are controversial. Evidence for the existence of inducible repair processes in response to ionizing radiation damage comes from several different types of experiment. These may be broadly categorized as (a) adaptive response of pre-exposed cells (see also [U2]), (b) refined analysis of survival in low-dose regions, and (c) direct molecular evidence for the inducibility of specific gene products.

147. Pre-exposure to low doses of tritiated thymidine (18-37 Bq ml^{-1}) or x rays (5-10 mGy) was found to decrease the frequency of chromosomal breaks in proliferating human lymphocytes irradiated subsequently with a higher dose (1.5 Gy) of x rays [O2, S34]. The aberration frequency is reduced to about 60% of cells not receiving pre-exposure [S30]. Pre-exposure to low concentrations of radiomimetic chemicals, such as hydrogen peroxide and bleomycin, can also reduce the effect of a subsequent high dose of x rays [C35, V4, W29]. This adaptive response is, however, stopped by 3-aminobenzamide, an inhibitor of poly(ADP-ribose) polymerase (paragraph 77), which is itself induced in response to DNA breakage by radiation [S34, W23]. These features of the adaptive response have led to the suggestion that low levels of DNA breakage act as a signal for a response mechanism leading to accelerated repair of radiation damage in mammalian cells.

148. Although increased resistance following low radiation doses has been observed in a number of laboratories, it occurs only in the lymphocytes of some individuals [B21, B26, H13, S22, S26]. Also, lymphocytes from several persons with

Down's syndrome [K16] and fibroblasts from homocystinuria patients [Z11] did not show the adaptive response. The adaptive response is usually found when lymphocytes are proliferating, not resting (G_0) [S31, W15], although there are data suggesting that pre-irradiation in G_0, followed by a challenge dose in G_2, gives increased resistance [C63, K16]. These observations have led to suggestions that the increased resistance is a result of cell-cycle perturbation in the lymphocyte populations of some individuals following the pre-exposure (i.e. the cells sampled following the second dose are in a more resistant phase of the cycle than if no pre-dose had been given). This idea has received some experimental support [A9, W27], but recently an adaptive response for chromosomal and cell-killing responses has been found in cells other than lymphocytes when irradiated in proliferating or non-proliferating states [I1, L27, M24, S23]. Thus, the causes of the adaptive response remain controversial, but further work with different cell types has outlined the responses affected and the conditions under which the response is observed.

149. Measuring chromatid breaks or chromosome-type exchanges in lymphocytes, the maximum effect on resistance to a second dose occurs within 5-6 h following the pre-exposure [S34, W15]; the phenomenon may persist for up to three cell cycles [C24, S34]. While pre-exposure to relatively high acute x-ray doses (e.g. >200 mGy) does not give the adaptive response [C46], when 500 mGy was given at low dose rate (e.g. 10 mGy min^{-1}) the adaptive response was found. Conversely, there appears to be a minimum dose rate for the response induction by low doses (e.g. for 10 mGy pre-dose, the dose rate must be >50 mGy min^{-1} for full effect) [S33]. A pretreatment with an acute dose of 20 mGy from x rays will confer resistance to chromosome breakage by 150 mGy alpha particles from radon [W28]; however, when acute high-LET radiation was used to give the pre-exposure, there was no increased resistance to a second dose [K16, W24].

150. Pre-exposure to low doses from x or gamma rays has also been shown in several different cell types to decrease the frequency of cell killing [L27, M24, S32], mutation [K13, R12, S20, U17, Z8], apoptosis [F26], and morphological transformation [A15, R33]. However, the last result is still controversial. Using a near-diploid mouse skin cell line (m5S), Sasaki [S23] showed that x-ray-induced cell killing and chromosomal and mutation responses, but not morphological transformation, are mitigated by acute pre-exposure to 20 mGy from x rays. A malignant derivative of m5S cells lacked the adaptive response, but this was restored along with morphological reversion by transfer into these cells of human chromosome 11. The study also showed that chemicals that either activate or inhibit protein kinase C, which has an important role in signal transduction, either mimicked or abolished, respectively, the adaptive response [S23]. There have been reports suggesting that capacity to rejoin radiation-induced DNA double-strand breaks is greater following low-dose pre-exposure [I4, Z8], as well as data implicating activation of antioxidant metabolism in the adaptive response {B65, Z14].

151. The repair of DNA base damage is also a potentially relevant process; a newly developed ultrasensitive assay for thymine glycols (paragraph 17) has shown that pre-exposure to 0.25 Gy from gamma rays increases the repair of this type of base damage from a subsequent dose of 2 Gy. The initial rate of removal of thymine glycols in human cellular DNA was found to be increased by a factor of 2 following pre-exposure [L60].

152. New methods to measure with improved accuracy the survival response of mammalian cells have led to the discovery that the dose-response curve may initially be steeper at low doses (<1 Gy) than predicted from the curve found at higher doses. This response has been characterized as an initial hypersensitivity at doses up to 0.5 Gy, followed by increased radioresistance of the cell population [L23, M17, W32]. The possibility that the hypersensitive response to low doses is the result of differentially sensitive fractions in cell populations has been rejected on the basis of two observations. First, survival curves for radiosensitive tumour cell lines and an ataxia-telangiectasia line appear to show no changes in sensitivity in the low-dose region. Preliminary data for two further radiosensitive lines, one with reduced DNA double-strand break repair and the other reduced excision repair, also show no increased radioresistance at low doses in comparison to their respective parental lines [S35]. Second, it is argued that the hypersensitivity seen would require one fraction of the cell population to have an unreasonably high level of sensitivity (e.g. about 7% of the cells to be >10 times more sensitive than ataxia-telangiectasia cells) [L24]. In parallel to the adaptive response of lymphocytes, recent data also show that pre-exposure to low doses from x rays or low concentrations of hydrogen peroxide increases radiation resistance in the hypersensitive region. Further, the additional resistance from pre-exposure was transitory, requiring time for development and diminishing after two or three cell cycles [M18]. Again, it has been found that low-dose hypersensitivity is absent with high-LET radiation [M17, M19].

153. Multi-laboratory experiments attempting to measure the dose response for chromosomal aberration induction in lymphocytes from two normal donors at low x-ray doses (down to 4 mGy) also appear to identify departures from fitted dose responses that have been attributed to the induction of repair processes [P15]. It was then suggested that the control value in these experiments was excessively high, biasing the result towards non-linearity [E5]. However, analysis of lymphocytes from individuals in Austria before and after exposure to fallout from the Chernobyl accident, where a peak twofold increase in radiation exposure was recorded, also showed dose-response curves departing from linearity (i.e. decreasing or levelling off) at annual doses between 0.3 and 0.5 mGy [P17]. Further *in vitro* studies with x rays and larger numbers of donors have not confirmed a significant departure from linearity at low acute doses (down to 20 mGy), although statistical variation in the small numbers of aberrations detected at lower doses do not allow conclusive statements on dose response [L37, L38]. These further studies did show an excess of multiply-damaged cells in some donors after

irradiation, leading to the possibility that a small subset of lymphocytes is especially sensitive to aberration induction by very low doses. Similar studies with 15 MeV D-T neutrons did not reveal significant departures from linearity at low doses [P16]. Similarly, a study of chromosomal aberration induction in Syrian hamsters injected with ^{137}Cs to give a whole-body dose of about 0.4 mGy, to mimic the Chernobyl fallout exposure, failed to reveal a significant increase above background values [L61]. Thus, the possibility that very low doses give a higher yield of chromosomal aberrations than expected on the basis of a linear extrapolation from high-dose data remains contentious.

154. Reports suggesting that low doses of ionizing radiation induce repair processes *in vivo* pre-date the cellular studies described above. Irradiation of mice with low doses of x or gamma rays has produced evidence of increased resistance to subsequent higher doses to both somatic and germ cells [C24, F6, W26]. Recent data show that the marked cytogenetic adaptive response of mouse germ cells does not influence the response of the somatic or germ cells of the offspring [C25]. Examination of chromosomal aberrations in the lymphocytes of people exposed occupationally to higher-than-average doses of alpha particles from radon (0.01-16 mGy) plus gamma rays (1-3 mGy) showed that the dose-effect curve was not related to that found at higher doses. Individuals subjected to the lowest doses gave a steep increase in aberration frequency with dose, while at higher doses the curve flattened out [P14]. Tuschl et al. [T33] showed that the people exposed to the higher doses (8-16 mGy a^{-1}) of alpha particles gave higher levels of repair (unscheduled DNA synthesis after UV light damage) in lymphocytes, compared to controls. Hospital workers exposed to low levels of x and gamma rays (maximum annual dose = 28 mSv) showed a reduction in the frequency of chromosome aberrations induced by a dose of 2 Gy to their blood lymphocytes, compared to non-exposed controls [B20]. However, it has been found that the lymphocytes of children living in areas contaminated by the Chernobyl accident showed no evidence of increased resistance to x rays [P9, U16], and separate reports suggests that fewer people from the region of the accident showed the adaptive response in lymphocytes compared with a control group [P19, M52].

155. A growing number of specific genes and proteins have been shown to be induced or repressed following irradiation, mostly using relatively high doses from x or gamma rays (2-6 Gy). While these do not as yet form coherent pathway(s) coordinating response to radiation, they do implicate genes and proteins involved in a variety of important molecular processes (see also Section II.B). Induced proteins include oncogenes/ transcription factors (c-jun, c-fos, interleukin-1, and egr-1), proteins involved in cell-cycle regulation (p53 and cyclins A and B), growth factors, and DNA-metabolizing proteins [PCNA, β-polymerase, and poly(ADP-ribose) polymerase], as well as the products of a number of unknown genes [B25, K15, W20]. The development of methods for rapidly screening hundreds or thousands of genes (micro-arrays) in one experiment, for changes in levels of gene expression, is beginning to show the extent of transcriptional response to radiation damage. For example, using an array of more than 600 genes, gamma irradiation of a myeloid cell line showed induction of 48 genes by factors of two or more, and many of these genes had not been previously reported as radiation-inducible [A25]. Considering those genes which respond differentially in the presence or absence of p53, it was found using micro-arrays that several genes encoding secreted proteins with growth inhibitory functions were upregulated in a p53-dependent fashion following gamma irradiation. Thus, the p53 response to radiation may also be involved in growth inhibitory effects on surrounding cells, as observed in "bystander" effects [K44]. Methods are being developed similarly for assaying large numbers of proteins, to reveal translational and post-translational responses, using 2-dimensional gel systems coupled to mass spectrometry [B64, C69]. These methods will also revolutionize the classification of normal tissue and disease states, in particular cancer, by permitting a molecular description of the complete profile of gene products present [G40]. Such classifications will be invaluable in understanding the mechanistic basis of cancer induction by agents such as ionizing radiation.

156. Increased or decreased levels of certain gene products have also been found after low doses of radiation, within the range inducing the adaptive response [B18]. Doses of less than 500 mGy gamma rays have been found to induce the expression of a variety of stress-response genes [P35, P36], and dose-responses of several of these genes were shown to be approximately linear between 20 and 500 mGy gamma rays [A26]. Some candidate gene products with a potential role in induced radiation resistance have been identified, including a member of the heat shock protein 70 family (PBP74 [S79]), a heat-shock related immunophilin protein (DIR1 [R37]), ribonucleotide reductase [S81], and the MAPK and PKC protein kinases [S80].

C. SUMMARY

157. When the cells of normal individuals are examined for radiosensitivity, as shown by their survival in culture, variation by a factor of 2 is seen; this factor may be extended to 3 or 4 with the use of low-dose-rate irradiation conditions. It has been suggested that this variation has a genetic basis, but it has been technically difficult to establish this. Additionally, a fraction of cancer therapy patients suffers from severe skin reactions, and cells from these patients commonly show a slightly elevated radiation sensitivity.

