SOURCES AND EFFECTS OF IONIZING RADIATION

United Nations Scientific Committee on the Effects of Atomic Radiation

UNSCEAR 1993 Report to the General Assembly, with Scientific Annexes

UNITED NATIONS
New York, 1993

NOTE

The report of the Committee without its annexes appears as Official Records of the General Assembly, Forty-eighth Session, Supplement No. 46 (A/48/46).

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UNITED NATIONS PUBLICATION
Sales No. E.94.IX.2
ISBN 92-1-142200-0

ANNEX E

Mechanisms of radiation oncogenesis

CONTENTS

INTRODUCTION

1. Basic information on cancer induction (oncogenesis) by radiation comes from epidemiological studies of exposed human populations. These give evidence of the relatively wide range of neoplasms involved and of the latency periods and the dose levels at which they are observed. It may be that the limits of resolution of such data are being reached, and for absorbed doses of less than 0.1-0.5 Gy of low linear energy transfer (LET) radiation, there may be increasing dependence on extrapolation procedures. Since most human exposures to radiation occur at doses substantially below 0.5 Gy, the validity of such extrapolations is a crucial factor in risk estimation.

2. The extrapolation of radiation response to low levels of dose is made with dose-effect models derived from physical and biophysical studies of the action of radiation on biological systems [C1, G1, U2]. The principal appeal of these models is that they attempt to describe dose-effect with simple linear or linear-quadratic equations. While in recent years considerable efforts have been made to validate such models of radiation action with respect to fundamental biological factors, such as the macromolecular structure of cellular targets and the influence of post-irradiation repair processes [C2, G2], the unqualified application of simple biophysical equations to a complex multifactorial biological process such as cancer induction is problematic. That is not to say that simple extrapolations and approximations from animal and cellular dose-effect data will not be possible, but rather that there is as yet insufficient knowledge to make them with confidence.

3. So that future extrapolation of high-dose epidemiological data may be made with confidence, it is crucial that a much more detailed picture be gained of the cellular and molecular processes that mediate oncogenic change in mammalian cells. Much of the mechanistic knowledge of radiation action is derived from studies on cell inactivation, chromosomal change, mutagenesis and the repair of deoxyribonucleic acid (DNA). These data will all continue to contribute towards a broad solution to the problem, but until they can be placed in the correct context through the identification of specific neoplasia-associated cellular events, their direct relevance to oncogenesis will remain uncertain.

4. There is strong evidence that oncogenesis is a multi-step process involving the accumulation of a series of genetic and epigenetic changes in a clonal population of cells, that different steps characterize different neoplasms and that the whole process is strongly influenced by genetic, physiological and environmental factors. The characterization of mechanisms of radiation oncogenesis is therefore a daunting challenge. Its importance to future estimates of cancer risk is, however, sufficiently great that the challenge must be accepted.

5. It is also important to stress that in addition to its obvious relevance to radiological protection, the further understanding of the mechanisms of radiation oncogenesis would contribute substantially to fundamental cancer research, a very active and rapidly developing field in which breakthroughs are being sought. This Annex outlines some of the principal problems, broad theories and experimental strategies regarding the mechanisms of radiation oncogenesis and attempts to anticipate future studies and their potential application in radiation risk estimation.

I. STRUCTURE AND FUNCTION OF MAMMALIAN CELLS

6. A brief description of the cell, its structure and functioning in the mammalian organism will serve as background information for the discussion to follow. Only those more basic aspects of the cell and of molecular biology that are relevant to this Annex are given here. More detailed information can be found in the references (e.g. [A1, C7, W1, W2, W5]).

A. CELLS AND TISSUES

7. The cell is the basic unit of all organisms. Each mammalian cell is bounded by a complex, semi-permeable lipid and protein membrane, which actively mediates the two-way flow of metabolites and presents protein receptor sites to allow the cell to interact with its environment. Contained within the membrane is a highly organized aqueous milieu, the cytoplasm, which contains numerous organelles, principally the nucleus containing the genetic material of the cell (see below), mitochondria (for the generation of biochemical energy), the endoplasmic reticulum (providing surfaces for biochemical reactions) and protein-nucleic acid complexes termed ribosomes (for protein synthesis). Proteins synthesized on ribosomes may act structurally, as catalysts of biochemical reactions (enzymes), or play a coordinating role in cell physiology (intracellular regulators, growth factors, cytokines and hormones).

8. At certain points in their life history cells are required to reproduce themselves; this is achieved by transit through the cell cycle. Most cells established in culture have cell cycle transit times of 18-24 h; *in vivo* cell cycle times range up to a week, these longer periods reflecting varying periods of quiescence in one phase of the cycle. In this cycle the genetic material of the cell is replicated and shared equally between the two identical daughter cells that result from the cell division that terminates the cycle.

9. Even at the single-cell level, the complexity of the biochemical and biophysical reactions that are required for maintenance of biological function is immense. In multicellular organisms, however, cells are required to act in concert in order to provide specialized tissue functions. Beyond that, tissue function itself is required to be interactive, so that the whole physiology of the organism is coordinated and responses to appropriate environmental conditions are available.

10. Although grossly simplistic, the above outline is sufficient to demonstrate the need for a highly complex network of information transfer, which must have its origins in single-cell function and response. The source of this lies in the cell nucleus, specifically in the nucleoprotein complexes that make up the microscopic bodies termed chromosomes.

B. CHROMOSOMES

11. Chromosomes are basic components in cellular reproduction. Before every somatic cell division, chromosomes are duplicated, and each daughter cell normally receives an identical set of chromosomes. Each mammalian species is characterized by a particular and constant chromosome number, size and morphology.

12. Each chromosome contains a single nucleic acid polymer, deoxyribonucleic acid (DNA), which is complexed along its length by proteins such as those of the histone family and others with roles in the regulation of chromosome structure and DNA metabolism. The DNA forms the crucial structural entity, but the whole nucleoprotein complex has a series of orders of structure progressing through secondary solenoid-like bodies (nucleosomes), which stack together to form tertiary chromatin fibres. The individual chromosome, the quaternary structure, is composed of a complex arrangement of these fibres maintained by matrix and scaffold proteins.

13. Although the maintenance of overall chromosome structure is crucial for DNA metabolic processes such as condensation, transcription, replication, recombination and repair, it is the DNA polymer itself that is the source of cellular information and, thereby, physiological control.

14. The information is encoded in a linear sequence of alternating aromatic (nucleic acid) base pairs. The pairing is achieved by a double-stranded helical arrangement, where the four bases, adenine (A), thymine (T), guanine (G) and cytosine (C), on one strand are covalently linked to a sugar-phosphate backbone and specifically pair (A with T, G with C) with the bases on the opposite strand through hydrogen bonding. The relatively weak hydrogen bonding between base pairs allows unwinding of the DNA duplex, which is necessary for some aspects of DNA processing. In particular, DNA replication through DNA polymerase enzyme activity has to occur with high fidelity each time the cell is preparing to divide. This fidelity is achieved by unwinding the DNA and replicating the base sequence on the two strands.

15. The base pair code in DNA is arranged in subunits of three bases, termed codons. Amino acids, the building blocks of protein, are specified by codons, and a string of codons is able to determine the

structure and thereby the function of a single protein (polypeptide chain). Such a functional string of DNA codons represents the basic unit of cellular information and hereditary, the gene. Three specific codons act as "stop" signals, thereby indicating the point at which the gene code is terminated. While all others specify an amino acid, the code is degenerate in that there is more than one codon for most amino acids, i.e. 61 coding units determine the 20 natural amino acids. Other non-coding DNA sequences associated with a given gene act to regulate its activity. In mammalian cells it may be estimated that there are approximately 100,000 genes, each of which depends for its correct function on maintaining a constant DNA base sequence. Changes in these sequences by base pair substitution, loss or addition can change gene function; such changes are termed genetic mutations. However, damage to the DNA does not always result in mutations, since cellular DNA repair enzymes are often able to restore both damaged DNA base and damaged sugar phosphate backbone.

16. Most cellular DNA does not code for proteins, and even allowing for base sequences that act to control gene expression, there appears to be much functional redundancy in the mammalian genome. This feature implies that DNA damage in certain chromosome regions will have little or no consequence for the cell, while in other regions damage may change the activity of key genes, leading to changes in cellular properties. Gene expression occurs when a single-stranded nucleic acid, ribonucleic acid (RNA), is synthesized (transcribed) from the gene. In mammalian genes only part of the linear base sequence actually codes. The coding segments (exons) are separated by non-coding segments (introns), which often constitute the major portion of the gene. Consequently, the primary RNA transcribed from the whole gene has to be appropriately cut and spliced before it is fully functional. This messenger RNA (mRNA) contains a string of codons complementary to those of the gene, and these are used by cellular ribosomes to construct the specified protein. Transcriptional processes and hence gene product availability are closely coordinated in cells by a series of interacting feedback loops.

17. In a given mammalian species, all cells contain the same genes; these cells may, however, differ in the relative activity of those genes. Developmental processes involve the selective functional activation and inactivation of many genes. While a core function is maintained by so-called housekeeping genes, specialized functions in tissues require the expression of appropriate sets of (luxury) genes in certain cell lineages. This reprogramming of the genome has to be stably maintained, since such specialized functions have to operate throughout the subsequent life-span of the cell. It may therefore be seen that this coordinated programming underlies cellular and tissue differentiation and is hence a central feature of normal organogenesis and tissue maintenance. Gene programming, which must be initiated early in embryogenesis, is believed to have its origins in gene activation/inactivation through secondary biochemical modification to DNA, such as DNA base methylation, changes in chromatin structure and/or stable protein binding [H16, M44]. Growth factors, hormones and cytokines also play a role in the mediation of differentiation in many tissues. Such stable changes in gene expression and cellular properties (phenotypes) in the absence of DNA base pair changes are essentially non-genetic and are often referred to as being epigenetic. The specific rearrangement of immunoglobulin germ-line DNA sequences is known to play a key role in the generation of immunocompetence in white blood cells; DNA rearrangement is not, however, believed to be a major mechanism of cell differentiation.

18. While fundamental knowledge of gene function, control and mutation may be traced back to pioneer work in micro-organisms [H1, J1], higher organisms have progressively more complex biological problems, including cancer development (oncogenesis). In a broad histopathological sense, cancer in mammalian tissues presents itself as a caricature of normal cell and tissue development. In some way, intrinsic biological control at both the cellular and tissue level has been subverted. Chapter II outlines some of the theories relating to oncogenesis, particularly those that are relevant to its induction by the exposure of mammalian cells to ionizing radiation.

II. PRINCIPAL THEORIES OF ONCOGENESIS

19. During the last decade there has been a remarkable increase in knowledge of the roles of chromosomal change, of specific cellular genes and of inherited mutations in human oncogenesis. However, huge gaps remain in the understanding of the problem. In the context of radiation oncogenesis, great uncertainty exists in the nature and consequences of the genetic/epigenetic events that mediate the inductive, promotional and progression processes, in the mechanisms and contribution of human genetic susceptibility and in differences in oncogenic mechanisms following exposure to radiation or chemical carcinogens.

A. INDUCTIVE AND DEVELOPMENTAL PROCESSES

1. DNA as a principal target for radiation action

20. The genetic material (DNA) of the cell is known to be damaged following exposure to ionizing radiation. Many induced DNA base damages have been identified (e.g. [F13]), and the induction of DNA strand breakage in cells has recently been reviewed [W12]. There is compelling evidence from a range of *in vitro* cellular studies that the main detrimental effects of radiation derive from its ability to damage cellular DNA. First, at the cellular level it has been shown through selective radioisotope irradiation that the cell nucleus contains the principal targets determining radiosensitivity (see, e.g. [H2, H3]). Secondly, radiation-induced chromosomal damage may be quantitatively correlated with cell inactivation (see, e.g. [J4, L1]) and, to a lesser extent, mutation [C3]. Thirdly, in highly radiosensitive mutant strains of both micro-organisms and cultured mammalian cells, there is a good correlation between radiosensitivity and genetic deficiency in the cellular processes that act on DNA damage [F1]. It has also been shown that the induction of DNA double-strand breaks (dsb) in cells by the introduction of restriction endonuclease enzymes mimics the chromosomal damage resulting from radiation exposure [N1] and that there is a quantitative correlation between DNA dsb induction/repair and cellular radiosensitivity [R1]. Although none of these observations exclude the involvement of non-DNA targets in some aspects of cellular response, they provide strong evidence for induced DNA damage as the principal mechanism of radiation action. Most of these data relate, however, to radiation-induced chromosome damage and cell inactivation. While the evidence is less extensive, *in vitro* cell transformation has been shown to be induced by DNA breaks produced in cells by restriction endonucleases [B9]; other evidence, outlined in paragraphs 162-165, provides additional support for a DNA target for *in vitro* cell transformation.

21. Microbial genetic techniques have also established a convincing correlation between the capacity of physical and chemical agents to induce somatic mutations in DNA and their activity as carcinogens (see, e.g. [A2]). This basic somatic mutation theory of cancer induction, although defining the principal macromolecular target for radiation oncogenesis as DNA, leaves unanswered important questions regarding the number of cells that need to be mutated in order to initiate the process, the number of gene mutations that are required, whether specific gene mutations in target cells are needed and whether these mutations are dominant or recessive in their action. In recent years, the application of modern methods of cell and molecular genetics to the characterization of neoplasms has led to a significant increase in the understanding of many of these questions.

2. The single-cell origin of neoplasia

22. In order to understand the molecular mechanisms of oncogenesis and the role of genetic and epigenetic change, it is most important to first consider the cellular derivation of disease. Evidence for the monoclonal origin of neoplasia comes from a number of sources.

23. In female mammals, all of whom are heterozygous for X-chromosome-linked genes, X-chromosome inactivation leads to a mosaic of somatic cells in tissues with cells of different clonal origin expressing different forms of the same gene product (i.e. isoenzymes), depending on which X-chromosome remains active. Neoplasms in female mammals have, in the vast majority of cases, been shown to have only a single form of the gene product (i.e. a single phenotype), implying that the neoplasm was of monoclonal origin [F1]. Studies utilizing restriction enzyme polymorphisms of characterized and anonymous DNA sequences, chromosomal markers and oncogene and immunoglobulin gene rearrangements have all confirmed the original X-chromosome biochemical studies [T1, W3]. Convincing evidence for the monoclonal origin of human and murine colorectal cancer has been provided by cytogenetic and molecular studies of benign and malignant manifestations of this disease [F8, P7]. Most, if not all, malignant human colorectal carcinomas arise from pre-existing benign adenomas [S17]. Thus, although normal colonic epithelium is polyclonal, adenomas arise within single pockets of epithelium, indicating that they were initiated within a monoclonal population of stem cells [F8, F9]. The characteristic cytogenetic and molecular features of such adenomas fully supports this contention. In addition, early pre-neoplastic haemopoietic cells carrying characteristic chromosomal rearrangements have been shown to convert in a monoclonal fashion to overt malignancy (paragraph 72); in animal studies, the monoclonal origin of chemically induced tumours is further supported by the finding of characteristic point mutations in *ras* proto-oncogenes of rodent skin papillomas/carcinomas and breast tumours (paragraphs 188 and 189). Studies on the *p53* gene in human tumours associated with chemical or sunlight exposure also support the monoclonal origin of neoplasia (paragraph 194).

24. Overall, it may be concluded that neoplasms with double phenotypes are rare and that neoplasia develops infrequently from a mixed population of normal cells. Double phenotypes have been recorded in some

induced animal neoplasms, usually where high doses of a potent carcinogen were employed. This probably reflects the coalescence of adjacent primary neoplasms, but there is some evidence that some double-phenotype polyploid neoplasms may originate through the fusion of two single-phenotype cells [T1].

25. Despite the compelling evidence that single neoplasms arise from single ancestral progenitor cells, some caution needs to be exercised with regard to the implications for oncogenic mechanisms. Since many rounds of cell reproduction and clonal evolution/selection occur between neoplastic initiation and final malignancy, the observations summarized above do not exclude initiation of a number of target cells, polyclonal pre-neoplasia and subsequent predominance of a single successful cancer clone. In these contexts, models of radiation carcinogenesis requiring induced damage in more than one cell have been proposed [M1], and it has been argued that radiation-induced murine acute myeloid leukaemia may not develop, as conventionally viewed, through a series of clonal progressions [M2]. It is inherently difficult to study clonal contributions to pre-neoplasia in most irradiated tissues and the problem is not one that will be easily resolved.

26. However, these qualifying remarks do not in the main detract from the conclusion that the majority of neoplasms originate from damage to single cells. In principle, therefore, the traversal of a single target cell by one ionizing track from radiation has a finite probability, albeit low, of initiating neoplastic change. On this basis, although only a proportion of induced pre-neoplastic cells may convert to malignancy, the initiation process may be expected to show a non-threshold response. This may be contrasted with a hypothetical process whereby neoplasia is initiated through damage accumulation in an adjacent set of target cells, producing, for example, a "field effect" in the tissue, resulting in aberrant differentiation. The predictions arising from this are (a) that a significant proportion of benign and malignant neoplasms will be polyclonal; (b) that the requirement for multi-cell damage will tend to produce a dose-effect relationship having a clear low-dose threshold. The current state of knowledge provides little general support for such hypotheses, and in the case of human bladder cancer, where field effects have been specifically postulated, recent molecular studies show multiple site tumours to be monoclonal in origin [S47]. Against this, some benign neurofibromas have, however, been shown to be of polyclonal origin [W6], and threshold-like responses have been reported for the induction of some animal tumours by radiation (see, e.g. [O5]). Related to this are problems regarding the role of multiple events ("hits"), genomic instability and cellular defense mechanisms in neoplastic development. These receive comment later in the Annex.

3. Genetic changes in oncogenesis

27. During the last decade, investigation of the genes directly involved in neoplasia has been one of the most rapid growth areas in cellular and molecular genetics. Consequently, only a very brief summary is possible (see [B1, B6, B18, H4, H46, S18, S63, W14, W20] for reviews). Although the association between chromosomal change and neoplasia was established in the early years of cancer research, the contribution of specific genetic changes to the neoplastic phenotype had to await the advent of recombinant DNA techniques, which allow the identification, isolation (cloning) and characterization of relatively small DNA sequences from whole genomic complexes.

(a) Proto-oncogene activation

28. In this area of research, a major breakthrough was achieved when it was shown that oncogenic DNA sequences (v-*onc*) in an avian virus had related proto-oncogene sequences (c-*onc*) in normal avian DNA [S1]. This approach initially identified c-*src* but was subsequently extended to other proto-oncogenes, e.g. c-*ras* (v-*ras* of rat sarcoma virus) and c-*abl* (v-*abl* of Abelson murine leukaemia virus). Using v-*onc* molecular probes, it has been possible to isolate and subsequently characterize cellular DNA sequences that encode a range of oncogenes [B1]. These and other cellular oncogenes have been characterized by other experimental approaches:

(a) by transferring DNA purified or cloned from a neoplasm into suitable host mammalian cells and establishing a correlation between the genomic integration of a specific donor DNA sequence and the acquisition of a quasi-neoplastic cellular phenotype (see, e.g. [C4, G3]);
(b) by identifying host DNA sequences that are consistently and specifically activated by the adjacent integration of oncogenic viruses (see, e.g. [M3, N2]);
(c) by isolating and characterizing DNA sequences that are located in the breakpoint region of chromosomal rearrangements consistently associated with specific neoplasms (see, e.g. [D1, H4, S63]);
(d) by using molecular hybridization techniques to identify new oncogenes that have conserved certain DNA sequences and are therefore part of a large gene family, some members of which are already known.

29. The characterization of oncogenes in human and animal neoplasms and in *in vitro* cellular systems initially leads to the following broad conclusions:

(a) altered expression of oncogenes may occur through point mutation [B6, B18], chromosomal

translocation and juxtaposition with another DNA sequence [H4, S63], insertion of relatively short and specific viral or genomic sequences [N2] or through gene amplification (generation of multiple oncogene copies) (see, e.g. [N7]); there is also evidence that epigenetic mechanisms, such as changes in the extent of DNA methylation, may also play a role in the expression of genes involved in neoplastic changes [F23, J5, S19];

(b) these mechanisms are often characteristic of particular oncogenes but are by no means mutually exclusive;

(c) the activation of particular oncogenes may characterize histopathologically related neoplasms, but equally, the same oncogene may play different temporal roles in different neoplasms [B6];

(d) a given neoplasm will often contain structural and/or activational changes in a number of known oncogenes, and there is evidence for oncogene cooperation in the development of neoplastic phenotypes [H12]; thus, long latency periods might be explained by the need to accumulate such changes;

(e) oncogene activation often generates a dominant phenotypic trait.

There is an ever-growing list of proto-oncogenes, many of which have known chromosomal locations and functions within the cell (see e.g. Table 1); some of these are referred to at various points in this Annex.

(b) Tumour suppressor gene inactivation

30. It may be argued, however, that despite their clear relevance to oncogenesis, dominantly acting oncogenes probably reflect only one aspect of the whole phenotype, determined in part by early scientific methods. In recent years, this view has received strong support from cytogenetic and molecular studies, which have revealed consistent loss of chromosomal regions and genes in a range of human neoplasms [M4, P1, S18, S19, S34]. Such specific losses during oncogenic development are suggestive of a major role for negative regulatory genes and recessive effects in oncogenesis.

31. The loss of negative regulatory signals from genes has been suspected for some years to be an important aspect of neoplastic development. Such genes, often collectively referred to as tumour suppressor genes, were originally proposed by Knudson to be specifically involved in the hereditary predisposition to certain autosomally determined childhood cancers, in particular retinoblastoma [K1, P12] of which 30%-40% are of the heritable type. Knudson's two hit hypothesis for the induction of such neoplasms requires the sequential mutation/loss of both copies of a specific suppressor gene. According to these proposals, the same mutant gene is involved in both familial and sporadic forms of the neoplasm. In the former case, the first mutation occurs pre-zygotically and is thus present in all somatic cells of the offspring. This is then followed by mutation of the second gene in target somatic cells of the affected organ. In the latter case (sporadic neoplasms), both mutations occur post-zygotically in the same target somatic cell. In general, tumour suppressor genes may be viewed as providing negative cellular effects, for the purposes of maintaining cells in a normal proliferative state and/or regulating normal cellular differentiation programmes.

32. The positive phenotypic role of oncogenes provides strong selection in experimental systems (paragraph 28) and was a major factor in their early identification and characterization. In contrast, such selection is far more difficult to achieve for suppressor functions, and as a consequence, research in this area did not make rapid progress until recently. Nevertheless, there is now persuasive evidence that suppressor genes play a normal role in cell cycle control, cell senescence, signal transduction and differentiation and that their loss can contribute to the development of a broad spectrum of neoplasms.

33. The function of tumour suppressor genes was first experimentally demonstrated in somatic cell hybrids produced from the *in vitro* fusion of normal and tumour cells (NT hybrids) [H29, S18]. In these studies, it was shown that genetic contributions from normal cells suppressed the neoplastic potential of the tumour cells, i.e. the malignant state was recessive and subject to negative regulation. As NT hybrids proliferated, they often lost chromosomes, and the loss of certain chromosomes was accompanied by a reversion to a neoplastic phenotype. Thus, tumour suppression was seen to be specific to certain chromosomes and, by implication, to specific genes. Recent technical advances allow the introduction of single normal chromosomes into tumour cells, and this microcell transfer technique has been used to map putative suppressor genes to specific human chromosomes (see, e.g. [S34, T3]).

34. The genetic pedigree analysis of cancer-prone families has also proved to be a powerful tool in the identification of tumour suppressor genes and their linkage on the human genome. Here it is important to recognize that for autosomal "loss of function" genetic traits associated with tumour suppression, the appearance of tumours in offspring is manifested as a dominant trait. Although the genes function in a recessive fashion at the cellular level, their dominance within family pedigrees reflects the high probability

that, in heterozygous offspring, the normal gene will be lost from a target somatic cell, thus initiating the specific tumour. These losses may, in some tumours, be evidenced by specific chromosome deletion events, and the identification of 13q deletions in retinoblastoma and 11p deletions in Wilms' tumour (see [S18, S19]) provided important early clues as to the location of the respective tumour suppressor genes. This approach has also been extended to familial adenomatous polyposis (FAP) [S20] and multiple endocrine neoplasia type 2 [L8], but here allelic losses do not always occur in the chromosomal region that is associated with the cancer predisposition locus.

35. Molecular approaches to suppressor gene identification in human tumours have centred on the analysis of the loss of specific chromosome regions where parental genetic contributions may be identified by the presence of DNA restriction fragment length polymorphisms (RFLPs); that is, where the two autosomal chromosome regions may be genetically distinguished in normal cells of the patient, it becomes possible to use molecular analysis to distinguish specific DNA losses through the loss of heterozygosity in tumours [M4, S19]. Thus, consistent DNA losses for a set of linked genes in a specific chromosome region may be taken as preliminary evidence for the presence of a tumour-specific suppressor gene in the region. This has proved to be an extremely powerful technique for resolving subchromosomal losses and has provided important information on the position and linkage of putative suppressor genes for a range of human tumour types [S18]. It is becoming increasingly clear that many different tumour types are characterized by specific losses, that the same losses are often apparent in different tumour types and that multiple losses are not uncommon. Parental effects on gene loss have also been observed in some tumours (paragraphs 58-60), and overall, it is now obvious that the loss of suppressor gene function plays a very important role in oncogenesis at both the initiation and progression levels [S18, S19].

36. From these crucial observations on the chromosomal linkage of tumour suppressor genes it has proved possible to obtain molecular clones representing the *RB*, *WT*, *p53* and *K-REV 1* genes (see [S18, S19]) and, more recently, candidate genes for human FAP characterized by colorectal carcinoma associated with chromosome 5q DNA sequence losses [F8, K15, N16]. A putative suppressor gene (*NF-1*) for neurofibromatosis has also been isolated [W6]. Table 2 provides a summary of the suppressor gene loci implicated in human tumorigenesis. For some of these it is now possible to suggest specific cellular functions and link these to oncogenic processes.

37. For *RB* and *p53* genes, a principal role of the protein products appears to be in the control of the cell cycle [C10, N23] (see also paragraphs 43-52). In the case of FAP, the closely linked and structurally related *APC* and *MCC* genes on 5q were candidate determinants of colorectal carcinogenesis, and it has been suggested that they may act in concert to control the proliferation of colonic epithelium. Both genes were found to be somatically mutated in sporadically arising tumours, and *APC* was found to carry point mutations in the germ line of FAP patients [K15, N16]. Moreover, the *APC* gene has been implicated in gastric and pancreatic tumours in man [N17] and, in the mouse, is also a germ line determinant of gastrointestinal tract cancer [S48]. *APC* somatic gene inactivation is principally associated with point mutation or deletion, but in one case of human colon cancer, L1 transposon insertion was detected [M46].

38. While *RB* gene function has provided a relatively straightforward example of recessive suppressor gene function, not only in eye tumours but also in other tumour types (see, e.g. [L21]), other tumour suppressor genes may not operate in such a simple manner, and multiple locus interactions may be a more common feature of such genes. There is evidence that predisposition to and development of Wilms' tumour is genetically complex [D12, F10], and recent studies, noted above, suggest that the phenotypic variation between FAP kindreds might be explained by the interaction between *APC* and *MCC* genes. In addition, some uncertainty still surrounds the genetics of the *p53* gene. This gene has been shown to have the properties of a suppressor gene in colorectal carcinoma [B19], and its germ line function in Li-Fraumeni patients (paragraph 127) supports this contention. However, *p53* in certain forms has also been shown to cooperate with *ras* in transforming primary rodent cells [H17], where it appears to act in a quasi-dominant fashion (see also [D15]).

39. In this context, the interaction between sequential gene losses and gene activation has been most clearly demonstrated in human colorectal cancer. Here mutations in at least four or five genes appear to be required for full malignant conversion, with fewer events being required for benign changes [F8, K15, N16]. There is also evidence that while there may be some degree of preference in the sequence of somatic cell genetic events in colorectal carcinogenesis, the overall accumulation of such changes may be more important [F8, F11]; these changes are illustrated in Figure I. Chromosome 5 genes may be *APC* and/or *MCC*; chromosome 18 losses may involve the *DCC* gene and chromosome 17 losses may involve the *p53* gene.

40. The escape from cellular senescence and the acquisition of cellular immortality is believed to be an important feature of the oncogenic process. Recent

studies suggest that cellular senescence is a process of quasi-differentiation, which results from recessive changes in growth-inhibiting genes [G8]. The *p53* gene has been implicated in the immortalization of some murine cells in culture [H39, R15], and a role for normal suppressor gene function in maintaining the senescence process now seems likely; thus, a loss of such genes could be viewed as an integral part of neoplastic development.

41. The immortalization, or life-span extension, of cells *in vitro* may be achieved by the introduction of viral or cellular oncogenes, and this may be linked with the function of these genes in controlling cytoplasmic signal transduction and/or cell proliferation (paragraphs 43-52). *In vivo*, many oncogenic viruses, while eliciting a chronic proliferative response in tissues, do not induce one-step oncogenesis, and further cellular mutations are needed. In the case of viral hepatocellular carcinoma, *p53* mutation appears to at least partially meet this need (paragraph 117). Similar conclusions may be formed from the observation that many immortalized human cell lines do not form tumours when transplanted into immunodeficient mice (see, e.g. [C17]). Thus while immortalization, or life-span extension, may well involve tumour suppressor gene changes and be an important step in the evolution of most tumours, it is not itself an unambiguous marker of tumorigenic potential.

42. In conclusion, it may be seen that both positive regulatory signals from activated oncogenes and the loss of negative signals from suppressor genes contribute, in an interactive fashion, to the development of neoplasia. As may be anticipated, oncogenes and tumour suppressor genes have a normal role to play in cell physiology, and it is through lack of or inappropriate expression, relatively minor structural change and combined effects that they enable the cell to evade the normal constraints of proliferation, migration and terminal differentiation and enter a phase of neoplastic evolution. The specific role of some oncogene and suppressor gene products is known. These include growth factor, growth factor receptor, transmembrane signalling protein, cytoplasmic message transducer, DNA binding protein and a range of regulatory proteins including transcriptional factors influencing all these functions [A13, B1, B6, B18, H4, H46, S18, S63, W14]. It may be envisaged, therefore, that in combination oncogene activation events, the loss of regulatory gene functions and epigenetic changes affecting gene activity can produce a cascade of inappropriate or defective gene expression, thus generating a metastable and grossly abnormal cellular phenotype. These changes may be brought about by a variety of different changes to DNA, point mutation, chromosome translocation/insertion, intragenic deletion, chromosomal deletion and non-mutational but stable changes to genes, such as DNA methylation.

(c) Cellular proliferative control and gene transcription

43. Cellular growth and proliferation are controlled through the constraints of the cell cycle [M27, N12]. Quiescent cells in the G_0 phase require a mitogenic stimulus to enter an active phase (G_1), where gene activity is greatly increased. At the end of the G_1 phase, DNA replication is initiated; this replication phase is termed S. Chromosome condensation, initiated at the end of the next (G_2) phase and proceeding through the prophase, is followed by assembly of the replicated chromosome on the mitotic apparatus; this M phase, and indeed the whole cycle, is completed when the mitotic cell divides, providing the two daughter cells with an equal share of cytoplasm and chromosomes. If mitotic stimulation continues, the daughter cells enter a second cycle via G_1; if not, they may fall back into a quiescent state.

44. The cell cycle is a highly conserved and ordered process (see [H50, M47, N23, S62]), which like most complex biological events is subject to both positive and negative regulation. At the single-cell level, mitogenesis and DNA replication/condensation need to occur at optimal points in cell development and be completed "on time", such that chromosomes segregate equally to the two daughter cells. Mistiming of cell cycle events would obviously have adverse physiological consequences for the dividing cell or would create genetic abnormalities in its clonal progeny. Equally, the rate of initiation and progression of neighbouring cells through the cycle must also be closely controlled so that tissue maintenance and development proceed without inappropriate clonal expansion.

45. It may be argued, therefore, that the expression of cell cycle defects may be related to some of the inherent characteristics of oncogenic transformation. While this relationship has been suspected for many years, it is only recently that insights have been gained into the molecular and biochemical control of the cell cycle and, particularly, the crucial roles played by some tumour-associated genes (see [B40, N18, R21]).

46. Studies with yeasts [M27, N12] initially provided evidence that the activity of two classes of proteins, cdc-like protein kinases and cyclins, was central to cell cycle control. Acting in concert as complexes, these proteins catalyse essential steps of cell cycle progression directly via phosphorylation of other nuclear proteins or indirectly via activation of secondary regulators. In general, these controls also apply in the case of animal cells [N23].

47. Cdc-like kinases are believed to act at a number of points in the cell cycle [M27, M28, N23], acting as master switches principally through their ability to interact with different members of the cyclin protein family. Cdc-kinases and cyclins form complexes involving the catalytic protein subunit of cdc2 and the regulatory subunit of cyclin. Such complexes are activated by dephosphorylation-phosphorylation steps and subsequently serve to activate secondary proteins which regulate, for example, chromosome replication and condensation, mitotic structures and nuclear membrane breakdown. According to current proposals (see [M27]), it is the timing of these phosphorylation reactions involving cdc2, together with cyclin complexing and breakdown, that provide biochemical and biophysical coordination. It is also clear that secondary regulators working with these complexes are equally important; it is at this point in the control chain that a strong connection with oncogenic processes has emerged [C10, N18].