158. Assays based on chromosomal damage in G_2 cells have also been used to estimate radiation sensitivity in normal individuals and in breast cancer patients, relative to individuals with known radiosensitivity disorders. The response of a relatively large fraction of breast cancer patients overlapped that of carriers of the ataxia-telangiectasia defect, suggesting that a number of genes involved in response to radiation damage may predispose to this form of cancer.

159. It has been suggested that individuals carrying one defective copy of the ataxia-telangiectasia gene (*ATM* heterozygotes) are at increased risk from cancer, especially breast cancer. A number of studies have recently tested this idea and come to the conclusion that *ATM* heterozygosity is not an important cause of breast cancer susceptibility (or severe response to radiotherapy), but commonly too few patients have been tested to exclude completely a role for *ATM*. Specific types of tumours, such as T-cell prolymphocytic leukaemia, are associated with high levels of mutation of the *ATM* gene (paragraph 59). Additionally, recent evidence suggests that the genes involved in familial susceptibility to breast and ovarian cancers (the *BRCA* genes, paragraph 72) are involved in DNA repair processes and lead to radiation sensitivity when defective in mice.

160. Repair processes affect the shape of survival curves, especially through differences in the processing of DNA double-strand breaks, as seen in specific repair-defective lines. The response to double-strand breaks probably also underlies operationally-defined measures of cellular recovery such as potentially-lethal damage repair and sublethal damage repair. High-LET radiations may not induce a higher frequency of DNA double-strand breaks than low-LET radiations, but high-LET radiation damage is much less repairable. The increase in RBE with LET, found for normal cells, is largely determined by the cell's ability to repair low-LET damage.

161. Evidence for the inducibility of repair processes in mammalian cells is fragmentary. Data from pre-exposures of cells to low radiation doses, as well as refined survival analyses at low doses (<1 Gy), show an altered response suggestive of an induction process but to date have failed to link this response to known inducible processes. However, recent experiments have shown that certain genes in at least two repair pathways are up-regulated following radiation damage (paragraphs 84 and 151). Additionally, refined methods for looking at protein structure and activity are beginning to reveal insights into more subtle modifications following irradiation. Some damage-response proteins may be activated by post-translational modifications (e.g. phosphorylation, paragraph 102), without any change in the amounts of these proteins, as part of a signalling mechanism promoting the repair of DNA damage. Other proteins have been shown to accumulate in the cell to form discrete foci following irradiation (paragraphs 71 and 74); these foci may represent repair protein complexes accumulating at sites of damage. While there has been little exploration of the dose dependence of these events, the data indicate that modification of cellular response mechanisms can occur following irradiation, raising the possibility that dose responses are altered as a result. Methods for data acquisition on the inducibility of gene products are presently being revolutionized by the introduction of micro-array and complementary techniques, so that rapid progress in this research area is to be expected. The potential importance of inducible repair processes in determining responses to low doses of ionizing radiation is considered further in Annex G, "*Biological effects at low radiation doses*".

IV. MECHANISMS OF RADIATION MUTAGENESIS

A. MUTATION AS A REPAIR-RELATED RESPONSE

162. The cellular processing of radiation-induced damage to DNA by enzymes may result in a return to normal sequence and structure (correct repair). Alternatively, the processing may fail or may cause alterations in DNA, with the consequence of lethality or inherited changes (mutations). It is also possible that some subtle forms of damage may be tolerated by the cell, particularly if it is non-replicating, and lead to persistent lesions in DNA. These lesions would have to be both chemically stable and not be substrates for repair enzymes and may include some minor types of damage such as methylated bases generated by non-enzymatic alkylation [L68].

163. It is likely that simple base damage or loss in the mammalian genome will commonly lead to base-pair substitutions. In recent years the defined production of single types of damage, at specific sites in DNA molecules *in vitro*, has given insights into their consequences. For example, DNA molecules carrying a single site of base loss (abasic site) have been shown to give rise to base-pair substitutions at that site, either when introduced on shuttle vectors [G8] or when the *RAS* gene was transfected and stable transformed clones selected [K7]. These substitutions (a form of point mutation) will often give rise to alterations in a gene product so that it works less efficiently or not at all.

164. Breaks in DNA, especially double-strand breaks, are thought to lead to larger alterations such as deletions and rearrangements. Some alterations are very large and are seen as chromosomal aberrations. While there is little formal proof of these relationships between breaks and mutation type, it has been shown that the transfer of restriction endonucleases into mammalian cells, causing site-specific DNA double-strand breaks, gives rise to mutations and chromosomal aberrations [T24]. Additionally, the processing of isolated double-strand breaks in defined DNA molecules by human cell extracts show that these may lead to large deletions of surrounding sequence [T10].

165. Reduction in the efficiency or fidelity of damage repair may lead to an increase in genetic change. This is seen strikingly in the recent discovery that loss of DNA mismatch repair capacity is involved in specific forms of cancer.

Mismatch repair is a form of base-excision repair (Section I.B) that removes bases that have been altered or incorrectly placed (by a polymerase) so that the two strands of DNA do not match in base sequence. The cancer connection was first noted as a high frequency of mutation of short-repeat-sequence (microsatellite) DNA in colon cancers, including hereditary non-polyposis colon cancer. It was rapidly established that the genes determining hereditary non-polyposis colon cancer co-localize with mismatch repair genes on human chromosomes 2 and 3 [B12, L15]. More genes with homology to these mismatch repair genes have since been identified, and at least two of these have also been shown to be mutated in the germ line of hereditary non-polyposis colon cancer patients [N10]. Colorectal cancer is one of the most common human cancers and perhaps the most frequent form of hereditary neoplasia; hereditary non-polyposis colon cancer accounts for as much as 5% of all cases of colon cancer, and the involvement of several genes in the phenotype may account for its prevalence [M20].

166. The high frequency of repeat-sequence mutation was recognized as characteristic of loss of the mismatch repair system (MutHLS), known for many years in bacteria and involving the concerted action of three mismatch enzymes to correct errors caused primarily during DNA replication (such as slippage of strands at repeat sequences) [M27]. Patients with hereditary non-polyposis colon cancer are therefore thought to have a mutator phenotype similar to mutant strains of bacteria with defects in mismatch repair. The role of inactive mismatch repair genes in humans is expected to be similar to the part played by tumour-suppressor genes; a germ-line mutation in one of the mismatch repair genes is followed by somatic mutation of the second gene copy during tumour development [H22, L36]. However, the mismatch repair genes are not thought to be directly involved in cancer; rather, they increase genetic instability and the probability that random mutations will affect those genes critical to cancer formation. This idea does not necessarily mean that mutations alone are sufficient for cancer formation; it is interesting to note that a subset of hereditary non-polyposis colon cancer patients carry numerous mutations in their cells and the expected defect in mismatch repair but have unexpectedly few tumours [P10]. Given the proposed mechanism, it is curious also that the increased cancer risk in hereditary non-polyposis colon cancer patients is selective; while other sites are affected, there is, for example, no increased risk for cancers of the breast and lung [W17]. Recent success at breeding transgenic mice defective in mismatch repair genes has shown that they are viable, but a large proportion develop lymphomas and sarcomas at an early age [B16, D9, R11]. While many of these mice die early, commonly succumbing to T-cell lymphoma, those that survive for more than 6 months develop gastrointestinal tumours, suggesting that these mice may be used as a model for human colon cancer [H50].

167. Cell lines lacking mismatch repair also show an increase in mutations at sites other than microsatellites. In colorectal carcinoma lines, shown to have high frequencies of microsatellite variation, the *HPRT* gene mutation rate was also found to be increased by a factor of more than 100 over the rate in normal human cells [B23, E8]. In one of these lines, known to have a defective human *MLH1* mismatch repair gene, about one quarter of the mutants had point mutations (frameshifts) at a hotspot within a run of guanine bases in the *HPRT* gene [B23]. An extensive study of one mismatch repair-defective line using the *APRT* gene similarly showed an elevation of frameshift mutations at sites of repeat base sequence, as well as AT → TA transversions at sites of secondary DNA structure [H23]. A potentially relevant site for mutations of this type is in the *BAX* gene, involved in promoting apoptosis (Section II.B.3); more than half of 41 colon carcinomas with microsatellite instability also had frameshift mutations in a run of eight guanines in the *BAX* gene [R19]. These findings are consistent with a role for mismatch repair in correcting base misalignments generated during DNA replication in normal cells. Interestingly, lack of mismatch repair also allows cells to become tolerant of certain forms of induced DNA damage. Alkylation of DNA bases normally produces cell-cycle arrest and/or death and can lead to cancer, but mismatch-repair-defective cell lines are highly resistant to the effects of alkyating agents [B28, K12]. Mismatch-repair-deficient mammalian cell lines also show a small but significant increase in resistance to gamma rays [F19], suggesting that repair of certain types of radiation-induced base damage is recognized by the mismatch repair system (e.g. 8-oxoguanine, which mispairs with adenine, paragraph 24). Mouse embryonic stem cells, heterozygous for a defect in the mismatch repair gene *Msh2*, showed resistance to low-dose-rate gamma radiation (0.004 Gy min^{-1}) but not to acute radiation (1 Gy min^{-1}). On the basis of this result, it was speculated that heterozygosity for mismatch repair may also contribute to tumorigenesis in a direct manner, without loss of the other gene copy [D20]. The mechanism of resistance is thought to follow from a reduction in abortive "repair": mismatch repair normally removes a base that is incorrectly incorporated opposite a damaged base but this repair is abortive, because the damaged base remains in place and may lead to lethality.

168. It is to be expected that the genes involved in other functions required for the maintenance of genome stability would lead to a similar effect of increasing mutation frequency, thereby affecting cancer rates. These functions would include the "proofreading" of DNA synthesis by polymerases and the regulation of DNA precursor synthesis. Indeed, it has been shown that mutations in the exonuclease domains of the DNA polymerase δ gene correlate with high mutation rates in some colorectal carcinoma cell lines lacking changes in mismatch repair genes [D6].

169. Repair enzymes may cause mutations by virtue of their imperfect response to damaged DNA, but in the last few years it has become apparent that many organisms have also retained specific enzymes that introduce mutations into damaged DNA. Thus the loss of certain repair functions can be antimutagenic, and indeed mutant strains of bacteria showing no increase in mutant frequency after treatment with DNA-damaging agents have been known for many years [W11]. Recent studies have defined these mutagenic or error-prone DNA repair processes in bacteria and yeast, and have

revealed that similar processes occur in other organisms, including humans [J23]. It has been found that genes coding for special types of DNA polymerases are responsible for many of the small mutations (base substitutions, frameshifts) occurring spontaneously and after treatment with DNA-damaging agents. In bacteria, for example, when DNA damage blocks the normal replication process, an "SOS" response is activated and more than 20 genes are induced [S83]. Among these genes are polymerases (e.g., DNA polymerase V) with a high affinity for damaged DNA, which are able to continue to synthesise DNA for a few bases in the presence of damage (translesion synthesis) and which commonly put in incorrect bases to give a mutation [R35, T45]. Similarly, in yeast, three genes (*REV1, REV3, REV7*) are required for much DNA-damage-induced mutagenesis, and these have been found to specify mutagenic DNA polymerase activity required for translesion synthesis [N23, N24]. A human gene named *hREV3* has recently been cloned through its homology to the yeast *REV3* gene and been found to have the properties of a mutagenic polymerase [G41]. As well as this error-prone activity, yeast cells have another specialized DNA polymerase (encoded by the *RAD30* gene) that can perform translesion synthesis in an error-free way. Strikingly, a human gene homologous to *RAD30* (*hRAD30)* has been found mutated in a variant form of the human sunlight-sensitivity disorder xeroderma pigmentosum (see paragraph 26). Since individuals with this disorder suffer a high frequency of skin cancer, this result suggests that error-free translesion synthesis is important to protect against sunlight-induced cancers [J24, M49]. While much remains to be learned about the operation of these specialized polymerases, it seems clear that maintaining a balance between error-free and error-prone pathways of translesion synthesis is important in the determination of rates of (point) mutation in cells.

B. THE SPECTRUM OF RADIATION-INDUCED MUTATIONS

170. Ionizing radiation can induce many types of mutation, from small point changes to very large alterations encompassing many genes. From recent studies of mammalian cells, in which a few genes have been examined in some detail, it is clear that radiation is most effective at inducing large genetic changes (large deletions and rearrangements). This mutation spectrum (proportion of different types of mutations) differs from that found spontaneously or the spectra induced by many other DNA-damaging agents (e.g. ultraviolet light, alkylating chemicals). These other agents tend to induce mostly point mutations, independent of the genomic region assessed. However, it has also been found that the proportion of radiation-induced large genetic changes can vary with the genomic region assessed.

171. The size of genetic changes in a given region of the genome is limited by the extent to which that region can tolerate change. The region may contain genes that are essential for the viability of the cell and organism; if an essential gene is altered or lost, the changes incurred will usually be lethal [E3, T16, T21]. Thus, although the initial occurrence of radiation-induced genetic changes may be similar at different sites in the genome, lethality will limit both the frequency and the apparent size of mutations recovered. Mutations will be recovered in some genes at low frequency, because they or the region they reside in can tolerate little change (certain genes have been found that, within experimental limits, show almost no radiation-induced mutation [T15]), while mutations in other genes may be detected at relatively high frequency because of cellular tolerance to large changes. Knowing this, it is possible to devise mutation detection systems that tolerate very large changes that would be inconsistent with survival in normal diploid cells. The most extreme example of high-frequency mutation detection is in a system devised by Waldren et al. [W12] that uses as the mutational target an accessory human chromosome 11 introduced into a Chinese hamster cell line. Since no genes on the human chromosome are essential for cell survival, very large changes including whole chromosome loss are tolerated, and mutant frequencies are about 100 times greater than for endogenous genes such as *HPRT*.

172. Much of the mutagenic response to ionizing radiation has been measured, for experimental ease, in regions of the genome that are present at the level of one copy per cell. This situation (monosomy) occurs naturally in parts of the genome such as the X and Y chromosomes in males; also, functional monosomy appears to be quite extensive through mechanisms that switch off gene expression in one copy (as in one copy of the X chromosome in females and in imprinted chromosomal regions). However, most chromosomes and genes are present as two copies per cell (disomic; see paragraph 7). Where the loss of function in one copy of a disomic gene is tolerated without harm (e.g. because of a recessive point mutation), it has been found that the other gene copy is mutated at relatively high frequency by ionizing radiation. Since the two gene copies differ in their functionality (that is, they are heterozygous), this mutation process is commonly termed loss of hetero-

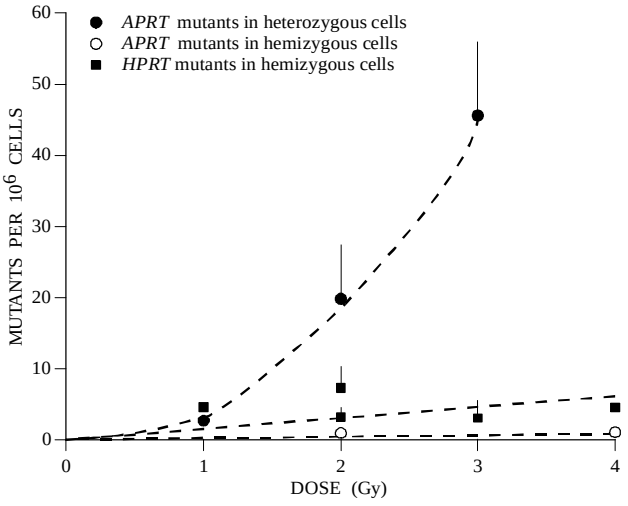

Figure V. Effect of gene location (for hemizygous genes) and copy number on mutant frequency in hamster cell lines carrying two copies of the gene (heterozygous) or single copy (hemizygous) [B11].

zygosity. The frequency of mutation in this disomic heterozygous situation has been shown experimentally to be higher than for the same gene in a monosomic situation [E3, M14, Y2], as illustrated in Figure V for the *APRT* gene, where the mutation frequency difference is about 20-fold [B11]. The reason for this higher mutation frequency in the disomic situation appears again to relate to tolerance of the frequent large genetic changes caused by radiation. Thus, the first copy, despite carrying a point mutation, still has the remainder of that genomic region intact (including any linked essential genes), and large deletions in the second copy can be tolerated because these do not lead to a complete loss of linked essential genes. In a monosomic chromosomal region, however, there is no other copy (or no functioning copy) present, and the mutant cell will not survive large genetic changes (Figure VI).

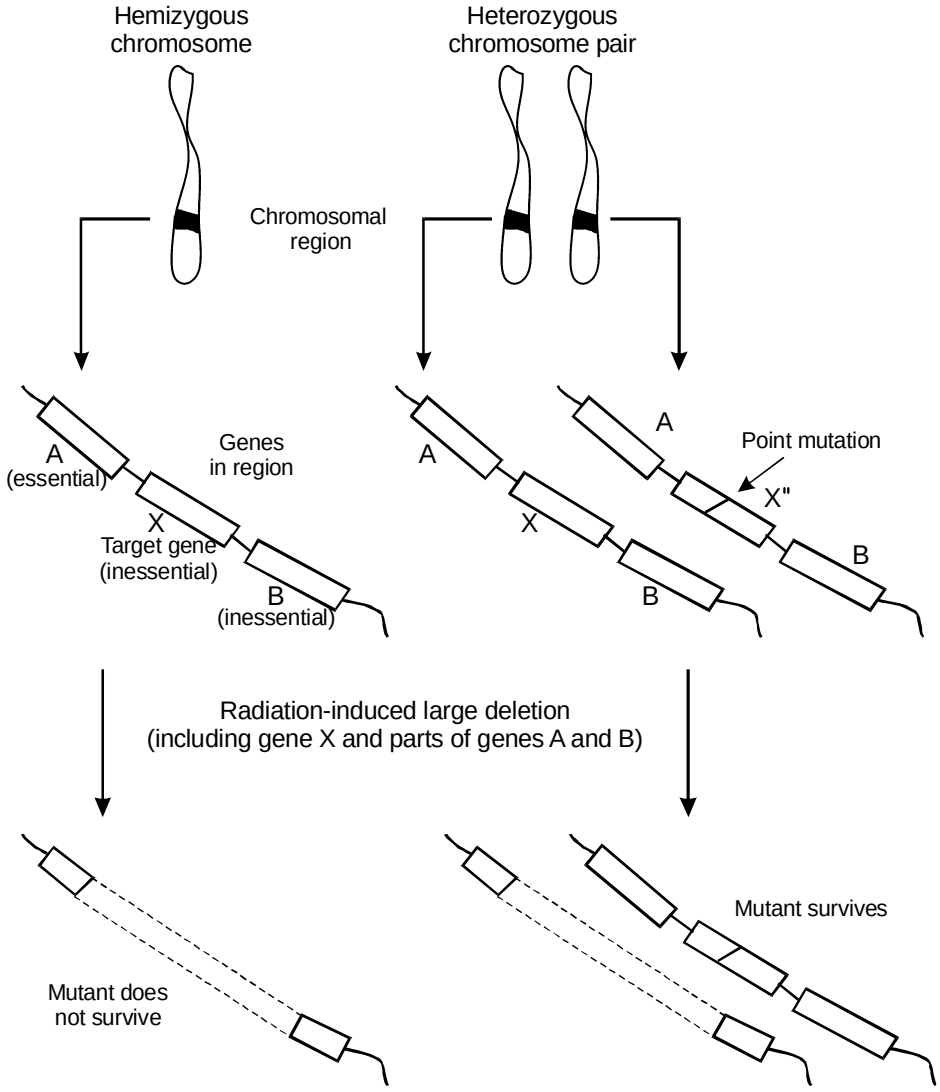

Figure VI. Consequences of radiation-induced deletion mutation in a hemizygous (monosomic) and heterozygous (disomic) gene.

Loss of function in gene X *is selected for, but the mutation also removes parts of adjacent genes, one of which is essential for cell viability. The mutation results in death of the cell carrying the mutation when the genetic region is hemizygous, but not when it is present in a heterozygous state.*

173. Where disomic genes are used to study radiation-induced mutation, the mutation frequency will also vary depending on which of the two copies (alleles) is used as the target. It is to be expected that in addition to the target gene, other linked genes will vary in their functional state (heterozygosity). Thus, if an active allele of the target gene is linked on one chromosome to the active allele of an essential gene, deletions of that chromosome will be severely limited. Conversely, linkage of the target gene to an inactive copy of the essential gene will not be limiting if the homologous chromosome carries the active essential gene and will allow the cell to survive a large deletion. Thus, a 10-fold difference in mutant frequency has been recorded for the two alleles of a heterozygous *TK* gene in lymphoblastoid cells [A11]. This difference in mutant frequency was associated with a class of slow-growth mutants carrying large genetic changes at the site of the mutable allele of the *TK* gene [A12].

174. High mutation frequencies may therefore mainly reflect tolerance to large genetic changes, although higher

frequencies may also arise from additional mutation mechanisms available in heterozygous cells (paragraph 183). Since ionizing radiation is relatively good at inducing large genetic changes, it will be very effective as a mutagen for disomic genes compared with its effectiveness for monosomic genes (also, in general, ionizing radiation is less effective than potent chemical mutagens when used to mutate monosomic genes). It may be that, for example, an individual inherits a point mutation in one copy of a tumour-suppressor gene, which in itself does not lead to deleterious effects because of the "good" copy still present. However, should this person be subjected to an agent like radiation, which is very effective at inducing large genetic changes, the other gene copy can be readily mutated along with much of the surrounding chromosomal region, because that region is shielded by the presence of the other gene copies. In this way radiation can be seen as a very effective mutagen for specific types of mutational change; indeed it could be argued that for recessive gene mutation, individuals are susceptible to agents such as radiation if they already carry point mutations in disomic genes. This phenomenon is an aspect of predisposition of individuals to specific genetic changes and should not be confused with radiosensitivity (paragraph 122).

175. The prediction that large genetic changes would be found in disomic target genes has been borne out in studies of mice [C19]. Screening progeny after x irradiation of male germ cells revealed that very large chromosomal changes (deletions, rearrangements, and complex changes) occur at high frequency in one copy of disomic regions of the genome; these large changes were consistent with reasonable viability and fertility in the progeny. As expected, when these mutant regions are not shielded by a second normal copy of the genes involved, they are invariably lethal to the mice.

C. MOLECULAR ANALYSIS OF RADIATION-INDUCED MUTATIONS

176. While it can be argued that ionizing radiation has a particular mutation spectrum, it has not been found that any one type of mutation is induced specifically by ionizing radiation. However, detailed analysis of mutations (and to some extent of chromosomal aberrations) has given some possible indicators of differences between spontaneously-occurring and radiation-induced mutations, and of differences between densely and sparsely ionizing radiation.

177. Sequence analysis of x- or gamma-ray-induced point mutations in mammalian cells has shown that a variety of types occur, from base-pair substitutions and frameshifts to small deletions. Analysis of substitutions in the *HPRT* and *APRT* genes has shown that all of the 6 possible types occur, although at different frequencies (Table 3) [G10, M12, N11]. A majority of the radiation-induced substitutions were transversions (alteration of the base from a purine to a pyrimidine or vice versa), while many spontaneously-occurring substitutions were G:C → A:T transitions. A larger proportion of frameshift mutations also occurred in the radiation-induced mutants than spontaneously, and a few more of the radiation-induced point mutations were multiple substitutions (more than one base change in close proximity). Using a small gene target transferred into mouse cells, little difference was found in the mutation spectra for spontaeously-occurring and x-ray-induced mutations [K18]. The frequency of small rearrangements and deletions was increased in both spontaneous and radiation-induced mutants, indicating differences in the balance of mutagenic mechanisms at the site of integration of the transgene compared with those at endogenous genes.