48. Current evidence (see [H51, M28, W13]) indicates that, amongst others, the *RB* tumour suppressor gene protein is phosphorylated by cdc2-like kinase but that it is the underphosphorylated form of Rb protein that is bound by viral oncoproteins and is found in quiescent (G_0) cells. The active form has also been shown to complex with various cellular nuclear proteins including a transcriptional regulator termed E2F. The Rb and a related protein termed p107 associate with E2F in different complexes that contain a cdc2 related kinase termed cdk2. Rb/E2F and E2F/p107/cdk2/cyclin E complexes are principally found in G_1 while an E2F/p107/cdk2/cyclin A complex is formed in S. Secondary control of proto-oncogenes (eg. *MYC*), cell cycle regulators (eg. *CDC2* and cyclin A) and genes involved in DNA replication and repair (eg. DNA polymerase α, thymidine kinase and thymidylate synthetase) is believed to be effected through E2F binding sites in their gene promotor regions.

49. Further evidence of the importance of Rb/E2F complexing in cell cycle control comes from studies showing that Rb protein regulates the transcription of *FOS* which, along with *JUN*, plays a crucial role in early mitogenic signal transduction pathways involving growth factors and other proto-oncogenes such as *SRC* and *RAS* [R12]; there is also evidence that Rb and transcription-activating Myc proteins interact directly, perhaps in a fashion parallel to that of Rb and E2F. Finally, and in accord with the above data, it has been shown that *RB* gene functions as both a growth and tumour suppressor in human bladder carcinoma cells [T19].

50. Together, these data indicate that normal Rb and Rb-related protein fulfil multiple roles in the control of the cell cycle, not only in regulating the response to early mitogenic signals to the cell but also in mediating the transitional phases of the cycle itself. The fundamental mechanism through which this is achieved centres on the repression of cell growth and division by the Rb binding of regulatory nuclear proteins, such as E2F and Myc, which drive proliferative responses. Mutational loss or inactivation of Rb in an appropriate target cell may therefore be viewed as a principal means of relaxing these controls, but the same relaxation may also be achieved through the activity of viral oncoproteins that interfere with the binding process.

51. The nuclear phosphoprotein product of the *p53* tumour suppressor gene is also suspected of playing a role in cell cycle regulation [L13]. The normal p53 protein is known to complex with the SV40 virus large T antigen, inhibiting its DNA helicase activity, preventing binding with αDNA polymerase and inhibiting viral DNA replication. These observations suggest that, in normal cells, p53 negatively regulates entry into the S phase of the cell cycle through influencing the assembly of late G_1 protein complexes that initiate DNA replication and/or acting as a transcriptional factor influencing critical gene expression.

52. It has been shown that normal p53 binds to specific DNA sequences in the genome having 2 copies of a 10 base pair repeat motif [E9, K12] and that normal p53 is a transcriptional regulator that is inhibited by SV40 large T antigen and by mutant p53 [F21, R21]. Further to this, it is becoming clear that in its role as a transcriptional regulator normal p53 may act as a damage-response protein providing for the arrest of DNA-damaged cells in the G_1 phase of the cell cycle [K26, L28]. This G_1 checkpoint control is believed to facilitate the repair of DNA damage including that induced by radiation; altered checkpoint control may underly the unusual post-irradiation DNA synthesis response recorded earlier in cells from Li-Fraumeni human patients carrying germ line *p53* mutations [P9]. An additional role for *p53* in the triggering of post-irradiation apoptosis (programmed cell death) has also been demonstrated [C32, L29, Y6]. Here, *p53* may be viewed as a primary regulator of a defence mechanism that acts to remove potentially damaged and abnormal cells before they become established in tissues. A defect in this mechanism would have obvious implications for the initiation and/or progression of neoplasia and the high frequencies of *p53* mutation seen in human tumours may testify to importance of this gene in maintaining the normal steady state functions of a range of human tissues.

53. In conclusion, although currently confined to only a few tumour genes, there is now compelling evidence that subversion of the control of the bio-

chemical pathways associated with the cell cycle is a major factor in oncogenic transformation. In view of the tumorigenic consequences of *RB* and *p53* germline mutations in man, it may also be argued that, in some cases, the loss of cell cycle control is intrinsic to tumour initiation. Coherent molecular and biochemical models are now emerging that explain how the protein products of some key genes of the tumour suppressor and proto-oncogene types interact with the other components of the cell cycle in order to effect fine control. Current models include tumour suppressor proteins with cyclins and transcription factors as the primary control switches, with proto-oncogenes fulfilling a secondary function. If, for the sake of argument, the loss of cell cycle control altered proliferative response, together with defective damage response, is regarded as a consistently early event in tumorigenesis, then irrespective of the physical or chemical nature of the carcinogenic initiator, it may be that mutational loss or inactivation of suppressor genes, such as *RB* or *p53*, is the most effective route for tumour initiation. While still uncertain, molecular studies on *p53* gene mutations in a variety of human solid tumours (see, e.g. [H30]) provide some support for this, as do the data that link *p53* tumour mutations with environmental exposure to carcinogens (paragraphs 194 and 223-226).

(d) Gene dosage, dominant negative effects and genomic imprinting

54. It may be argued that the sequential mutation of target somatic cells is the most important feature of oncogenesis. These changes must occur within an evolving clone of cells, and in order to arise with a reasonable probability at the early phases of the disease, it may be necessary for that clone to be expanding at an abnormally high rate. It may be expected, therefore, that many neoplasia-initiating events will, in appropriate *in vivo* circumstances, provide target cells with some degree of proliferative or selective advantage. For dominantly acting oncogenes, such as those of the *RAS* family, a specific point mutation may satisfy this requirement [B6, B18]. Similarly, the activation of *MYC* and *ABL* proto-oncogenes through, for example, chromosome-specific translocation [H4, N3, N9] could similarly provide a one-step growth stimulus to appropriate target cells. For autosomal recessive genes (tumour suppressor genes), the loss of function of one gene copy will, in principle, only reduce gene product availability to 50%. Such a moderate reduction of gene product availability as a consequence of this change in gene number (usually termed gene dosage) would not obviously provide the appropriate proliferative stimulus to the cell. In familial neoplasms such as retinoblastoma, the loss of the first *RB* suppressor gene occurs pre-zygotically, thus obviating the need for initial clonal expansion (paragraph 31), but since *RB* gene loss also characterizes non-familial retinoblastoma and osteosarcoma, there remains the considerable problem of explaining apparently recessive "loss of function" genes, which at the cellular level may have some degree of dominance.

55. The problem of single autosomal gene loss and phenotypic effect has persisted for some years, and although it is still to be fully resolved, there are a number of mechanisms that would explain it.

56. For some autosomal loci involved in tumorigenesis, gene dosage (one versus two copies of the gene) may be critical, such that loss of even 50% of the gene product in a mutated cell will give rise to a phenotypic change associated with a degree of proliferative/selective growth advantage [F8]. In some individuals this effect might be emphasized by otherwise minor structural differences between alleles, i.e. germ-line mutations of very low penetrance (paragraph 127). Alternatively, particular forms of structural gene mutations may produce so-called dominant negative effects, whereby an abnormal protein determined by the mutated gene interferes with the function of the normal protein specified by its non-mutant homologue [H18]. It has been suggested that such dominant negative effects underlie the oncogenic functions of *p53* suppressor gene mutations (see [D15, W14]). Although gene transfer studies indicated that in some tumorigenic cells wild type *p53* is dominant to mutated *p53* [C18], more recent investigations show that oncogenic forms of *p53* inhibit *p53*-regulated gene expression, thus providing a basis for the selection of *p53* mutant cells during tumorigenesis [K17]. It may be seen, therefore, that gene dosage or dominant negative effects can underlie the action of some tumour suppressor gene mutations, but considerable uncertainty still surrounds the implications of single allelic mutation for tumour initiation.

57. A further possible solution to the problem has recently emerged from molecular studies on sporadic tumours thought to be initiated through suppressor gene loss. For sporadic tumours associated with *RB* and other putative tumour suppressor genes (retinoblastoma, osteosarcoma, rhabdomyosarcoma, neuroblastoma and Wilms' tumour) it has been observed that major chromosome segment loss events occur preferentially from the maternally inherited chromosome and that mutant paternal loci are usually retained in the neoplasm (see [F23, H16, R7, R8]). These results run counter to the normal expectation that in sporadically arising neoplasms there should be an equal probability that somatic cell gene loss will occur from maternal and paternal chromosomes. A process termed genomic imprinting [H19, M14] has been invoked to explain these findings.

58. Genomic imprinting is a poorly understood epigenetic process by which differential expression of certain autosomal chromosomal regions is imposed in somatic cells of the offspring following some form of differential chromosomal modification of male and female gametes. In such situations the successful embryonic/neonatal development of the offspring is thought to be dependent upon the inheritance of equal parental genetic contributions for that chromosomal region. The molecular mechanisms of such imprinting effects are not well understood, but current proposals [B41, H16, R8] include the differential methylation of DNA in imprinted regions, such that the activity of the hypomethylated genes inherited from one parent significantly exceeds that of the hypermethylated genes inherited from the other. Thus, some developmentally associated autosomal recessive genes do not make equal phenotypic contributions to the cell.

59. On the basis of this hypothesis and for the examples cited in paragraph 57 it may be seen that an inactivating mutation in the more active paternally derived suppressor gene of the target somatic cell could reduce gene product availability to a level that deregulated cellular proliferation/differentiation and resulted in the excessive clonal expansion of pre-neoplastic cells. This expanding clonal population carrying the first suppressor mutation may then complete sufficient cell divisions to allow for the probability of spontaneous loss of the second, less active suppressor gene copy of maternal origin. According to the hypothesis [R7, R8], this second mutational event greatly increases the probability that the cell clone will progress towards malignancy.

60. Although an imprinting effect on gene mutability has also been suggested for the *RB* gene in the initiation of osteosarcoma [T5], the above hypothesis does account for the molecular findings in sporadic tumours, and if it is a more general phenomenon, there may be important implications for radiation oncogenesis. Specifically, an increasing number of potentially imprinted chromosomal regions and genes involved in differentiation and development are being identified in man and the mouse [B41, C19, H19, H52]. The involvement of negative regulatory genes in normal cell differentiation and development may mean that some potential tumour suppressor genes are imprinted in specific tissues and, therefore, the loss of one copy may be sufficient to initiate neoplastic change. This, together with the finding of a DNA deletion mechanism for radiation mutagenesis (paragraph 154), implies that radiation may be an efficient "single event" initiating agent for certain neoplasms associated with the loss of tumour suppressor gene function. However, the whole process of imprinting remains poorly understood. The recent observation of imprinting-like effects on the t(9:22) translocation in human chronic myelogenous leukaemia and *N-MYC* amplification in neuroblastoma indicates that such effects may also extend to certain proto-oncogene activation events [F23, H40, R16].

(e) Chromosomal fragile sites

61. Chromosomal rearrangement and deletion is a major feature of oncogenic development, and there is an ongoing debate as to the significance of specific sites of chromosomal instability (fragile sites, c-*fra*) [H25, H26, L9, M5, S21]. These sites, classified according to their frequency in the human population and their inducibility by different chemical treatments of cells, have been suggested as possible predisposing factors in oncogenesis. Some fragile sites are also believed to be preferential targets for the clastogenic action of DNA-damaging agents, including ionizing radiation [Y1]. Despite statistical analyses showing no overall association between common fragile sites and cancer-associated chromosome breakpoints [M48, S21], an association with rare and distamycin A inducible fragile sites remains, particularly with respect to certain leukaemias [H20, L9, S21]. Overall, it appears that the expression of DNA damage is non-uniformly distributed within the mammalian genome, that certain highly recombinogenic sequences are preferentially involved in induced chromosomal changes and that certain classes of these may contribute towards oncogenesis.

62. The molecular structures of fragile sites are not known, although it seems likely that they represent certain reiterated (repeat) DNA sequences that may recombine at a high frequency. In this context, it has been suggested that interstitial telomere-like repeat sequences may be highly recombinogenic [H21]. These DNA repeat sequences, in their normal terminal position on chromosomes, buffer against chromosomal erosion and instability during DNA replication [M15, M29, S22]. However, in some lower organisms, telomere-like repeat (TLR) sequences are highly recombinogenic; it is this feature, together with limited chromosome aberration and mapping data, that initially prompted the speculation that, when located in interstitial sites, they may represent a subclass of mammalian c-*fra* [H21]. Further support for telomere sequence instability has come from both microbial [K18] and *in vitro* mammalian cell studies [F19], but other investigators [I2] failed to find an association between a specific human c-*fra*, *FRA2B*, and a TLR sequence array located in the same chromosomal region.

63. Cytogenetic evidence of telomere-associated chromosomal instability has been obtained in studies with some human leukaemias (see, e.g. [S35]), and

chromosome break healing mechanisms involving the *de novo* addition of telomeric sequences have been developed from studies with lower eukaryotes (see, e.g. [H31]). Under certain circumstances it appears that these DNA repeat sequences are subject to considerable modification in somatic cells, perhaps reflecting their structure and/or the lack of telomerase enzymes, which can act to extend the repeat arrays [B20]. This apparent instability is not, however, restricted to somatic cells, since studies in the mouse clearly show the arrays to be hypervariable within mouse strains, a feature that might be explained by their capacity to initiate DNA recombination and DNA replication slippage or as a consequence of the repeat sequence modification by telomerase, which is believed to occur during gametogenesis [S36, S37]. A possible factor in telomere-like sequence instability is the unusual secondary structure that these sequences adopt *in vitro* [B20, B42, K19]. Such secondary structures, if they were to occur *in vivo*, might also contribute to the fragile site properties suggested above. The contention that such sites may be particularly prone to radiation-induced breakage and rearrangements and that some such events are involved in radiation oncogenesis is supported by recent studies on the nature of the chromosome 2 deletions and rearrangements that are believed to initiate murine myeloid leukaemogenesis (paragraph 183). Alpha-particle-induced chromosomal instability has recently been demonstrated in murine haemopoietic cells [K25], but this has yet to be specifically linked with fragile site expression or neoplastic change.

64. Telomeric DNA sequences are also believed to play a role in cell senescence [G8], and it has been shown that the ageing of human fibroblasts is accompanied by telomeric shortening [H22]. This may be viewed as increasing inherent chromosomal instability and could be a factor in age-related carcinogenesis. There is no doubt that the current intense interest in the involvement of telomeric and other genomic repeat sequences (see, e.g. [B10, B42, K20, S49]) in chromosomal fragility and rearrangement will yield data of direct relevance to molecular mechanisms of chromosomal instability and their role in oncogenesis.

4. Multistage cellular development in oncogenesis

65. The concept of multistage oncogenesis, originally proposed by Berenblum and Schubik in 1948, has been a most valuable and durable concept. In modern developments of this theory, oncogenesis is divided, albeit imprecisely, into three phases: initiation, promotion and progression.

(a) Tumour initiation and other early events in oncogenesis

66. The initiation of oncogenesis may be most simply viewed as one or more stable cellular events arising spontaneously or induced by exposure to a carcinogen, which predisposes carrier cells to subsequent neoplastic conversion. In the case of neoplasms induced in man or experimental animals by single acute doses of a carcinogen, the agent is assumed to act as an initiator by damaging a specific cellular target in a stable and irreversible fashion [B6, B18, Y2]. Throughout this Annex it is argued, on the basis of animal and human data, that specific somatic mutations in target genes are initiating events for neoplasia.

67. In the preceding Section, cytogenetic and molecular findings on suppressor gene mutations and proto-oncogene activation were discussed in respect of their possible roles in oncogenesis, including initiation. In subsequent Sections, data from experimental studies will be discussed, with an emphasis on the molecular nature of induced initiating events. Here it is sufficient to emphasize that at low doses of ionizing radiation, it is knowledge of the induction of early initiating events that may be most important to the understanding of radiation effects. That is not to say however that radiation plays no part in the other stages of oncogenesis.

68. In principle, it appears that neoplasia may be initiated either through proto-oncogene activation or suppressor gene inactivation. Thus, a multiplicity of induced somatic mutations may contribute towards human radiation oncogenesis, and even for a single histopathological form of neoplasia, there may be a number of possible initiating events, albeit with different probabilities of contributing towards overt malignancy. The necessity for a single clone to accumulate further somatic mutations before malignant conversion must be rate-limiting and implies that only a minority of initiated cells progress beyond the pre-neoplastic phase (paragraph 219).

69. At this early phase, aberrant pre-neoplastic clones may be lost through, for example, metabolic insufficiency, non-specific cell selection, the suppressive effects of neighbouring cells, terminal differentiation, cell senescence, programmed cell death (apoptosis) or cell surveillance mechanisms. Thus, the capacity of an initiated cell clone to expand in a relatively undifferentiated state may be viewed as a crucial aspect of the early phase of the disease (paragraph 54). In this respect, the manifestation of benign tissue dysplasias (e.g. papillomas, adenomas and haemopoietic hyperplasia) may be viewed as clonal expansion, limited perhaps by a combination of the factors noted above.

70. Specific examples of tumour-initiating mutations associated with *RAS*, *RB*, *WT*, *p53*, *NF-1* and *APC* genes are discussed elsewhere in this Annex, and the evidence that mutation of these genes in appropriate normal target cells predisposes to malignant conversion is relatively strong. For other tumour mutations it is not yet possible to conclude that they initiated the tumour, only that they are present at an early pre-neoplastic phase, before full clinical progression of disease is manifested.

71. In human neoplasia, particularly leukaemias and lymphomas, early phases of neoplastic development are frequently associated with consistent chromosomal rearrangements, such as the 9:22 translocation in chronic myeloid leukaemia (CML), the 5q-deletion in myelodysplastic syndrome and the 14:18 translocation in follicular lymphoma [H11, S63]. In such cases the progression of early indolent disease is usually accompanied by the selective clonal expansion of neoplastic subpopulations containing further chromosomal abnormalities. In general, in both haemopoietic and solid tumours, the more advanced and aggressive the disease the greater the degree of chromosomal change. Tables 3 and 4 illustrate consistent chromosomal changes recorded in human leukaemias/lymphomas and solid tumours, respectively (see [S45]). In the case of the 9:22 translocation in CML it has been established that a *BCR-ABL* fused gene is produced at the translocation breakpoint; this has tyrosine kinase activity, which produces a stable mitogenic stimulus to the cell. Of particular note are observations of different forms of *BCR-ABL* fusion in acute and chronic myelogenous leukaemia and a likely correlation between mitogenicity of the *BCR-ABL* gene product and the relative aggression of the two myeloid neoplasms (see [G24, N9]). For the 14:18 translocation in follicular lymphoma, molecular studies show that a newly recognized proto-oncogene *BCL-2* is upregulated by juxtaposition with an active immunoglobulin heavy chain gene (see [N9]). It has recently been established that such overexpression of *BCL-2*, rather than being mitogenic, serves the purpose of blocking programmed cell death [H23] and extending the *in vivo* life-span of B-cells (see also [E11]). Thus, certain gene activation events in tumorigenesis do not simply increase proliferation rates but instead block or prolong the normal differentiation pathway. Other examples of chromosomal translocations in acute B-cell and myeloid neoplasms are the t(15;17) in some acute myelogenous leukaemia (involving the *PML* and retinoic acid receptor genes), the t(1;19) in pre-B ALL (involving the *PBX* and *E2A* genes) and the t(17;19) in B-cell ALL (involving the *HFL* and *E2A* genes) (see [E10]). In addition, the 11q breakpoint gene thought to be involved in t(4;11) and t(9;11) acute leukaemias has significant homology to the *trx* transcription factor gene of *Drosophila* [D13].

72. Characteristic chromosomal rearrangements are also seen in human T-cell leukaemias; many of these involve specific sites on chromosomes 7 and 14. In peripheral lymphocytes of ataxia-telangiectasia, clonal rearrangements of this type have been observed in a number of patients showing no other evidence of malignancy. Although often present at relatively high frequencies, these clones do not usually convert to frank leukaemia; in a few cases, however, malignant progression of these clones has been observed (see [T6]). Molecular studies have now established that many of these rearrangements in T-cell neoplasms involve very specific recombination between T-cell receptor (*TCR*) genes and other chromosomal regions encoding transcription factors such as *HOX 11* (see [H32, R3]). The activation of these factors through *TCR* translocation will tend to alter the developmental programmes of T-cell precursors and could explain the early clonal expansion observed in some ataxia-telangiectasia patients. A more complete discussion of many of the chromosomal events noted here is provided by Solomon et al. [S63].

73. In any consideration of the mechanism of low doses of radiation to initiate oncogenesis it is important to attempt to establish the relative probability with which a single ionizing track will intersect a given DNA target, causing a tumour-initiating mutation. This is considered in the following for gain-of-function and loss-of-function mutations.

74. Activation of proto-oncogenes through gain-of-function mutations appears to occur via two principal mechanisms. For proto-oncogenes such as *RAS*, the DNA base-pair (bp) changes required for activation are very restricted, and hence the molecular target (for direct effects) will be small, perhaps only ~10 bp [B6, B18]. For the gene-specific chromosomal translocations involving the juxtaposition of proto-oncogenes such as *ABL*, *BCL-2* and *HOX 11* with other relatively specific activating genes, the molecular target may be larger, perhaps 10^2-10^4 bp [H4, H32, R3, S63]. However, for these events it may in principle be necessary to damage DNA at two specific sites rather than one, thus effectively reducing overall target size. Relative to the size of the whole genome, ~10^9 bp, such gain-of-function, gene-activating mutations would seem therefore to present very small targets for single-track radiation action. Nevertheless, on a target-size basis it has been proposed that the primary event in human CML can be a radiation-induced *BCR-ABL* translocation [H41].

75. This situation may be contrasted with the loss-of-function mutations characteristic of the tumour suppressor roles of genes such as *RB*, *WT*, *APC* and *p53*. Loss-of-function of tumour suppressor genes may occur through point mutation, small intragenic deletion

or larger deletions spanning whole chromosome segments (~10^7 bp) [H30, S18, W14]. The principal limit to the size of the DNA deletions associated with suppressor gene mutation will be the degree to which the cell can sustain viability following substantial genetic losses. Since this will vary with the location of the target gene in relation to essential DNA sequences and, correspondingly, with the genetic background of the cell itself, it is impossible to predict more precisely the target sizes for these deletions. It should also be noted that in the case of dominant negative effects, such as those postulated for *p53*, deletion of the whole gene may not be effective (paragraph 56).

76. In spite of these uncertainties and qualifications made in paragraphs 158 and 159, this simple biophysical argument would predict that radiation-induced loss-of-function mutations may dominate the spectrum of potential initiating events for carcinogenesis. On the basis of the figures given above, the probabilities between "loss" and "gain" events may differ by perhaps two orders of magnitude. It remains to be seen how realistic these estimates might be, but some support for this argument has come from molecular studies on radiation-induced somatic cell mutation (paragraph 154).

77. It may be concluded that tumour-initiating mutations probably vary in form, but on the basis of relative target sizes, it seems likely that tumour suppressor gene mutations may be the predominant form in radiation oncogenesis. Alone, such initiating events in normal target cells will not be sufficient to produce a malignant phenotype, which would require both clonal expansion of initiated cells (promotion) and the accumulation of further epigenetic and genetic changes (promotion plus progression). The point of malignant commitment in a developing neoplasm is therefore difficult to specify; indeed, there may not be an "all or nothing" transition but rather an increasing probability that pre-neoplastic cells will bypass normal cellular constraints and convert to frank neoplasia. Consequently, while oncogenesis may be operationally subdivided into initiation, promotion and progression, these definitions grossly simplify a genetically complex process of cell development that will vary between neoplasms.

(b) Tumour promotion

78. In experimental animal systems, promoters are identified as agents that, alone, have low oncogenic potential but that are able to greatly enhance the yield of neoplasms induced by prior exposure to a sub-carcinogenic dose of an initiator [C11, S2, Y2]. Agents that strongly promote oncogenesis generally do so at low concentrations, but in contrast to initiators, repeated or chronic exposure is usually necessary. A third distinguishing feature is that, unlike initiation, promotional effects are usually reversible. In these respects promotion has the properties of an epigenetic process, involving metastable changes in gene expression and cellular/tissue responses that have dramatic consequences for the initiated cell and its clonal progeny. In the majority of experimental animal systems, initiation and promotion procedures produce an increase in pre-neoplastic lesions or benign neoplasms, and for most promoters there appear to be no dramatic effects on oncogenic progression. The whole question of carcinogenic interaction between radiation and chemicals, including initiation/promotion, has been discussed in depth by Streffer et al. [S2] and Trosko et al. [T12].

79. Neoplastic promotion following ionizing radiation has been studied in both experimental animal systems and *in vitro* transformation systems [H6, K10, L3, S2, T12]. However, much of the detailed knowledge of mechanisms of promotion has come from chemical initiation/promotion studies with rodent skin carcinomas [C11, Y2], coupled with detailed biochemical investigations on the cellular consequences of promoter exposure [N5, N14, T11, T12, W4].

80. The nature of promoter action has, until recently, been obscure. The term promoter is an operational definition encompassing a diversity of chemical entities ranging from the classical phorbol esters through phenobarbital and bile acids to growth factors, hormones and ill-defined dietary components. However, tissue wounding and stress [S23] should also be included in any broad definition. Clearly, not all these factors will operate in all tissues, and it is likely that a range of biochemical pathways can be involved. It is through studies with the phorbol esters, such as TPA (12-*o*-tetradeconylphorbol-13-acetate), diterpenes, indole alkaloid and polyacetate promoters, that an understanding of certain aspects of promotional mechanisms has emerged. High affinity receptors for these promoters have been identified in mammalian cells [N5, W4]. In the case of TPA, that receptor, or part of it, is the calcium- and lipid-dependent enzyme protein kinase C.

81. Protein kinase C, through its ability to phosphorylate and activate a range of cellular proteins and induce the expression of cellular genes, plays a crucial role both in signal transduction across cell membranes into the cytoplasm and in subsequent cellular responses. In this respect, protein kinase C is at the crossroads of a number of biochemical pathways that are known to mediate cellular proliferative response to hormones, growth factors and cytokines. Promoter-mediated activation of protein kinase C will tend to enhance these pathways through a cascade of protein

phosphorylation and gene expression events, a principal outcome of which will be a disturbance in tissue homeostasis. Although diverse in their structure, most tumour promoters share the basic property of inducing a degree of tissue hyperplasia and inflammation; this histopathological feature probably stems from the disturbance of tissue homeostasis.

82. Endogenous promotion is likely to play a far greater role in human oncogenesis than extrinsic chemical factors, and in this respect it is important to recognize that protein kinase C is normally stimulated by increasing cellular diacyl glycerol (DAG) levels through lipid turnover [N5]. Since DAG levels have been shown to be increased following the action of a variety of cellular growth factors and cytokines (see, e.g. [M16, M17]), it follows that these factors probably play a major role in oncogenic promotion. This view is also consistent with observations indicating that the promotional wounding response in Rous sarcoma virus tumorigenesis is mediated by transforming growth factor-ß [S23].

83. However, it has become increasingly clear that tumour promotional pathways are not solely mediated through the biochemical pathways involving protein kinase C and that changes in cellular communication and oxidative metabolism [C21, T11, T12] may also occur in response to exposure to many tumour promoters.

84. The maintenance of tissue homeostasis, which requires cells to establish a critical balance between proliferation and differentiation, is known to involve not only responses to high molecular weight systemic factors (growth factors and hormones) but also lower molecular weight (≤1,000 daltons) ions and metabolites that are exchanged between neighbouring cells linked by so-called gap junctions at cell membranes [L14]. In facilitating cellular communication and biochemical coupling, these junctions are believed to play an important role in the local coordination of cell proliferation.

85. The establishment of gap junctions is determined by a family of conserved mammalian genes, the activity of which appears to be controlled by both systemic factors and intracellular processes mediated through signal transduction pathways. A critical link between gap junction communication and oncogenesis was established by the observation of the stable loss of coupling in many tumour cells [K13, T12]. However, such losses in tumour cells may often be selective, in that tumour cells remain coupled to each other but lose the ability to communicate with normal cells [N13, Y7]. Thus, it became possible to postulate that the signals exchanged between normal cells (homologous coupling) adequately regulate certain proliferation/ differentiation responses but that the selective loss of heterologous coupling allows tumour cells to become more autonomous and less receptive to tissue regulation.

86. The relevance of cellular communication in tumour promotion became obvious when it was shown that a wide range of tumour promoters had the capacity to induce transient dysfunction in gap-junction-mediated processes [M30, N14, Y7]. Additional studies show such inhibition of coupling to be part of the normal proliferative response in tissues, in that inhibition occurs when cells are drawn from a quiescent phase in order to complete tissue growth or repopulation [P10]. It seems likely, therefore, that in some tissues gap junction formation and loss is a secondary component of the complex cellular machinery that drives the cell cycle (paragraphs 48-50).

87. Thus, albeit in a transient fashion, the inhibition of gap junction communication by promotional stimulation may be viewed as a means whereby one layer of cellular proliferative control is removed; this would tend to elicit a mitogenic response in all undifferentiated stem-like cells in tissue. The magnitude of that response may not, however, be uniform, in that cells carrying tumour-initiating mutations in genes central to cell cycle control, such as *RB*, might be expected to respond most strongly. The involvement of tumour-associated mutations affecting cell adhesion proteins, e.g. DCC (deleted in colon cancer) [F11], in gap-junction mediated processes is also possible.

88. The potential complexity of tumour promotion mechanisms is further increased by the finding that some, but not all, promoting agents induce bursts of oxidative metabolism in exposed cells, which lead to the generation of short-lived, free chemical radicals [C21]. Such radicals are highly reactive within the cell and are able to induce damage in a range of macromolecular structures, including cell membranes and chromosomes.

89. In the case of TPA exposure, it has been established that induced radicals such as superoxides, peroxides and arachidonic acid metabolites are able to attack cellular DNA, inducing a range of cytogenetic abnormalities [C21, D11, P11]. On the basis of these observations it has been proposed that TPA-generated chemical radicals act at an early stage of promotion (stage 1) and convert carcinogen-initiated cells to a state in which they are more sensitive to proliferative stimulation (stage 2 promotion) [F15].

90. In the mouse skin papilloma/carcinoma system, TPA has been shown to be a "complete" promoter in that it fulfils both stage 1 and stage 2 promotional requirements; in contrast, the related phorbol ester

RPA fulfils only stage 2 requirements, i.e. it is not clastogenic. The importance of chromosome damage in the stage 1 conversion phase was further established by studies showing that at low doses the alkylating clastogenic agent, methyl methane sulphonate acted synergistically with RPA to effect complete promotion [F16]. Since the conversion stage of promotion involves chromosomal damage, it may centre on changes in gene activity or chromosomal instability; the specificity of such events has not, however, been established.

91. The cytogenetic observations noted above require that a much broader view be taken of the involvement of genetic changes in the promotional phases of oncogenesis. The boundary between initiation and promotion is becoming increasingly artificial, defined only by the tumour system under consideration, the physico-chemical nature of the insult applied and the cellular mechanism being sought. Here it is sufficient to say that, mechanistically, both initiation and promotion probably involve a combination of genetic and epigenetic cellular events and together they drive a clonal population of cells through a pre-neoplastic phase to a point where malignant conversion is assured. Perhaps they differ most clearly in the temporal requirements for phenotypic change and, on current belief, the larger contribution that stable mutagenic change makes to the initiation process.

92. Ionizing radiation is a powerful clastogen and would certainly induce many of the forms of cytogenetic damage currently associated with the conversion stage of tumour promotion. What may be questioned is the extent to which radiation might induce the epigenetic changes associated with stage 2 promotion. As noted below, it seems that, in general, radiation acts as only a weak promotor of neoplastic change.

93. Through its ability to damage tissues and induce a mitogenic response in repopulating cells, radiation could be regarded as a stage 2 promoting agent. Such effects will, however, only be significant at relatively high doses, where substantial cell inactivation has occurred. Thus, it may be argued that for radiological protection considerations, this form of promotional activity is of minor importance. This view may be tempered, however, by recent evidence of upregulation of protein kinase C and proto-oncogene gene products by relatively low doses of radiation [S50, W7, W21], and it will be important to establish whether such biochemical responses extend to other promotional processes, particularly, perhaps, those determining intercellular communication.

94. Recent experimental studies using the mouse skin papilloma/carcinoma system have highlighted some of the difficulties faced in approaching the question of interaction between radiation and other carcinogenic agents. Here it has been shown that beta irradiation induces resistance to chemically induced papilloma but not carcinoma formation [M31]. These observations may imply that radiation induces a DNA repair process that acts differentially on the chemically induced lesions driving promotion-dependent and -independent skin carcinogenesis. However, given the complexity of the promotional processes described here, other explanations should not be excluded. In the same experimental system, chronic beta irradiation lacked action as a complete or stage 2 tumour promoter but did show weak but positive action in stage 1 promotion [M37].