Table 3
Comparison of spontaneous and radiation-induced point mutation spectra

Type of mutation	APRT *gene / hamster CHO cells [M12]*		HPRT *gene / human TK6 cells [N11]*	
	Spontaneous	*Induced by gamma rays (2.5–4 Gy)*	*Spontaneous*	*Induced by x rays (2 Gy)*
Base substitution Transition Transversion	55 (71%) 31 (40%) 24 (31%)	19 (66%) [a] 6 (21%) 13 (45%)	10 (55%) 6 (33%) 4 (22%)	19 (54%) 7 (20%) 12 (34%) [a]
Frameshift	6 (8%)	5 (17%)	3 (17%)	8 (23%)
Small deletion or rearrangement	16 (21%)	5 (17%)	5 (28%)	8 (23%)
Total	77	29	18	35

a Includes two tandem substitutions.

178. Radiation-induced point mutations were found at sites widely distributed within the gene, while spontaneously-occurring point mutations tended to cluster at certain sites [G10, M12, N11]. Differences in the types of base damage responsible and in the randomness of damage induction are likely to explain these differences.

179. Smaller numbers of large genetic changes have been sequenced because of the difficulty of locating and cloning the breakpoints of large deletions and rearrangements. No new or specific mechanism has been found for the induction of these larger changes. In the hamster *APRT* gene, present in the hemizygous state, the large gamma-ray-induced deletions tend

to have short direct or inverted repeat sequences around their breakpoints and to fall into regions rich in adenine-thymine base pairs. Insertions, however, involved repetitive sequences and were accompanied by short deletions [M13]. It was notable in these studies that large changes extending downstream of the *APRT* gene are not found, suggesting that an essential gene is present in this region.

180. In the human *HPRT* gene, also in the hemizygous state, large deletions are found to extend both upstream and downstream of the gene over a region of about 2 Mb [M16, N9]. Radiation-induced large genetic changes tend to eliminate the whole *HPRT* gene, making their analysis difficult. Therefore a series of flanking markers has been identified to delimit these changes and ultimately to map the positions of the breakpoints. Sequence analysis of a few deletion mutations has again shown the presence of short direct repeats at some breakpoints induced by both x rays and alpha particles, as well as other sequence features in adjacent regions [M15, S18]. The large deletions induced by radiation frequently include sequences adjacent to *HPRT*, and it has been suggested that this may be a signature of radiation-induced events as opposed to spontaneous events [N17]. This type of mutation was equally common with gamma-ray doses of 0.2 or 2 Gy d^{-1}. Use of methods amplifying specific regions of the genome to measure rapidly the frequency of rearrangements following x- or gamma-irradiation, followed by sequencing the breakpoints, has given little indication of the involvement of specific sequence features or clustering of breakpoints [F22, F23].

181. Shuttle vector systems consist of small defined DNA molecules that may be mutated in mammalian cells but rescued into bacteria for rapid analysis of sequence changes. While these facilitate the molecular analysis of induced mutations, they generally have the drawback that the target genes are very small and are flanked by essential sequence. This means that only point mutations are detectable, and the major class of radiation-induced mutations (large deletions and rearrangements) cannot be analysed. However, Lutze and Winegar [L19] devised a large shuttle vector based on Epstein-Barr viral sequence that could be maintained episomally in human lymphoblastoid cells and could detect changes of up to 8 kb or so. Using this vector, the mutation spectra of both x rays (150-600 Gy) and alpha particles (3 Gy) from radon gas were studied [L19, L20]. A larger proportion of deletions was found among alpha-particle mutations (64%) than among x-ray mutations (13%), but in a subsequent study with lower doses of x rays, more deletions were found (41% at 100 Gy, 33% at 20 Gy) [L21]. For both x rays and alpha particles, these deletions were large (>2.4 kb), and their breakpoints were clustered in specific regions of the shuttle vector. The breakpoints were commonly associated with short direct sequence repeats of up to six base pairs. Use of the same vector system in a lymphoblastoid cell line defective in double-strand-break repair gave a slightly higher frequency of deletions after x irradiation, in association with an additional class of small deletion mutations (<2.4 kb) [L21].

182. The presence of sequence features such as short direct repeats of a few base pairs at large deletion junctions suggests that illegitimate recombination (see Section I.B) has driven the mutation process. Recently it has been possible to reconstruct the process of illegitimate recombination in cell-free conditions to show that the process can be associated with a DNA double-strand break. DNA molecules are broken at a specific site, using an endonuclease, and exposed to extracts from human cells for a brief period. Analysis of the extract-treated DNA shows that while the majority of broken molecules are correctly rejoined, a small fraction (about 0.5%) are mis-rejoined to give a deletion. The mis-rejoin mechanism involves the pairing of short direct sequence repeats, situated either side of the break, so that the intervening sequence (including one of the repeats) is deleted [T10]. This mechanism has been found in both radiation-induced mutations and in germinal and somatic-cell mutations in humans. A model for this process is shown in Figure IV. In addition to simple deletion, this cell-free system detected a small fraction of more complex changes, e.g. a large deletion associated with the insertion of several hundred base pairs.

183. In heterozygotes, in addition to the tolerance of large changes, additional mechanisms of mutation may occur. The two gene copies may undergo mitotic recombination or non-disjunction, leading to the appearance of mutations. However, where loss of heterozygosity is measured simply by the presence or absence of the active (wild-type) gene, it is difficult to distinguish mutations occurring by these additional mechanisms from large deletions. In a study of mutation in the human *TK* gene, using both linked marker analysis and densitometry to assess gene copy number, about 50% of the spontaneous mutants appeared to involve recombination, while x-ray-induced mutants were mainly deletions [L29]. Recombination was similarly found to be involved in spontaneous mutation of the *APRT* gene in human cells, but this mechanism was also detected in a number of gamma-ray-induced mutants [F8].

184. While it is difficult to compare somatic-cell mutation data with animal germ-cell data, some recent molecular analyses of the genes used in mouse specific-locus tests indicate possible similarities in the types and mechanisms of mutation. For example, with some dependence on both cell stage and radiation quality, a large proportion of *albino* (*c*) locus mutations on mouse chromosome 7 recovered from germ-cell irradiations are large deletions [R14]. Molecular analysis of radiation-induced mutations at the *c* locus shows that a genomic region of 1.5-2 Mb around the tyrosinase gene may be deleted in mutants without leading to inviability in homozygotes [R13]. This target size is very similar to that of the human *HPRT* gene region used in somatic-cell mutation studies. Also, mutation frequencies per unit dose of low-LET radiation at the *c* locus in mouse germ cells and at the *HPRT* locus in human somatic cells are in order-of-magnitude agreement [T19], given that mutations at the *c* locus occur with about average induced frequency relative to other loci in specific-locus tests [S29]. Comparisons of this type emphasize that the mechanisms of radiation mutagenesis are similar in

somatic and germ cells, and that if measurements are made under similar conditions of mutation tolerance, then the mutation frequencies will be comparable.

185. Comparison of two human lymphoblastoid cell lines derived from the same original cell line has given further insights into the mechanisms of radiation mutagenesis. Line TK6 is more sensitive to the lethal effects of x rays than line WIL2-NS, but the converse is true for mutation response to x rays (although not to chemical mutagens). The increased frequency in WIL2-NS at the hemizygous *HPRT* gene was modest (factor of 4 at 2 Gy), while the increase for the heterozygous *TK* gene is by a factor of 20-50, depending on the *TK* allele used as the target [A13]. Molecular studies have revealed that all *TK* mutants in WIL2-NS arose by loss of the active allele and linked markers (over a 5 Mb region), while point mutations and less extensive deletions were common in the TK6 line. Two copies of the *TK* gene were present on a majority of karyotypes in the WIL2-NS mutants, as expected if these arose from mitotic recombination rather than deletion, while the converse was true for TK6 mutants [X1]. It seems likely, therefore, that the WIL2-NS line has a higher frequency of recombination, leading to a greater ability to survive x-ray damage but incurring a concomitant increase in mutation. Analysis of the p53 status (see paragraph 112) of these two cell lines has shown that p53 protein levels are four times higher in WIL2-NS than in TK6, because of a mutation in exon 7 of the *TP53* gene in WIL2-NS (TK6 is wild-type) and that apoptotic death is substantially delayed in WIL2-NS [X2, Z7].

186. In a study of B-cell precursors from *Tp53*-knockout mice, it was also found that high frequencies of *HPRT* mutations occurred following x irradiation but that these resulted from a preferential survival of mutant clones rather than from a p53-dependent increase in mutation rate [G26]. It was concluded that loss of p53 function allows mutated cells to survive that would otherwise be eliminated by apoptosis. Other recent studies with cells from p53-deficient mice have similarly concluded that they do not have an intrinsically higher-than-average mutation rate [C68, N12, S21].

187. It has been suggested that a relatively unique feature of radiation mutagenesis is the induction of complex genetic changes [M11]. For example, a radiation-induced deletion may be associated with a rearrangement at the same site, and the rearrangement may be an exchange, inversion, or insertion. Additionally, complex changes may be seen as chromosomal exchanges involving several sites in the genome following a single acute radiation treatment [S15]. However, because of their large size and complexity, these changes have been difficult to analyse in cellular genes at the molecular level. Further molecular analyses will be necessary to establish whether or not large mutations and chromosomal aberrations are formed by similar mechanisms. It is clear from studies with one or two genetic regions, and especially the region containing the human *HPRT* gene (Xq26), that genetic changes identified at the molecular level as mutations can extend to sizes that are visible in the light microscope [S17]. However, the breakpoints of such very large mutations and chromosomal aberrations have not been sequenced.

D. EFFECT OF RADIATION QUALITY

188. In general, high-LET radiation induces a higher frequency of mutants in rodent and human cells, per unit dose, than low-LET radiation [B17, B19, C22, C30, F7, G18, H20, L31, M22, M25, S88, S89, T16, T18, T32, W22]. The RBE varies with LET, peaking at 100-200 keV μm^{-1}, with values as high as 7-10 found for alpha particles and heavy ions in this LET range [C22, T16]. In some mouse cell lines, a relatively small RBE of 2-3 has been found for *HPRT* mutant frequency with alpha particles in the peak range, and when cell survival is taken into account, there is no difference in the effectiveness of alpha particles and x rays. This result appears to be due to the very high effectiveness of low-LET radiation on these cells, as reflected in increased cell killing and mutagenesis for a given dose compared with other rodent lines [B19, I2]. That is, the mutagenic effectiveness of low-LET radiation is more variable than that of high-LET radiation, presumably because cell lines have different abilities to repair low-LET radiation damage (while high-LET damage is less repairable; see Section III.B). An example of this variability is shown in Figure VII [T28].

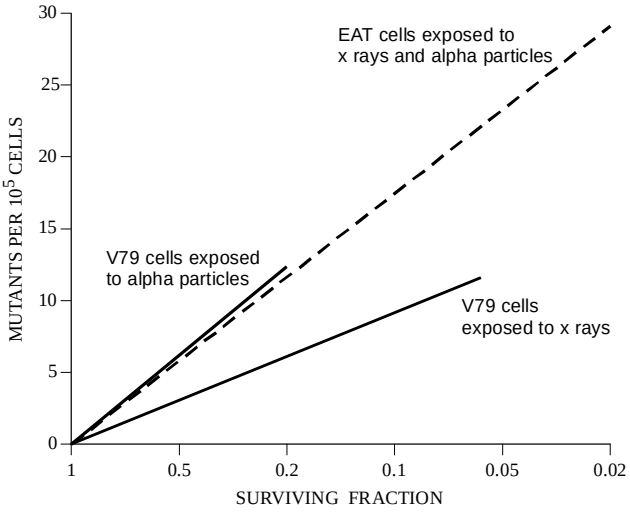

Figure VII. Fitted curves of mutant frequencies in Chinese hamster V79 cells [T18] and mouse ascites (EAT) cells [I20] induced by x rays and ^{238}Pu alpha particles (see paragraph 172).