95. The suppression of cellular transformational processes by so-called anticarcinogens or antipromoters has been studied using *in vitro* transformation systems. Protease inhibitors, such as antipain and chymostatin, appear to strongly inhibit radiation transformation in an irreversible fashion, suggesting that they may act at the level of DNA damage modification [K10]. However, since such inhibitors also suppress the promotional activity of TPA, there may also be involvements in cell surface receptor-promoter binding processes or the subsequent protein-kinase-C-mediated message transduction process. Other chemical factors, such as retinoids, ascorbic acid, lymphotoxin and vitamin E, have also been shown to have anticarcinogenic and/or antipromotional activities (see [C11, K10, T20, Y5]). Some of these factors may also act *in vivo* [C11, C15, C16, L15]. In the case of d-limonene it has been suggested that induced changes in the intracellular location of GTP-binding proteins such as $p21^{ras}$ might underlie the anticarcinogenic action [C27].

96. While no single molecular mechanism of antipromotion may exist, at the cellular level, promotional processes are most consistently characterized by the transient loss of intercellular communication. Relevant to this is recent evidence showing that strongly antipromoting agents such as retinoids markedly increase intercellular communication [H48, M45]. *In vitro* antipromotion by ascorbic acid may, however, involve oxygen radical removal from cells [Y10].

97. In conclusion, the promoter enhancement of appropriate biochemical pathways in carcinogen-initiated cells can be viewed as interacting with the stable biochemical sequelae of the initiating event in a target cell in a manner that elicits a supranormal proliferative response. Such a mechanism, depending on the strength of the promoter and the duration of exposure, would tend to establish initiated clones in their host tissues rapidly and efficiently, thus increasing the frequency and proliferative capacity of

pre-neoplastic lesions. Until these transient changes are stabilized through perhaps further clonal mutations, they are, in principle, reversible. Assuming gap junction dysfunction to be a pivotal feature of promotion [T12], then upregulation of junction formation could be regarded as an important mechanism of antipromotion [H48]. On the basis of current experimental knowledge, this may be achieved using exogenous agents such as retinoids but, *in vivo*, endogenous processes involving the regulation of tumour suppressor genes and other cell cycle control factors may be expected to be of greater importance.

(c) Malignant progression

98. During the life history of a neoplasm there is often a progressive tendency towards increased malignancy. This is most frequently seen as a stepwise change in both tumour histopathology and aggression [F3, N3]. In the case of solid tumours, an extended blood supply may be recruited; subsequently, metastatic properties emerge, allowing the neoplasm to spread to distant sites. Phenotypically, neoplastic progression appears to be the most complex of the three phases, and considerable histopathological, cellular, cytogenetic and molecular variation may be seen, even within a single progressing neoplastic clone. Importantly, progression through clinically defined phases is generally, in the absence of clinical intervention, irreversible. This, together with other features discussed later, is suggestive of sequential somatic mutation and consequent selective clonal proliferation.

99. Although the histopathological manifestations of neoplastic progression have been well-documented [F3], it is only recently that some understanding has been gained of the underlying cellular and molecular mechanisms. The apparently stepwise transitions that characterize the progression from benign pre-neoplastic lesions to aggressively metastatic neoplasms are thought to represent clonal evolution and selection processes driven by genetic and epigenetic cellular changes [K6, N3, N9, S19].

100. At the cytogenetic level these changes are often evidenced by secondary chromosomal translocations, deletions or duplications. Some of these, particularly those appearing at low clonal frequency, probably represent "cytogenetic noise" and may be regarded as neutral. Others appear more consistently in dominant clones and are likely to involve the activation, amplification or loss of specific genes. These may be viewed as positively contributing towards clonal selection, dominance and, thereby, neoplastic progression. Examples of consistent secondary cytogenetic changes in human neoplasms are the 8:14 translocation in some cases of acute lymphocytic leukaemia (*MYC* oncogene activation), the trisomy of chromosome 7 in advanced melanoma (*ERB B* gene dosage?) and the appearance of homologously staining regions and double-minute chromosomes in neuroblastoma (*N-MYC* amplification) [H11, N3]. Secondary mutations potentially relevant to neoplastic progression do not always involve chromosomal change, and in some neoplasms, *RAS* mutations are believed to occur during progression and contribute towards tumour aggression (see, e.g. [R11, V2]). Such studies have also highlighted the genetic polymorphisms in tumours associated with genomic instability [R11]. The polyclonal evolution of tumours may also be studied using experimental molecular markers; for example, using plasmid-transfected mouse fibrosarcoma cells, it has been possible to identify individual clones that have acquired properties associated with preferential metastasis [E5].

101. Secondary chromosomal changes and oncogene activation events have also been characterized in a number of animal neoplasms induced by ionizing radiation or chemical carcinogens and have received comment elsewhere in this Annex. Animal models of neoplastic induction are also beginning to demonstrate the possible roles of induced DNA damage in oncogenic progression. Neoplastic progression in chemically initiated murine skin has been shown to be enhanced by treatment with radiation [B11]. This observation may be consistent with the specific losses from chromosome 7 that have been shown to contribute to the progression of murine skin carcinomas [B12, B29].

102. Studies of this type should extend the knowledge of differences in the genetic events that mediate neoplastic initiation and progression and should indicate the potential contribution of physical and chemical agents in the two phases. In this respect, it is important to recognize that in the case of protracted or fractionated exposures, ionizing-radiation-induced cellular damage may, in principle, contribute to both neoplastic initiation and progression. Parallel but more complex considerations apply in the case of combined exposure to radiation and chemical carcinogens [S2].

103. There is growing evidence that neoplastic progression may be greatly influenced by the acquisition, at a relatively early stage in the process, of intrinsic genomic instability. Benign lesions usually contain few cells with mitotic abnormalities, but these cells usually increase in frequency as the neoplasm progresses. This is often accompanied by increases in ploidy, chromosomal breakage, non-disjunction and sister chromatid exchange [S13], i.e. all the features of the development of abnormalities in DNA metabolism.

104. While the mechanisms underlying these putative defects are still obscure, the consequences are crucial to the understanding of neoplastic progression. Intrinsic chromosomal instability will greatly increase the frequency of spontaneous and induced genetic and epigenetic change within the evolving neoplastic clone. This provides the dynamic heterogeneity at the cellular level that is the hallmark of clonal neoplastic progression. Loss of cell cycle control, established during earlier phases of the neoplastic process (paragraphs 43-53), may play a role in the genetic instability that characterizes tumour progression. It is also possible that mechanisms of genomic instability involving the expression of recombinogenic sites and telomere-like repeat sequences in DNA may contribute to this process; for some human leukaemias there is some evidence for telomere sequence instability [S35] (paragraphs 61 and 64), and one form of heritable colon cancer is characterized by widespread instability of dinucleotide repeat sequences (see paragraph 122).

105. The principal phenotypic characteristic of the malignant progression of many tumours is the ability to spread (metastasize) from the primary tumour mass and to establish secondary growth foci (metastases) at other sites. Figure II provides a schematic representation of the steps involved in the spread of tumours. Such tumour dissemination requires primary tumour cells to acquire a range of new properties, particularly those that determine the relationship between the tumour and its host tissues (see [A4, D5, D6, F3, F5, H33, H42, K2, K5, L5, L6, N6, S14, V3, V4]). Metastasizing cells are first required to invade normal tissues and penetrate blood and lymphatic systems. Subsequently, penetrating cells need to be able to survive passage in these circulatory systems, exit the systems and then establish themselves in surrounding normal tissue. To what extent have experimental approaches succeeded in resolving the complex cellular and molecular processes involved in metastatic growth?

106. The molecular strategies so successful in identifying proto-oncogene and tumour suppressor gene activities in earlier phases of the neoplastic process have not proved to be wholly satisfactory when applied to metastatic mechanisms. While transfer of activated *ras* oncogenes to cell lines such as C3H10T½ and 3T3 has apparently resulted in one-step metastasis [E6], the complexity of the process *in vivo* makes it highly unlikely that invasive properties could simply emerge through single gene mutations. It may be, therefore, that direct approaches to the identification of metastasis genes using such atypical rodent cell lines will tend to give a misleading impression of the process.

107. Indirect approaches have compared gene activities in primary and metastatic tumours of the same origin or type. Using these strategies some evidence for *RAS* gene activation during metastatic progression has emerged (see, e.g. [V2]), but for tumours *in situ* it has proved difficult to obtain clear correlations between levels of *RAS* gene activity and the invasive capacity of the tumour. Indeed in some studies with human colonic tumours, directly conflicting results have been obtained [H33]. Similar conflict is evident in respect to *FOS* gene activity in some experimental mammary tumours. Overall, with the exception of consistent *MYC* gene amplification in certain solid tumours (small cell lung carcinoma and neuroblastoma) and *HER-2/NEU* gene amplification in many mammary tumours, there is a marked lack of correlation between known oncogene activation events and clinical staging criteria [H33]. There is some evidence that the activity of the protein kinase C gene [G11] is one determinant of the metastatic process, but, again, the available data do not allow simple correlations to be established. However, although general correlations between metastasis and tumour gene activity have yet to be established, some comment is possible on specific aspects of the problem.

108. The initial step in metastasis is the attachment of the primary tumour cell to the extracellular stromal tissue matrices and basement membranes [L10]. Cadherin proteins are believed to play a role in cell adhesion, and an inverse correlation has been suggested between E (epithelial) cadherin expression in tumour cells and the loss of cellular differentiation associated with increased metastatic properties [H42, S51]. In addition, so-called integrin glycoproteins are also believed to act as cell surface receptors for cellular attachment, and it has been shown that many meta-stasizing tumours strongly express these molecules at cell surfaces [C22, W15]; there may also be a relationship between cancer development and the synthesis of certain extracellular matrix proteins such as tenascin [K27]. Following such attachment, tumour cells then use a variety of proteolytic enzymes to digest the matrix in order to penetrate normal tissues; of particular current interest is the involvement of metalloproteinases (MP) in this process. The activity of these enzymes is regulated by a specific tissue inhibitor, TIMP, which has been shown to have anti-metastatic activity [S38], and an important role for TIMP in suppressing malignant phenotypes is now suspected [H33, H42]. A specific protease encoded by the transin gene is also known to be expressed during the skin papilloma to carcinoma progression in the mouse [B11]. The metastatic behaviour of some tumours has been linked to the expression of variant CD44 glycoprotein (see [H42]). The finding that CD44 serves to activate both B and T lymphocytes has led to the suggestion that mimicry of lymphocyte behaviour may be an important aspect of the metastatic process [A8].

109. More recently, the invasive characteristics of human breast carcinoma have been directly linked to the expression of a metalloproteinase termed stromelysin-3 [B21]. Of particular importance is the observation that this matrix-digesting enzyme is expressed in stromal rather than tumour cells, with stromelysin-3 gene expression being confined to tissue surrounding only invasive mammary tumours. Thus, the acquisition of metastatic properties appears to involve the specific local stimulation of normal breast tissue through signals received from the tumour; these data provide one of the clearest examples of the intimate relationship between normal and tumour cells during tumour progression.

110. Such interactions are also a crucial aspect of tumour blood supply recruitment. Once a solid tumour has expanded beyond a few millimetres diameter, it becomes necessary for the tumour mass to be served by new blood vessels. Blood supply recruitment from normal tissues (angiogenesis) is known to be an active process mediated by the secretion of angiogenic factors such as fibroblast growth factor, epidermal growth factor, transforming growth factor and angiogenin from the tumour [F17]. In some cases, other cytokines produced by infiltrating leucocytes are also believed to play a role.

111. Cell to cell communication through the establishment of gap junctions has been shown to be selectively lost in many progressing tumours, and the expression of connexins have been analysed in a rat liver tumour system [S65]. Communication between normal cells is believed to be part of the regulatory mechanism for cell proliferation, and its loss may result in the relaxation of the controls that restrict invasive growth. Since a number of oncogenes have been shown to downregulate gap junction formation [T11], the appearance of activated forms of these genes may enhance tumour progression via the loss of cellular communication.

112. Other phenotypic changes, such as increased cell mobility, may also contribute towards the metastatic spread of tumours. The molecular signals that increase tumour cell mobility and thereby promote invasiveness are poorly understood. Secretion of autocrine mobility stimulating factor has, however, been shown to correlate with the invasive properties of human bladder carcinoma [G12], and a so-called scatter factor involved in epithelial cell motility has been shown to be identical with hepatocyte growth factor, the receptor of which is coded by the *MET* proto-oncogene (see [H42]). Some genes involved in the cellular control of metastasis have been isolated by cDNA procedures based on the over- or underexpression of certain mRNA species in metastasizing tumours. Of particular note is the chromosome 17 encoded gene *NM23*, which appears to function as a metastasis suppressor [L22, S39]; the loss of *NM23* expression has been correlated with poor survival in breast cancer [L23].

113. In conclusion, from a clinical viewpoint, the acquisition of metastatic properties is perhaps the most critical aspect of the neoplastic process. Since much of the lethality of human malignancy derives from secondary metastatic growth, tumour progression is a major determining factor in the judgement of tissue weighting in radiological protection. For example, skin tumours, most of which only rarely metastasize to distant sites, have low lethality, and this is reflected in a much lower tissue weighting than that given to breast or lung where, largely as a consequence of secondary growths, the lethal fraction is very much higher. Clearly, an understanding of the cellular and molecular mechanism of the metastatic process will be of long-term value in making informed judgements on such weighting factors.

114. In spite of the difficulties experienced in resolving the complex mechanisms involved, some specific aspects of the metastatic process are now becoming clearer. DNA and gene transfer studies have yet to provide broad guidance on tumour gene involvement, but the overall approach remains valid: some studies (e.g. [R13]) do show promise; cell hybridization [C23] and cDNA screening procedures are also proving to be valuable [S39]. However, perhaps the most critical aspect of the metastatic cascade is the complex interaction between invasive tumour cells and the surrounding stromal structures and cells. In this area significant progress is being made in understanding the underlying mechanisms; it is to be hoped that this will also contribute to the design of more effective therapeutic procedures.

5. Viral involvement

115. Viruses are believed to influence the appearance of neoplasms in experimental animals and man by a number of different mechanisms [N2, O1, P13, W16, Z3] and, worldwide, may account for around 15% of cancer incidence in man [Z3]. Viral oncogenesis is currently believed to proceed via the following routes:

(a) through suppression of host systems for the elimination of tumour cells (e.g. avian reticuloendotheliosis and feline leukaemia virus);
(b) by stimulating cell proliferation through the specific interaction of viral and cellular proteins, either transiently or in a persistent manner (e.g. human papilloma virus and cytomegalovirus);
(c) through the transduction of acquired and activated viral oncogenes and growth-regulating genes to host cells (e.g. Rous sarcoma and Epstein-Barr viruses);

(d) through site-specific integration into the genome of host cells, resulting in the activation or inactivation (insertional mutagenesis) of critical host genes (e.g. avian leukaemia and hepatitis B viruses).

The molecular mechanisms of some of these processes have been studied in detail, but while both DNA and RNA viruses have been implicated in the aetiology of a number of human and animal neoplasms (e.g. anogenital cancer, skin cancer, liver cancer, leukaemias and lymphomas), the overall extent of viral involvement in human oncogenesis remains uncertain. Also, in many instances it is clear that the viral component of disease represents only part of a more complex picture involving interaction with other risk factors (Table 5).

116. In recent years a close association between certain human viruses and specific host genes has become apparent, and the following examples serve to illustrate some of the mechanisms currently believed to operate in human viral oncogenesis.

117. First, a transforming protein (E7) of human papilloma virus 16 (HPV 16, associated with anal and cervical papillomas) has been shown to bind the Rb suppressor protein; a second HPV 16 protein (E6) complexes with the p53 suppressor protein in the cell. Thus, the transforming potential of this virus may be mediated by dual inhibition of tumour suppressor activity [W9]. Secondly, human cytomegalovirus (HCMV) infection, possibly associated with Kaposi's sarcoma and cervical cancer, has been shown to lead to upregulation of cellular *FOS*, *JUN* and *MYC* proto-oncogenes. This occurs prior to the onset of viral protein synthesis and may be mediated by a hit and run process, whereby the interaction of viral particles with cell surfaces triggers cellular proliferation [B13]. Thirdly, the Epstein-Barr virus (EBV) gene *BCRF1* has been shown to be homologous to the cytokine synthesis inhibitory factor gene *IL-10*, suggesting that this oncogenic herpes virus uses a captured cytokine gene to enhance its survival in the host, thus potentiating its oncogenic properties in respect to Burkitt's lymphoma [M18]. Fourthly, human T-lymphotrophic virus (HTLV) has been shown to be associated with a unique form of adult T-cell leukaemia/lymphoma (ATL), with the two main foci of infection being in Japan and the Caribbean (see [B35]). The mechanisms of T-cell oncogenesis following HTLV infection are uncertain but may involve a combination of immunosuppressive effects [J6, K23] and T-cell proliferative dysregulation elicited by virally encoded proteins such as the products of the *tax* and *rex* genes [Z3]. Finally, hepatitis virus and aflatoxin B1 (a mutagenic food contaminant) appear to be strongly interactive factors in the induction of human hepatocellular carcinoma (HCC), which is prevalent in southern Africa and eastern Asia [B22]. The insertional mutagenesis of host genes by hepatitis B virus has been demonstrated, and recent evidence strongly suggests that the aflatoxin component of risk derives from the induction of target liver cell mutations that inactivate the *p53* tumour suppressor gene [B23, H34]. The still uncertain role of germ-line retroviral elements in human tumorigenesis has been reviewed recently [L24].

118. These observations provide evidence that the oncogenic potential of many human viruses derives from their capacity to provide a chronic growth stimulus to cells, often by the interaction of viral oncoproteins with cell cycle control proteins [N18], but that direct mutational damage may underlie the action of other viruses. The apparent synergy between hepatitis B virus and a specific environmental DNA damaging agent (aflatoxin B1), noted above, together with data on retroviral involvement in some radiation-induced animal neoplasms (see paragraph 180) and the inducibility of virus-like elements in mice by radiation [P14], suggests that viral processes may well synergistically influence a component of human radiation oncogenesis; there is, however, only preliminary evidence for this in respect to laryngeal carcinoma associated with papilloma virus infection (see [W16]).

B. HUMAN SUSCEPTIBILITY TO RADIOGENIC CANCER

1. Homozygous deficiencies in DNA repair, cell inactivation and chromosome breakage

119. There is increasing awareness of the strong influence of germ-line gene mutations in human oncogenesis (paragraphs 31-34). In addition to the familial traits associated with specific DNA sequence loss and organ-specific neoplasms (e.g. retinoblastoma, Wilms-aniridia, multiple endocrine neoplasia and familial adenomatous polyposis), there is, in the context of this Annex, the most important problem of cancer susceptibility through deficiencies in DNA metabolism [C12, F1, H5]. The clearest example of this is found in the genetically complex autosomal recessive trait xeroderma pigmentosum (XP). In this genetic disorder, defects in the repair of DNA photoproducts appear to be causally linked with a high incidence of skin neoplasia in the sun-exposed regions of affected patients [B14, C12]. Human and rodent genes involved in the repair of UV-induced damage have been isolated and characterized (see, e.g. [T13, V7]), and there is much new information on the cellular and molecular mechanisms of UV carcinogenesis (see, e.g. [A6, B36]). A detailed description of these mechanisms is, however, outside the scope of this Annex.

120. For ionizing radiation, direct links between the epidemiological, genetic and mechanistic aspects of oncogenesis are less well established. However, potentially relevant DNA repair genes are now being identified and isolated (see, e.g. [K11, T15]), and studies on the autosomal recessive human genetic disorder ataxia-telangiectasia continue to provide potentially important information on the association between cellular radiosensitivity, DNA repair deficiency and cancer proneness [G7, G13]; a candidate gene for ataxia-telangiectasia group D has recently been isolated [K29].

121. *Cellular radiosensitivity - cell inactivation.* Although largely restricted to the inactivation of cultured skin fibroblasts, *in vitro* clonogenic techniques have highlighted the relatively wide range of low-LET cellular radiosensitivity in the normal human population [A3, A12, C13, L11]. These data, for both acute and chronic exposures, show that only cells from ataxia-telangiectasia and Nijmegen break syndrome (NBS) homozygotes clearly fall outside the normal range of radiosensitivity and that patients with a variety of other putative DNA repair deficiencies are contained within the relatively broad normal distribution. Cell inactivation is, however, only a crude surrogate for overall cellular radiosensitivity and obviously fails to reflect the genetic complexity of oncogenic processes. The lack of correlation between intrinsic radiosensitivity in fibroblast and T-lymphocyte cell strains also casts some doubts on the predictive value of studies on cell inactivation [G15]. Consequently, the data cited can only be used to provide comment on a single aspect (DNA damage and repair) of the problem; they should not be used alone as an indicator of the distribution of neoplasia susceptibility in a radiation-exposed human population. In spite of these reservations, the overall approach has yielded much useful information (see [A12, G9]).

122. *Chromosomal radiosensitivity and fragile sites.* The quantitative estimation of radiation-induced chromosomal abnormalities also identifies ataxia-telangiectasia and NBS homozygous genetic disorders as being abnormally radiosensitive [T6, T7]. While these data are somewhat more relevant to oncogenesis than cell inactivation, here again it may be argued that they are of limited value to radiological protection. This view may, however, be tempered by recent observations suggesting that a range of human disorders predisposing to cancer are characterized by cell-cycle-dependent chromosomal radiosensitivity [S24]. This is a potentially important observation that demands further study. The potential relevance to oncogenesis of specific chromosomal sites of fragility and enhanced recombination is discussed in paragraphs 61-64. For both ionizing radiation and chemical agent exposure of cells, there is growing evidence for the expression of chromosomal sites of preferential induced breakage; in some studies an association with fragile sites and cancer-specific chromosome breakpoints has also been suggested [D7, M19, S25, S26, Y1]. Of particular note are the proposed mutagen sensitive sites (MSS) of human ch5q, which at the cytogenetic level, appear to correspond to the 5q breakpoints that characterize myelodysplastic syndromes (MDS) [M20, Y1]. Since these myeloid disorders and neoplasms are believed to be significantly radiogenic [M21, R9, V5], confirmation of 5q breakpoint concordance for MSS and MDS would be of some importance. Losses from the 5q region in MDS are now believed to centre on the interferon regulatory factor-1 gene [W24]. Recent studies on site-specific chromosomal breakpoints in radiation-induced murine acute myeloid leukaemias (paragraphs 182 and 183) add support to the contention that heritable predisposition to breakage at fragile sites on certain chromosomes may influence susceptibility to radiation leukaemogenesis. The most striking example of genomic instability associated with human cancer predisposition is, however, provided by hereditary non-polyposis colorectal cancer (HNPCC), which is determined by a 2p15-16 locus [P16]. This is believed to account for 4%-13% of all colorectal cancers in industrial nations and is therefore more common than FAP, which accounts for around 1% of such cancers. Contrary to normal expectations based on the HNPCC locus being a tumour suppressor gene, HNPCC tumours do not show characteristic DNA losses in the 2p15-16 region [A16]. Instead, such tumours exhibit widespread alterations in short dinucleotide $(CA)_n$ repeat sequences suggestive of genomic instability mediated through a dominant defect in a DNA replication factor. Such instability can be viewed as a means whereby mutation rates for $(CA)_n$-associated genes are elevated, thus enhancing tumour development. The finding of an excess of other tumour types in HNPCC kindreds indicates that such mechanisms may not be restricted to colonic neoplasms.

123. Although not associated with oncogenic processes, the recent characterization of the X-linked fragile site (*FRA X*) mutation associated with heritable mental retardation in man allows some comment on the genetics of chromosome fragility. The *FRA X* mutation confers not only X-chromosome fragility in somatic cells but also appears to be unstable in germ cells and subject to structural modification that is dependent on its parental route of inheritance [O2, Y8]. This feature appears to contribute to the bizarre genetics of *FRA X* inheritance and expression [R17, S53] and, if it were to apply to cancer-related fragile sites, would tend to disguise the underlying genetic basis of familial predisposition. These *FRA X* data also

highlight the potential importance of DNA repeat sequences and chromosomal instability for certain disease states [R17], a theme that is echoed elsewhere in this Annex.

2. Heterozygous carriers of genetic traits

124. While the overall picture of human genetic influences on cancer susceptibility is becoming clearer, the information available is based largely on studies with highly penetrating dominant mutations and homozygous mutations at autosomal loci. The frequency of such clearly recognizable disorders in the population is low, and while there are obvious implications for affected individuals and their families, the overall contribution to cancers in the population may be relatively small. In contrast, for autosomal recessive traits, the frequency of carrier heterozygotes will be much higher. In the case of the ataxia-telangiectasia disorder, epidemiological studies point towards increased risk of spontaneous neoplasms, particularly breast cancer [P2, S4, U2], and it is possible that such carriers of the ataxia-telangiectasia mutation represent a human subpopulation at increased risk of radiation-induced neoplasia.

125. Similar considerations may apply to the familial traits associated with organ-specific neoplasia. Cytogenetic and molecular studies with these imply that cancer proneness is often associated with the loss of one germ-line copy of a tumour suppressor gene. The spontaneous loss of the other copy in a target somatic cell would then explain the elevated frequency of organ-specific neoplasia and the apparent phenotypic dominance of these traits. An explanation that demands the reduction of target gene number in cells from two to one also implies that certain tissues of affected individuals would be at a considerably elevated risk of radiation-induced neoplasia. Some aspects of therapy-related neoplasia in familial cancer patients have been discussed [S5, U3]. Although the paucity of data precludes detailed analysis, there is evidence of an increased yield of therapy-related second tumours in familial retinoblastoma patients [E12], an observation that is consistent with the known involvement of the *RB* gene in the initiation of tumours other than retinoblastoma, including osteosarcoma [T5]. With respect to radiogenic neoplasms in dominant familial traits, intriguing observations have also been made with basal cell nevus syndrome (BCNS), where there is unambiguous evidence of radiotherapy-induced multiple skin neoplasms [S5]. Although there is evidence for post-irradiation repair/recovery defects in BCNS cells (see [A3, L11, N21]), the disorder may be heterogenous, and at this stage of knowledge it is not possible to directly link cellular repair observations with the clinical manifestation of radiogenic skin carcinoma.

126. The frequency of known highly penetrating human monogenic diseases that are possibly associated with elevated susceptibility to radiation oncogenesis is low. Examples of the estimated frequencies of occurrence of some of these cancer-prone human mutations are listed in Table 6. Consequently, in terms of the whole population, the highly penetrating mutations do not appear to present a significant problem for risk estimation. However, more frequent mutations, such as ataxia-telangiectasia heterozygotes, (estimated frequency of ~1%), who show increased incidences of breast cancer, with a relative risk of 6.8 claimed in one study [P2, S4, S66], together with a possible but still contentious increase in radiosensitivity [S64], could make significant contributions to population risks.

127. However, it is most important to recognize that cancer is essentially a multifactorial genetic disease and that genes determining cancer susceptibility will differ markedly in the probability of expression (penetrance), as measured by the appearance of one or more tumours in members of families carrying the appropriate mutations. The often-cited retinoblastoma (*RB*) and Wilms' tumour (*WT*) gene mutations are highly penetrating and express as bilateral childhood tumours in a high proportion of carriers. Mutations of lower penetrance have been suspected for many years. However, since they tend to express, perhaps as single adult tumours, in many fewer carriers, they also would be much more difficult to detect in the population, even if they occurred, overall, at a higher frequency. This view has received some support from the finding of relatively low penetrance *p53* germ-line mutations in rare cancer-prone Li-Fraumeni syndrome (LFS) patients [S41] and mutations associated with predisposition to breast and ovarian cancer [H35, K21, S52]. One of these breast cancer genes, *BRCA1*, encoded on chromosome 17, is strongly associated with early onset disease and appears to act as a tumour suppressor. Such heritable forms of breast cancer may account for around 5% of the total in industrialized countries. Since one in ten women develops breast cancer in her lifetime, perhaps as many as one in 200 carry genes that predispose to this neoplasm [K21]. However, with the exception of *BRCA1* most of these mutations are probably of low penetrance. In the case of *p53*, cancer predisposing germ-line mutations are by no means restricted to LFS families [M38, T17] and include one family showing abnormal expression of wild-type p53 protein [B30].

128. Recent observations on the effects of genomic imprinting on the expression of tumour suppressor genes (paragraphs 57-60) are also relevant to patterns of heritable tumour susceptibility. Genomic imprinting

may be considered to be a process whereby the dominance of mutant gene expression is modified according to whether it is inherited from the mother or the father [H19, H43, S27]. In the case of imprinted cancer-susceptibility genes, the expected Mendelian patterns of tumour incidence in affected families will tend to be distorted, depending critically on parental routes of inheritance. An illustration of the inheritance pattern of a human disease with imprinting effects is shown in Figure III. The term maternal imprinting is used to imply that there will be no phenotypic expression of the abnormal allele when transmitted from the mother, and paternal imprinting is used to imply that there will be no phenotypic expression when transmitted from the father. Because there will be a phenotypic effect only when the gene or the chromosome segment in question is transmitted from one or the other parent, there are a number of unaffected carriers. There are equal numbers of affected males and affected females or of unaffected male and unaffected female carriers in each generation [H19] (see also Annex G, "Hereditary effects of radiation").

129. In addition to the imprinting-like effects seen for the *RB*- and *WT*-related genes in man, it may be that this process influences the expression of other tumour suppressor and growth-factor genes with known or suspected involvement in oncogenesis [C23, F23, H19]. The inheritance pattern for familial glomus tumours in man has been shown to be consistent with imprinting [V6]; clinical manifestations, including neoplasia, in the human Beckwith-Wiedermann syndrome are also believed to involve imprinting effects mediated by paternal inheritance of both copies of a region from the short arm of chromosome 11 (paternal disomy/maternal deficiency) (see [L16]). It may be reasonably predicted that other less-well-defined "cancer families" will, in detailed studies, show pedigree distributions indicative of imprinting-like effects. The observation of parent-of-origin effects on leukaemogenic translocations may also have implications for the genetics of haemopoietic neoplasms [H40].

130. In conclusion, based on the frequency of the known highly penetrating mutations, it might be concluded that genetic susceptibility to cancer is not a major factor in the formulation of radiation risk estimates. However, the increasing appreciation of partial effects in heterozygotes, the variable penetrance of mutations, and epigenetic modifying factors should sound a note of caution on this conclusion.

131. An overall genetic contribution to cancer risk in the human population of around 20% has been suggested (see [B24]). Although considerable uncertainty exists, this suggested value implies that as knowledge accrues it should be possible to begin to consider individual risk. For future epidemiological investigation of possible genetic effects on radiation carcinogenesis it will be important to selectively consider familial history of neoplasia along with other relevant factors, such as age of onset in relation to age at exposure. In this specific context, although other explanations are possible, the recent and unexpected findings of an elevated relative risk of early onset breast cancer among the survivors of the atomic bombings in Japan [L30] might accord with the apparently high genetic component of this disease (see paragraph 127). It should be emphasized, however, that the full establishment of such relationships demands extensive investigations of familial cancer incidences and, where possible, molecular analysis of relevant germ- line DNA sequences in order to ascertain the carrier status of the affected individual. If progress in this most important area of human genetics can be maintained, it may be necessary to modify current views on the expected distribution of induced cancers in human populations.

3. Systemic factors

132. Although there is, at present, a paucity of informative data, the identification of initiating events for the principal radiation-induced neoplasms is of critical importance for the further development of mechanistic models of oncogenesis and the validation of dose-effect relationships. Armed with knowledge of the target cell and the initiating event, it may be possible to make informed judgements on the effects of post-irradiation repair processes, on dose rate and on radiation quality effects. However, since oncogenic processes involve far more than initiation, it is of considerable importance to gain a further understanding of the factors that influence the expression and development of neoplastic change, i.e. age, sex, dietary and hormonal factors. In this context, animal models of radiation oncogenesis can be of great value in gaining a broad understanding of these factors (see, e.g. [F2]), and there is also valuable information to be obtained from human population studies.

133. Hormonal status is known to be a major factor in the appearance of tumours in breast, ovarian, testicular and prostate tissues in man and may also be implicated in the progression of other hormone-sensitive or hormone-producing tumours, such as those arising in adrenal and thyroid tissues [L17, M32]. Since, however, hormones and other cytokines play a ubiquitous role in the development and maintenance of all tissues, their action should not be considered to be restricted to the tumour types noted above. On current knowledge and with few exceptions, the principal oncogenic role of such systemic factors centres on their action as tumour promoters or co-carcinogens [M32].

134. The influence of systemic endocrine factors in human radiation oncogenesis is most strongly evidenced by age- and sex-related effects on breast cancer incidence [U1]. These observations imply that hormonal status is a major determinant of the development of this neoplasm and that initiated cells may remain dormant in breast tissues for long periods. Cellular and molecular studies outlined elsewhere (paragraph 189) support this contention and are also broadly consistent with current views on the mechanisms of neoplastic promotion and progression (paragraphs 78-114) that are believed to mediate oncogenesis. Cellular interactions involving hormones and growth factors are also known to influence neoplastic yield in experimental systems through promotional and selection processes; there can be little doubt that such effects are crucial to neoplastic yields in man and may be determined by physiological changes deriving from both genetic and environmental influences.

135. Diet is thought to be a significant factor in carcinogenesis, with fat intake perhaps the most important determinant of cancer in different human populations [C15]. Promotional mechanisms centred on dietary lipid effects on the activity of endocrine systems, on prostaglandin synthesis, on immune functions and on bile acid production have been proposed for some neoplasms and may be supported by epidemiological and animal studies [C15]. Although cellular and molecular data relating directly to these proposals have yet to be presented, it may be relevant that the promotional pathways involving protein kinase C are known to involve cellular lipid turnover (paragraph 82), and it is possible that their activity is influenced by lipid or lipid metabolite availability in tissues. Non-genotoxic chemicals present in the diet or in the environment have also been proposed as factors in human carcinogenesis [C16]. Through their capacity to elicit a chronic proliferative stimulus to cells, it is possible that many of these may act as relatively non-specific tumour-promoting agents; the role of induced mitogenesis and endogenously induced DNA damage in the initiation of oncogenesis has also been considered (see, e.g. [A10, A11, C16]). Although somewhat outside the scope of this Annex, the rate at which cells are believed to sustain and repair endogenous DNA damage, in relation to that small amount of additional damage induced by a low dose of radiation, has received detailed comment [B43, B44, L27, S66, W27] and is of considerable importance to the relationship between DNA repair and radiation carcinogenesis (see also paragraphs 154-159).

136. The possible cellular mechanisms of anti-tumorigenic agents, such as retinoic acid, have been outlined in paragraphs 95-97. Animal studies, particularly with the mouse skin papilloma/carcinoma system, clearly demonstrate the anti-tumorigenic action of retinoic acid *in vivo* [R14], and recently, the retinoic acid treatment of tumour-sensitive transgenic mice carrying an active germ-line copy of a v-*ras* oncogene has been shown to dramatically delay or even completely inhibit the appearance of promoter-induced papillomas [L15].

137. Hormone promotion (paragraph 80), increasing genomic instability (paragraph 104) and intrinsic progressional processes (paragraphs 98-114) have been mentioned as possible factors in age-related carcinogenesis. Also, for familial tumours such as retinoblastoma and some breast and colon tumours, early onset of induced malignant disease would, for genetic reasons, be anticipated. For the early onset (childhood) acute lymphocytic leukaemia, however, a different mechanism may operate; it has been proposed that this disease results from two sequential mutations in haemopoietic target cells [G6]. The first of these is postulated to occur *in utero*, where rapid cell proliferation is required for haemopoietic development, and the second during the neonatal period, when a clonal population of these cells is expanding in response to a primary antigenic stimulus.

138. Animal models provide the basis for quantitative *in vivo* studies on radiation oncogenesis and dose-effect relationships (see, e.g. [E2, M1, M2]), and spontaneous/induced ratios for malignancy strongly influence views on the choice of radiological risk models (i.e. relative vs. absolute risk) (see, e.g. [S12]). Where there is a paucity of human epidemiological data, as in the case of leukaemogenesis by bone-seeking alpha-emitters, animal studies can provide the preliminary data. For example, the finding of induced acute myeloid leukaemia in low dose ^{224}Ra exposed mice at a higher frequency than that of osteosarcoma may have important implications for human radiation risk estimates [H9, H10]. The inhomogeneity of dose, which is inherent in bone-seeking and other internalized radioisotopes, highlights a major systemic uncertainty in radiation oncogenesis, i.e. the identity and *in vivo* distribution of target cells for oncogenesis. The pattern of isotope distribution and decay in relation to organ specific target cells is probably the major determinant of oncogenesis by these isotopes, yet very little is known about target cell identity and even less about distribution.

139. In conclusion, the highly interactive multi-step nature of oncogenesis demands that systemic factors will greatly influence the probability that a carcinogen-initiated cell in tissue will complete all steps and give rise to an overt malignancy. Positive factors such as dietary and hormonal/growth factor-mediated promotional mechanisms may tend to drive the process forward, while terminal cellular differentiation, programmed cell death, cellular communication, cellular surveillance and dietary/endogenous anti-oncogenic

compounds may restrict or even abrogate tumour development. Any specific judgement on the probability that a single tumour-initiated stem cell will progress to malignancy requires a greater knowledge of the complex interplay between these factors than is currently available. In general, however, it seems certain that the negative factors greatly outweigh the positive and that, although only crudely estimated, perhaps less than one in a million initiated cells complete the full transition to overt malignancy (paragraph 218).

4. Immunodeficiency and cell surveillance mechanisms

140. Some human genetic immunodeficiency diseases, such as the Wiskott-Aldrich syndrome [P3], are characterized by susceptibility to certain neoplasms. However, the role of host immune-response in oncogenesis is a problem that has yet to be satisfactorily resolved (see, e.g. [S43]). In the context of oncogenic mechanisms, some specific comment is necessary.

141. In the case of oncogenic DNA viruses, such as Epstein Barr virus (EBV) that carry transforming genes, there is evidence that immune functions efficiently suppress oncogenesis [P4] and that EBV-carrying lympho-proliferative disorders normally only develop where there is evidence of host immunodeficiency. It has been suggested [K2] that the rather unusual spectrum of human neoplasms that develop in the case of inherited or acquired immuno-deficiency reflects the involvement of oncogenic DNA viruses in tumour aetiology. In such cases the expression of "foreign" viral genes in neoplastic cells could be seen as providing specific targets for host immune functions. Viral oncogenesis often depends on the persistent proliferation of the virus in host tissues (paragraphs 115-118), and this is obviously influenced by the degree to which the host is able to mount an effective immune response against the highly specific "non-self" viral proteins.

142. For oncogenesis mediated by the insertion of a viral sequence close to cellular oncogenes the situation is less clear. The target gene may be activated by the insertion of only a short, non-coding viral sequence (the viral long terminal repeat or enhancer) [J2]. This would not tend to generate immunogenicity in the cell. If, however, the whole viral genome were inserted, the expression of virally encoded proteins could elicit an immune response to eliminate the carrier cell [K2]. The activation of normally silent transposons has been demonstrated in human testicular tumours [B34], and in one case of colon cancer the target *APC* gene had been inactivated by transpositional insertion [M46]. The implication of such activational and insertional events for immune response is however uncertain.

143. In the case of oncogene activation by endogenous processes, i.e. chromosome translocation, gene amplification or point mutation, either normal or minimally modified proteins have been seen to be expressed (paragraphs 28 and 29). Chaemeric fusion proteins (paragraph 71 and Table 1) are a possible exception to this. Consequently, such mechanisms would not, in general, provide strongly antigenic gene products to act as high-affinity "non-self" targets for B- and T-cell mediated immune mechanisms. This conclusion accords with the low immunogenicity seen in most spontaneous neoplasms [K2]. Oncogenic processes associated with gene losses would not obviously have any direct consequences for conventional immune response.

144. T-cell mediated immune recognition depends on the precise presentation of antigens on target cell surfaces through the action of proteins, collectively termed the major histocompatibility complex (MHC), and there is evidence that during the course of their development many tumours develop antigenic determinants that can elicit immune reactions. Tumour antigens have been characterized in a range of human and animal tumours [G14], but the contention that they are capable of stimulating an effective immune reaction may be questioned. Melanoma probably represents the best antigenically characterized human tumour, and around 40 different tumour antigens have been identified using monoclonal antibody techniques. These include antigens of MHC, pigment, growth factor receptor and cell membrane/matrix origin [H36]. A feature of these antigens that illustrates the central problem of tumour immunology is that none are truly tumour-specific; all are expressed, albeit in some cases weakly, by other normal tissues. The lack of specificity of the vast majority of tumour antigens may well underlie the apparent failure of T- and B-cell mediated immune systems to mount an effective response to neoplastic cells arising in tissues. As noted above, the virally-associated human tumours represent a clear and wholly understandable exception to this.

145. Thus, while T- and B-cell mediated immune systems may under some circumstances modify the development of non-viral human tumours, clear evidence that they have the capacity to eliminate early pre-neoplastic cells in tissues is lacking. Indeed, if such efficient systems were to be available, it would be necessary to seek explanations as to why such cells can remain dormant in tissues for long periods prior to promotional stimulation by systemic factors such as hormones (paragraphs 78-97). Also, from an experimental point of view, an explanation is needed for the fact that very large studies with mice, genetically deficient in T-cell immunity, have failed to provide any evidence of elevated tumour susceptibility (see [B25, S42]).

146. Immunodeficiency is a characteristic feature of the ataxia-telangiectasia human genetic disorder [R10]. While the immune defect in the disease may contribute to the increased frequency of lymphoreticular neoplasia in ataxia-telangiectasia, chromosomal rearrangements that are characteristic of the predominant T-cell leukaemias suggest that the underlying defect in DNA metabolism resulting in misrecombination of T-cell receptor genes [R3] may be a more important determinant of neoplasia incidence.

147. There is also evidence that the skin plays a significant role in the body's immune system and that Langerhans' cells in skin originate in bone marrow and are immunocompetent [S28]. With regard to immune effects in carcinogenesis, it has been shown that immunosuppressed patients show an excess of squamous cell carcinomas [K7], and clinical observations have led to suggestions that a depression of the cell-mediated immune function in skin, as a consequence of UV-sensitivity, may contribute towards the incidence of skin neoplasia in XP patients [B14].

148. While there is relatively good understanding of the nature and extent of T- and B-cell mediated immune reactions, the same is not true of the potential defence mechanism provided by natural killer (NK) cells; these appear to recognize abnormal cells without MHC involvement and are far less discriminating in their action than T-cells [B2]. Recognizing certain cell surface receptors that are not necessarily antigenic, NK cells bind and then secrete membrane-perforating proteins that lead to target cell lysis [Y3]. Thus cells misplaced in tissues, cells over-expressing certain membrane proteins and/or cells with conformational membrane changes may be eliminated. The accidental elimination of normal cells by NK cells is known to occur [Y3], and this relatively low specificity of action may provide NK cells with a capacity to eliminate pre-neoplastic cells showing characteristic changes at membrane surfaces. In this general context, NK cells have been shown to exert selective inhibitory effects during post-irradiation haemopoiesis [P8], and a regulatory role for lymphoid cells in selectively preventing the self-renewal and accumulation of early neoplastic cells has also been proposed [G4].

149. The overall extent of NK or NK-like cell function in the elimination of tumour cells and the development of malignancy in man remains uncertain. However, in animal systems it has been shown that the transplantation of cloned NK cells to NK-deficient host mice leads to resistance to radiation-induced thymic lymphoma and the inhibition of lung nodule development following implantation of melanoma tumour cells [W17]. The selective activation of NK cells has also been shown experimentally to inhibit tumour metastasis [H37]. The implications of these observations for radiation carcinogenesis in man remain to be established, but they do provide evidence for the existence of cell-mediated processes that defend against tumour growth and metastasis [H38, Y3]. In spite of this, their apparent lack of specificity poses questions on the overall efficiency of their scavenging capacity.

150. In conclusion, it has been argued that with the exception of a possible viral component, T- and B-cell mediated immune response may not play a major role in moderating human radiation oncogenesis. However, specialized immune functions in certain organs and the existence of non-immunogenic cell surveillance mechanisms imply that a proportion of early pre-neoplastic cells may be eliminated before they become established. Other mechanisms defending against tumour induction or development, including DNA repair, programmed cell death, terminal differentiation and phenotypic suppression, are noted in other Sections of this Annex. Together, these will reduce the probability that a specifically damaged target cell will progress to frank malignancy. An estimate of this probability, while of considerable importance to radiological protection, is extremely difficult to make. Nevertheless, in paragraph 219 a first approximation calculation is illustrated.

III. EXPERIMENTAL INVESTIGATIONS OF CELLULAR AND MOLECULAR MECHANISMS OF RADIATION ONCOGENESIS

151. Given the complexity of the cellular genetic events involved in oncogenesis, how should the principal questions regarding the mechanisms of radiation oncogenesis be framed? With a view to exploring experimental strategies, these questions may be grouped as follows:

(a) what is the nature of radiation-induced initiating events? Is DNA the principal cellular target, and if so, what is the effect of post-irradiation DNA repair on the fate of these potentially initiating lesions?

(b) what is the identity, distribution and radiosensitivity of target cells for the major induced neoplasms?

(c) what are the consequences of an initiating event to a given target cell, and how does this event

interact with or determine promotional or progressional events in order to give rise to the overtly neoplastic cellular phenotype?

(d) in what ways do genetic, hormonal and/or environmental factors affect the expression of initial oncogenic damage and the subsequent progression towards malignancy?

(e) how closely can experimentally derived data on oncogenic change be related to other biological/biophysical effects of radiation and, crucially, to *in vivo* human oncogenesis.

152. Unambiguous answers to most of the above questions are not yet possible. The questions serve here only as a framework for discussion. Nevertheless, some specific comment is possible from both *in vitro* and *in vivo* approaches to the problem.

A. EPIDEMIOLOGICAL STUDIES

153. Epidemiological studies of human groups exposed to low-LET radiation show that a range of neoplasms are represented in excess and, broadly, that these do not differ markedly from those arising spontaneously in the population (see, e.g. [U1]). That is not to say that different tissue sensitivities or characteristic mechanisms do not occur for radiation oncogenesis but rather that no unique neoplastic signature of human radiation exposure is, as yet, apparent (paragraphs 223-227). This may be contrasted with the organ-specific neoplasms that characterize exposure to certain chemical agents, e.g. asbestos and mesothelioma, vinyl chloride monomer and hepatic angiosarcoma, benzene and leukaemia and aflatoxin and hepatocellular carcinoma [B22, T16] (paragraphs 91-93). The basis of these observations, although uncertain, may be associated with the evidence that, through energy deposition and chemical radical interaction in DNA, radiation is able to induce a diversity of genomic lesions, ranging from damage to single bases to gross DNA deletions and rearrangements. Again, this may be contrasted with some chemical agents, which have characteristic chemical specificities in their interaction with DNA and also target certain organs (paragraphs 188-203). Thus, the epidemiological characteristics of radiation oncogenesis would be explained, if the spectrum and distribution of induced cellular initiating events was not grossly different from that arising spontaneously and if low-LET radiation simply increased the frequency of the commonly occurring neoplasms, albeit with different levels of excess in different target organs. Although attractive in its simplicity, this hypothesis may need to be modified in the light of molecular information on induced somatic cell mutation. As noted later in Chapter IV, the physiological and biochemical processes governing the uptake, distribution and excretion of radioactive isotopes can lead to dose-inhomogeneity and, subsequently, organ-specific neoplasia. There is limited information on such carcinogenic effects in man [U1, U2]; and Annex F, "Influence of dose and dose rate on stochastic effects of radiation" summarizes some of the relevant animal data.

B. MOLECULAR STUDIES OF MUTAGENESIS AND REPAIR: POSSIBLE IMPLICATIONS FOR NEOPLASTIC INITIATION

154. If, as implied in previous paragraphs, the majority of neoplasms are initiated through gene- and cell-specific somatic mutation, then the molecular characteristics of radiation-induced gene mutation may be informative. Detailed studies of radiation-induced mutation at the *HPRT*, *APRT*, *TK* and *DHFR* loci of mammalian cells show that the principal mechanism of radiation-induced mutation is through gross genetic change, usually DNA deletions [E1, H7, M6, M33, N20, S29, T2, T8, U11]. These data in no way exclude radiation mutagenesis through point mutation, which has been convincingly demonstrated at the *APRT* locus [G5, M33], but rather suggest that ionizing radiation is a relatively weak point mutagen. The molecular analysis of spontaneously arising somatic cell mutants suggests that point mutation may be the predominant spontaneous mutagenic event, although spontaneous deletion mutants have also been characterized [M33, S29, T2].

155. It is, however, important to recognize that most of these data relate to mutation induction in a limited selection of genes and, generally, at relatively high doses and dose rates (see [T8]). In recent studies, it has been shown that *tk* mutations in a murine (L5178Y) cell line are induced in a dose-rate-dependent fashion but, apparently, with an increase in the proportion of multilocus mutations correlating with decreased dose rate [E4]. In this study, mutations at the *hprt* locus, which are thought to be single-locus events, showed no such dose-rate dependence. In contrast, other studies (see [F22, M22]) show that induced *hprt* mutation rates decrease with dose rate or dose fractionation. It has also been demonstrated [M22] that the specific fraction of full *hprt* gene deletion in Chinese hamster ovary cells is not altered by post-irradiation repair processes, suggesting that DNA repair acts with equal fidelity on the majority of potentially mutagenic lesions (see also Annex F, "Influence of dose and dose rate on stochastic effects of radiation"). This latter study also provided preliminary evidence for *hprt* breakpoint hot spots in the 3' segment of the gene. Since oxygen concentration is known to affect the spectrum of radiation-induced

DNA lesions, it may be an additional and important variable, particularly for DNA base damage that might give rise to point mutations.

156. There is growing evidence that spontaneous and radiation-induced gene deletions in cultured mammalian cells may involve preferential DNA breakage at certain short repeat sequences [M33, M39, M49]. The mechanism for the induction of such deletions remains uncertain but could include the induction of DNA double-strand breaks, followed by recombination and/or replication slippage past secondary DNA structures produced by the pairing of the repeats [M33]. The potential importance of this mechanism for oncogenesis is evidenced by the finding of similar short, direct repeats at the breakpoints of deletions in the *RB* gene in human retinoblastoma, and it also appears that the repeat sequences involved in gene deletions vary according to the locus that is being studied [C25]. The catalytic properties of such repeat sequences, while still to be fully resolved, could therefore be a significant factor in the relative radiosensitivity of different tumour genes and, through this, could be a determinant of the inducibility of the neoplasms with which they are associated. At present the information available is largely restricted to small intragenic deletions, and it is of some importance to determine the relevance of the DNA deletion mechanism noted above for the gross chromosomal losses that characterize some radiogenic neoplasms [B42].

157. A similar picture with respect to gross genetic damage also emerges from molecular studies on the nature of the DNA-repair defect in radiosensitive ataxia-telangiectasia human cells. These data imply that misrepair of DNA double-strand scissions may be the major determinant of ataxia-telangiectasia radiosensitivity and that the misrepair takes the form of DNA deletion and/or rearrangement around the site of the scission [C6, D2] (see also Annex F, "Influence of dose and dose rate on stochastic effects of radiation"). Some of the uncertainties about the relevance of this misrepair mechanism for the ataxia-telangiectasia phenotype have been resolved by the use of a DNA repair assay utilizing cell-free extracts [G16, N15]. Here it has been shown that, whereas nuclear extracts of normal and ataxia-telangiectasia cell lines do not differ markedly in the efficiency with which they rejoin enzyme-induced double-strand breaks in plasmid DNA, they do differ in the fidelity with which certain forms of breaks are repaired; from the data presented it appears likely that the nature of the DNA sequence at the breakpoint may influence repair fidelity. Such *in vitro* approaches may have considerable potential for resolving the molecular mechanisms of oncogenic initiation and could, for example, be used as model systems to explore the importance of double-strand breaks and DNA repeat sequences in the induction of DNA deletions in cloned tumour suppressor genes such as *RB*, *APC* and *p53*. Homologous recombination is thought to be involved in some cases of tumour suppressor gene mutagenesis, and model *in vitro* approaches to this mechanism and its induction by radiation have been described [B31].

158. DNA misrepair may also result in the appearance of DNA translocations as well as deletions, and it is relevant that the predominant, spontaneously arising T-cell neoplasms in ataxia-telangiectasia patients often involve specific chromosomal rearrangement [T6] centred on incorrect recombination of DNA sequences associated with the assembly of mature T-cell receptor genes [R3]. Owing to the apparently small DNA target involved, the efficient induction of such precise translocations by radiation may be questioned (paragraphs 74 and 75). It may be, however, that as a consequence of specific recombination affinity, radiation-induced molecular damage outside the target sequence may catalyse site-specific misrecombination [B42]. Again, this problem could, in principle, be approached using *in vitro* molecular strategies.

159. Overall, the above molecular studies may be used to argue that radiation-induced somatic cell mutations associated with the initiation of oncogenesis are, perhaps, more likely to be specific DNA deletions and/or rearrangements than point mutations. However, there is insufficient evidence to be dogmatic about this important aspect of oncogenic initiation, and it may be that the genomic domain within which a target gene is located is a major determinant of the predominant molecular mechanism of induced genetic change. It may also be oversimplistic to view mutagenic mechanisms as the principal determinant of oncogenesis. A significant factor in the emergence of an established neoplasm will be the degree of proliferative advantage and/or selection associated with early pre-neoplastic events. Thus, while ionizing radiation may be a weak point mutagen for a given gene, the selective advantage conferred to a target cell by a specific point mutation in that gene may in some circumstances outweigh the relatively low overall frequency of such mutations induced within the target cell population. In conclusion, recent attempts to relate specific mutations in radiation-associated human tumours to the mutagenic action of ionizing radiation [V10] highlight the pressing need for more detailed information on the mechanisms of radiation mutagenesis and repair. The induction of cellular repair by low doses of ionizing radiation has been demonstrated in certain experimental systems, but the implications of this for oncogenic processes remain obscure (see Annex F, "Influence of dose and dose rate on stochastic effects of radiation").

C. *IN VITRO* STUDIES WITH CELLULAR SYSTEMS

1. Conventional systems

160. The early work of Reznikoff et al. [R2] provided strategies and techniques for the development of *in vitro* oncogenic cell transformation systems. These clonogenic systems, such as that based on C3H10T½ cells, seek to characterize and quantify radiation-induced changes in quasi-normal cells that mimic or are associated with neoplastic cellular phenotypes, e.g. loss of contact inhibition, growth in semi-solid medium [H6]. The positive and negative arguments on the relevance of these *in vitro* techniques to oncogenesis *in vivo* have been discussed [L2], and it is sufficient here to make only a few general points about their role in the understanding of oncogenic mechanisms.

161. First, irrespective of the detailed cell biology of the systems, *in vitro* studies that relate radiation exposure to the induction of a clonogenic, neoplasia-associated cellular phenotype should be viewed as being potentially informative. Secondly, the characteristics of the established rodent cell systems allow rapid assay of the transformed phenotype and ready manipulation, for the purpose of addressing questions of dose effect, dose rate, radiation quality, post-irradiation repair and tumour promotion. Also of great potential benefit is the possibility that such techniques could be applied to cultured human cells.

162. For the induction of the *in vitro* transformed cellular phenotype there is compelling evidence that the principal target is genomic DNA. The gross morphological cellular changes that signal transformation may be produced by DNA-mediated oncogene transfer, and there is a good correlation between the ability of the given agent to induce cell transformation and its capacity to induce DNA-damage (see also paragraphs 20 and 21). In addition, there is evidence that *in vitro* transformation progresses by at least a two-step process and that many agents and conditions that influence *in vivo* oncogenesis have parallel effects on *in vitro* cell transformation [H6, K10, L3].

163. There remain, however, many uncertainties regarding the true relevance to *in vivo* oncogenesis of quantitative and qualitative studies with many existing *in vitro* systems. The principal *in vitro* cellular systems are based on immortalized rodent cell lines, and quantitative, radiation-induced transformation of normal human diploid cells has yet to be convincingly demonstrated. In the established rodent cell systems there has been considerable controversy on the shape of dose-effect curves [L2], on the influence of radiation quality and, recently, on the existence of a reverse dose-rate effect for high-LET radiations (see [C14]). Overall, the relative simplicity of these systems is appealing, but their direct application to quantitative aspects of human radiological risk is perhaps premature. Some of these aspects are discussed in Annex F, "Influence of dose and dose rate on stochastic effects of radiation".

164. Mechanistic studies of *in vitro* cell transformation indicate that initiating events may, in some instances, be induced at a surprisingly high frequency in an irradiated population of C3H10T½ cells [K3]. Evidence has also been presented that, at low doses, ^{125}I incorporated into DNA is extremely effective at inducing the transformation of BALB/3T3 cells [L18]. Indeed, at ^{125}I levels producing only ~30 decays per cell, the high transformation frequency observed (10^{-4} per surviving cell) implies that only a very small number of unspecified DNA sites need to be damaged to convert the cell to a neoplastic phenotype. Similar conclusions are also emerging from *in vivo/in vitro* studies with rat mammary and thyroid clonogens [C5]; such high-frequency induction is, however, difficult to reconcile with the overall picture of specific gene targets for neoplastic initiation that is emerging from studies with human tumours. The molecular mechanisms underlying this process remain obscure, but establishing the generality of such high frequency induced events is of obvious importance. The molecular aspects of the radiation-induced transformed phenotype have been studied using DNA-mediated gene transfer techniques, and although dominantly acting transforming DNA sequences have been shown to be carried by overtly transformed C3H10T½ cells, their identity has yet to be fully established [B3, K22]. The presence of unidentified transforming sequences has been demonstrated in post-irradiation mass cultures of C3H10T½ cells [L2], and recently, DNA transfer techniques have been successfully employed in the molecular cloning of a transforming gene from a radiation-induced C3H10T½ transformant [F20]; it remains to be seen however whether this gene represents the principal target for radiation-induced initiation of C3H10T½ transformation. In addition, *myc* oncogene rearrangement has been characterized in a radiation transformed C3H10T½ clone [S6] and *myc* and *raf* gene expression has been shown to be increased in another such transformed clone [L25].

165. While it is possible that the identification of characteristic gene activation events in *in vitro* transformed cells may allow comment on the broad mechanisms of radiation oncogenesis, it is more likely that such identification will be of greatest value in the understanding the systems themselves. With conventional rodent *in vitro* transformation systems, doubt is likely to remain about the true significance of single cell *in vitro* response to the more complex interactive processes that mediate *in vivo* oncogenesis.

2. Novel systems

166. While quantitative and qualitative data from conventional cell transformation systems will continue to be useful, more attention is now being focused on the potentially more relevant systems that utilize human cells, cells of epithelial origin and cells from haemopoietic tissues (see [C9]). It is already apparent that the conventional criteria for *in vitro* cell transformation, i.e. loss of contact inhibitions/focus formation, growth in semi-solid media, immortalization and relatively rapid progression to an overtly oncogenic phenotype, do not apply generally in cellular systems. Cellular and molecular studies of human neoplasms strongly suggest that different transformational processes may operate in different cell lineages, and this should be reflected in the *in vitro* systems that are employed. The most significant progress that could be anticipated would be the design of *in vitro* cellular systems that were linked, by cytogenetic and molecular studies, to specific *in vivo* neoplastic and pre-neoplastic conditions. It cannot be expected that such systems will be simple or quantitatively precise [H13], but they should reveal more about oncogenic mechanisms than the currently favoured systems based on rodent fibroblast cell lines.

167. New developments in *in vitro* cell transformation have recently been reviewed [C9], and it is sufficient here to highlight a few of the most important findings. Studies with human diploid fibroblasts have been discussed by McCormick et al. [M9], with particular emphasis on the lack of clear evidence for induced malignant transformation in these cells. The immortalization of human fibroblasts, unlike that of cultured rodent cells, appears to be a very rare event and probably represents a rate-limiting step in the pathway towards malignancy. It has been shown, however, that a rare, radiation-induced, immortalized human fibroblast strain may be transformed to a fully malignant phenotype by the introduction of an activated H-*ras* oncogene [N8, N11]. In a series of subsequent experiments [M40] it has been shown that intermittent *in vitro* exposure (total dose 28 Gy) to gamma rays over 50 passages resulted in the immortalization of human fibroblasts but that final malignant conversion could not be demonstrated until the 547th passage (2,800 d in culture). These studies provide a clear demonstration of the multi-step nature of *in vitro* malignant transformation in human fibroblasts and, unfortunately, the difficulties that are faced in obtaining quantitative estimates of induced transformation with these cells.

168. A human hybrid cell system has also been developed in order to quantify radiation effects on a putative tumour suppressor gene encoded on chromosome 11. The quantitative, cellular and molecular aspects of this system are currently under investigation [R4], and good progress may be anticipated. Although based on immortalized strains of human cells and used so far for characterization and chemical carcinogenesis studies, mammary epithelial [S54], kidney epithelial [T18], skin keratinocyte [B7, F6, R18, R19], colonic epithelial [W25] and urinary epithelial cell [R5] systems also show interesting potential for both mechanistic and quantitative studies.

169. Cytogenetic and molecular studies of chemically transformed variants of the SV-HUC human uroepithelial cell line have been particularly rewarding [K15]. It has been shown that chromosome 3p deletions are characteristic of the high-grade carcinomas produced in transplanted nude mice following *in vitro* chemical transformation. What is particularly noteworthy is that loss of heterozygosity for 3p encoded DNA sequences have been reported in a variety of human carcinomas, thus for the first time providing a putative link between *in vitro* induced cellular damage and carcinogenesis *in vivo*. An extension of this approach to radiation-induced transformation should be encouraged, but it should be recognized that, at present, the assay system as developed does not lend itself to detailed quantitative studies and that the viral immortalization process may influence the transformational response.

170. Novel cell transformation systems that include *in vivo* and *in vitro* phases have been developed for mammary [C5], thyroid [M23] and tracheal [T9] tissues of rodents. In the mammary system, transplantation of dissociated mammary tissue to subcutaneous fat pads has allowed the quantification of differentiating mammary clonogens and, through this, estimates of radiosensitivity. A tissue-dependent post-irradiation repair process has been characterized, as has the induction of dysplastic (pre-neoplastic) lesions resulting from radiation damage to clonogens. The promotional effects of elevated prolactin and glucocorticoid deficiency are evident in these studies, and it is also apparent that neoplastic initiation is a relatively common cellular event [C5].

171. A fat pad transplantation technique also underlies the *in vivo in vitro* studies on thyroid carcinogenesis. The radiation induction of pre-neoplastic lesions was shown to be dependent on hormonal status (thyroid-stimulating hormone), and again, initiating events were observed at a surprisingly high frequency [C5]. Cell-cell interactions have been shown to influence strongly the expression of oncogenesis in both mammary and thyroid systems. Similar findings have been made using an *in vivo in vitro* model of tracheal epithelial transformation in the rat [T10]. Here, direct cell-cell contact in tissues and a diffusible factor from cultured cells have been shown to suppress the expression of early oncogenic changes. In the case of *in vitro*

suppression the factor is believed to be transforming growth factor ß (TGF ß), which may exert its effect through the induction of terminal differentiation [T10]. Thus, the emergence of initiated cells and their subsequent proliferative advantage may depend on their ability to escape from TGF ß mediated terminal differentiation.

172. Despite the increasing knowledge of the complex interactions between growth promoting and inhibitory factors in the regulation of haemopoiesis and associated cell lineage differentiation [D8, M24], transformation systems for cells of haemopoietic origin are at a relatively early stage of development. However, the availability of growth factor-dependent haemopoietic cell lines [D9, G10, S30] and their oncogenic transformation by specific haemopoiesis-related genes [B15, L12] are providing powerful tools with which it may be possible to relate transforming events *in vitro* to those that characterize haemopoietic neoplasms *in vivo*. In the case of osteosarcomagenesis, an *in vitro* system for murine mandibular condyles is being developed to study the cellular and molecular events that mediate skeletoblast differentiation and oncogenic transformation [S15]. In this system it has been shown that *fos* expression precedes the *in vitro* osteogenic differentiation of cartilage cells [C28].

D. *IN VIVO* STUDIES

1. General experimental strategies

173. It has been argued in previous paragraphs that the most plausible molecular form of neoplastic initiation is specific gene mutation that predisposes appropriate target cells to subsequent malignant conversion; studies with familial human neoplasms have also shown that, under some circumstances, it is possible to gain detailed information on the identity of tumour-initiating events in man. However, the central problem for cellular and molecular studies using inducing agents and experimental animal systems is that during the post-irradiation latent period, a number of genetic/epigenetic changes will have accumulated within the evolving neoplastic cell population, and many of them will be represented in the final malignant clone that is available for study. What criteria can be used to determine which of these changes was induced by the carcinogen and is therefore the initiating event?

174. Two main experimental strategies have evolved to approach this crucial question:

(a) to use molecular techniques to search for consistent molecular change in specific genes of the induced neoplasm and attempt to relate any characteristic DNA changes to the known DNA-damaging properties of the inducing agent;
(b) to identify candidate initiating events as consistent molecular and/or chromosomal changes in the overt induced neoplasm and subsequently trace the induction of these events back to the immediate post-treatment proliferation of cells in the target organ.

175. The first strategy has been effectively employed in the putative identification of *ras* proto-oncogenes as principal targets for early events in chemically induced mammary, hepatic and skin carcinomas in rodents (paragraphs 188 and 189). In general, the success of this approach to chemical oncogenesis depends on the induced initiating events being base-pair specific changes in a restricted set of codons of a known gene (see, e.g. [B37]). In this case the chemical specificities of the carcinogen will be immediately apparent and informative. The relative lack of DNA damage specificity following exposure to ionizing radiation may mean that this strategy is less appropriate to radiation oncogenesis. Consistency of molecular genetic change in a given neoplasm infers a major contribution of that change to the success of the malignant clone but need not be informative on the stage in clonal evolution at which the change took place. Point mutation within a limited set of *ras* gene codons has been demonstrated in radiation-induced murine lymphomas [D3, D4, N19], but alone, these data are insufficient to identify *ras* activation as an initiating event for this neoplasm. More recently, however, it was shown that *K-ras* codon mutation spectra in tumours induced by gamma rays are different from those in tumours induced by neutrons [S31]. These data were used to argue that differences in physical properties between the two radiation qualities might influence mutational spectra and hence that *K-ras* activation might represent an initiating event for radiation lymphomagenesis. There is, however, some uncertainty in the biophysical interpretation of these data, and it remains possible that *ras* mutations play a greater role in the development of lymphoma than in its initiation. Even greater problems of interpretation surround other observations of structural and functional changes in oncogenes of radiation-induced animal neoplasms (paragraphs 179-187). In no case is it possible to be confident about the identity of the radiation-induced initiating event (e.g. [F4, S7]). In this area, however, the implementation of polymerase chain reaction techniques (PCR) for rapid gene mutation analysis [E8, R11] during early post-irradiation cell proliferation periods may be expected to have a major impact on future research.