189. A study of 19 non-smoking people living in houses with a range of radon concentrations (21-244 Bq m^{-3} in the United Kingdom suggested that there was a correlation of *HPRT* mutant frequency in blood T-lymphocytes with radon concentration. These data suggested that alpha particles in this LET range have a considerably higher effectiveness than found previously in high-dose experiments with human somatic cells. While the estimation of

alpha-particle dose to circulating blood lymphocytes is difficult, the authors estimated an annual doubling dose of 2.1 mSv from their results [B29]. A number of problems attend such measurements, not the least of which is the sample size. In a follow-up study by the same authors with a total of 65 persons from 41 houses in the same town, no significant correlation was found between mutant frequency and radon levels, even for people living in the same house [C34]. In a study in Belgium of 24 people living in houses with radon concentrations exceeding 100 $Bq\,m^{-3}$, a negative association between radon concentration and *HPRT* mutation frequency was found [A10]. This result led the authors to suggest the induction of repair processes by low levels of radon.

190. The *TP53* tumour-suppressor gene is commonly mutated in many different types of cancers, with a frequency ranging from <10% to >50% depending on tumour type. Analysis of mutations in the *TP53* gene in lung tumours from uranium miners initially gave some hope that these would provide a means of fingerprinting high-LET radiation damage. Vahakangas et al. [V2] sequenced exons 5-9 of the *TP53* gene in 19 lung tumours from miners working in New Mexico. They found several point mutations, although these lacked a class of base substitution (G:C → T:A transversions) and were not at the hotspots described for lung cancer. In contrast, in a larger study of 52 lung tumours from Colorado uranium miners exposed to a dose that was, on average, five times higher, Taylor et al. [T12] sequenced the same exons and found that about half of the substitutions were of the G:C → T:A type in codon 249 (codon = base-pair triplet encoding one subunit of the p53 protein). This transversion is very rare among *TP53* mutations in lung tumours (many of which are presumed to be smoking-associated) and was suggested to be a potential marker for radon-associated lung cancer. At present, apart from the difference in average dose, it is difficult to see why these two *TP53* mutation studies gave such contradictory results. One possibility is that the transversion mutations seen are a result of some other agent in the environment of the mine; if the mine is damp, then fungal toxins similar to aflatoxin B1, known to induce this type of mutation, might be present [V3]. Follow-up experiments looking specifically for the codon 249 transversion in lung cancers from individuals exposed to high domestic radon levels in the United Kingdom [L48] and from German uranium miners [B45] failed to find any examples of this mutation.

191. In a study of thymic lymphomas in RF/J mice induced by gamma rays or by neutrons (0.44 MeV), a similar frequency of lymphomas was associated with mutations activating *RAS* oncogenes (24% for gamma rays, 17% for neutrons). Of the gamma-ray set, 89% were K-*RAS*-activated, with the majority (7 of 8) having a GAT → GGT mutation in codon 12. However, in the neutron set no particular mutation site predominated, and one lymphoma contained a K-*RAS* gene activated by a point mutation in codon 146, a site not previously associated with any human or animal tumour [S36].

192. There are conflicting data on possible differences in the spectrum of large genetic changes induced by high-LET radiation relative to those induced by low-LET radiation. Early cytogenetic studies with diploid human fibroblasts suggested that a greater proportion of *HPRT* mutants induced by high-LET radiation carried large genetic changes than did x-ray-induced mutants [C37, T19]. Studies of specific-locus mutations in mouse spermatogonial stem cells had also concluded, based on a number of criteria, that ^{239}Pu-induced mutants carried more severe genetic damage than mutants induced by low-LET radiation [R16]. However, classification of *HPRT* mutations by molecular analysis in both diploid human cells and hamster cells has not shown differences in the proportions of large deletions to point mutations at doses of low- or high-LET radiation giving about 20% cell survival [A8, G16, S18, T20]. A confounding factor (commonly underestimated) in determining mutation spectra can be the non-independence of mutants when grown in bulk culture after irradiation [T22], but in the most recent studies [A8, S18] the experimental design ensured the independence of all mutants. Other studies have reported differences: irradiation with 190 keV μm^{-1} Fe ions gave 82% large deletions of the *HPRT* gene in human TK6 cells relative to 54% for x rays [K21]. High doses of radiation from incorporated ^{125}I, giving 1% survival of human TK6 cells, were reported to increase the frequency of deletions or rearrangements when compared with lower doses of ^{125}I or with x rays [W22]. It was noted that these high-dose mutations commonly showed loss of part of the *HPRT* gene rather than the entire gene. It has similarly been reported in recent studies with the same cell line that the average size of radon-induced deletions of the *HPRT* gene is not as great as that induced by x rays [B19, C29]. This feature has also been noted for mutations induced at the hemizygous DHFR gene in hamster cells; that is, the frequency of total-gene deletions was much higher for gamma-ray- than for alpha-particle-induced mutations, while the reverse was true for intragenic deletions [J11]. In the latter study, it was also found that some alpha-particle-induced deletions shared common breakpoint sites, indicating a non-random distribution. Use of the heterozygous *TK* gene as a target, also in TK6 cells, showed again that the proportion of deletions was similar for x rays and high-LET radiation (4.2 MeV neutrons and argon ions), but that high-LET radiation induced a category of large rearrangements not found for x rays [K20]. It has also been claimed that high LET radiations induce non-contiguous deletions that are not found with x or gamma rays (see also paragraph 208) [S90]. A number of these studies, therefore, suggest there could be differences in the types of large genetic changes induced by high-LET as opposed to low-LET radiation. Much more careful analysis is required before any general statement can be made about the extent or nature of these possible differences.

193. The mutagenic effects of a single alpha-particle traversal were measured with a high-frequency detection system (paragraph 171). Using a microbeam to localize 90 keV μm^{-1} alpha particles to cell nuclei, a dose-dependent increase in mutant frequency was found for traversals of 1-8 particles, with a twofold increase for 1 alpha particle traversal (cell surviving fraction = 0.8) [H42]. Molecular analysis of

these mutants gave some support for the view that higher doses of high-LET radiation induce larger genetic changes, but this system is unusual in being able to sustain loss of the whole target chromosome without lethality.

194. It has been proposed that the ratio of specific forms of chromosomal aberration may be a fingerprint of exposure to high-LET radiation [B13, S71]. A low ratio (about 6) of interchromosomal to intrachromosomal exchanges was found in several studies of aberration induction by high-LET radiation, while a value of ≥ 15 was found for x or gamma rays. However it was subsequently demonstrated [S54], using a simple two-dimensional chromosome model allowing the prediction of exchange sites relative to track structure, that more densely clustered tracks are not predicted to give a significantly lower ratio of aberration types than random scattering of tracks. More critical assessment of experimental data also does not support this theory [L54, S66]; in particular, reexamination of extensive plant data has ruled out both this theory and any LET-dependence in the ratio of interstitial deletions to inter-arm exchanges [S72]. A different high-LET fingerprint has more recently been proposed by Lucas [L55], who suggested that the proportion of unrejoined ("incomplete") chromosome aberrations will be greater with high-LET radiation, because the local density of DNA double-strand breaks is greater than for low-LET radiation and competition between broken ends leads to a greater chance of incomplete exchange events. Published data are cited that give a ratio of incomplete exchanges to complete exchanges (translocations) of about 9 for low-LET radiations and about 2 for high-LET radiations, with "mixed-LET" radiations giving intermediate values. While this theory has yet to receive critical appraisal in the literature, it may suffer from the same problems as the earlier theory, insofar as only certain sets of data give large differences between high- and low-LET radiations, partly because of difficulties in correctly defining incomplete aberrations. There may be further difficulties with the idea that competition between broken ends will necessarily lead to more incomplete aberrations and with the simple assumption that incomplete aberrations represent unrejoined breaks.

E. NOVEL MECHANISMS OF GENETIC CHANGE

195. Most of the processing of DNA damage by cellular repair enzymes is completed within a few hours of irradiation, including the fixation of mutations. However, there is evidence that cellular responses continue to occur for much longer periods, over many cell generations. These responses include delayed cell death and genetic changes. While these responses may in part be attributed to the time taken for the cell to recover from irradiation, there are in principle several reasons why persistent effects could occur. These include persistence of the damaging agent; persistence of certain forms of DNA damage, i.e. lack of repair; the repair of damage leading to rearrangements of the genome, which themselves upset the correct functioning of the cell (e.g. "position effects" on blocks of genes); and the induction of a long-lived metabolic disturbance in somatic cells, such that enzymatic activities (e.g. DNA polymerases) involved in the fidelity of maintaining the genome do not function properly.

196. It has been known for many years that cells may take some time to die following irradiation [E2, J7], but more recently emphasis has been placed on lethal mutations that may take effect many cell generations after irradiation [B53, G9, S16]. These two phenomena may in part be aspects of the same response. Delay in cell death in fibroblastic cell lines may be explained by the time taken for cells to show the effects of loss of essential genes through chromosome fragmentation (see paragraph 110). As cells divide, they segregate broken chromosomes (often seen as micronuclei), and the encoded gene products are diluted out of daughter cells, eventually causing death [J7]. In the same way, lethal mutations may cause late-onset death in cells; however, they can arise through more subtle effects on the genome than simple chromosomal fragmentation and loss. Thus, all types of mutation, from point mutation to large genetic changes, may represent lethal mutations if they lead to loss of essential gene products. However, to account for the high frequency of lethal mutations at long times after irradiation, it has also been proposed that some form of persistent genetic instability can be induced by radiation.

197. In addition to delayed death, the mutation frequency in inessential genes such as *HPRT* has been found to be persistently elevated in a large fraction of clones surviving irradiation [C20]. In these experiments, cells irradiated with x rays (12 Gy) were grown as separate clones and examined for both the proportion of cells surviving and for mutant frequency at different times after irradiation. It was found that clones showing reduced survival also commonly had elevated mutant frequencies at the *HPRT* gene for as many as 50-100 cell generations following irradiation. The mutant frequency in individual clones was highly variable but sometimes exceeded $1\ 10^{-3}$. These delayed mutations appeared to be predominantly point mutations [L34].

198. Chromosomal aberrations have also been found to persist following irradiation. One-cell mouse embryos irradiated with x rays or neutrons showed an approximately linear increase in the frequency of chromosomal aberrations per cell in the first, second, and third mitoses post-irradiation. The relatively high frequency of aberrations, especially for neutrons, and the occurrence of chromatid-type aberrations on the third mitosis following irradiation suggested that new aberrations were being produced in post-irradiation cell cycles [W40, W41]. Similar results were obtained with x irradiation of two-cell mouse embryos [W42]. Delayed chromosomal aberrations were not observed in embryos treated with restriction endonucleases, suggesting that lesions other than DNA double-strand breaks are responsible for this effect [W48]. A significant increase in chromosome- and chromatid-type aberrations was also found in cell cultures derived from foetal skin biopsies of mice x irradiated as zygotes [P25]. The study of chromosomal aberrations in lymphocyte and fibroblast clones surviving x irradiation has similarly given evidence of persistent genetic effects [H30, M34, P33]. Analysis of clones

for two months following irradiation showed that >20% had sporadic aberrations as well as transmissable clonal karyotype alterations [H31].