176. The second experimental strategy has been somewhat more successful, particularly in providing comment on the status of characteristic chromosomal changes in radiation-induced haemopoietic neoplasms.

177. Trisomy of chromosome (ch) 15 is a characteristic cytogenetic feature of radiation-induced murine thymic lymphoma. Cytogenetic studies of pre-neoplastic animals strongly suggest that the appearance of the extra ch15 copy is associated with the later stages of neoplastic development. In contrast, the early appearance of a ch 1:5 translocation in a significant proportion of pre-neoplastic animals suggests that this is a better candidate for a lymphoma-initiating event [M7]. There is, as yet, no information regarding the involvement of specific genes in either of these chromosomal changes.

178. The deletion and rearrangement of ch2 is a consistent feature of radiation-induced acute myeloid leukaemia in the mouse [B4, B16, H8]. Using an *in vitro* irradiation and transplantation technique, similar ch2 rearrangements were characterized in rapidly proliferating haemopoietic cell clones within five days of irradiation, and it was concluded that these may represent candidate initiating events for murine acute myeloid leukaemia [B16, S8]. More recently, a strong statistical concordance has been established between radiation-induced ch2 breakpoints in irradiated murine haemopoietic cells and those in acute myeloid leukaemia [B16]. In addition to strengthening the link between ch2 deletion/rearrangement and leukaemic initiation, these data also suggest that induced breakage may occur at specific radiation-sensitive sites on this chromosome. Stable chromosomal changes have also been recorded in irradiated and repopulating haemopoietic cells in the rat [M35].

2. Molecular studies of induced animal neoplasms

179. The molecular aspects of radiation oncogenesis in animal models of thymic lymphoma, myeloid leukaemia and osteosarcoma have recently been reviewed [J2] and are outlined here briefly, together with some observations on rodent skin, mammary and lung carcinogenesis. This whole area has also been explored in a number of recent symposia (see [B28, E7]).

180. *Thymic lymphoma.* Studies of thymic lymphomagenesis in mice have provided evidence that an indirect mechanism is involved [K4]. It has been repeatedly shown that thymic lymphomas may develop from immature lymphoid cells present in non-irradiated thymuses that are subcutaneously grafted into split-dose-irradiated, thymectomized recipients [K4, M8]. Since weakly leukaemogenic activity is often present in cell-free extracts of primary lymphomas and its potency increases with serial passage, a viral role in lymphomagenesis was suspected [L4]. Although recombinant retroviral complexes have been reproducibly detected in radiation-induced lymphomas [J2], the specific role of these and their derivation from germ-line ecotropic provirus remains uncertain [J2, N4]. Some evidence for proviral induction by radiation and integration into common genomic domains in lymphomas and derivative cell lines has recently been obtained, but the genes within these domains are not known [J2, S55]. The weight of evidence now tends to favour a role for provirus in lymphoma development rather than initiation, and there are studies that imply that radiation leukaemogenesis in NFS mice does not involve C- and B-type retroviruses [O3]. Indeed, recent evidence suggests that infection by activated viruses is not necessary to explain the indirect induction of radiogenic lymphomas [S56, S57]. It has been proposed that the depletion of T-cell precursors in the bone marrow, together with the depletion of thymic lymphocytes, is sufficient for regenerating thymus cells to undergo pre-neoplastic changes, possibly due to an aberrant expression of genes involved in the growth and differentiation of thymus cells [M50, S56, S57].

181. It has been shown that the injection of syngeneic bone marrow cells into split-dose-irradiated mice can prevent lymphoma development, possibly as a consequence of the rapid repopulation of recipient thymus by donor lymphoid cells [K4, S9]. Overall, it may be concluded that complex cellular processes mediate radiation lymphomagenesis and that direct radiation damage to target cells may not be a prerequisite for the appearance of oncogenic events. In this respect, four interactive factors may be invoked:

(a) activation of potentially leukaemogenic virus from germ-line provirus and subsequent recombination to yield more potent virus;
(b) thymic involution and regeneration, giving a population of virus-susceptible cells;
(c) impairment of cell surveillance mechanisms;
(d) impairment of bone marrow and thymocyte cell function, leading to reduced thymic repopulation and altered gene expression.

In addition, codon mutations in *N-* and *K-ras* oncogenes, T-cell growth factor changes together with specific chromosomal rearrangements have been detected in radiation-induced lymphomas, and some of these have been suggested as early events in lymphomagenesis (paragraph 175, [N19]); in contrast, recent data tend to argue against the involvement of *p53* mutations in radiation-induced and chemically-induced lymphomas [B33]. Attempts to characterize and isolate target cells for radiation lymphomagenesis are also under way [M26].

182. *Acute myeloid leukaemia.* There is little evidence to suggest that radiation-induced murine acute myeloid leukaemia proceeds via an indirect (viral) mechanism. In contrast, the specific deletion/

rearrangement of chromosome 2 is a consistent cytogenetic feature of induced murine acute myeloid leukaemia [B4, B16, H8, H44], and evidence has been obtained that ch2 rearrangement is induced directly by x rays in multipotential haemopoietic cells, in some cases generating a cellular phenotype associated with preferential haemopoietic recruitment and/or proliferative advantage [B16, S8]. It has also been shown that ch2 events may be observed in bone marrow cultures established from irradiated mice long before the anticipated appearance of acute myeloid leukaemia [T4] and that the induction of ch2 rearrangement is an early step in leukaemogenesis but is not sufficient for the development of myeloid malignancy [H27, H28]. Preliminary evidence for deletion of the homeobox gene, *hox* 4.1, and activation of the cytokine gene, interleukin-1ß (*Il-1ß*), has been obtained in some ch2-rearranged acute myeloid leukaemias [B5, S10, S11]. However, the evidence presented for the involvement of these genes in leukaemic initiation was not compelling, and more recently, detailed molecular analyses of the *Il-1* genomic region on ch2 failed to provide evidence of the structural rearrangements predicted in acute myeloid leukaemia [S32, S33]. In these analyses it was found, however, that some acute myeloid leukaemias show very similar methylation pattern changes in the *Il-1* region, and it was suggested that these may be associated with certain forms of ch2 rearrangement and deletion. Other studies have shown that the ch2-encoded *his-1* locus is involved in virally induced myeloid leukaemia in the mouse and may represent another candidate gene for the initiation of acute myeloid leukaemia [A9].

183. Cytogenetic studies with radiation-induced murine acute myeloid leukaemias and irradiated normal haemopoietic cells have yielded evidence that ch2-encoded fragile sites are involved in leukaemogenic initiation [B16]. These investigations also suggest that the interstitial ch2 sites in question have a strong recombination affinity with the terminal (telomeric) ends of other chromosomes, implying that they may indeed be telomere-like repeat sequences (paragraphs 62 and 63); it was also suggested that the expression of these sites might be influenced by genomic imprinting. More recently, interstitial telomere-like sequences of an inverted repeat form have been cytogenetically mapped close to the relevant ch2 breakpoints, and the use of telomeric sequences as molecular probes has provided some evidence that germ-line variation at certain telomere-like repeat sequences may be associated with genetically determined leukaemogenic radiosensitivity in the mouse [S43]. Following these studies, it has been suggested that the telomere sequences at ch2 fragile sites may promote the formation of recombinogenic secondary DNA structures that are highly radiosensitive [B32]. Finally, while *ras* mutation is not a common feature of murine myeloid leukaemias, the activation of *N-ras* has been reported in radiation-induced myeloid leukaemias in the dog [G17].

184. *Osteosarcoma.* Although weakly oncogenic viruses have been isolated from radiation-induced murine osteosarcomas, there is no evidence for common genomic insertion sites nor is there an obligate requirement for such viruses in osteosarcomagenesis [J2, V8]. A non-essential role for such viruses in oncogenic progression appears, perhaps, to be more likely. Molecular studies have revealed the amplification of a ch15 domain encoding c-*myc* and the *Mlvi-1* proviral integration site in up to 30% of ^{224}Ra-induced osteosarcomas, and evidence for altered expression of *K-ras*, *myc*, *sis*, *abl*, *bas* and *fos* has been reported in some tumours [J2, S59]. Structural and/or expressional changes have also been reported in the *p53* gene of some osteosarcomas, but to date, there is no evidence for consistent structural changes in the retinoblastoma (*rb*) gene [J2, S58]. Although specific comment on the status of these molecular changes in radiation osteosarcomagenesis is not yet possible, the human evidence of *RB* and *p53* gene involvement in the early phases of the human neoplasm [C29, T5] demands further studies with murine osteosarcomas.

185. *Skin carcinomas.* Ionizing radiation induces skin carcinomas in both the mouse and the rat [J3, S7], but little is known of the molecular mechanisms involved. Recently, it has been shown that the activation of *K-ras* and *myc* oncogenes is frequently observed in primary rat skin carcinomas induced by ionizing radiation [S7]; the status of these changes in the oncogenic process remains uncertain, but *myc* seems most likely to function relatively late in skin carcinogenesis [G18]. In murine studies it has been shown that the gene encoding the transin protease is overexpressed in carcinomas but not in benign papillomas. It was inferred from these observations that transin gene expression might be involved in neoplastic progression by enhancing tumour invasiveness [B11].

186. *Mammary carcinomas.* The induction of mammary carcinomas in mice has been studied by isolating mammary epithelial cells at intervals following irradiation *in vivo* and subsequently selecting clonal cell lines *in vitro*. After low *in vitro* passage, isolated cell lines produced normal ductal outgrowth in transplanted mice, but with increasing passage cells became increasingly tumorigenic and invasive. Chromosomal abnormalities and *myc* (but not *ras*) gene expression, increased with passage and tumorigenic potential. On the basis of studies on *rb* and *p53* gene expression it was suggested that alteration of expression of tumour suppressor genes might be an early event in the oncogenic transformation of mammary epithelial cells

[E7]. This *in vitro* system has also been used to investigate the acquisition of angiogenic activity in irradiated mammary cells and its temporal relationship with tumorigenic potential [U12]. Although *ras* proto-oncogene involvement in chemically induced mammary carcinoma in rodents is becoming understood (see [G19]), the situation regarding ionizing radiation has not been resolved.

187. *Lung carcinomas.* Lung carcinogenesis has been studied in a number of animal species [M41], and in contrast to the situation in humans it seems at present unlikely that either the *rb* or *p53* gene plays a major role in induced lung neoplasia [G20, M41]. Studies on *ras* gene changes in plutonium-induced rat lung tumours imply that the activation of this gene may be a relatively early event in the generation of proliferative pulmonary lesions [S60], while in the dog, the expression of the epidermal growth factor receptor gene was elevated in a significant proportion of lung tumours and proliferative foci arising after exposure to plutonium [G21].

IV. COMPARATIVE ASPECTS OF ONCOGENESIS BY RADIATION AND CHEMICALS

188. It has been suggested that ionizing radiation may principally, but not exclusively, initiate oncogenesis through mechanisms involving DNA deletion and/or rearrangement. While many chemical agents also induce gross (chromosomal) damage in mammalian cells, the mechanisms of induction are fundamentally different from those of radiation [C3, E3]. In addition, molecular studies of induced somatic mutation show the majority of chemical agents to act principally as point mutagens [S29, T2]. Although the mechanisms of chromosome aberration induction by radiation and by the majority of chemical agents differ in terms of cell cycle dependence, the genomic distribution of damage and the DNA repair/recombination processes involved, there may not be major differences in the final stable forms of many induced chromosomal changes in surviving cells. It should be emphasized, however, that the distribution of these changes within the genome may be expected to be different for radiation and chemical agents. In spite of these uncertainties it appears that the major mechanistic difference at the cellular level between radiation-induced and chemically induced oncogenesis is likely to centre on their relative efficiencies for the induction of neoplasms that are principally initiated through point mutation and activation of proto-oncogenes. Chemical and physical carcinogenesis has been reviewed recently [H46], and the strongest experimental evidence for the existence of such an initiating mechanism for chemical oncogenesis comes from studies of *ras* proto-oncogene activation in some rodent neoplasms. In many chemically induced skin, mammary and hepatic tumours, codon-specific point mutations responsible for *ras* activation correspond to those predicted by the known base-pair-specific mutagenic action of the agent used as the initiator, i.e. *ras* mutation appears to be the initiating event [B6].

189. Recent studies [K8] have extended these observations by showing that following nitrosomethylurea exposure, *ras* activating events may be detected in normal rat mammary glands only two weeks after treatment, i.e. at least two months before the onset of overt neoplasia. It appears, therefore, that these specific chemically induced somatic mutations remain dormant in tissue until promotional, hormone-mediated proliferative responses mobilize *ras* activated cells and allow them to progress towards neoplasia. Broadly, these and other data, e.g. [B37, M42, S61], although currently limited to only a few neoplasms and chemical agents, identify candidate initiating events as point mutations and further strengthen the link between mutagenic and oncogenic mechanisms. However, other studies indicate that *ras* activation is not the only potential initiating event for rat mammary carcinomas and that different initiating mechanisms may have different carcinogen dose and promotional dependencies [Z1, Z2]. Although still at an early stage of development, molecular studies on *in vitro* transformed cells are also consistent with the existence of different molecular mechanisms for radiation and chemical carcinogenesis [C9]. However, for molecular events in a given neoplasm that follow initiating damage, i.e. those contributing towards neoplastic progression, radiation-induced and chemically induced neoplasms may be expected to share some common genetic changes [N7].

190. Two major issues emerge from the suggested mechanistic differences that may distinguish radiation and chemical oncogenesis. First, since different neoplasms may depend for their initiation on different forms of molecular damage to the potentially oncogenic DNA sequence, e.g. point mutations rather than DNA rearrangement or deletion, then differences in

the predominant induced neoplasias following human exposures to radiation and chemical carcinogens may be expected. Secondly, if radiation and chemical agents induce characteristically different spectra of initiating lesions, then different genetically determined post-exposure cellular DNA-repair processes will tend to operate to modify and/or remove that damage. Since the efficiency and fidelity of these repair systems will be a significant determinant of induced oncogenesis, the above differences in repair imply that human genetic susceptibility to induced neoplasia by radiation and chemicals will not necessarily correspond. Although this speculation is broadly supported by the lack of cellular cross-sensitivity between radiation and chemicals in the majority of human genetic diseases showing hypersensitivity to DNA damaging agents [A2, F4, H5], the mutations in these disorders will not be fully representative of those having effects on mutagenesis and oncogenic initiation. This is an important problem in human cancer susceptibility, of which there is insufficient knowledge.

191. Tomatis [T16] has reviewed those chemical agents that are known to have carcinogenic activity in man, and among these are agents for which exposure would occur occupationally (e.g. asbestos, aromatic hydrocarbon derivatives and vinyl chloride monomer), medically (e.g. cyclophosphamide and phenacetin) or environmentally (e.g. aflatoxins, nitrosamines, soots, tars and oils). The majority of these agents do not act directly but require specific endogenous biochemical modification in order to generate the carcinogenic species (see [H46]).

192. Certain workers in the plastics industry are occupationally exposed to vinyl chloride monomer (VCM). The enzyme-mediated activation of VCM (the procarcinogen) to carcinogenic metabolites such as chloroethylene oxide and 2-chloroactelaldehyde occurs principally in the liver. Such activation produces tissue-specific exposure to the direct carcinogen and in the case of VCM produces a very clear excess of the rare hepatic neoplasm, angiosarcoma, in exposed workers [B8].

193. Occupational exposure to aromatic hydrocarbon compounds, in particular benzene, has been associated with the development of haemopoietic neoplasms [T16]. Most polycyclic aromatic hydrocarobons, such as benzo(a)pyrene (BP), are not directly carcinogenic; however, through the action of cytochrome P450-linked mixed function oxidases and epoxide hydrases, BP may be converted to active carcinogens, such as BP 7,8-diol,-9,10 epoxide (see [W18]). Studies with the mouse skin papilloma/carcinoma system suggest that the carcinogenic action of this diol epoxide derivative centres on its capacity to form adducts on the guanine (G) residues of DNA. Misrepair of these adducts results in base-pair substitutions at G residues, and these have been linked with the *ras* proto-oncogene activation events that are believed to initiate mouse skin carcinogenesis (see paragraphs 188-189).

194. As noted earlier, aflatoxin B1 (AFB), present in some fungally contaminated foods, is associated with the prevalence of human liver cancer in parts of southern Africa and eastern Asia [B22]. AFB is metabolized, principally in the liver, by the mixed oxidase enzyme system to produce several products, including a highly reactive 2,3-epoxide derivative. This reacts with the 7 position of guanine residues in DNA and represents a major DNA adduct in the liver of AFB-exposed rats (see [W18]). The importance of this reaction for human liver carcinogenesis has recently been demonstrated by molecular studies on tumour DNA from hepatocellular carcinoma (HCC) patients from regions where the disease is prevalent, probably as a consequence of AFB contamination. These studies revealed that the G→T base substitutions at codon 249 of the *p53* suppressor gene, which might be predicted from AFB epoxide action dominated the *p53* mutational spectrum observed [B23, H34]. As well as highlighting the importance of *p53* suppressor gene mutations for the initiation of HCC, these studies provide the first clear links between environmental carcinogen exposure, procarcinogen activational processes and gene-specific tumour mutations in man. An essentially similar approach with the *p53* gene has been used to study the involvement of specific UV photoproducts in sunlight-mediated human skin carcinogenesis [B36]. In essence, the specific form of *p53* mutation in HCC and skin tumours is providing a signature of human AFB and UV exposure, respectively, [H46]; the potential implications of these data are discussed in paragraphs 221 and 222. However, it should be noted that in the case of the mouse, liver carcinogenesis following chemical exposure may not be associated with consistent *p53* mutation [G20].

195. The *p53* point mutations observed in HCC and other solid tumours [C31, H30, H46, L13] also serve to illustrate the point that the initiation of oncogenesis by chemical agents is in no way restricted to proto-oncogene activation but may also occur via the mutation of tumour suppressor genes. In such cases, the expectation is that those mutations will tend to cluster in the regions of the gene specifying certain active sites in the protein product. Detailed analyses of *p53* gene mutations in human tumours by polymerase chain reaction techniques have provided ample evidence of this [H30, L13] (Figure IV). In the distribution illustrated in the Figure, the horizontal axis represents the 393 codons of the human *p53* gene from N-terminal (NH_2) to C-terminal (COOH) ends. The vertical bars represent the relative proportion of

mutations that occur at each codon of the *p53* gene, as sequenced from 94 different primary tumours, xenografts or cell lines derived from tumours (brain, breast, colon, oesophageal and lung tumours and neurofibrosarcomas, osteosarcomas, rhadomyosarcomas and T-cell lymphomas). The highest percentage is at position 273, which accounted for 13% of all mutations [L13]. Tumour types and *p53* mutations have been tabulated [C31, H30], and further information on tumours of the thyroid and cervix is available [C30, I1, K24]. Some studies show that *p53* mutation can, however, arise at the malignant conversion stage of oncogenic development indicating that *p53* mutational spectra will not always be informative on carcinogenic initiation. Nevertheless, current *p53* mutational analyses have been most informative, and the same approach will doubtless yield important information on the mutational damage sustained by other tumour suppressor genes, such as *APC* [K15, N16], thought to be involved in the initiation of other tumours.

196. Since many environmental chemical carcinogens are subject to specific cellular activation and/or degradative processes, human genetic heterogeneity with respect to these processes may be an important factor in determining the specific dose of the carcinogen to target cells [A7, W19]. These activation/degradative processes will also affect the dose of the carcinogen to different target tissues and thereby influence the spectrum of induced neoplasms.

197. For external radiation, the induction of potentially oncogenic cellular lesions may be viewed as a more direct and genetically less complex process influenced principally by the LET and the penetrative properties of the radiation, by DNA metabolic functions in target cells and, particularly for high-LET radiation, by biophysical factors such as the cross-section of the cellular nucleus of target cells and by the packaging density of the DNA. If, as implied, a significant genetic determinant of the radiation initiation of oncogenesis resides in the activity and fidelity of DNA repair processes, then it may be that there is a greater degree of human genetic variation in cancer susceptibility following exposure to low-LET radiation than there is following high-LET radiation. This speculation derives from observations of the apparent lack of (correct) cellular repair following high-LET irradiation of cultured human cells. If high-LET induced genomic lesions are inherently difficult to repair correctly [C8], then less genetic variation and less influence of repair functions may be anticipated in the human population. The main qualification to this speculation is that it is based on studies of radiation-induced potentially lethal lesions and may not apply in full to potentially mutagenic or oncogenic damage.

198. In contrast, for internal radiation, the chemical and biochemical properties of the isotope will determine *in vivo* metabolic routes for uptake, transport, distribution and excretion (see, e.g. [P5]). All these processes will have genetic determinants, and in this respect, oncogenesis by isotopes taken into the body has some parallels with chemical oncogenesis and may be subject to a greater degree of genetic heterogeneity than predicted for external radiation.

199. For radon, a combination of physical, physicochemical and physiological factors will interact to determine radon inhalation, its concentration in respiratory compartments and the subsequent dose distribution in tissues. The active carcinogenic species following radon exposure is, however, likely to be the daughter nuclides in the decay chain. Hence, the underlying mechanism of radon carcinogenesis will be subject to a series of complex interactive factors involving not only initial radon uptake and distribution but also the biokinetics and half-lives of the radon daughters (see Annex A, "Exposures from natural sources of radiation").

200. More straightforwardly, some alpha-emitting actinides have a high affinity for specific glycoproteins present on bone surfaces and may indeed be bound by metal transport proteins. Consequently, the greatest radionuclide deposition and, thereby, accumulation of alpha-particle dose often occurs at active endosteal bone surfaces. Metabolic factors therefore determine that the principal somatic cells at risk are osteogenic cells at bone surfaces and haemopoietic cells in peripheral marrow close to bone surfaces. Clear evidence for radium-induced osteosarcomas has been obtained in both man and experimental animals. However, alpha particles from some actinides deposited in bone appear to be only weakly leukaemogenic [H9, H10, S16]. It is possible that this latter observation reflects the hypersensitivity of multipotential haemopoietic cells to the lethal effects of track traversals by single alpha particles, in which case there will be a low probability of survival for irradiated target cells; equally, however, the spatial relationship between sites of deposition of radionuclides and target cells and the alpha-particle track LET may also be critical [B26, S16]. In this latter context it is important to recognize that alpha-emitting actinides distribute differently between bone volume and bone surface, and it is, therefore, not appropriate to generalize on the *in vivo* dose to different target cell populations.

201. In the case of radionuclides taken up in particulate form, the action of macrophages in the engulfment, dissolution and nuclide redistribution in tissues is of prime importance. These factors will undoubtedly influence the relationships between dose and target tissues [S46]. In human patients receiving colloidal alpha-emitting thorotrast as a contrast medium, distribution of dose to tissues will have been

subject to cell-mediated effects. In these patients there is evidence of both the accumulation of the isotope in liver and a dramatically increased risk of liver cancer [U1, U2]. Studies have been initiated to relate *p53* mutations in these tumours to potential radiation induced damage [W26].

202. It should, however, be stressed that many of the arguments presented here relate to genetic and physiological effects on the initiation of oncogenesis. Genetic and physiological heterogeneity with respect to promotional and progressive processes in tumour development must be of importance to the overall incidence of induced neoplasia in the population and could indeed be the dominant factors. The relative roles of radiation and chemical agents in these processes are noted in paragraphs 78-114 and discussed in detail elsewhere (see, e.g. [S2, T14]). Here it is sufficient to suggest, that for a highly interactive multi-step disease such as cancer, it is only possible to generalize on the relative roles of radiation and chemical agents in the steps that may currently be defined on the basis of incomplete mechanistic knowledge. Better knowledge should make the task somewhat easier, but it is important to recognize the current extent of ignorance.

203. In conclusion, the mechanisms of oncogenic initiation by radiation and chemicals differ substantially and may be subject to different genetic and physiological factors. Consequently, the determination of relative risk from radiation and chemicals will not be an easy task. A report of the United States National Council on Radiation Protection and Measurements [N10] considers in detail the comparative aspects of the carcinogenicity of ionizing radiation and chemicals, and it is clear from that report and the outline presented here that the determination of relative risk from radiation and chemicals is not at all straightforward. For radiation and those chemical agents with known mechanisms of action on DNA, it may be possible to make some assessments on the basis of molecular dosimetry. In such cases the abundance of initial mutagenic/carcinogenic lesions in the DNA of cells of the target organ following exposure to a carcinogen may provide a crude experimental indicator of potency. However, it should be recognized that for this strategy to have a firm scientific base, it is necessary to know the different spectra of mutagenic lesions and their repair/misrepair characteristics and, perhaps most importantly, to have a much better understanding of the mechanistic differences that characterize the oncogenic processes that drive the induction of different neoplasms. In such a future experimental approach to comparative carcinogenicity it will be essential to be able to relate animal and *in vitro* data to man. In this respect, it should be noted that the current quantitative knowledge of human risk from exposure to ionizing radiation greatly exceeds that for any chemical carcinogen.

V. FUTURE PERSPECTIVES

204. It will have become obvious that although some understanding of the mechanisms of radiation oncogenesis is beginning to emerge, the complexity of the whole process is such that rapid progress on a broad front should not be anticipated. Nevertheless, it should be noted that the main component of understanding has come within the last decade and owes much to the application of modern methods of cell and molecular biology. It may be that the best prospects for the future lie in the strategic implementation of these modern methods, with particular emphasis on the full integration of radiation oncogenesis research into the continually expanding field of cancer biology. A few of the many potential growth points and future needs in research on radiation oncogenesis are briefly discussed in the following Sections.

A. IMPLICATIONS OF ADVANCES IN RELATED RESEARCH

205. During the last few years much progress has been made in elucidating mechanisms of DNA-repair and mutagenesis; some of these advances have been discussed in other Sections. Of particular note is the anticipated availability of molecular probes for genes that determine human sensitivity to radiation and other genotoxic agents (e.g. [F1, G7, H45, K11, K28, T15]). When available, these will find use in the elucidation of human genetic heterogeneity with regard to radiosensitivity, with the final prospect of identifying subpopulations that may carry elevated risks of oncogenesis. In this context, the identification and molecular cloning of tumour genes associated with cancer susceptibility (Table 6) is also of crucial importance. Studies on the latter have largely focused on the dominantly expressing genes of high penetrance, such as *RB*, *WT* and *APC*, but the prospect of identifying less evident genes of low penetrance, which may be more frequent in the population, is probably of greater importance. In addition to increasing the knowledge of oncogenic mechanisms at the human population level, such information may have significant social and economic implications.

206. The characterization of genes involved in mammalian DNA repair and radiosensitivity may also provide the means of identifying (or of generating

through genetic manipulation) radiosensitive, DNA-repair deficient mutants of the mouse. The availability of such mutant strains would provide a very powerful tool for detailed whole animal studies on the relationship between DNA-repair and oncogenesis. Recent studies imply that severe combined immunodeficient (*scid*) mice may have an inherent defect in DNA metabolism that confers cellular radiosensitivity [B27, F12, M10]; mouse mutants of this type could provide valuable models for radiation oncogenesis studies. In a similar way, genetically manipulated mice (transgenics) carrying germ line copies of activated oncogenes (see, e.g. [H15, H24, L26, M11, R20, V9]) may also have an important future role to play in understanding the mechanisms of radiation oncogenesis. In this context, genetically engineered murine models of retinoblastoma and of *p53* deficiency in mice may be particularly valuable [D14, W11]. Other so-called tumour suppressor gene "knock-out" mice will doubtless be available in the near future.

207. Recently, important advances have been made by the successful long-term repopulation of the haemopoietic systems of *scid* immunodeficient mice with engrafted human haemopoietic cells [K28, M12, M13]. These *scid*-human chimaeras may provide the means for studying haemopoietic and leukaemogenic response in an experimental animal system. This general approach, i.e. the use of mouse-human chimaeras, may become an important focus of future studies on human radiation oncogenesis, in that it may allow the study of a range of transplanted human cells in murine host tissues.

208. On a more practical note, two relatively recently developed techniques may have application in the direct assay of radiation-associated somatic cell changes in radiation-exposed human groups. First, a novel technique now allows small samples of human blood to be assayed for the frequency of certain somatic mutations; this technique has been employed to examine residual *HPRT* and glycophorin gene mutations in atomic bomb survivors in Japan [A15, H14, H49, L7]. The technique may have broader application [U13], particularly if it becomes possible to identify gene-specific protein variants associated with haemopoietic neoplasms and to use flow-sorting techniques on blood samples in the same way as recently described for glycophorin A locus variants in bomb survivors [K9]. Secondly, a very powerful molecular technique, polymerase chain reaction (PCR) (see, e.g. [E8, F7, R11]), now allows rapid and specific gene analysis in samples comprising very few cells (conventional methods require much larger samples). If specific genomic changes associated with the initiation of human haemopoietic neoplasms are identified, this technique could, in principle, be employed in conjunction with new automated cytogenetics [P6] and *in situ* chromosome hybridization to detect pre-leukaemic conditions in radiation-exposed individuals.

209. Future molecular approaches to the detection of pre-neoplasia may not however be restricted to the easily accessible haemopoietic and lymphatic systems. In circumstances where a small number of exfoliate cells can be obtained from tissues, polymerase chain reaction techniques have sufficient resolving power to detect specific tumour-associated gene mutations, and this approach has been employed to identify, for example, *p53* gene mutations in cells present in the urine of bladder cancer patients [S44], as well as the *BCR-ABL* gene fusions in pre-leukaemias [M43]. The use of such new molecular and biochemical markers of neoplasia clearly have great potential for the early diagnosis of neoplasia, thereby allowing for early and more effective clinical intervention (see [M34]).

210. However, although the power and speed of polymerase chain reaction techniques may, in principle, be sufficient to contribute to the evaluation of the consequences of human radiation exposure, such as occurred in the immediate vicinity of the Chernobyl accident, it may be argued that there is, as yet, insufficient fundamental knowledge to effectively use the techniques as a screening procedure (see paragraphs 222 and 223).

211. Similar considerations apply in the case of automated cytogenetic screening for stable cytogenetic events, although here there can be no doubt that, if they had been available at the time, they would have greatly facilitated cytogenetic analyses in atomic bomb survivors in Japan (see, e.g. [A5, B17]). Despite these reservations, with increasing technical innovation, the collection and maintenance of archival neoplastic material from radiation-exposed human populations should be given serious consideration. The potential benefit to knowledge that would accrue from such coordinated procedures should not be underestimated.

B. METHODOLOGICAL AND CONCEPTUAL ASPECTS

212. Over many years, a substantial number of experimental animal models of induced neoplasia have been developed. In the long term these models will be most informative if quantitative studies on induced neoplasia are coupled with cellular, cytogenetic and molecular studies of the mechanisms of induction. It may, therefore, be argued that where possible, murine models of neoplasia should be favoured, since for the mouse well-established cytogenetics are available, a detailed genetic map, information on mouse-man genetic homologies, information on the cellular and

molecular aspects of developmental biology and an ever-increasing number of relevant recombinant DNA probes. It is not likely that an equivalent data resource will become available for another mammalian species. This having been said, the rat offers considerable advantages for the study of some model neoplasms, e.g. those of the lung. Where such benefits outweigh technical difficulties, it is obvious that the biological relevance of the animal system should take priority.

213. The degree of commitment that is required to resolve oncogenic mechanisms in whole animal systems is so considerable that animal neoplasms having the greatest relevance to human radiogenic neoplasms should be emphasized. Detailed histopathological comparisons of both neoplastic and pre-neoplastic conditions are crucial to the choice of such animal models. At present mechanistic studies are emphasizing induced leukaemias and osteosarcomas, and it is in these areas that the most rapid progress may be anticipated. However, with a view to representing the whole problem, it will be necessary to approach the broader task of understanding inductive mechanisms for a range of solid tumours, such as those of the breast, liver, kidney, lung and colon. Studies using *in vivo in vitro* transformation systems are already making an important contribution in this area (paragraphs 170-172), and a new dominant mutation predisposing mice to gastro-intestinal tract neoplasms [M25] may be of great value. Since this predisposing gene in the mouse is the homologue of the human *APC* gene (colorectal cancer susceptibility) [S48], there is also the prospect of exploring genetically determined susceptibility to radiation oncogenesis using animal models. Recent findings regarding rodent susceptibility loci for mammary [G23], kidney [W23], liver [B39] and myeloid [S43] neoplasms tend to heighten expectations in this important area of research. The further cytogenetic and molecular study of therapy-related neoplasms in man (see, e.g. [B38, R9, V5, W22]) also holds considerable promise and should be encouraged, particularly since the approach may not only provide direct information on early events in radiation oncogenesis but could also, in principle, contribute towards an understanding of human cancer susceptibility following radiation.