199. Genetic instability in clones of cells surviving irradiation has also been described for alpha-particle irradiation of cultured haematopoietic stem cells from CBA/H mice [K8]. In this case, chromosomal aberrations were measured in cell clones surviving 3 Gy from x rays or 0.25-1 Gy from alpha particles (0.5 Gy from ^{238}Pu alpha particles corresponds to an average of one track per cell). About 50% of the clones surviving alpha-particle irradiation carried aberrations; these were mostly non-identical chromatid-type aberrations, suggesting that they had arisen many generations after irradiation. The frequency of such delayed aberration induction in x-ray survivors was only about 2% [K8]. This form of chromosomal instability in bone marrow cells was also found to be transmissible *in vivo*, by transplanting male cells irradiated with alpha particles into female recipients [W38]. The repopulated haemopoietic system showed instability persisting for up to one year. Alpha particles have also been shown to induce similar delayed chromosomal effects in the bone marrow of two out of four normal humans [K9]. It was suggested that the lack of effect in some individuals reflects genetic determinants that vary in the human population, and additional studies of other inbred mouse strains have also been found to show varying levels of this form of genetic instability. The alpha-particle-induced instability was, however, found to be independent of the p53 status of the cell [K32].

200. Heavy ion (neon, argon, or lead) irradiations have also been found to induce chromosomal instability in cultured human fibroblasts [M35, S53]. Analysis of mass cell cultures for up to 25 passages following irradiation showed that the frequency of aberrations declined at first but then increased until >60% of the cells showed aberrations. In contrast to clonal cell populations, these aberrations showed that the telomeric regions of specific chromosomes (1, 13, and 16) were involved. In a study of alpha particles of different LET, the frequency of micronuclei was found to be increased over that in unirradiated hamster cells seven days after irradiation, even at a dose giving one alpha-particle traversal per cell nucleus [M36]. Calculations indicate that the target size for this effect exceeds the size of the nucleus, suggesting that direct DNA damage by radiation tracks is not causal.

201. It has been speculated that these events indicate that ionizing radiation may induce an "untargeted" mechanism of mutagenesis in cells, as a result of the epigenetic alteration of enzymatic pathways controlling genomic stability [L18]. The idea that DNA may not require a direct hit from radiation to have an increased frequency of genetic changes has received support from the measurement of non-mutational responses. With reference to the process of carcinogenesis in particular, the work of Kennedy et al. [K10, K11] suggests that radiation may induce high-frequency events that predispose the cell towards further (spontaneously-occurring) changes in the process of malignant cell transformation. Furthermore, a study of the induction of sister-chromatid exchanges in immortalized hamster cells by very low doses of ^{238}Pu alpha particles claimed that induction could be measured at doses (0.3 mGy) where <1% of the cell's nuclei were traversed by an alpha-particle track [N8]. Subsequent studies of primary human fibroblasts confirmed this finding; low doses of alpha particles give three times as many sister-chromatid exchanges [D13] or a five times higher *HPRT* gene mutation frequency [N25] than predicted from the number of nuclei traversed by the particle tracks, giving a considerably larger target size for this effect than expected from nuclear dimensions [D13]. Similarly, the use of a physical barrier to protect one part of a cell population from alpha-particle irradiation showed the expected reduction in killing of mouse bone-marrow stem cells but did not reduce chromosomal instability [L63]. These data are considered as evidence for the existence of a "bystander" effect; that is, damage signals may be transmitted from irradiated to neighbouring unirradiated cells. In a refinement of this type of experiment, an alpha-particle microbeam was used to give precise irradiation of the cytoplasm of cells, without damaging their nuclei. Under these conditions, an average increase of three-fold was found in the mutation frequency in a sensitive human-hamster hybrid cell line (paragraph 171), and the mutation spectrum was similar to that occurring spontaneously [W51]. One explanation of this phenomenon is that reactive oxygen species are generated by radiation in the whole cell and perhaps also in the surrounding medium, and these species or stable reaction products (e.g. lipid peroxides) diffuse into the nucleus and persist to cause chromosomal effects. Evidence for this idea has been found with x or neutron irradiation of bone marrow cells; various indicators of persistent oxygen radical activity were found in cell cultures seven days after irradiation [C40]. It has also been shown that alpha particles can produce factors in culture medium or in cells that cause increases in sister-chromatid exchanges, as noted above; these factors are inhibited by superoxide dismutase, an enzyme that catalyses the conversion of superoxide ions produced in water radiolysis [L17]. Similarly, in the microbeam experiments, reactive oxygen species were implicated in the mutagenic effects of cytoplamsic irradiation [W51]. Further, in human fibroblasts, the modulation of proteins involved in the p53-dependent pathway (Section II.B.2) following very low doses of alpha particles occurs in more cells than have been traversed by an alpha-particle track, apparently through cell-cell contact [A24]. The potential consequences of bystander effects are discussed further in Annex G, "*Biological effects at low radiation doses*".

202. In an attempt to link the delayed appearance of chromosomal aberrations to cancer-proneness, Ponnaiya et al. [P24] measured this form of instability in strains of mice differing in their sensitivity to radiation-induced mammary cancer. Strikingly, cells from the more sensitive strain (BALB/c) showed a marked increase in the frequency of chromatid aberrations after 16 population doublings, while the less sensitive strain (C57BL/6) showed no increase in aberrations over the control level.

203. Higher-than-average frequencies of specific types of chromosomal aberrations have been found after irradiation of

strains of mice that are prone to certain types of cancer. These aberrations are not necessarily the delayed effects of irradiation but nonetheless may indicate that radiation damage to specific parts of the genome contributes disproportionately to cancer induction. As an example, interstitial deletions of mouse chromosome 2 are consistently associated with radiation-induced acute myeloid leukaemia in several inbred strains of mice. The chromosome breakpoints involved in these chromosome 2 deletions are non-randomly distributed and can be detected at high frequency at early times following irradiation, suggesting that they may be an early event in leukaemogenesis [B54, B55, H43, H45, R28, T23]. Investigation of two mouse strains showing large differences in susceptibility to radiation-induced thymic lymphomas showed that at early times following x irradiation the incidence of specific chromosomal aberrations, especially trisomy 15, was much higher in the cancer-prone strain than in the resistant strain [C21]. These examples suggest that certain chromosomal regions may be more sensitive to radiation-induced genetic changes that are associated with cancer, although it is difficult to quantify this contribution precisely without a better understanding of the development of aberrant clones of cells in animal tissues.

204. In some mutation systems very high frequencies of mutation occur both spontaneously and after irradiation. With radiation induction, the amount of initial damage (e.g. the numbers of DNA double-strand breaks per unit dose) in the mutational target may not in these cases be sufficient to account for the numbers of mutations induced. While it is possible to consider that every type of damage (paragraph 15, Table 1) may sometimes lead to mutation, these examples of high-frequency mutation have usually been proposed to arise from a form of genetic instability. For instance, somatic mutation frequencies of coat colour in *Pink-eyed unstable* mice are linear with x-ray dose down to 10 mGy and occur at least 100 times more frequently per unit dose than germ-line mutations in the mouse specific-locus tests [S57]. The *Pink-eyed unstable* male mice have a reduction in the pigment of the coat and eyes, caused by gene duplication interfering with normal pigment production; deletion of this duplication is scored as the mutation, giving normal pigmentation seen as black spots on the grey coat.

205. The spontaneous frequency of mutation at tandem-repeat (VNTR or "minisatellite") DNA sequences in the germ line of mice is also very high (1%-10% in offspring). The mechanism of mutation at these hypervariable loci does not appear to involve unequal exchange between homologous chromosomes [W30] but rather some form of complex gene conversion process [J10]. The incorporation of human tandem-repeat sequences into yeast cells, to study the mechanisms of variability, has confirmed that such sequences are destabilized in meiosis and that this process depends on the initiation of homologous recombination at a nearby DNA double-strand break [D23]. Irradiation of mouse spermatogonial stem cells by x rays increased the mutation frequency in offspring relative to controls [D10]. More extensive experiments at different stages of spermatogenesis using both single- and multi-locus probes showed no increase in mutation frequency in post-meiotic spermatids but gave a doubling dose of 0.33 Gy for premeiotic spermatogonial and stem cells. The dose-response for mutation induction by 0.5-1 Gy x rays, combined for spermatogonial and stem cells, was linear [D19]. In contrast, in a separate study using one of the same probes at different germ-cell stages, the irradiation of spermatogonia gave a non-significant increase in mutation frequency, while the frequency in irradiated spermatids was significant. The increase in mutation frequency was not linear with dose, showing little increase above 1 Gy [F5, S19]. Similar data were reported for ^{252}Cf irradiations (35% gamma rays, 65% neutrons): spermatids again showed the highest induced mutation frequency, and a single dose (1 Gy) to spermatogonia was significantly mutagenic. The RBE for these mutations with ^{252}Cf was 5.9 for spermatogonia, 2.6 for spermatids, and 6.5 for spermatogonial stem cells [N15]. More work is required to reconcile the discrepancies that are seen for different germ-cell stages. Recently a striking result has been found from tandem-repeat mutation studies following irradiation of male mice with 0.5 Gy ^{252}Cf neutrons, and the mating of these mice to unirradiated mice through two generations to yield second-generation (F_2) progeny. Remarkably, the F_2 mice showed an elevated frequency of mutation in the repeat sequences inherited from both male and female lines (6-fold and 3.5-fold, respectively), suggesting that genetic instability can be transmitted through the germline [D24]. The induced mutation frequency at these hypervariable sequences seems to be too high, by two orders of magnitude, for direct damage by radiation at the sites of mutation; it was proposed that some indirect mechanism of mutation induction is responsible [D19, F5, S19, D24].

206. Human spontaneous germ-line mutation frequencies at tandem-repeat loci can also be high (1%-7% per gamete). In a pilot study of children of survivors of the atomic bombings (mean gonad dose, generally to only one of the parents = 1.9 Sv) and matched controls, mutation frequency was measured at six tandem-repeat loci. The average mutation frequency was similar in the two groups, at 1.5% per gamete per locus for the exposed gametes and 2% for the unexposed gametes [K26]. A further study of the same children using a probe to detect multiple-repeat loci again showed no increase in mutation rate for the exposed group [S67]. However, a twofold increase in the frequency of mutation at tandem-repeat loci has been reported for children of parents resident in the Mogilev district of Belarus, which was heavily contaminated in the Chernobyl accident [D14]. Four tandem-repeat loci were tested, three of which showed increases by a factor of 1.7-2.0, while one locus (in which only one mutation was found) showed a reduction in the exposed group. The 79 families tested were compared to a control Caucasian population from the United Kingdom, which showed a similar overall distribution of repeat lengths at these loci. In a follow-up study using five additional tandem-repeat loci and including a further 48 families from Mogilev, the same general twofold increase was found when compared with the same (United Kingdom) control population [D1]. The mean dose to the exposed population was calculated to be 27.6±3.3 mSv from ^{137}Cs; some evidence for a dose-response relationship was obtained by dividing the exposed group into

those receiving >20 mSv (mutation rate = 0.024) and those receiving <20 mSv (rate = 0.018), compared with the control rate of 0.011. While it is clear that there is a general increase in the mutation rate for several different genomic sites in the exposed population, difficulties of interpretation exist because of the geographical disparity of the control group and the possibility that other environmental agents may be responsible for the increased mutation rate [S67]. As a direct test of the involvement of tandem-repeat loci in radiation-induced carcinogenesis, normal and tumour DNA from post-Chernobyl thyroid carcinomas was examined for mutations in three loci [N22]. Mutations were found in 3 of 17 tumours (18%), with one of these having mutations in all three loci, while none of 20 sporadic thyroid cancers from patients without a history of radiation exposure showed tandem-repeat mutations.

207. Recent studies of "hypermutation" in non-dividing cultures of bacteria placed under stress have also suggested that recombination processes are involved in the formation of small deletions at high frequency [R29]. It is suggested that when bacteria are starved of nutrients, for example, mutations arise at a very high rate in order to survive (if sufficient mutations occur, one of these may be sufficiently favourable to allow the cells to adapt to the prevailing conditions). Evidence supports a model in which cells enter a transient hypermutable state in which DNA double-strand breaks initiate homologous recombination activity (see Figure Id), priming error-prone DNA synthesis. It seems likely that the errors (mutations) arise as a result of a down-regulation of the mismatch repair system (paragraph 166) [H44]. Similarly, specific types of oxidative base damage (e.g. 8-oxoguanine, paragraph 24) may lead to small deletion mutations when mismatch correction is compromised [B56]. The significance of these findings is that the intrinsic mutability of cells is modifiable, and this principle may apply equally to many types of cell, perhaps including those involved in carcino-genesis [R30].