214. Of great importance is the prospect of being able to bridge experimentally between species. One strategy providing for such bridging would be to develop further the organ-specific *in vivo in vitro* approaches to rodent radiation oncogenesis. These experimental systems currently incorporate an *in vitro* phase to reflect the cellular mechanisms and quantitative radiation responses that underlie the *in vivo* induction of relevant radiogenic respiratory, mammary, thyroid and bone neoplasms. If it becomes possible to extend the *in vitro* approach to human tissues, perhaps using transplantation to immune-suppressed mice (paragraph 206), then a direct mechanistic and quantitative comparison between rodent and human *in vitro* phases could be achieved. Such studies, together with quantitative estimates of tumour induction in the whole animal, would then provide for direct extrapolation to mechanisms and risk in man. Existing rodent *in vivo in vitro* transformation systems, as well as providing a potential for the identification of initiating events for the neoplasm in question, have already highlighted the crucial role of hormonal and growth factor effects in the inductive process. The experimental elucidation and quantification of parallel effects in human tissues would be of substantial importance. Thus, while it may not be possible to realize the full theoretical potential of this strategy, it does provide a major goal for future studies and one that unites *in vivo* and *in vitro* approaches to the problem.

215. One important methodological implication of recent advances in cancer research concerns the limitations of current *in vitro* and *in vivo* models of induced neoplastic change for determining the loss of specific DNA sequences in induced tumours. Most of the existing rodent-based animal models of tumorigenesis utilize inbred or closely related hybrid strains of animals; similarly, animal cell culture systems from the mouse, the rat and the hamster are usually of inbred animal strain origin.

216. It has been shown that in outbred human populations, the loss of specific genes from tumours is most easily determined by the molecular analysis of loss of DNA heterozygosity [S19]. Highly inbred animal systems do not allow for this, since for any given autosomal gene the two copies are, by definition, identical, and distinguishing the complete deletion of one of them is not straightforward. Thus, while for the purpose of providing reproducible quantitative estimates of radiation-induced neoplasia it is logical to employ inbred animal strains, this choice can be seen to impose important methodological limitations to the resolution of the molecular mechanisms of oncogenesis. The extension of a selected number of animal and cellular models of radiation tumorigenesis to cross-bred animals, while not a trivial task, now seems to be essential. This will allow the gene deletion mechanism strongly suspected of being involved in radiation oncogenesis to be approached not only in respect to specific candidate genes but also using the more generally applicable new method of determining loss of heterozygosity by analysing highly dispersed and polymorphic DNA repeat sequences, such as polypurine-pyrimidine tracts [C25]. It is notable that this whole approach is already being successfully implemented for animal studies of chemical carcinogenesis (e.g. [B27]). The coupling of these techniques with the rapidly improving techniques of fluorescence *in situ*

hybridization (FISH) for single gene location and "painting" of chromosomes [F18, L19] should further enhance the prospects for resolv-ing mechanisms of radiation oncogenesis at both the molecular and cytogenetic levels.

217. In spite of the optimistic note sounded above, it is most likely that an increased understanding of the molecular mechanisms of oncogenesis will derive from detailed studies of neoplastic phenotypes induced experimentally at relatively high total doses. To what extent will it be possible to provide specific comment on the quantitative aspects of tumour induction at the low doses that are of prime concern in radiological protection?

218. The principal problem in providing any answer to this question concerns the great uncertainties involved in judging the probability with which a single neoplasia-initiated cell in tissue gives rise to a malignancy. As noted elsewhere, the current lack of understanding of the interactive factors that determine this probability is such that even a first approximate calculation is highly dependent on a series of necessary biological assumptions. Nevertheless, it is useful to at least illustrate such an estimation.

219. The assumptions made are as follows:

(a) the human body contains a total of 10^{14} cells;
(b) between one and ten cells per 10,000 have stem-like properties and, as such, are potential targets for induced neoplasia, i.e. 10^{10}-10^{11} target cells per individual;
(c) the typical neoplasia-initiating event centres on single gene inactivation/loss mutations in one of ten possible genes in target cells;
(d) the average acute low-LET induced mutation rate (per cell) for these genes is similar to that observed *in vitro* for the *HPRT* gene i.e. ~10^{-5} Gy^{-1};
(e) an acute low-LET exposure of 1 Gy to a population generates a 10% excess risk of malignancy, i.e. one excess tumour for ten exposed individuals;
(f) 1 Gy of low-LET radiation generates 1,000 electron tracks in each cell.

It may then be inferred that:

(a) one excess malignancy occurs within 10^{11}-10^{12} target cells receiving 1 Gy (i.e. 10×10^{10}-10^{11});
(b) the rate of target gene inactivations with ten possible target genes per cell is 10^{-4} Gy^{-1} (i.e. 10×10^{-5});
(c) the number of initiating mutations within these target cells giving rise to a single malignancy is 10^{7}-10^{8} (i.e. $10^{-4} \times 10^{11}$-10^{12});
(d) the probability that a single track intersection of a target cell will give rise to an excess malignancy is 10^{-14}-10^{-15} (i.e. 10^{-11}-$10^{-12} \div 1{,}000$).

In respect to low-LET background radiation of, say, 1 mGy per year, each cell will receive, on average, one track intersection per year. Therefore:

(a) the probability per year of malignancy will be around 10^{-4} [i.e. 10^{10}-10^{11} (target cells) $\times$ 10^{-14}-10^{-15} (probability of malignant conversion)];
(b) for 50 years of exposure this gives a lifetime risk of 5×10^{-3} (50×10^{-4}), i.e. 0.5%;
(c) assuming a natural cumulative frequency of malignancy of 20%, then 1 in 40 (20% ÷ 0.5%) cancers in the population are due to low-LET natural background radiation.

It should be noted that the illustrative calculations above take no account of dose and dose-rate effects for low-LET radiation and that (c) above does not depend on the assumptions made in respect to target cell numbers or on the probability of malignant conversion.

220. The realism of such calculations is, however, highly questionable, depending as it does on a series of biological estimates of frequency, each of which may be incorrect by an order of magnitude or more. However, the exercise points out some questions that need answers from research in cellular and molecular biology if the problems of estimating risk at low doses are to be solved. The most important questions centre on (a) the number of target cells in different tissues; (b) the number of relevant target genes in cells (this may well be dependent on cell type and tissue); (c) the mutagenic mechanism of initiation and the induced mutation rate for different target genes; (d) the mechanisms governing the probability that a given initiated cell will progress to full malignancy.

221. Further to this, epidemiological observations of constant relative cancer risk over time in the atomic bomb survivors in Japan [L20], together with the experimental data outlined in this Annex, imply that radiation acts principally at an early phase in oncogenesis, probably at the initiation stage. Thus, for excess cancer in an irradiated population, the efficiency with which the initiating mutation is induced may be a major factor in the contributions specific neoplasms make to that excess. In this respect, the absence of evidence for any measurable excess of chronic lymphocytic leukaemia (CLL) might be explained by a stringent requirement for CLL initiation by gene-specific chromosome translocation, which has been shown to be involved in the early development of some human T-cell neoplasms [R3]. It has been argued in paragraphs 74 and 75 that such events present very small molecular targets for radiation action, and, on this basis, the T-cell leukaemogenic

potential of radiation might be expected to be low. Experimental evidence for the induction of BCR-ABL gene fusion by high dose x-irradiation has, however, been presented [I3]. Other explanations for this epidemiological observation are also possible. For example, a high sensitivity of T-cell targets to radiation-induced apoptotic death might efficiently remove initiated cells from irradiated target T-cell populations; an explanation of this general form has recently been offered in respect of the differential cancer incidence in the large and small bowel [P15]. It is also important to consider whether cellular and molecular techniques have the potential to distinguish radiation-induced neoplasms from those arising spontaneously or from other forms of carcinogen exposure.

222. As noted in paragraphs 188 and 189, the specificity of many genotoxic chemical agents in their interaction with nucleic acids may provide a characteristic mutational signature in DNA, which infers that cellular exposure to this agent has taken place. In cases where target genes for neoplasia induction have been identified it thus becomes possible to catalogue the DNA base sequence of such mutant genes in human tumours, with a view to identifying characteristic DNA base changes indicative of the action of agents of known mutagenic and carcinogenic potential.

223. Inactivating mutations in the *p53* tumour suppressor gene are strongly suspected of being involved in the early development of a wide range of human solid tumours, and recent analyses of *p53* gene structure in human carcinomas are beginning to provide evidence that tumours of divergent aetiologies arising at various sites do not exhibit the same spectra of mutational change to this gene [C31, H30, H46] (Figure IV). Characteristic *p53* mutations are most evident for hepatocellular carcinoma associated with environmental exposure to aflatoxin B1 [B23, M34, O4] and skin tumours associated with sunlight exposure [B36], but within this rapidly developing field of molecular epidemiology (see also [M34]), parallel advances in relation to other chemical carcinogens and other target genes should be anticipated.

224. Ionizing radiation does not, however, show the same degree of mutagenic specificity as chemical agents and, on current evidence, it may tend to act principally through DNA deletion/ rearrangement rather than base pair change. Nevertheless, in a recent study [V10] rare *p53* gene deletions and base pair changes were shown to characterize radon-associated lung cancer in uranium miners; it may be that this unusual mutational spectrum is associated with alpha-particle-induced damage to the *p53* gene of target respiratory cells. Mutations of the *p53* gene have also been characterized in radiation-associated human sarcomas [B38], and *K-ras* mutation has been shown to be more frequent in radiation-associated follicular carcinomas of the thyroid than in spontaneously arising neoplasms [W22]. In neither case, however, is it possible to be certain that these mutations were specifically induced by the radiation. Even greater uncertainty surrounds the interpretation of the *MOS* gene polymorphisms in normal tissue of radium exposed individuals [H47].

225. The molecular characterization of DNA base pair changes in tumour-associated genes using polymerase chain reaction (PCR) as employed in many of the above studies is a flexible, rapid and reasonably straightforward procedure [E8]. In contrast, the molecular characterization of DNA deletions, particularly those that extend outside the gene of interest, is more difficult, because it demands a detailed knowledge of gene and gene-flanking sequences. While small intragenic DNA deletions will tend to encompass or disturb the reading frame of those sequences encoding functional protein sites, for deletions resulting in loss of the whole gene there is no *a priori* reason why the deletion breakpoints should be characteristic of that gene or of the mutagenic agent in question.

226. There is, however, limited evidence that radiation-induced DNA deletions in mammalian cells may involve specific breakpoints. Whether such deletion breakpoints might be characteristic of radiation-induced damage remains an open question, but the presence of DNA repeat sequence motifs at some DNA sequence breakpoints in somatic cell mutants [M33] and, albeit less certainly, in some neoplasia-associated chromosomal changes [B16, B32] does allow for this possibility. However, on current knowledge, it seems highly unlikely that DNA events of this or other types will be unique to radiation-induced damage (see, e.g. [A14, M33]); if so, this will tend to limit the general utility of molecular analyses for the purposes of discriminating between radiation-induced, chemically induced and spontaneously arising neoplasms in human populations. Nevertheless, the rate of progress in this whole area is such that at present it is prudent to reserve judgement on this crucial issue. Indeed, the unusual *p53* mutation spectrum observed in the lung tumours of uranium miners [V10] hints at the utility of this approach in respect to the *p53* gene.

227. From a predictive viewpoint, the identification of initiating events for radiation oncogenesis is not limited to its potential to discriminate between tumours of inductive and spontaneous origins. Even if it only becomes possible to identify in a more general

fashion the principal categories of induced DNA damage and target genes associated with radiation oncogenesis, there still remain important implications for future research.

228. First, the provision of such data may allow the design of quantitatively reliable cellular systems that mimic or reflect the relevant molecular events. Secondly, it would become possible to specifically explore the post-irradiation repair of the relevant initial DNA lesions (see [W10]), particularly if these were to involve some form of gene or DNA sequence specificity. Thirdly, using Monte Carlo simulations (see, e.g. [N22]) of radiation track structure, it might become possible to link the induction of the initial, potentially oncogenic damage with specific energy loss events in DNA, thus allowing a biologically more realistic microdosimetric extrapolation of radiation effects.

229. However, as noted in paragraph 5, a resolution of the mechanisms of radiation oncogenesis would also provide an essential input into the whole field of cancer research. It is only through such integration that the whole of the complex multi-stage oncogenic process will be better understood, allowing the formulation of realistic cellular and molecular models to replace, or at least complement, the existing empirical approaches to cancer risk projection.

CONCLUSIONS

230. Ionizing radiation induces a broad spectrum of neoplasms in both man and experimental animals. The basic mechanisms of the induction, promotion and progression processes are not yet well understood; however, some points from considerations in this Annex can be summarized.

231. Point mutation, chromosomal translocations and deletions may all play roles in the initiation and progression of neoplasia. Some of these changes are shared by different neoplasms. Others appear to be restricted to certain tumour types.

232. Studies on human susceptibility to neoplasia associated with inherited defects in DNA metabolism or the loss of tumour suppressor genes are making important contributions to the understanding of oncogenic mechanisms and the different modes of inheritance of cancer-proneness in the human population.

233. Neoplastic initiation as a consequence of specific somatic mutation is thought to provide target cells with some degree of proliferative dysregulation. These events may not be phenotypically expressed as pre-neoplastic conditions until a promotional proliferative signal is received.

234. Point mutations or chromosomal translocations that activate proto-oncogenes or mutations that lead to loss of function of tumour suppressor genes may be considered as potential initiating events for oncogenesis. Relative target sizes for the induction of these events by radiation would tend to favour tumour suppressor genes as the most radiosensitive targets.

235. Strong evidence for tumour suppressor genes as targets for oncogenic initiation has been obtained in studies of germ-line mutations that predispose to cancer. Some of these genes appear to play a central role in the control of the cell cycle.

236. Neoplastic promotion arises mainly as a consequence of induced changes in gene expression in initiated cells. It may occur through the action of specific chemicals, hormones and growth factors on cell surface receptors, and the ensuing proliferative responses may favour the establishment of pre-neoplastic clones in tissues. In some cases the enzyme protein kinase C is thought to mediate promotional processes; changes in intercellular communication may be an important aspect of promotion, but the induction of endogenous DNA damage is also a feature of some strong promoting agents.

237. Neoplastic progression is a complex, multifaceted process that appears to involve a series of subsequent genetic changes within the evolving pre-neoplastic clone of cells; these changes may include changes in growth rate, growth factor response, invasiveness and metastatic potential. The development of intrinsic genomic instability in neoplastic cells may provide the cellular heterogeneity in tumours that drives the progressional process.

238. Both physical and biological factors influence radiation oncogenesis, as shown in experimental animal systems, but few specific details of cellular mechanisms have emerged from quantitative studies. *In vitro* cell transformation studies have highlighted some aspects of cellular oncogenic mechanisms (frequency of potentially initiating events; effects of LET, dose rate and repair; and neoplastic promotional mechanisms). While *in vitro* studies on specific gene involvement in radiation-induced cell transformation

have yet to yield detailed information on the molecular mechanisms of oncogenic initiation by radiation, novel transformation systems, already making an impact in chemical carcinogenesis, show considerable potential.

239. Cell mutagenesis and DNA repair data may be used to argue that oncogenic initiation following ionizing radiation may occur more frequently through DNA rearrangement/deletion than through point mutation, but this may well depend on the gene in question. Chromosomes or cellular oncogenes have been shown to be changed in a number of radiation-induced experimental neoplasms, but the temporal position and role of most of these changes are uncertain. In the case of murine acute myelogenous leukaemia, the induced initiating event is thought to be a specific chromosomal rearrangement/deletion leading to the loss of critical genes. In contrast, other studies suggest that point mutations in *ras* proto-oncogenes may be initiating events for radiation-induced murine thymic lymphomas. Some progress is also being made in the molecular characterization of radiation-induced osteosarcomas and tumours of the skin, breast and lung.

240. There are a number of mechanistic differences between chemical and radiation oncogenesis, including differences in DNA damage induction/repair and the activational/degradative processes that influence the carcinogenicity of many chemicals. An important difference in oncogenic mechanisms may derive from the relative efficiency with which chemical carcinogens and radiation induce point mutations. It is suggested that radiation and chemical oncogenesis are subject to differing degrees of human genetic variation.

241. Molecular studies with certain human tumours have drawn attention to the possibility that specific point mutation in tumour genes may serve as a signature of prior exposure to chemical carcinogens. On current knowledge, the same approach may not be equally informative for ionizing radiation exposures, but the possible preferential involvement of DNA repeat sequences in radiation-induced DNA deletion and of certain DNA bases in point mutations may provide a focus for further study.

242. The development of new methods of investigation promises further advances in understanding. Novel *in vitro* cell transformation systems and cellular/molecular studies with these and with neoplasms induced in outbred animals may be a productive area for the study of oncogenic mechanisms. Recent advances in the construction of transgenic and chimaeric mice, new cellular cytogenic and molecular approaches to the assay of *in vivo* somatic cell changes, and studies on radiation-associated human tumour cells appear to have great potential.

243. In order to take advantage of modern methods of cell and molecular biology and to anticipate further technical advances, tumour material obtained from radiation-exposed human populations should be systematically stored. This material may prove to be a very important resource for future molecular studies of oncogenic mechanisms.

Table 1
The function and chromosomal location of some human oncogenes
[A13, B1, C7, M36, S63]

Classification	*Oncogene*	*Chromosomal location*	*Product/function* [a]
Growth factor	*SIS*	22q 12-13	PDGF β chain
	HST-1	11q 13	FGF family
	INT-2	11q 13	FGF family
Tyrosine kinase	*ERB B1*	7q 12-13	EGF receptor
	ERB B2/NEU	17q 21	EGF receptor-like
	FMS	5q 34	CSF-1 receptor
	KIT	4q 11-12	SCF receptor
	SRC	20q 12-13	Expressed in nerve cells
	YES	18q 21	Expressed in spleen and brain
	FPS/FES	15q 25-26	Expressed in granulocytes/monocytes
	FYN	6q 21	Bound to T-cell receptor
	LCK	1q 32-35	Bound to CD4/CD8 complex
	BCR/ABL	22q 11/9q 34	Chaemeric protein (CML)
Serine/threonine kinase	*MOS*	8q 11	Oocyte maturation
	RAF/MIL	3p 25	Function downstream of *RAS*
GTP binding protein [a]	*H-RAS*	11p 15	Message transduction, mutated in many tumour types
	K-RAS	12p 12	
	N-RAS	1p 13-22	Amplified in neuroblastoma
Regulatory factors	*JUN*	1p 31-32	Binds Fos (transcription factor)
	FOS	14q 21-31	Binds Jun (transcription factor)
	MYB	6q 22-24	Expressed in erythroblasts
	MYC	8q 24	Transcription/replication factor
	N-MYC	2p 23-24	Amplified in neuroblastoma
	ERBA	17q 11-21	Thyroid hormone receptor
	PML/RARα	15q 21/17q 11	Chaemeric protein (transcription factor)
	HFL/E2A	17q 22/19p 13	Chaemeric protein (transcription factor)
	PBX/E2A	1q 23/19p 13	Chaemeric protein (transcription factor)
	HOX11	10q 24	Transcription factor
Others	*BCL-2*	18 q 21	Control of apoptosis

[a] GTP: guanosine triphosphate; PDGF: platelet-derived growth factor; FGF: fibroblast growth factor; EGF: epidermal growth factor; CSF: colony stimulating factor; SCF: stem cell factor; CML: chronic myelogenous leukaemia.

Table 2
Suppressor genes in human tumours
[W14]

Detected by cell hybridization or chromosome transfer	
Chromosomal location	*Tumour type*
1p	Neuroblastoma
3p	Renal carcinoma
6	Endometrial carcinoma
9	Endometrial carcinoma
11	Neuroblastoma; cervical carcinoma; Wilms' tumour

Detected through loss of heterozygosity or direct molecular probing	
Chromosomal location	*Tumour type*
1p	Melanoma; multiple endocrine neoplasia type 2; neuroblastoma; medullary thyroid carcinoma; pheochromocytoma; ductal cell carcinoma
1q	Breast carcinoma
3p	Small cell lung carcinoma; adeno carcinoma of lung; cervical carcinoma; von Hippel-Lindau disease, renal cell carcinoma
5q	Familial adenomatous polyposis; colorectal carcinoma
9q	Bladder carcinoma
10q	Astrocytoma; multiple endocrine neoplasia type 2
11p	Wilms' tumour; rhabdomyosarcoma; breast carcinoma; hepatoblastoma; transitional cell bladder carcinoma, lung carcinoma
11q	Multiple endocrine neoplasia type 1
13q	Retinoblastoma; osteosarcoma; small cell lung carcinoma; ductal breast carcinoma; stomach carcinoma; bladder carcinoma; colon carcinoma
17p	Small cell lung carcinoma; colorectal carcinoma; breast carcinoma, osteosarcoma; astrocytoma squamous cell lung carcinoma
17q	Neurofibromatosis type 1
18q	Colorectal carcinoma
22q	Neurofibromatosis type 2; meningioma; acoustic neuroma; pheochromocytoma

Table 3
Consistent chromosomal changes in leukaemias and lymphomas
[S45]

Neoplasm	*Chromosome aberration*
Leukaemias	
Chronic myeloid leukaemia	t(9;22)(q34;q11)
Acute myeloid leukaemia M1 M2 M3 M4 with abnormal eosinophils M5a M1, M2, M4 with increased basophils M1, M2, M4, M5, M6	 t(9;22)(q34;q11) t(8;21)(q22;q22) t(15;17)(q22;q12) inv(16)(p13q22) t(9;11)(p22;q23) t(6;9)(p23;q34) Monosomy 5/del(5q) Monosomy 7/del(7q) Trisomy 8
Chronic lymphocytic leukaemia	t(11;14)(q13;q32) Trisomy 12
Acute lymphocytic leukaemia	t(9;22)(q34;q11) t(4;11)(q21;q23)
Acute B-cell leukaemia	t(8;14)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11)
Acute T-cell leukaemia	inv(14)(q11q32) t(14;14)(q11;q32) t(8;14)(q24;q11) t(10;14)(q24;q11) t(11;14)(p13;q11)
Lymphomas	
Burkitt's lymphoma	t(8;14)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11)
Small non-cleaved cell lymphoma, large cell immunoblastic lymphoma	t(8;14)(q24;q32)
Follicular small cleaved cell lymphoma	t(14;18)(q32;q21)
Small cell lymphocytic lymphoma	Trisomy 12
Small cell lymphocytic transformed to diffuse large cell lymphoma	t(11;14)(q13;q32)
Polycythaemia vera	
Polycythaemia vera	del(20q)

Table 4
Consistent chromosomal changes in solid tumours
[S45]

Neoplasm	*Chromosome aberration*
Alveolar rhabdomyosarcoma	t(2;13)(q37;q14)
Bladder carcinoma	Structural changes of 1 i(5p) Structural changes of 11
Breast carcinoma	Structural changes of 1 t/del(16q)
Ewing's sarcoma, Askin's tumour/neuroepithelioma	t(11;22)(q24:q12)
Kidney carcinoma	t/del(3)(p11-21) t(5;14)(q13;q22)
Large bowel cancer	Structural changes of 1 Trisomy 7 Structural changes of 17
Lipoma	t(12)(q13-14)
Malignant melanoma	t/del(1)(p12-22) t(1;19)(q12;p13) t/del(6q)/i(6p) Trisomy 7
Meningioma	Monosomy 22
Mixed salivary gland adenoma	t(3)(p21) t/del(8)(q12) t/del(12)(q13-15)
Myxoid liposarcoma	t(12;16)(q13-14;p11)
Neuroblastoma	del(1)(p31-32)
Ovarian carcinoma	t(6;14)(q21;q24) Structural changes of 1
Prostatic carcinoma	del(7)(q22) del(10)(q24)
Retinoblastoma	Structural changes of 1 i(6p) del(13)(q14)/-13
Small cell lung carcinoma	del(3)(p14p23)
Synovia sarcoma	t(X;18)(p11;q11)
Testicular teratoma/seminoma	i(12p)
Uterine carcinoma	Structural and numerical changes of 1
Wilms' tumour	Structural changes of 1 t/del(11)(p13)

Table 5
Viral associations in human oncogenesis
[W16]

Virus (type)	*Associated tumours*	*Other risk factors*
T-cell viruses (RNA)	T-cell leukaemia/lymphoma	[a]
Lentiviruses (RNA)	Kaposi's sarcoma, lymphoma	Concurrent viral infections Immune deficiency
Herpes virus (DNA)		
Epstein-Barr virus	Burkitt's lymphoma	Malaria
	Immunoblastic lymphoma	Immune deficiency
	Nasopharyngeal carcinoma	Dietary components HLA genotype
Herpes simplex	Cervical neoplasia (?)	Papilloma viruses, tobacco
Cytomegalovirus	Kaposi's sarcoma (?)	Immune deficiency
	Cervical neoplasia (?)	HLA genotype
Hepatitis B viruses (DNA)	Liver cancer	Aflatoxin, alcohol, tobacco
Papilloma viruses (DNA)	Cervical and anal neoplasia	Tobacco, herpes virus and immune deficiency
	Laryngeal carcinoma	X rays, tobacco
	Skin carcinoma	Sunlight, genetic factors influencing skin pigmentation

[a] Considerable uncertainty.

Table 6
Estimates of the frequency of some cancer-prone human mutations

Mutation	*Principal neoplasms*	*Phenotypic manifestation*	*Chromosomal location*	*Frequency per live births*
Ataxia-telangiectasia (homozygotes)	Leukaemias, lymphomas	Autosomal recessive	11/a/	~ 1 per 100,000
Ataxia-telangiectasia (heterozygotes)	Mammary carcinomas	Autosomal recessive	11/a/	1 per 100 [a]
Retinoblastoma	Retinoblastoma, osteosarcoma	Autosomal dominant	13q	~ 1 per 20,000
Wilms-aniridia	Nephroblastoma	Autosomal dominant	11p	~ 1 per 30,000
Basal cell nevus syndrome	Skin carcinoma, medulloblastoma	Autosomal dominant	9q	< 1 per 50,000
Neurofibromatosis	Neurofibromas, CNS tumours	Autosomal dominant	17q	~ 1 per 5,000
Familial adenomatous polyposis	Colorectal carcinomas	Autosomal dominant	5q	~ 1 per 10,000
Non polyposis colorectal cancer	Colorectal carcinomas	Autosomal dominant	2p	[b]
Familial breast cancer	Mammary and ovarian carcinomas	Autosomal dominant	17q	[c]
Li-Fraumeni syndrome	Wide range of malignancies	Autosomal dominant	17p	< 1 per 50,000

[a] The number and location of ataxia-telangiectasia genes has yet to be conclusively determined; the number of genetic complementaion groups in ataxia-telangiectasia is the major determinant of heterozygote frequency.
[b] Accounts for around 14% of colorectal cancers in the population.
[c] It has been estimated that as many as 1 in 200 women carry one of a number of genes that predispose breast cancer (paragraph 127).

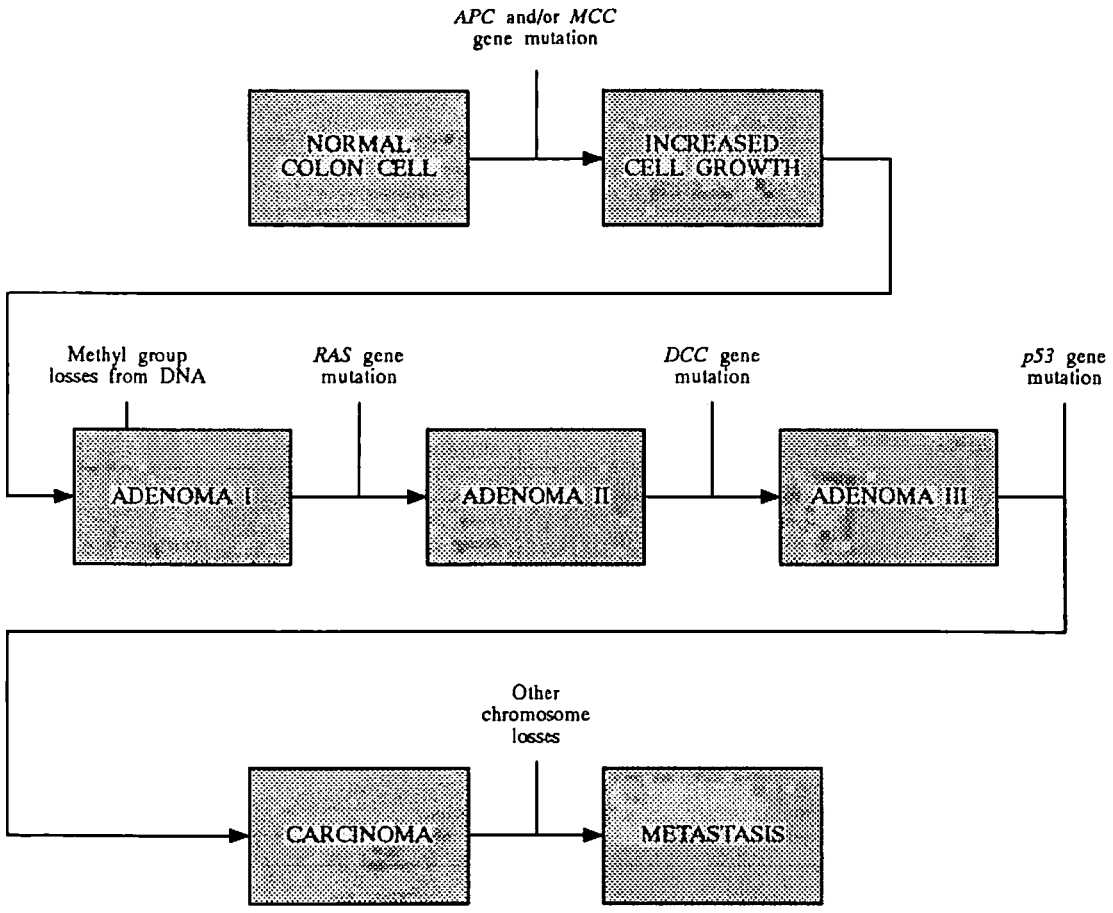

Figure I.
Multi-step colorectal carcinogenesis.
[F8]

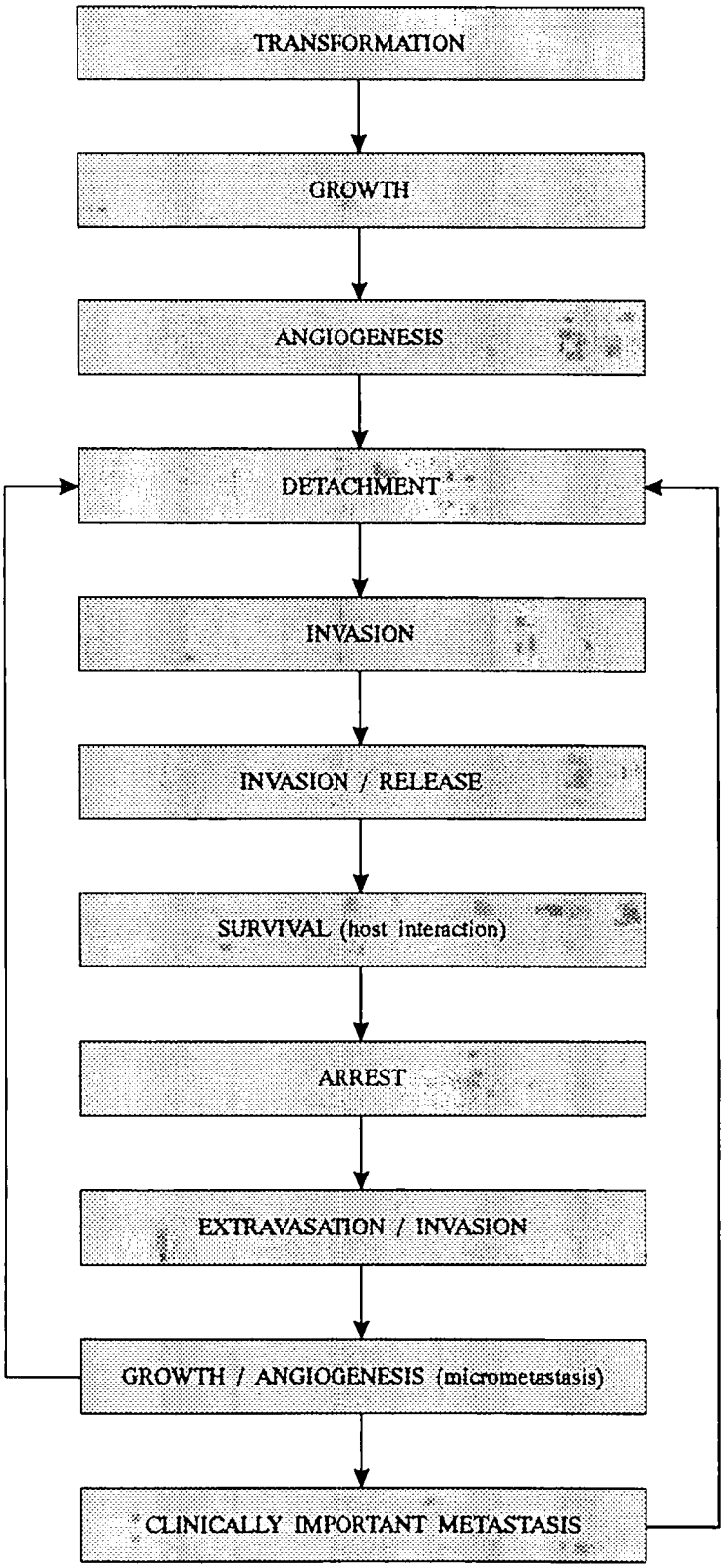

Figure II.
Possible steps involved in the metastatic spread of solid tumours.
[H42]

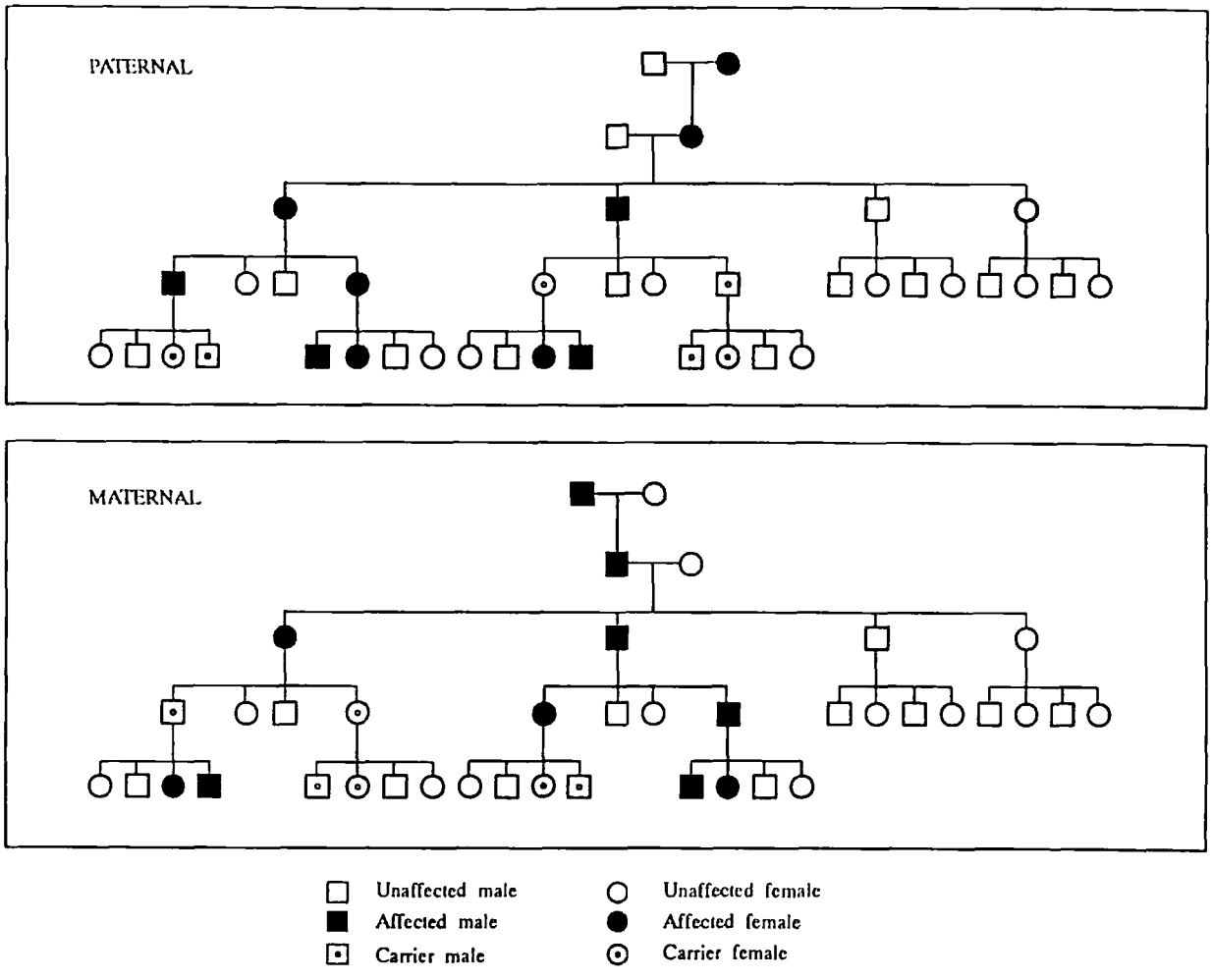

Figure III.
Idealized pedigrees for maternal and paternal genomic imprinting.
[H19]

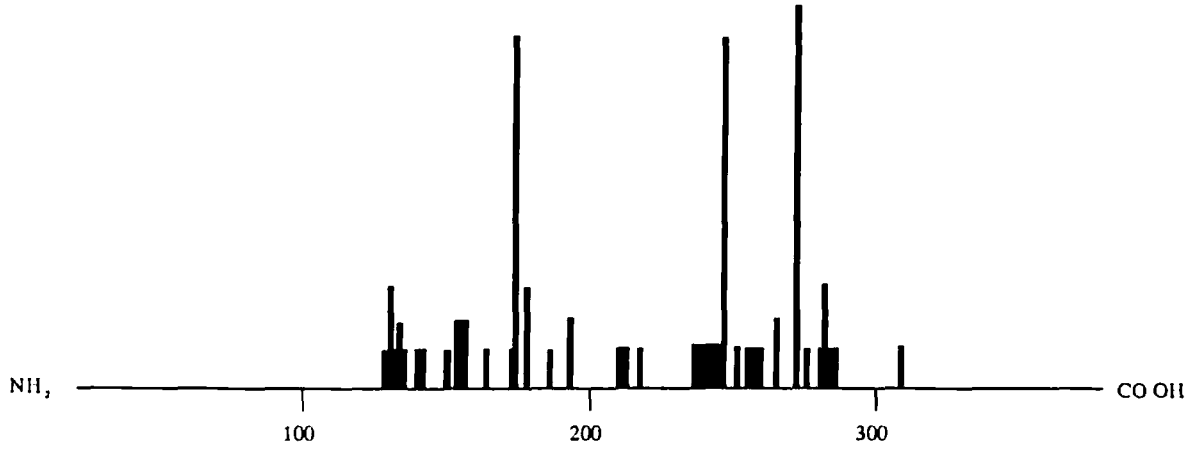

Figure IV.
The distribution of *p53* tumour cell gene mutations in man observed in a wide range of human neoplasms. The horizontal axis represents the 393 condons of the *p53* gene from the NH_2 to the CO OH terminals.
[L13]

References

A1 Alberts, B. (ed.). Molecular Biology of the Cell. Garland, New York, 1988.

A2 Ames, B.N. and J. McCann. Validation of the salmonella test. Cancer Res. 41: 4192-4195 (1981).

A3 Arlett, C.F. The radiosensitivity of cultured human cells. p. 424-430 in: Radiation Research. Volume 2. (E.M. Fielden et al., eds.) Taylor and Francis, London, 1987.

A4 Anisimov, V.N. Carcinogenesis and aging. Adv. Cancer Res. 40: 365-415 (1983).

A5 Awa, A.A. Chromosome aberrations in A-bomb survivors in Hiroshima and Nagasaki. p. 180-190 in: Chromosome Aberrations: Basic and Applied Aspects. (G. Obe and A.T. Natarajan, eds.) Springer Verlag, Berlin, 1990.

A6 Anathaswamy, H.N. and W.E. Pierceall. Molecular mechanisms of ultraviolet radiation carcinogenesis. Photochem. Photobiol. 52: 1119-1136 (1990).

A7 Ayesh, R., J.R. Idle, J.C. Richie et al. Metabolic oxidation phenotypes as markers for susceptibility to lung cancer. Nature 312: 169-170 (1984).

A8 Arch, R., K. Wirth, M. Hofman et al. Participation in normal immune responses of a metastasis-inducing splice variant of CD44. Science 257: 682-685 (1992).

A9 Askew, D.S., C. Bartholomew, A.M. Buchberg et al. His-1 and His-2: Identification and chromosomal mapping of two common sites of integration in a myeloid leukaemia. Oncogene 6: 2041-2047 (1991).

A10 Ames, B.N. Endogenous DNA damage as related to cancer and ageing. Mutat. Res. 214: 41-46 (1989).

A11 Ames, B.N. and L.S. Gold. Too many rodent carcinogens: mitogenesis increases mutagenesis. Science 249: 970-971 (1990).

A12 Arlett, C.F. Human cellular radiosensitivity - the search for the diagnostic holy grail or a poisoned chalice. Adv. Radiat. Biol. 16: 273-292 (1992).

A13 Aaronson, S.A. Growth factors and cancer. Science 254: 1146-1153 (1991).

A14 Akagi, T., K. Morota, H. Iyehara-Ogawa et al. Mutational specificity of the carcinogen 3-amino-1-methyl-5H-pyrido[4,3-b]-indole in mammalian cells. Carcinogenesis 11: 841-846 (1990).

A15 Akiyama, M., K. Nakamura, M. Hakoda et al. Somatic cell mutation in atomic bomb survivors. J. Radiat. Res. (Suppl): 278-282 (1991).

A16 Aaltonen, L.A., P. Peltomaki, F.S. Leach et al. Clues to the pathogenesis of familial colorectal cancer. Science 260: 812-815 (1993).

B1 Bishop, J.M. The molecular genetics of cancer. Science 235: 305-311 (1987).

B2 Bonavida, B. and S.C. Wright. Multistage model of natural killer cell mediated cytotoxicity involving NKCF as a soluble cytotoxic mediators. Adv. Cancer Res. 49: 169-187 (1987).

B3 Borek, C., A. Ong and H. Mason. Distinctive transforming genes in x-ray transformed mammalian cells. Proc. Natl. Acad. Sci. (USA) 84: 794-798 (1987).

B4 Breckon, G., A. Silver and R. Cox. Consistent chromosome changes in radiation-induced murine leukaemias. p. 179-184 in: Kew Chromosome Conference III. (P.E. Brandham and M.D. Bennett, eds.) HMSO, London, 1988.

B5 Blatt, C. and L. Sachs. Deletion of a homeobox gene in myeloid leukaemias with a deletion of chromosome 2. Biochem. Biophys. Res. Commun. 156: 1265-1270 (1988).

B6 Barbacid, M. Ras genes. Annu. Rev. Biochem. 56: 779-878 (1987).

B7 Borek, C. Oncogenes in human cell transformation in vitro. p. 223-230 in: Cell Transformation Systems and Radiation Induced Cancer in Man. (K. Chadwick, B. Barnhart and C. Seymour, eds.) Adam Hilger, Bristol, 1989.

B8 Bartsch, H. and R. Montesano. Mutagenic and carcinogenic effects of vinyl chloride. Mutat. Res. 32: 93-114 (1975).

B9 Bryant, P.E. and A.C. Riches. Chromosome damage and oncogenic transformation in mouse 10T1/2 cells following restriction endonuclease treatment. p. 309-314 in: Cell Transformation Systems and Radiation Induced Cancer in Man. (K. Chadwick, B. Barnhart and C. Seymour, eds.) Adam Hilger, Bristol, 1989.

B10 Boehm, T., L. Mengel-Gaw, U.R. Kees et al. Alternating purine-pyrimidine tracts may promote chromosomal translocations seen in a variety of human lymphoid tumours. EMBO J. 8: 2621-2631 (1989).

B11 Bowden, G.T., D. Jaffe and K. Andrews. Biological and molecular aspects of radiation carcinogenesis in mouse skin. Radiat. Res. 121: 235-241 (1990).

B12 Bremner, R. and A. Balmain. Genetic changes in skin tumour progression: correlation between presence of a mutant ras gene and loss of heterozygosity on mouse chromosome 7. Cell 61: 407-417 (1990).

B13 Boldogh, I., S. AbuBakar and T. Albrecht. Activation of proto-oncogenes: An immediate early event in human cytomegalovirus infection. Science 247: 561-563 (1990).

B14 Bridges, B.A. Some DNA repair-deficient syndromes and their implications for human health. p. 47-57 in: Environmental Mutagens and Carcinogens. (T. Sugimura et al., eds.) A.R. Liss, New York, 1982.

B15 Boettiger, D., S. Anderson and T.M. Dexter. Effect of src infection on long term marrow cultures: increased self-renewal of haemopoietic progenitor cells without leukaemia. Cell 36 (3): 763-773 (1984).

B16 Breckon, G., D. Papworth and R. Cox. Murine radiation myeloid leukaemogenesis: a possible role for radiation sensitive sites on chromosome 2. Genes, Chrom. Cancer 3: 367-375 (1991).

B17 Bender, M.A., A.A. Awa, A.L. Brooks et al. Current status of cytogenetic procedures to detect and quantify previous exposures to radiation. Mutat. Res. 196: 103-159 (1988).

B18 Bos, J.L. The ras gene family and human carcinogenesis. Mutat. Res. 195: 225-271 (1988).

B19 Baker, S.J., S. Mankowitz, E.R. Fearon et al. Suppression of human colorectal carcinoma cell growth by wild type p53. Science 250: 912-915 (1990).

B20 Blackburn, E.H. Structure and function of telomeres. Nature 350: 569-572 (1991).

B21 Basset, P., J.P. Bellocq, C. Wolf et al. A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature 348: 699-704 (1990).

B22 Bannasch, P., D. Keppler and G. Weber (eds.). Liver Cell Carcinoma. Kluwer Academic, Dordrecht, 1989.

B23 Bressac, B., M. Kew, J. Wands et al. Selective G to T mutations of p53 gene in hepatocellular carcinoma in southern Africa. Nature 350: 429-431 (1991).

B24 Bodmer, W.F. Inherited susceptibility to cancer. p. 98-124 in: Introduction to the Cellular and Molecular Biology of Cancer. (L.M. Franks and N.M. Teich, eds.) Oxford University Press, Oxford, 1991.

B25 Beverley, P. Immunology of cancer. p. 406-433 in: Introduction to the Cellular and Molecular Biology of Cancer. (L.M. Franks and N.M. Teich, eds.) Oxford University Press, Oxford, 1991.

B26 Breckon, G. and R. Cox. Alpha particle leukaemogenesis. Lancet 335: 656-657 (1990).

B27 Biedermann, K.A., J.R. Sun, A.J. Giaccia et al. Scid mutation in mice confers hypersensitivity to ionizing radiation and a deficiency in DNA double-strand break repair. Proc. Natl. Acad. Sci. (USA) 88: 1394-1397 (1991).

B28 Broerse, J.J. and D.W. van Bekkum (eds.). The relevance of animal models of radiation carcinogenesis in the light of development in molecular biology. Radiat. Environ. Biophys. 30: 161-257 (1991).

B29 Bianchi, A.B., N.M. Navone, C.M. Aldaz et al. Overlapping loss of heterozygosity by mitotic recombination on mouse chromosome 7F1-ter in skin carcinogenesis. Proc. Natl. Acad. Sci. (USA) 88: 7590-7594 (1991).

B30 Barnes, D.M., A.M. Hanby, C.E. Gillett et al. Abnormal expression of wild type p53 protein in normal cells of a cancer family patient. Lancet 340: 259-263 (1992).

B31 Benjamin, M.B. and J.B. Little. X rays induce inter-allelic homologous recombination at the human thymidine kinase gene. Mol. Cell. Biol. 12: 2730-2738 (1992).

B32 Bouffler, S.B., A. Silver, D. Papworth et al. Murine radiation myeloid leukaemogenesis: the relationship between interstitial telomere-like sequences and chromosome 2 fragile sites. Genes, Chrom. Cancer 6: 98-106 (1993).

B33 Braithwaite, O., W. Bayona and E.W. Newcomb. p53 mutations in C57BL/6J murine thymic lymphomas induced by gamma-irradiation and N-methyl-nitrosourea. Cancer Res. 52: 3791-3795 (1992).

B34 Bratthauer, G.L. and T.G. Fanning. Active LINE-1 retrotransposons in human testicular cancer. Oncogene 7: 507-510 (1992).

B35 Blattner, W.A. (ed.). Epidemiology of HTLV-I and associated diseases. p. 251-265 in: Human Retrovirology: HTLV. Raven Press, New York, 1990.

B36 Brash, D.E., J.A. Rudolph, J.A. Simon et al. A role for sunlight in skin cancer: UV-induced p53 squamous cell carcinoma. Proc. Natl. Acad. Sci. (USA) 88: 10124-10128 (1991).

B37 Beer, D.G. and H.C. Pitot. Proto-oncogene activation during chemically induced hepatocarcinogenesis in rodents. Mutat. Res. 220: 1-10 (1989).

B38 Brachman, D.G., D.E. Hallahan, M.A. Beckett et al. p53 gene mutations and abnormal retinoblastoma protein in radiation-induced human sarcomas. Cancer Res. 51: 6393-6396 (1991).

B39 Buchman, A., R. Bauer-Hofmann, J. Mahr et al. Mutational activation of the c-Ha-ras gene in liver tumours of different rodent strains: correlation with susceptibility to hepatocarcinogenesis. Proc. Natl. Acad. Sci. (USA) 88: 911-915 (1991).

B40 Bourne, H.R. and H.E. Varmus. Oncogenes and cell proliferation. Curr. Opin. Genet. Dev. 2: 1-3 (1992).

B41 Barlow D.P. Methylation and imprinting: From host defense to gene regulation. Science 260: 309-310 (1993).

B42 Bouffler, S., A. Silver and R. Cox. The role of DNA repeats and associated secondary structures in genomic instability and neoplasia. BioEssays 15: 409-412 (1993).

B43 Billen, D. Response to comments of K.F. Baverstock and J.F. Ward. Radiat. Res. 126: 388-389 (1991).

B44 Billen, D. Spontaneous DNA damage and its significance for the "negligible" dose controversy in radiation protection. Radiat. Res. 124: 242-245 (1990).

B45 Baverstock, K.F. Comments on commentary by D. Billen. Radiat. Res. 126: 383-384 (1991).

C1 Curtis, S.B. The cellular consequences of binary misrepair and linear fixation of initial biophysical damage. p. 312-317 in: Radiation Research. Volume 2. (E.M. Fielden et al., eds.) Taylor and Francis, London, 1987.

C2 Curtis, S.B. Lethal and potentially lethal lesions induced by radiation - a unified repair model. Radiat. Res. 106: 252-270 (1985).

C3 Cox, R. Comparative mutagenesis in cultured mammalian cells. p. 33-46 in: Progress in Environmental Mutagenesis. (M. Alacevic, ed.) Elsevier, Amsterdam, 1980.

C4 Cooper, G.M. Cellular transforming genes. Science 218: 801-806 (1982).

C5 Clifton, K.H., K. Kamiya, K.M. Groch et al. Quantitative studies of rat mammary and thyroid clonogens, the presumptive cancer progenitor cells. p. 135-146 in: Cell Transformation Systems and Radiation Induced Cancer in Man. (K. Chadwick, B. Barnhart and C. Seymour, eds.) Adam Hilger, Bristol, 1989.

C6 Cox, R., P.G. Debenham, W.K. Masson et al. Ataxia-telangiectasia: a human mutation giving high frequency misrepair of DNA double strand scissions. Mol. Biol. Med. 3: 229-244 (1986).

C7 Cooper, G.M. Oncogenes. Jones and Bartlett, 1990.

C8 Cox, R. A cellular description of the repair defect ataxia-telangiectasia. p. 141-153 in: Ataxia-telangiectasia. (B.A. Bridges and D.G. Harnden, eds.) John Wiley & Sons, Chichester, 1982.

C9 Cox, R. and J.B. Little. Oncogenic cell transformation in vitro. Adv. Radiat. Biol. 16: 137-155 (1992).

C10 Cooper, J.A. and P. Whyte. RB and the cell cycle: entrance or exit. Cell 58: 1009-1011 (1989).

C11 Colburn, N.H., E. Farber, B. Weinstein et al. American cancer society workshop conference on tumour promotion and anti-promotion. Cancer Res. 47: 5509-5513 (1987).

C12 Cleaver, J.E. DNA repair in man. p. 61-85 in: Genetic Susceptibility to Environmental Mutagens and Carcinogens. (A.D. Bloom and L. Spatz, eds.) March of Dimes, New York, 1989.

C13 Cox, R. and W.K. Masson. Radiosensitivity in cultured human fibroblasts. Int. J. Radiat. Biol. 38: 575-576 (1980).

C14 Charles, M., R. Cox, D. Goodhead et al. CEIR forum on the effects of high-LET radiation at low doses/dose rates. Int. J. Radiat. Biol. 58: 859-885 (1990).

C15 Cohen, L.A. Diet and cancer. Sci. Am. 257: 42-50 (1987).

C16 Cohen, S.M. and L.B. Ellwein. Cell proliferation in carcinogenesis. Science 249: 1007-1011 (1990).

C17 Christian, B.J., L.J. Loretz, T.D. Oberly et al. Characterization of human urocpithelial cell immortalized in vitro by simian virus 40. Cancer Res. 47: 6066-6073 (1987).

C18 Chen, P.-L., Y. Chen, R. Bookstein et al. Genetic mechanisms of tumour suppression by the human p53 gene. Science 250: 1576-1578 (1991).

C19 Cattanach, B.M. and C.V. Beechey. Chromosome imprinting phenomena in mice and indications in man. Chromosomes Today 10: 135-148 (1990).

C20 Collins, M. Death by a thousand cuts. Curr. Biol. 1: 40-142 (1991).

C21 Cerutti, P.A. Pro-oxidant states and tumour promotion. Science 227: 375-381 (1985).

C22 Cheresh, D.A. and J.R. Harper. Arg-Gly-Asp recognition by a cell adhesion receptor requires its 130 kDa alpha subunit. J. Biol. Chem. 262: 1434-1437 (1987).

C23 Collard, J.G., M. Van de Poll, A. Scheffer et al. Location of genes involved in invasion and metastasis on human chromosome 7. Cancer Res. 47: 6666-6670 (1987).

C24 Cattanach, B.M. Chromosome imprinting and its significance for mammalian development. in: Genome Analysis Series. Volume 2. (K. Davis and S. Tighleman, eds.) Cold Spring Harbour Laboratory Press, Cold Spring Harbour, 1991.

C25 Canning, S. and T.P. Dryja. Short, direct repeats at the breakpoints of deletions of the retinoblastoma gene. Proc. Natl. Acad. Sci. (USA) 86: 5044-5048 (1989).

C26 Cox, R.D. and H. Lehrach. Genome mapping: PCR based meiotic and somatic cell hybrid analysis. BioEssays 13: 193-198 (1991).

C27 Crowell, P.L., R.R. Chang, Z. Ren et al. Selective inhibition of isoprenylation of 21-26-kDa proteins by the anticarcinogen d-limonene and its metabolites. J. Biol. Chem. 266: 17679-17685 (1991).

C28 Closs, E.L., A.B. Murray, J. Schmidt et al. c-fos expression precedes osteogenic differentiation of cartilage cells in vitro. J. Cell Biol. 111: 1313-1323 (1990).

C29 Chandar, N., B. Billig, J. McMaster et al. Inactivation of p53 gene in human and murine osteosarcoma cells. Br. J. Cancer 65: 208-214 (1992).

C30 Crook, T., D. Wrede, J.A. Tidy et al. Clonal p53 mutation in primary cervical cancer: association with human-papillomavirus-negative tumours. Lancet 339: 1070-1073 (1992).

C31 Caron de Fromental, C. and T. Soussi. Tp53 tumour suppressor gene: a model for investigating human mutagenesis. Genes, Chrom. Cancer 4: 1-15 (1992).

C32 Clarke, A.R., C.A. Purdie, D.J. Harrison et al. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature 362: 849-852 (1993).

D1 de Klein, A., A.G. van Kessel, G. Grosveld et al. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature 300: 765-767 (1982).

D2 Debenham, P., M. Webb, M. Jones et al. Molecular studies on the nature of the repair defect in ataxia-telangiectasia and their implication for cellular radiobiology. J. Cell Sci. (Suppl.) 6: 1977-1983 (1987).

D3 Diamond, L.E., E.W. Newcomb, L.E. McMorrow et al. In vivo activation of mouse oncogenes by radiation and chemicals. p. 532-537 in: Radiation Research. Volume 2. (E.M. Fielden et al., eds.) Taylor and Francis, London, 1987.

D4 Diamond, L.E., I. Guerrero and A. Pellicer. Concomitant K- and N-ras gene point mutations in clonal murine lymphomas. Mol. Cell. Biol. 8: 2233-2236 (1988).

D5 Doherty, P.C., B.B. Knowles and P.J. Wettstein. Immunological surveillance of tumours in the context of major histocompatibility complex restriction of T-cell function. Adv. Cancer Res. 42: 1-55 (1984).

D6 Defrensne, M.P., A.M. Rongy, R. Greimers et al. Cellular aspects of radiation leukaemogenesis in C57BL/Ka mice. Leuk. Res. 10: 783-789 (1986).

D7 Dubos, C., E.V. Pequignot and B. Dutrillaux. Localization of γ-ray induced chromatid breaks using a three consecutive staining technique. Mutat. Res. 49: 127-131 (1978).

D8 Dexter, T.M. and H. White. Growth without inflation. Nature 344: 380-381 (1990).

D9 Dexter, T.M., J. Garland, D. Scott et al. Growth of factor-dependent haemopoietic precursor cell lines. J. Exp. Med. 152: 1036-1047 (1980).

D10 Duesberg, P.H. Retroviruses as carcinogens and pathogens: expectations and reality. Cancer Res. 47: 1199-1220 (1987).

D11 Dutton, D.R. and G.T. Bowden. Indirect induction of a clastogenic effect in epidermal cells by a tumour promotor. Carcinogenesis 6: 1279-1284 (1985).

D12 Dowdy, S.F., C.L. Fasching, D. Aranjo et al. Suppression of tumorigenicity in Wilms' tumour by the p15.5-p14 region of chromosome 11. Science 254: 293-295 (1991).

D13 Djabali, M., L. Selleri, P. Parry et al. A trithorax-like gene is interrupted by chromosome 11q23 translocations in acute leukaemias. Nat. Genet. 2: 113-118 (1992).

D14 Donehower, L.A., M. Harvey, B.L. Slagle et al. Mice deficient for *p53* are developmentally normal but susceptible to spontaneous tumours. Nature 356: 215-219 (1992).

D15 Dittmer, D., P. Sibani, G. Zambetti et al. Gain of function mutations in p53. Nat. Genet. 4: 42-46 (1993).

E1 Evans, H.H., J. Mencl, M.F. Horng et al. Locus specificity in the mutability of L5178Y mouse lymphoma cells: The role of multilocus lesions. Proc. Natl. Acad. Sci. (USA) 83: 4379-4383 (1986).

E2 Elkind, M.M. Cell killing in radiation tumorigenesis. p. 513-518 in: Radiation Research. Volume 2. (E.M. Fielden et al., eds.) Taylor and Francis, London, 1987.

E3 Evans, H.J. Molecular mechanisms in the induction of chromosomal aberrations. p. 57-74 in: Progress in Genetic Toxicology. (D. Scott et al., eds) Elsevier, Amsterdam, 1977.

E4 Evans, H.H., M. Neilsen, J. Mencl et al. The effect of dose-rate on X-radiation-induced mutant frequencies and the nature of DNA lesions in mouse lymphoma L5178Y cells. Radiat. Res. 122: 316-325 (1990).

E5 Enoki, Y., O. Niwa, K. Yokoro et al. Analysis of clonal evolution in a tumour consisting of psv2neo-transfected mouse fibrosarcoma clones. Jpn. J. Cancer Res. 81: 141-147 (1990).

E6 Egan, S.E., G.A. McClarty, L. Jarolim et al. Expression of H-ras correlates with metastatic potential: evidence for direct regulation of the metastatic phenotype in 10T½ and N1H 3T3 cells. Mol. Cell. Biol. 7: 830-837 (1987).

E7 Elkind, M.H., J.S. Bedford, S.A. Benjamin et al. Oncogenic mechanisms in radiation-induced cancer (meeting report). Cancer Res. 51: 2740-2747 (1991).

E8 Erlich, H.A., D. Gelfand and J.J. Sninsky. Recent advances in the polymerase chain reaction. Science 252: 1643-1651 (1991).

E9 El-Deiry, W.S., S.E. Kern, J.A. Pietenpol et al. Definition of a consensus binding site for p53. Nat. Genet. 1: 45-49 (1992).

E10 Editorial. Keeping track of the translocations. Nat. Genet. 2: 85-86 (1992).

E11 Ellis, R.E. Negative regulators of programmed cell death. Curr. Opin. Genet. Dev. 2: 635-641 (1992).

E12 Eng, C., F.P. Li, D.H. Abramson et al. Mortality from second tumours among long-term survivors of retinoblastoma. J. Natl. Cancer Inst. (in press, 1993).

F1 Friberg, E.C. and P.C. Hanawalt (eds.). Mechanisms and Consequences of DNA Damage Processing. A.R. Liss, New York, 1988.

F2 Fry, R.J.M. and B.A. Carnes. Age, sex and other factors in radiation carcinogenesis. p. 195-206 in: Low Dose Radiation: Biological Bases of Risk Assessment. (K.F. Baverstock and J.W. Stather, eds.) Taylor and Francis, London, 1989.

F3 Foulds, L. Neoplastic Development. Volume 2. Academic Press, New York, 1975.

F4 Frazier, M.E., T.M. Seed, L.L. Scott et al. Radiation-induced carcinogenesis in dogs. p. 488-493 in: Radiation Research. Volume 2. (E.M. Fielden et al., eds.) Taylor and Francis, London, 1987.

F5 Folkman, J. Tumour angiogenesis. Adv. Cancer Res. 43: 175-204 (1985).

F6 Fusenig, N.B., P. Boukamp and D. Breitkreuzt. Transformation of human skin epithelial cells in vitro: concepts and stages of transformation. p. 45-56 in: Cell Transformation Systems and Radiation Induced Cancer in Man. (K. Chadwick, B. Barnhart and C. Seymour, eds.) Adam Hilger, Bristol, 1989.

F7 Farr, C.J., R.K. Saiki, H.A. Erlich et al. Analysis of RAS gene mutations in acute myeloid leukaemia by polymerase chain reaction and oligo-nucleotide probes. Proc. Natl. Acad. Sci. (USA) 85: 1629-1633 (1988).

F8 Fearon, E.R. and B. Vogelstein. A genetic model for colorectal tumourigenesis. Cell 61: 759-767 (1990).

F9 Fearon, E.R., S.R. Hamilton and B. Vogelstein. Clonal analysis of human colorectal tumours. Science 238: 193-197 (1987).

F10 Franke, U. A gene for Wilms' tumour? Nature 343: 692-694 (1990).

F11 Fearon, E.R., K.R. Cho and J.M. Nigro et al. Identification of a chromosome 18q gene that is altered in colorectal cancers. Science 247: 49-56 (1990).

F12 Fulop, G.M. and R.A. Phillips. The scid mutation in mice causes a general defect in DNA repair. Nature 347: 479-482 (1990).

F13 Fuciarelli, A.F., B.J. Wegher, W.F. Blakely et al. Yields of radiation-induced base products in DNA: effects of DNA conformation and gassing conditions. Int. J. Radiat. Biol. 58: 397-415 (1990).

F14 Fialkow, P.J. Clonal origin of human tumours. Ann. Rev. Med. 30: 135-176 (1979).

F15 Furstenburger, G., V. Kinzel, M. Schwartz et al. Partial inversion of the initiation-promotion sequence of multi-stage tumourigenesis in the skin of NMRI mice. Science 230: 76-78 (1985).

F16 Furstenburger, G., B. Schurich, B. Kaina et al. Tumour induction in initiated mouse skin by phorbol esters and methyl methanesulphonate: correlation between chromosomal damage and conversion in vivo. Carcinogenesis 10: 749-752 (1989).

F17 Folkman, J. and M. Klagsbrun. Angiogenic factors. Science 235: 442-447 (1987).

F18 Fuscoe, J., J. Gray, E. Hildebrand et al. Human chromosome-specific DNA libraries: Construction and availability. Biotechnology 4: 537-552 (1986).

F19 Farr, C., J. Fantes, P. Goodfellow et al. Functional reintroduction of human telomeres into mammalian cells. Proc. Natl. Acad. Sci. (USA) 88: 7006-7010 (1991).

F20 Fryer, G.A. and I. Gurvits. Isolation and identification of an oncogene induced by gamma irradiation in C3H 10T½ cells. p. 146 in: Radiation Research: A 20th Century Perspective. Volume 1. (J.D. Chapman et al., eds.) Academic Press, San Diego, 1991.

F21 Farmer, G., J. Bargonetti, H. Zhu et al. Wild-type p53 activates transcription in vitro. Nature 358: 83-86 (1992).

F22 Furuno-Fukushi, I., A.M. Ueno and H. Matsudaira. Mutation induction by very low dose-rate gamma rays in cultured mouse leukaemia cells L5178Y. Radiat. Res. 115: 273-280 (1988).

F23 Feinberg, A.P. Genomic imprinting and gene activation in cancer. Nat. Genet. 4: 110-113 (1993).

G1 Goodhead, D.T. Biophysical models of radiation action. p. 306-311 in: Radiation Research. Volume 2. (E.M. Fielden et al., eds.) Taylor and Francis, London, 1987.