208. Other reported phenomena may also be connected to the induction of genetic instability following irradiation. One of these is coincident mutation: in cells selected for radiation-induced mutation at one genomic site, a high frequency of mutations occurs at other sites. Li et al. [L16] found two mutations at tandem-repeat loci among 50 x-ray-induced *TK* gene mutants, a frequency of 4 10^{-2} and far in excess of expectation based on current knowledge of radiation-induced gene mutation frequencies. No second-site mutations were found in 70 unirradiated clones. Perhaps the clearest example of coincident mutations was found in an extensive study of x-ray-induced mutation in the fungus *Neurospora crassa* [D5]. In a chromosomal region containing 21 genes, about 10% of the radiation-induced mutants had mutations in more than one gene following a single acute dose. However, a yeast cell study has revealed a possible mechanistic basis for non-targeted mutations that occur relatively close to the initial site of damage, without invoking genetic instability [S52]. A DNA double-strand break was placed at a single site in one yeast chromosome, using a site-specific endonuclease, and mutations were measured in a gene situated adjacent to the break site. After the break had undergone recombination repair, it was found that the adjacent gene had sustained a 300-fold increase in the frequency of point mutations. This result suggests that the break repair process is error-prone, presumably because of a lack of fidelity in DNA repair synthesis, and extends over distances of several hundred DNA base pairs.

209. It is difficult to know how the reports of delayed genetic effects and instability following both low- and high-LET radiation may apply to humans. As indicated above (paragraphs 201-203), a high frequency of radiation-induced genetic or epigenetic changes may contribute to cancer incidence, at least in those instances where an accumulation of somatically-stable changes is required. However, it is still not clear whether genetic instability or a higher-than-normal mutation rate is a necessary for the development of tumours [T38]. In the case of inherited genetic alterations, where germ cells are the target, measurements of mutation frequency made at short intervals after irradiation could underestimate the induced mutation frequency. However, in the case of alpha-particle irradiation, mice injected with ^{239}Pu and subsequently mated to tester stocks (specific-locus method) over many weeks were not found to show increasingly high levels of mutation [R16]. It is, of course, possible that some potential increase in mutations may be balanced by selection against sperm carrying an increased load of genetic damage due to instability. The type of mutation induced may be important; if these are point mutations, they could have a greater chance of transmission and could lead to dominant genetic effects in offspring. The potential importance of genetic instability in carcinogenesis, especially at low radiation doses, is discussed further in Annex G, "*Biological effects at low radiation doses*".

F. MUTATION FREQUENCIES AND CONSEQUENCES

210. Radiation-induced mutations are always measured against a background of spontaneously-occurring mutations. The mechanisms of spontaneous mutation are numerous; the chemical reactivity of DNA leads to instability, and there are inherent errors in replicating a very large molecule. Some of these mechanisms will overlap those of radiation damage; for example, oxidative damage from metabolic processes in aerobic organisms will give both base damage and strand breaks (paragraph 19). This indicates why many of the types of mutation that occur spontaneously are similar to those formed by ionizing radiation. The cell requires efficient repair processes to cope with endogenous damage; if unrepaired, the damage will lead to base-pair substitutions as well as some larger changes. The increase in mutant frequency found after exposure to ionizing radiation is likely to come from both the additional load of damage similar to that occurring spontaneously (such as DNA base damage and loss) and more complex radiation-induced damage that cannot be handled easily by the cell's battery of repair enzymes (Section I.B).

211. As was stressed in Section IV.B, an important influence on the observed frequency of mutation is the tolerance of the genome site to large changes. When large changes are tolerated, the spectrum of mutations induced by ionizing radiation shows that at least half (and commonly more than half) of the mutations measured shortly after irradiation are large deletions and rearrangements. This spectrum may change with time after irradiation, but as yet there are too few data to comment meaningfully on this possibility. Mutation frequencies have been measured for a few target genes, which have been chosen for their ease of use and especially for their presence in a hemizygous state. While these measurements seem unlikely to represent the genome as a whole (see Section IV.E), they do give some idea of the variation in frequencies and the reasons for this variation. An interesting comparison can be made of the hamster *APRT* and *HPRT* genes; since both of these code for inessential enzymes of similar function (purine salvage) they should both detect all types of mutant. However, the *APRT* gene is thought to have an essential gene situated downstream, which limits the possibility of detecting very large deletions (paragraph 179). Table 4 shows that the frequencies of both spontaneous and radiation-induced *APRT* mutations are lower than those for *HPRT* by more than an order of magnitude.

Table 4
Mutant frequency and spectrum in two different genes of hamster cells

Agent	*Mutant frequency (10^{-6} cells)*	*Number analysed*	*Deletions/rearrangements (%)*
***APRT* (autosomal hemizygous)** [M13]			
None	0.13	125	7.2
EMS [a]	430	48	0
Gamma rays	1.5–3.0 [b]	85	22.3
***HPRT* (X-linked hemizygous)** [T20]			
None	6.2	44	18
EMS [a]	690	56	0
Gamma rays [c]	38.8	48	71

a Ethylmethane sulphonate, an alkylating agent.
b Doses 2.5 and 4 Gy.
c Dose 5 Gy (both EMS and gamma-ray doses gave 20% cell survival).

212. Whether available mutation systems are sensitive enough to yield useful data on genetic damage in situations of practical importance is questionable. There has been some controversy over attempts to measure, for example, the frequency of *HPRT* gene mutations in the blood lymphocytes of radiotherapy technicians and patients. It was concluded that some of these studies are better at revealing the variables involved in measuring mutation at low doses than at giving reliable data on mutation frequencies (reviewed in [T22]). One study [N16], however, included molecular analysis of the mutations found in lymphocytes of radioimmune therapy patients and showed that a higher proportion with large genetic changes occurred than in controls, with some dose dependence for the fraction of mutants with large changes due to cumulative ^{131}I activity. Studies of the survivors of the atomic bombings [H32, H33] reported a slight increase, about 10% per Gy, in *HPRT* mutant frequency as estimated dose increased. This frequency increase is considerably lower than found in freshly-irradiated lymphocytes, suggesting that *HPRT* mutants are selected against over the long time period involved. However, it has recently been found that a significant increase in *HPRT* mutant frequency could be detected in combined data from 142 liquidators involved in the Chernobyl accident (24% increase in the liquidator group relative to Russian controls) after adjustment for age and smoking [T49].

213. The problem of selection against mutant cells seems to be less severe in some more recently developed mutation assays. An assay based on the loss of one copy of the cell-surface marker glycophorin A, encoded by the *GPA* gene, in human erythrocytes gave dose-dependent increases in mutant frequency in survivors of the atomic bombings, and the frequencies per unit dose were similar to those found in human cells irradiated with low-LET radiation under laboratory conditions [K1, K33, L44, L45]. In contrast to the *HPRT* data, there was a positive correlation between the frequencies of chromosomal aberrations and *GPA* mutants in exposed individuals; additionally, survivors with malignant solid tumours showed a significantly higher mutant frequency than those without cancer. This assay will measure chromosome loss (by, for example, non-disjunction) as well as mutation and recombination events in individuals already heterozygous for the *GPA* gene (about half of the human population), but it has the drawback that no molecular analysis of the mutations is possible (mature erythrocytes lack nuclei) [G24]. The *GPA* mutation system has also shown dose-dependent mutation induction in individuals exposed to doses of up to 6 Gy during or following the Chernobyl accident, and the slope of the dose response was very similar for these measurements and the *GPA* mutants measured in survivors of the atomic bombings (at about 25 10^{-6} mutants Gy^{-1}) [J18]. This result has received support from subsequent measurements of *GPA* mutation in liquidators [W53] or

people living in the vicinity of Chernobyl [L71] at the time of the accident. However, use of the *GPA* system to measure mutation in more than 700 Estonian and Latvian workers involved in the Chernobyl clean-up, with estimated median doses of about 100 mGy, did not detect a consistent increase in mutant frequency [B39]. The mutagenic effects of 5.3 MeV alpha particles (average LET = 140 keV μm^{-1}) in a human exposed to ^{232}Th over a 43-year period following thorotrast injection were measurable using the *GPA* gene assay [L11]. These data showed a fivefold increase in *GPA* mutants and correlated to large increases in chromosomal aberration frequency in lymphocytes from the same individual. Other data associated with the consequences of the Chernobyl accident are considered in the Annex J, *"Exposures and effects of the Chernobyl accident"*.

214. Where enough measurements have been made, the average frequency of gene mutation induced by low-LET radiation is similar in cells from different somatic tissues and species. This can be illustrated by a plot of induced *HPRT* mutant frequency against surviving fraction (Figure VIIIa), where differences in intrinsic radiosensitivity are taken into account [T13, T14]. The plot also indicates that there is some consistency in the relationship between mutation and killing, suggesting that these responses derive from similar types of damage, a constant fraction of which is converted to mutations [T17]. It should be noted that this relationship does not say anything about the absolute mutant frequencies for different tissues or organisms; these may differ substantially, as seen, for example, in the response of different germ-cell stages in the mouse [S29].

215. The mutation frequency/survival plot can also be used to show that both the type of radiation and cellular parameters can influence the effectiveness of mutation induction. Thus, for *HPRT* mutation, densely ionizing radiation shows an increase in the effectiveness by a factor of no more than 2; i.e. the RBE for mutation is about twice that for cell killing (Figure VIIIb) [C22, T16, T18]. Similarly, x rays vary in effectiveness with the phase of the cell cycle; an increase in the effectiveness of mutation induction by factors of 2-3 is found relative to cell killing, especially in G_1/S phase [B14, J6]. It is tempting to speculate that the repair systems operational at this point in the cycle (see Section II.B.1) are more prone to recombinational errors.

216. Mutation induction is also subject to dose-rate effects; in general, the effectiveness of low-LET radiations is reduced by factors of 2-4 at low dose rates [T22]. However, it has also been found in specific conditions that the mutagenic effectiveness may remain the same or may increase at low dose rates relative to high dose rates [T22]. For example, if TK6 lymphoblastoid cells are exposed to low dose rates, no change in mutagenic effectiveness is found [K36], while their more radiation-resistant counterpart WIL2-NS (paragraph 185) shows an approximately twofold reduction in effectiveness [F11]. Lack of alteration in mutagenic effectiveness with dose rate has also been seen for radiation-sensitive (DNA-repair-deficient) cell lines [F12]. In addition, at dose rates of low-LET radiation of $\leq$0.5 mGy min^{-1}, rodent cell lines may show no dose-rate effect [E10, E11, F13] or an increased (inverse) dose-rate effect [C44, C45]. Also, inverse dose-rate effects have been seen with low dose rates of high-LET radiation [K37, N18].

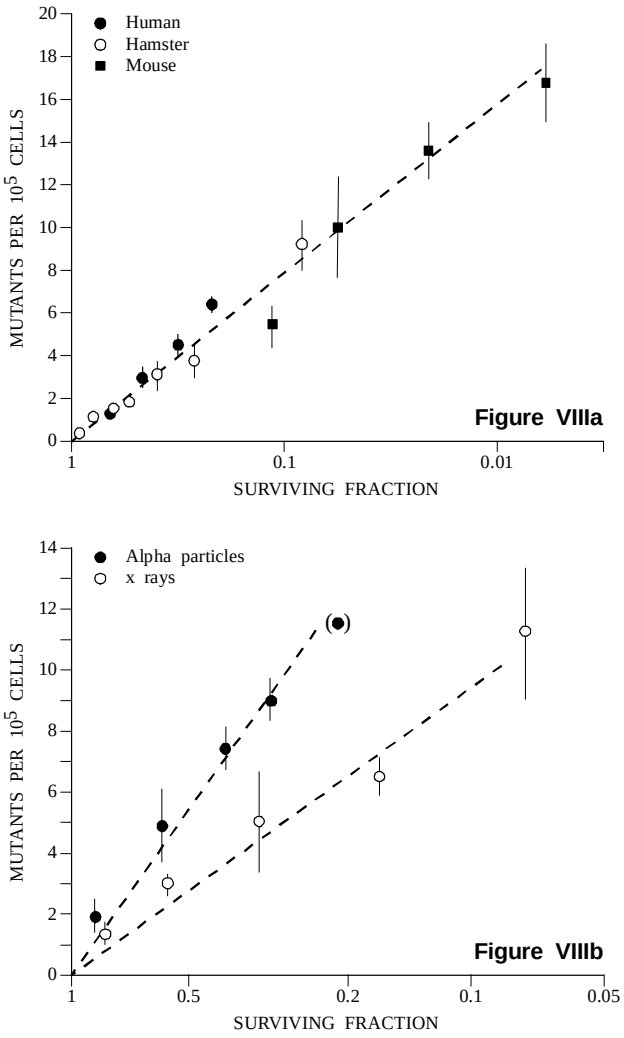

Figure VIII. Mutation-survival relationship for *HPRT* genes.
VIIIa: Similarity of induced mutant frequency in cell lines from human, mouse and hamster [T17];
VIIIb: The increased effectiveness of densely ionizing radiation (^{238}Pu alpha particles) compared to x rays in hamster cells [T18].

217. Some of these dose-rate data point to specific cellular processes influencing the response; in particular, it appears that the response is dependent on the capacity of cells to repair damage to DNA [T22]. It has long been thought that cellular repair capacity is responsible for the reduction in mutagenic effectiveness with dose rate, as seen for example in classical mouse germ-cell data [R6, R7]. Where little or no dose-rate effect is found, the repair capacity of the genetic region or of the whole cell is therefore likely to be impaired. This situation may also apply in some germ-cell stages; spermatozoa of both *Drosophila* and the mouse show no dose-rate effect [R6, T31]. The finding that very low dose rates give little relative effect, or an inverse effect, in some somatic cell lines may indicate that the full repair capacity of a cell requires a minimum level of insult (damage induced per unit

time) before it is brought to bear on that damage. However, it should be noted that chronic irradiation of mouse spermatogonial stem cells has shown no further reduction in specific-locus mutation frequency below 8 mGy min^{-1} with dose rates down to 7 μGy min^{-1} [R17].

G. SUMMARY

218. DNA damage caused by ionizing radiations leads to various types of mutation: the smaller mutations, such as base-pair substitutions, appear to result from damage to single bases, while larger changes, such as large deletions and rearrangements, probably arise from DNA double-strand breakage. The spectrum of radiation-induced mutations is dominated by the larger molecular changes if the genetic site assessed will tolerate these without lethality. In view of the propensity to induce large genetic changes, it may be supposed that the average radiation-induced mutation has greater consequences in terms of extensive alterations of the genome than the average spontaneous mutation or those induced by other agents. As yet no distinct mutational fingerprint has been identified for ionizing radiations, although there is a suggestion that the more complex types of molecular change may be overrepresented in the spectrum. High-LET radiations induce a higher mutation frequency per unit dose, but there are insufficient data to establish whether the mutation spectrum is different from that for low-LET radiations.

219. Attempts by cellular enzymes to repair DNA damage are intimately linked to mutation formation. The molecular mechanisms of radiation-induced mutation include illegitimate recombination, but where genes are heterozygous there is evidence that homologous recombination is involved (giving loss of heterozygosity, commonly seen in some cancers as loss of tumour-suppressor gene function). Some forms of radiation-induced base damage will be repaired by mismatch repair pathways; loss of mismatch repair function can lead to hypermutability, which is linked to specific forms of cancer such as hereditary non-polyposis colon cancer. The involvement of repair processes in mutation formation suggests that the intrinsic mutability of cells is not fixed but will vary with their repair capacity; this concept may have important consequences for the process of carcinogenesis.

220. There are several reports of persistent genetic effects in cells and animals following irradiation, suggesting that genetic instability is induced. A possible explanation for this phenomenon is that stable oxidative reaction products persist and can continue to give genetic damage in subsequent cell generations, although other explanations are possible (paragraph 195). In some mutation systems very high frequencies of mutation (hypermutability) occur spontaneously and after irradiation; on the basis of target size, it is unlikely that these mutations are induced directly by radiation damage at the sites of mutation. Hypermutability also suggests that some form of genetic instability is induced by radiation treatment, but at present it is difficult to know how these data relate to responses such as cancer induction.

221. Methods have been established for measuring mutations with some accuracy in human somatic cells, such as blood lymphocytes, but most mutation systems lack the sensitivity to be used as indicators of genetic damage in cases of low-dose radiation exposure. Some success has been found in measuring mutant frequencies in cells of individuals exposed to relatively high doses of radiation, such as atomic bomb survivors and Chernobyl recovery operation workers.

CONCLUSIONS

222. DNA repair processes have evolved in all biological organisms to combat the deleterious effects of damage to their genetic material. Attempts to repair DNA damage also cause many types of mutation, through inability to properly restore the DNA sequence. Many of the genes involved in the repair of DNA damage in human cells have now been cloned, and their functional analysis has led to considerable progress in our understanding of the ways in which cells and organisms respond to radiation damage. Several different pathways of repair are required to cope with damage from ionizing radiation (Figure III), and the consequences of losing repair capacity can be drastic. The importance of the repair gene function has been revealed in particular by the development of methods to knock out specific genes in experimental animals. Using these methods, it has been found that the loss of repair gene activity is often lethal in early stages of development (Table 2). Where the knockout animals survive the loss of repair capacity, they are commonly very prone to cancer. These findings reinforce more limited studies with rare individuals in the human population, which have suggested for some time that radiosensitivity is linked to cancer proneness. The converse finding, that mice lacking homologues of the human breast-cancer-predisposing (*BRCA*) genes are radiosensitive, is a further important illustration of this link. Loss of repair gene function is considered to lead to cancer proneness primarily through an increase in genetic instability, although the mechanistic details of this process remain to be elucidated. The precise contribution of loss of repair gene function to the risk of radiation-induced cancer in the population is unknown at present; if calculations are restricted to those rare individuals with recognized repair syndromes, then this contribution will be small, but it is already known that a more subtle variation in these genes occurs widely in the human population.

223. Cellular survival assays indicate a twofold variation in the response of individuals in the general population to acute radiation exposure. This variation may become three- or fourfold when irradiation is given at low dose rates. Specific groups of individuals may show a consistently elevated sensitivity; for example, individuals heterozygous for the ataxia-telangiectasia-mutated (*ATM*) gene, who constitute

about 1 percent of the population, show enhanced radiosensitivity especially when tested in a chromosome-damage assay. This finding has recently been reinforced by similar data with transgenic mice heterozygous for ATM deficiency. Strikingly, using the chromosome-damage assay, one study has shown that about 40 percent of breast cancer patients show a similar enhancement of radiation sensitivity. Recent evidence shows that the enhanced radiosensitivity of breast cancer patients has a genetic basis; it has been suggested that the *ATM* gene is involved in predisposition to breast cancer, but the evidence for this is controversial. It is possible that small reductions in the efficiency of any one of a number of genes involved in radiation response, due to subtle mutations or polymorphisms, will account for the existence of radiosensitive groups of individuals.

224. The analysis of DNA repair processes has revealed that they are part of a complex response system in our cells, which includes genes involved in recognizing and signalling the presence of damage and genes operating checkpoints to ensure that cells do not progress through the cell cycle if they carry DNA damage. The functional analysis of repair genes involved in radiation response has also revealed a link between radiosensitivity and immune dysfunction, because some of the gene products involved in the repair of radiation-induced DNA double-strand breaks also assemble functional immune genes. Thus, radiosensitivity in humans can arise from the loss of a broader spectrum of gene functions than was initially recognized.

225. A reduction in DNA repair capacity may have several consequences for the cellular radiation response, including alteration in the shapes of dose-response curves, the loss of low-dose-rate sparing, and a loss of relative effectiveness for high-LET radiations. It is probable that these consequences arise mainly from an alteration in the ability to repair complex forms of DNA damage, particularly those involving double-strand breaks, since this type of damage is extremely hazardous to the cell. DNA repair capacity is therefore an important component of radiation dose-response, and it is clear that it needs to be considered as a variable in modelling radiation action, especially when attempting to extrapolate to low dose exposure. However, before the effects of repair pathways can be modelled accurately, there is still considerably more to learn about the way in which different pathways contribute to the overall response to radiation. At present it is not known how cells control the use of different repair pathways in responding to damage, and in particular how a balance is achieved between correct (error-free) repair and repair leading to mutation induction. Additionally, more information is needed on the relationship between DNA repair and apoptosis in different cells and tissues to predict the outcome of radiation exposure.

226. There is experimental evidence that pre-irradiation with low doses (5-10 mGy) can increase the resistance of cells to a subsequent higher dose, and that a more sensitive cellular response to radiation exists at low doses than at higher doses. These observations have led to some controversy; they suggest that under some circumstances a resistance factor can be induced by low radiation doses, but the mechanistic basis of the observations has proved difficult to establish. It has also been found in separate studies that a number of different genes and proteins are induced or repressed by radiation, although few of these appear to be involved directly in the repair of DNA damage. To date little of this characterization has been carried out in a systematic fashion, because it has been conditioned by the availability of cloned genes and proteins. However, new technology based on large-scale gene sequencing coupled to micro-arraying of sequences is beginning to revolutionize this type of study by enabling the assay of hundreds or thousands of gene products at one time. By examining the levels of many gene products from cells before and after irradiation, as well as with time elapsed following irradiation, it will be possible to see how the damage response is coordinated. These methods also permit a molecular description of the differences in levels of gene products present in normal and diseased tissues, including tumour tissue, facilitating an understanding of the mechanistic basis of cancer induction by agents such as ionizing radiation.

227. Many different types of DNA damage are caused by ionizing radiations, ranging from isolated single-strand damage at sites of single ionizations to complex DNA alterations at sites of clustered ionizations. The more complex forms of damage may be unique to the interaction of ionizing radiations with DNA, compared with damage occurring spontaneously or that caused by other DNA-damaging agents. Several attempts have been made to establish whether radiation damage can lead to a distinct fingerprint of genetic changes, but this has proved elusive. It is clear, for example, that the spectrum of gene mutation arising from radiation damage has differences from the spontaneous mutation spectrum, but the overlap in the two spectra is considerable. Again, new technologies based on fluorescent *in situ* hybridization, the use of reporter genes with fluorescent tags, and comparative genomic hybridization will allow a refined view of radiation-induced genetic changes and the hope of future distinctions.

228. Many genetic changes caused by radiation occur within a few hours of giving the dose, but there is experimental evidence to show that some forms of change may occur after much longer times and following many cell divisions. In addition, at some sites in the human genome, the frequency of radiation-induced genetic changes is much higher than would be expected based on direct damage to DNA by radiation tracks. These observations broaden our knowledge of the mechanisms of radiation action, but elucidation is required further before the consequences of these mechanisms for radiation risk can be understood.

229. It is clear that much more knowledge of the structure of the human genome, in particular the disposition of the genes within it and their responses to radiation, is required before it will be possible to predict the average frequency of mutation induced by a given dose of radiation. Additionally, the range of repair capacities present in the human population, including carriers of defective repair genes, will have to be considered to predict mutability on an individual basis. A more complete knowledge of these response mechanisms will allow greater accuracy in the prediction of radiation-induced carcinogenic and hereditary effects.

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