G2 Goodhead, D.T. Saturable repair models of radiation action in mammalian cells. Radiat. Res. 104: 558-567 (1985).

G3 Graham, F.L., A.J. van der Eb and H.L. Heijneker. Size and location of the transforming region in human adenovirus type 5 DNA. Nature 251: 687-691 (1974).

G4 Grossman, Z. and R.B. Herberman. Immune surveillance without immunogenicity. Immunol. Today 7: 128-131 (1986).

G5 Glickman, B.W., E.A. Drobetsky, J. deBoer et al. Ionizing radiation induced point mutations in mammalian cells. p. 562-567 in: Radiation Research. Volume 2. (E.M. Fielden et al., eds.) Taylor and Francis, London, 1987.

G6 Greaves, M.F. Speculations on the cause of childhood acute lymphoblastic leukaemia. Leukaemia 2: 120-125 (1988).

G7 Gatti, R.A., I. Berkal, E. Boder et al. Localization of an ataxia-telangiectasia gene to chromosome 11q 22-23. Nature 336: 577-580 (1988).

G8 Goldstein, S. Replicative senescence: the human fibroblast comes of age. Science 249: 1129-1133 (1990).

G9 Gentner, N.E. and D.P. Morrison. Determination of the proportion of persons in the population-at-large who exhibit abnormal sensitivity to ionizing radiation. p. 253-262 in: Low Dose Radiation: Biological Bases of Risk Assessment. (K.F. Baverstock and J.W. Stather, eds.) Taylor and Francis, London, 1989.

G10 Greenberger, J.S., M.A. Sakakeemy, R.K. Humphries et al. Demonstration of permanent factor-dependent multipotential (erythroid/neutrophil/basophil) haemopoietic progenitor cell lines. Proc. Natl. Acad. Sci. (USA) 80: 2931-2935 (1983).

G11 Gopalakrishna, R. and S.H. Barsky. Tumour promotor-induced membrane-bound protein kinase C regulates haematogenous metastasis. Proc. Natl. Acad. Sci. (USA) 85: 612-616 (1988).

G12 Guirguis, R., E. Schiffman, B. Lin et al. Detection of autocrine mobility factor in urine as a marker of bladder cancer. J. Natl. Cancer Inst. 80: 1203-1211 (1988).

G13 Gatti, R.A. and M. Swift (eds). Ataxia-telangiectasia: Genetics, Neuropathology and Immunology of a Degenerative Disease of Childhood. A.R. Liss, New York, 1985.

G14 Ghosh, B.C. and L. Ghosh. Tumour Markers and Tumour Associated Antigens. McGraw-Hill, New York, 1988.

G15 Green, M.H.L., C.F. Arlett, J. Cole et al. Comparative human cellular radiosensitivity III. γ radiation survival of cultured skin fibroblasts and resting T-lymphocytes from the peripheral blood of the same individual. Int. J. Radiat. Biol. 59: 749-765 (1991).

G16 Ganesh, A., P. North and J. Thacker. Repair and misrepair of site-specific DNA double strand breaks by human cell extracts. Mutat. Res. 299:3-4: 251-259 (1993).

G17 Gumerlock, P.H., F.J. Meyers, B.A. Foster et al. Activated c-N-ras in radiation-induced acute nonlymphocytic leukaemia: twelfth codon aspartic acid. Radiat. Res. 117: 198-206 (1989).

G18 Garte, S.J., F.J. Burns, T. Ashkenazi-Kimmel et al. Amplification of the c-myc oncogene during progression of radiation-induced rat skin tumours. Cancer Res. 50: 3073-3077 (1990).

G19 Gould, M.N., K.H. Clifton, K. Kamiya et al. Quantitative and molecular comparison of initiation frequency of mammary carcinogenesis by radiation and chemical carcinogens. Radiat. Environ. Biophys. 30: 221-223 (1991).

G20 Goodrow, T.L., R.D. Storer, K.R. Leander et al. Murine p53 intron sequences 5-8 and their use in polymerase chain reaction/direct sequencing analysis of p53 mutations in CD-1 mouse liver and lung tumours. Mol. Carcinog. 5: 9-15 (1992).

G21 Gillett, N.A., B.L. Stegelmeier, G. Kelly et al. Expression of epidermal growth factor receptor in plutonium-239-induced lung neoplasms in dogs. Vet. Pathol. 29: 46-52 (1992).

G22 Gazzolo, L. and M. Duc Dodon. Direct activation of resting T lymphocytes by human T-lymphotropic virus type 1. Nature 326: 714-717 (1987).

G23 Gould, M.N. and R. Zhang. Genetic regulation of mammary carcinogenesis in the rat by susceptibility and suppressor genes. Environ. Health Perspect. 93: 161-167 (1991).

G24 Gishizky, M.L. and O.N. Witte. Initiation of deregulated growth of multipotent progenitor cells by bcr-abl in vitro. Science 256: 836-839 (1992).

H1 Hayes, W. The Genetics of Bacteria and their Viruses. Blackwells, Oxford, 1964.

H2 Hofer, K.G., C.R. Harris and J.M. Smith. Radiotoxicity of intracellular Ga-67, I-125 and H-3. Int. J. Radiat. Biol. 28: 225-241 (1975).

H3 Hofer, K.G. and R.L. Warters. Cell lethality after selective irradiation of the DNA replication fork. Radiat. Environ. Biophys. 24: 161-174 (1985).

H4 Haluska, F.G., Y. Tsujimoto and C.M. Croce. Oncogene activation by chromosome translocation in human malignancy. Annu. Rev. Genet. : 321-345 (1987).

H5 Hanawalt, P.C. and A. Sarasin. Cancer prone hereditary diseases with DNA processing abnormalities. Trends Genet. 2: 124-129 (1986).

H6 Hall, E.J. and T.K. Hei. Oncogenic transformation with radiation and chemicals. Int. J. Radiat. Biol. 48: 1-18 (1985).

H7 Hei, T.K., E.J. Hall and C.A. Waldren. Mutation induction and relative biological effectiveness of neutrons in mammalian cells. Radiat. Res. 115: 281-291 (1988).

H8 Hayata, I. Partial deletion of chromosome 2 in radiation induced myeloid leukaemia in mice. p. 277-293 in: Radiation-Induced Chromosome Damage in Man. (I. Ishihara and M.S. Sasaki, eds.) A.R. Liss, New York, 1983.

H9 Humphreys, E.R., J.F. Loutit, I.R. Major et al. The induction by Ra-224 of myeloid leukaemia and osteosarcoma in male CBA mice. Int. J. Radiat. Biol. 47: 239-247 (1985).

H10 Humphreys, E.R., J.F. Loutit and V.A. Stones. The induction by Pu-239 of myeloid leukaemia and osteosarcomas in female CBA mice. Int. J. Radiat. Biol. 51: 331-339 (1987).

H11 Heim, S. and F. Mitelman. Primary chromosome abnormalities in human neoplasia. Adv. Cancer Res. 52: 2-43 (1989).

H12 Herrlich, P. and H. Ponta. Nuclear oncogenes convert extracellular stimuli into changes in the genetic programme. Trends Genet. 52: 112-116 (1989).

H13 Hall, E.J. Finding a smoother pebble: A workshop summary. p. 401-412 in: Cell Transformation Systems and Radiation Induced Cancer in Man. (K. Chadwick, B. Barnhart and C. Seymour, eds.) Adam Hilger, Bristol, 1989.

H14 Hakoda, M., M. Akiyama, S. Kyouizumi et al. Increased somatic cell mutant frequency in atomic bomb survivors. Mutat. Res. 201: 39-48 (1988).

H15 Hanahan, D. Dissecting multistep tumorigenesis in transgenic mice. Annu. Rev. Genet. 22: 479-519 (1988).

H16 Holliday, R. A different kind of inheritance. Sci. Am. 260: 40-48 (1989).

H17 Hinds, P.W., C. Finlay and A.J. Levine. Mutation is required to activate the p53 gene for cooperation with the ras oncogene and transformation. J. Virol. 63: 739-746 (1989).

H18 Herskowitz, I. Functional inactivation of genes by dominant negative mutations. Nature 329: 219-222 (1987).

H19 Hall, J.G. Genomic imprinting: Review and relevance to human diseases. Am. J. Hum. Genet. 46: 857-873 (1990).

H20 Hori, T., E. Takahashi, T. Ishihara et al. Distamycin A-inducible fragile sites and cancer proneness. Cancer Genet. Cytogenet. 34: 177-187 (1988).

H21 Hastie, N.D. and R. Allshire. Human telomeres: fusion and interstitial sites. Trends Genet. 5: 326-330 (1989).

H22 Harley, C.B., A.B. Futcher and C.W. Greidner. Telomeres shorten during ageing of human fibroblasts. Nature 345: 458-460 (1990).

H23 Hockenberry, D., G. Numez, C. Milliman et al. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 348: 334-336 (1990).

H24 Hanahan, D. Transgenic mice as probes into complex systems. Science 246: 1265-1275 (1990).

H25 Hecht, F. and B.K. Hecht. Fragile sites and chromosome breakpoints in constitutional rearrangements. I. Amniocentesis. Clin. Genet. 26: 169-173 (1984).

H26 Hecht, F. and B.K. Hecht. Fragile sites and constitutional chromosome rearrangements. II. Spontaneous abortions, stillbirths and newborns. Clin. Genet. 26: 174-177 (1984).

H27 Hayata, I., T. Ichikawa and Y. Ichikawa. Specificity in chromosomal abnormalities in mouse bone marrows induced by the difference of the conditions of irradiation. Proc. Jpn. Acad., Ser. B 63: 289-292 (1987).

H28 Hayata, I. Leukaemogenesis and chromosomal abnormalities: Experimental animals. Acta Haematol. Jpn. 48: 181-187 (1985).

H29 Harris, H. The analysis of malignancy by cell fusion: the position in 1988. Cancer Res. 48: 3302-3306 (1988).

H30 Hollstein, M., D. Sidransky, B. Vogelstein et al. p53 mutations in human cancers. Science 253: 49-53 (1991).

H31 Harrington, L.A. and C.W. Greidner. Telomerase primer specificity and chromosome healing. Nature 353: 451-454 (1991).

H32 Hatano, M., C.W.M. Roberts, M. Minden et al. Deregulation of a homeobox gene, Hox 11, by the t(10;14) in T-cell leukaemia. Science 253: 79-82 (1991).

H33 Hart, I.R., N.T. Goode and R.E. Wilson. Molecular aspects of the metastatic cascade. Biochim. Biophys. Acta 989: 65-84 (1989).

H34 Hsu, I.C., R.A. Metcalf, T. Sun et al. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature 350: 427-428 (1991).

H35 Hall, J.M., M.K. Lee, B. Newman et al. Linkage of early onset familial breast cancer to chromosome 17q21. Science 250: 1684-1689 (1990).

H36 Herlyn, M. and H. Koprowski. Melanoma antigens: immunological and biological characterisation and clinical significance. Annu. Rev. Immunol. 6: 283-308 (1988).

H37 Hanna, N. Inhibition of experimental tumour metastasis by selective activation of natural killer cells. Cancer Res. 42: 1337-1342 (1982).

H38 Herberman, R.B. and J.R. Ortaldo. Natural killer cells: their role in defenses against disease. Science 214: 24-30 (1981).

H39 Harvey, D.M. and A.J. Levine. p53 alteration is a common event in the spontaneous immortalization of primary BALB/c murine embryo fibroblasts. Genes Dev. 5: 2375-2385 (1991).

H40 Haas, O.A., A. Argyriou-Tirita and T. Lion. Parental origin of chromosomes involved in the translocation t(9;22). Nature 359: 414-416 (1992).

H41 Holmberg, M. Is the primary event in radiation-induced chronic myelogenous leukaemia the induction of the t(9;22) translocation? Leuk. Res. 16: 333-336 (1992).

H42 Hart, I.R. and A. Saini. Biology of tumour metastasis. Lancet 339: 1453-1457 (1992).

H43 Hall, J.G. Genomic imprinting and its clinical implications. N. Engl. J. Med. 326: 827-829 (1992).

H44 Hayata, I., M. Seki, K. Yoshida et al. Chromosomal aberrations observed in 52 mouse myeloid leukaemias. Cancer Res. 43: 367-373 (1983).

H45 Hoeijmakers, J.H. and D. Bootsma. DNA repair: two pieces of the puzzle. Nat. Genet. 1: 313-314 (1992).

H46 Harris, C.C. Chemical and physical carcinogenesis: advances and perspectives for the 1990s. Cancer Res. 51 (Suppl.): 5023s-5044s (1991).

H47 Hardwick, J.P., R.A. Schlenker and E. Huberman. Alteration of the c-mos locus in "normal" tissues from humans exposed to radium. Cancer Res. 49: 2668-2673 (1989).

H48 Hossain, M.Z., L.R. Wilkens, P.P. Mehta et al. Enhancement of gap junctional communication by retinoids correlates with their ability to inhibit neoplastic transformation. Carcinogenesis 10: 1743-1748 (1989).

H49 Hakoda, M., M. Akiyama, Y. Hirai et al. In vivo mutant T cell frequency in atomic bomb survivors carrying outlying values of chromosome aberration frequencies. Mutat. Res. 202: 203-208 (1988).

H50 Hartwell, L.H. and T.A. Weinert. Checkpoints: controls that ensure the order of cell cycle events. Science 246: 629-634 (1989).

H51 Helin, K. and E. Harlow. The retinoblastoma protein as a transcriptional repressor. Trend, Cell Biol. 3: 43-46 (1993).

H52 Haig, D. and C. Graham. Genome imprinting and the strange case of the insulin-like growth factor II receptor. Cell 64: 1045-1046 (1991).

I1 Ito, T., T. Seyama, T. Mizuno et al. Unique association of p53 mutations with undifferentiated but not with differentiated carcinomas of the thyroid gland. Cancer Res. 52: 1369-1371 (1992).

I2 Ijdo, J.W., A. Baldini, R.A. Wells et al. FRA 2B is distinct from inverted telomere repeat arrays at 2q13. Genomics 12: 833-835 (1992).

I3 Ho, T., T. Seyama, T. Mizuno et al. Induction of BCR-ABL fusion genes by in vitro X-irradiation. Jpn. J. Cancer Res. 84: 105-109 (1993).

J1 Jacob, F. and E.L. Wollman. Sexuality and the Genetics of Bacteria. Academic Press, New York, 1961.

J2 Janowski, M., R. Cox and G. Strauss. The molecular biology of radiation-induced carcinogenesis: thymic lymphoma, myeloid leukaemia and osteosarcoma. Int. J. Radiat. Biol. 57: 677-691 (1990).

J3 Jaffe, D. and G.T. Bowden. Ionizing radiation as an initiator: effects on proliferation and promotion time on tumor incidence in mice. Cancer Res. 47: 6692-6696 (1987).

J4 Joshi, G.P., W.J. Nelson, S.H. Revell et al. X-ray induced chromosome damage in live mammalian cells and improved measurements of its effects on their colony forming ability. Int. J. Radiat. Biol. 41: 161-181 (1982).

J5 Jones, P.A. and J.D. Buckley. The role of DNA methylation in cancer. Adv. Cancer Res. 54: 1-24 (1990).

J6 Jacobson, S., V. Zaninovic, C. Mora et al. Immunological findings in neurological diseases associated with antibodies to HTLV-1. Ann. Neurol. 23 (Suppl 1): 196-200 (1988).

K1 Knudson, A.G. Genetics of human cancer. Annu. Rev. Genet. 20: 231-251 (1986).

K2 Klein, G. and E. Klein. Evolution of tumours and the impact of molecular oncology. Nature 315: 190-195 (1985).

K3 Kennedy, A.R., J. Cairns and J.B. Little. Timing of the steps in transformation of C3H10T1/2 cells by x-irradiation. Nature 307: 85-86 (1984).

K4 Kaplan, H.S. On the natural history of the murine leukaemias. Cancer Res. 27: 1325-1340 (1967).

K5 Kasid, U., A. Pfeifer, R.R. Weichselbaum et al. The ras oncogene is associated with a radiation resistant human laryngeal cancer. Science 237: 1039-1040 (1987).

K6 Kerbel, R.S., C. Waghorne, C. Korczak et al. Clonal dominance of primary tumours by metastatic cells. Cancer Surveys 7(4): 597-629 (1988).

K7 Kinlen, L. Immunologic factors. p. 494-505 in: Cancer Epidemiology and Prevention. (D. Schottenfeld and J. Fraumeni, eds.) Saunders, Philadelphia, 1982.

K8 Kumar, R., S. Sukumar and M. Barbacid. Activation of ras oncogenes preceding the onset of neoplasia. Science 248: 1101-1104 (1990).

K9 Kyoizumi, S., N. Nakamura, M. Hakoda et al. Detection of somatic mutations at the glycophorin A locus of erythrocytes of atomic bomb survivors using a single beam flow sorter. Cancer Res. 49: 581-588 (1989).

K10 Kennedy, A.R. Initiation and promotion of radiation-induced transformation in vitro: relevance of in vitro studies to radiation-induced cancer in human populations. p. 263-277 in: Cell Transformation Systems and Radiation Induced Cancer in Man. (K. Chadwick et al., eds.) Adam Hilger, Bristol, 1989.

K11 Komatsu, K.S., S. Kodama, Y. Okumura et al. Restoration of radiation resistance in ataxia-telangiectasia cells by the introduction of normal human chromosome 11. Mutat. Res. 235: 59-63 (1990).

K12 Kern, S.E., K.W. Kinzler, A. Bruskin et al. Identification of p53 as a sequence-specific DNA-binding protein. Science 252: 1708-1710 (1991).

K13 Kanno, Y. Modulation of cell communication and carcinogenesis. Jpn. J. Physiol. 35: 693-707 (1985).

K14 Klingelhutz, A.J., S.-Q. Win, E.A. Bookland et al. Allelic 3p deletions in high grade carcinomas after transformation in vitro of human uroepithelial cells. Genes, Chrom. Cancer 3: 346-357 (1991).

K15 Kinzler, K.W., M.C. Nilbert, L.-K. Su et al. Identification of FAP locus genes from chromosome 5q21. Science 253: 661-665 (1991).

K16 Kastan, M.B., O. Onvekwere, D. Sidransky et al. Participation of p53 protein in the cellular response to DNA damage. Cancer Res. 51: 6304-6311 (1991).

K17 Kern, S.E., J.A. Pietenpol, S. Thiagalingam et al. Oncogenic forms of p53 inhibit p53-regulated gene expression. Science 256: 827-830 (1992).

K18 Katinka, M.D. and F.M. Bourgain. Interstitial telomeres are hotspots for illegitimate recombination with DNA molecules injected into the macronucleus of Paramecium primaurelia. EMBO J. 11: 725-732 (1992).

K19 Kang, C., X. Zhang, R. Ratliff et al. Crystal structure of four stranded *Oxytricha* telomeric DNA. Nature 356: 126-131 (1992).

K20 Krowczyska, A.M., R.A. Rudders and T.G. Kronftiris. The human minisatellite consensus at breakpoints of oncogene translocations. Nucleic Acids Res. 18: 1121-1127 (1990).

K21 King, M.-C. Breast cancer genes: how many, where and who are they? Nat. Genet. 2: 89-90 (1992).

K22 Krolewski, B. and J.B. Little. Molecular analysis of DNA isolated from the different stages of x-ray induced transformation in vitro. Mol. Carcinog. 2: 27-33 (1989).

K23 Kramer, A. and W.H. Blattner. The HTLV-1 model and chronic demyelinating neurological diseases. p. 204-214 in: Concepts in Viral Pathogenesis. (A.L. Notkins and M.B.A. Oldstone, eds.) Springer Verlag, New York, 1989.

K24 Kaelbling, M., R.D. Burk, N.B. Atkin et al. Loss of heterozygosity on chromosome 17p and mutant p53 in HPV-negative cervical carcinomas. Lancet 340: 140-142 (1992).

K25 Kadhim, M.A., D.A. Macdonald, D.T. Goodhead et al. Transmission of chromosomal instability after plutonium α particle irradiation. Nature 355: 738-740 (1992).

K26 Kuerbitz, S.J., B.S. Plunkett, W.V. Walsh et al. Wild type p53 is a cell cycle checkpoint determinant following irradiation. Proc. Natl. Acad. Sci. (USA) 89: 7491-7495 (1992).

K27 Kawakatsu, H., R. Shiurba, M. Obara et al. Human carcinoma cells synthesize and secrete tenascin in vitro. Jpn. J. Cancer Res. 83: 1073-1080 (1992).

K28 Kyoizumi, S., M. Akiyama and Y. Hirai. Spontaneous loss and alteration of antigen receptor expression in mature CD4 T-cells. J. Exp. Med. 171: 1981-1999 (1990).

K29 Kapp, L.N., R.B. Painter, L.C. Yu et al. Cloning of a candidate gene for ataxia-telangiectasia group D. Am. J. Hum. Genet. 51: 45-54 (1992).

L1 Lloyd, D.C., R.J. Purrott, G.W. Dolphin et al. The relationship between chromosome aberrations and low-LET radiation dose to human lymphocytes. Int. J. Radiat. Biol. 28: 75-90 (1975).

L2 Little, J.B. The relevance of cell transformation to carcinogenesis in vivo. p. 396-413 in: Low Dose Radiation: Biological Bases of Risk Assessment. (K.F. Baverstock and J.W. Stather, eds.) Taylor and Francis, London, 1989.

L3 Little, J.B. Influence of noncarcinogenic secondary factors on radiation carcinogenesis. Radiat. Res. 87: 240-250 (1981).

L4 Lieberman, M. and H.S. Kaplan. Leukaemogenic activity of filtrates from radiation-induced lymphoid tumors of mice. Science 130: 387-388 (1959).

L5 Lapis, K., L. Liotta and A. Rabson (eds.). Biochemistry and Molecular Genetics of Cancer Metastasis. Martinus Nijhoff, The Hague, 1986.

L6 Ling, V., A.F. Chambers, J.F. Harris et al. Quantitative genetic analysis of tumour progression. Cancer Metastasis Rev. 4: 173-194 (1985).

L7 Langlois, R.G., W.L. Bigbee, S. Kyouizumi et al. Evidence for increased somatic cell mutations at the glycophorin A locus in atomic bomb survivors. Science 236: 445-448 (1987).

L8 Landsvater, R.M., C.G.P. Mathew, B.A. Smith et al. Development of multiple endocrine neoplasia type 2A does not involve substantial deletions of chromosome 10. Genomics 4: 246-250 (1989).

L9 Le Beau, M.M. Chromosomal fragile sites and cancer specific breakpoints - A moderating viewpoint. Cancer Genet. Cytogenet. 31: 55-61 (1988).

L10 Liotta, L.A. Tumour invasion and metastases: role of the basement membrane. Am. J. Pathol. 117: 339-348 (1984).

L11 Little, J.B. and J. Nove. Sensitivity of human diploid fibroblast cell strains from various genetic disorders to acute and protracted radiation exposure. Radiat. Res. 123: 87-92 (1990).

L12 Lang, R.A., D. Metcalf, N.M. Gough et al. Expression of a haemopoietic growth factor cDNA in a factor dependent cell line resulting in autonomous growth and tumourigenicity. Cell 43(2): 531-542 (1985).

L13 Levine, A.J., J. Momand and C.A. Finlay. The p53 tumour suppressor gene. Nature 351: 453-456 (1991).

L14 Loewenstein, W.R. Junctional intercellular communication: the cell to cell membrane channel. Physiol. Rev. 61: 829-913 (1981).

L15 Leder, A., A. Kuo, R.D. Cardiff et al. v-Ha-ras transgene abrogates the initiation step in mouse skin tumorigenesis: effects of phorbol esters and retinoic acid. Proc. Natl. Acad. Sci. (USA) 87: 9178-9182 (1990).

L16 Little, M., V. Van Heyningen and N. Hastie. Dads and disomy and disease. Nature 351: 609-610 (1991).

L17 Lupulesco, A. Hormones and Carcinogenesis. Praegar Press, New York, 1982.

L18 LeMotte, P.K., S.J. Adelstein and J.B. Little. Malignant transformation induced by incorporated radionuclides in BALB/3T3 mouse embryo fibroblasts. Proc. Natl. Acad. Sci. (USA) 79: 7763-7767 (1982).

L19 Lucas, J., A. Awa, Y. Kodama et al. Rapid translocation frequency analysis in human decades after exposure to ionising radiation. Proc. Natl. Acad. Sci. (USA) 89: 53-63 (1992).

L20 Land, C.E. and W.K. Sinclair. The relative contributions of different organ sites to the total cancer mortality associated with low dose radiation exposure. p. 31-58 in: Annals of the ICRP. ICRP Publication 22. Pergamon Press, Oxford, 1991.

L21 Lee, E.Y., H. To, J.Y. Shew et al. Inactivation of the retinoblastoma susceptibility gene in human breast cancers. Science 241: 218-221 (1988).

L22 Leone, A., U. Flatlow, C.R. King et al. Reduced tumour incidence, metastatic potential and cytokine responsiveness of nm23-transfected melanoma cells. Cell 65: 25-35 (1991).

L23 Liotta, L.A., P.S. Steeg and W.G. Stetler-Stevenson. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 64: 327-336 (1991).

L24 Leib-Mosch, C., R. Brack-Werner, T. Werner et al. Endogenous retroviral elements in human DNA. Cancer Res. 50 (Suppl.): 5636s-5642s (1990).

L25 Leuthauser, S.W.C., J.E. Thomas and D.L. Guernsey. Oncogenes in X-ray-transformed C3H10T½ mouse cells and in X-ray-induced mouse fibrosarcoma (RIF-1) cells. Int. J. Radiat. Biol. 62: 45-51 (1992).

L26 Laviguer, A., V. Maltby, D. Mock et al. High incidence of lung, bone, and lymphoid tumors in transgenic mice overexpressing mutant alleles of the p53 oncogene. Mol. Cell. Biol. 9: 3982-3991 (1989).

L27 Loeb, L.A. Endogenous carcinogenesis. Cancer Res. 49: 5489-5496 (1989).

L28 Lane, D.P. p53, guardian of the genome. Nature 358: 15-16 (1992).

L29 Lowe, S.W., E.M. Schmitt, S.W. Smith et al. p53 is required for radiation-induced apoptosis in mouse thermocytes. Nature 362: 847-849 (1993).

L30 Land, C.E., M. Tokunaga, S. Tokuoka et al. Early onset breast cancer in A-bomb survivors. Lancet 342: 237 (1993).

M1 Mole, R.H. Dose response relationships. p. 403 in: Radiation Carcinogenesis. (J.D. Boice and J.F. Fraumeni, eds.) Raven Press, New York, 1985.

M2 Mole, R.H. Radiation-induced acute myeloid leukaemia in the mouse. Leuk. Res. 10: 859-865 (1986).

M3 Moreau-Gachelin, F., A. Tavitian and P. Tambourin. Spi-1 is a putative oncogene in virally induced murine erythroleukaemias. Nature 331: 277-280 (1988).

M4 Monpezat, J.P., O. Delattre, A. Bernard et al. Loss of alleles on chromosome 18 and on the short arm of chromosome 17 in polyploid colorectal carcinomas. Int. J. Cancer 41: 404-408 (1988).

M5 Mitelman, F. and S. Heim. Consistent involvement of only 71 of the 329 chromosomal bands of the human genome in primary neoplasia-associated rearrangements. Cancer Res. 48: 7115-7119 (1988).

M6 Moore, M.M., A. Amtower, G.H.S. Strauss et al. Genotoxicity of γ-irradiation in L5178Y mouse lymphoma cells. Mutat. Res. 174: 149-154 (1986).

M7 McMorrow, L., E.W. Newcomb and A. Pellicer. Identification of a specific marker chromosome early in tumour development in γ-irradiated C57BL/6J mice. Leukaemia 2: 115-119 (1988).

M8 Muto, M., T. Sato, I. Hayata et al. Reconfirmation of indirect induction of radiogenic lymphomas using thymectomized, irradiated B10 mice grafted with neonatal thymuses from Thy1 congenic donors. Cancer Res. 43: 3822-3827 (1983).

M9 McCormick, J.J. and V.M. Maher. Towards an understanding of the malignant transformation of human fibroblasts. Mutat. Res. 199: 273-292 (1988).

M10 Malynn, B.A., T.K. Blackwell, G.M. Fulop et al. The scid defect affects the final step of the immunoglobulin VDJ recombinase mechanism. Cell 54: 453-460 (1988).

M11 Muller, W.J., E. Sinn, P.K. Pattenhale et al. Single step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. Cell 54: 105-115 (1988).

M12 McCune, J.M., R. Namikawa, H. Kaneshima et al. The SCID-hu mouse: murine model for the analysis of human haematolymphoid differentiation and function. Science 241: 1632-1639 (1988).

M13 Mosier, D.E., R.J. Gulizia, S.M. Baird et al. Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature 335: 256-259 (1988).

M14 Monk, M. Genomic imprinting. Genes Dev. 2: 921-925 (1988).

M15 Murray, A. Telomeres: all's well that ends well. Nature 346: 797-798 (1990).

M16 Metcalf, J.C., T.R. Hesketh, G.A. Smith et al. Early response pattern analysis of the mitogenic pathway in lymphocytes and fibroblasts. J. Cell Sci. 3 (Suppl.): 199-228 (1985).

M17 Margolis, B., A. Zilberstein and C. Franks et al. Effect of phospholipase C-γ overexpression on PDGF-induced second messengers and mitogenesis. Science 248: 607-610 (1990).

M18 Moore, K.V., P. Vieira, D.F. Fiorentino et al. Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCR F1. Science 248: 1230-1234 (1990).

M19 Maraschin, J., B. Dutrillaux and A. Animas. Chromosome aberrations induced by etoposide (VP-16) are not random. Int. J. Cancer 46: 808-812 (1990).

M20 Mittelman, F., Y. Manolova, G. Manolov et al. High resolution analysis of the 5q-marker chromosome in refractory anemia. Hereditas 105: 49-54 (1986).

M21 Moloney, W.C. Radiogenic leukaemia revisited. Blood 70: 905-908 (1987).

M22 Morgan, T.L., E.W. Fleck, B.J.F. Rossiter et al. Molecular characterization of X-ray induced mutations at the HPRT locus in plateau phase Chinese hamster ovary cells: the effect of dose, dose fractionation and delayed plating. p. 207-214 in: Cell Transformation Systems and Radiation Induced Cancer in Man. (K. Chadwick, B. Barnhart and C. Seymour, eds.) Adam Hilger, Bristol, 1989.

M23 Mulcahy, R.T., M.N. Gould and K.H. Clifton. Radiogenic initiation of thyroid cancer: a common cellular event. Int. J. Radiat. Biol. 45: 419-426 (1984).

M24 Metcalf, D. Molecular Control of Blood Cells. Harvard University Press, Boston, 1988.

M25 Moser, A.R., H.C. Pitot and W.F. Dove. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 247: 322-324 (1990).

M26 Muto, M., E. Kubo, H. Kamisaku et al. Phenotypic characterization of thymic prelymphoma cells of B10 mice treated with split-dose irradiation. J. Immunol. 144: 849-853 (1990).

M27 Murray, A.W. and H.W. Kirschner. What controls the cell cycle. Sci. Am. 264: 56-63 (1991).

M28 Marx, J. The cell cycle: spinning farther afield. Science 252: 1490-1492 (1991).

M29 Moyzis, R.K. The human telomere. Sci. Am. 265: 34-41 (1991).

M30 Murray, A.W. and D.J. Fitzgerald. Tumour promotors inhibit metabolic co-operation in co-cultures of epidermal and 3T3 cells. Biochem. Biophys. Res. Comm. 91: 395-401 (1979).

M31 Mitchel, R.E.J., N.J. Gragtmans and D.P. Morrison. Beta-radiation-induced resistance to MNNG initiation of papilloma but not carcinoma formation in mouse skin. Radiat. Res. 121: 180-186 (1990).

M32 Moolgavkar, S.M. Hormones and multistage carcinogenesis. Cancer Surveys 5: 635-648 (1986).

M33 Meuth, M. The structure of mutations in mammalian cells. Biochim. Biophys. Acta 1032: 1-17 (1990).

M34 Marx, J. Zeroing in on individual cancer risk. Science 253: 612-616 (1991).

M35 Muxinova, K.N. and G.S. Mushkacheva. Cellular and molecular mechanisms for the changes of the haemopoietic system in case of long-term irradiation. Energoatomizdat, Moscow, 1990.

M36 Muramatsu, M. Introduction to Molecular Medicine. Nankoda, Tokyo, 1992.

M37 Mitchel, R.E.J. and A. Trivedi. Chronic exposure to ionizing radiation as a tumor promoter in mouse skin. Radiat. Res. 129: 192-201 (1992).

M38 Malkin, D., K.W. Jolly, N. Barbier et al. Germline mutations of the p53 tumor-suppressor gene in children and young adults with second malignant neoplasms. N. Engl. J. Med. 326: 1309-1315 (1992).

M39 Monnat, R.J., A.F.M. Hackman and T.A. Chiaverotti. Nucleotide sequence analysis of human hypoxanthine phosphoribosyl transferase (HPRT) gene deletions. Genomics 13: 777-787 (1992).

M40 Mihara, K., L. Bai, Y. Kano et al. Malignant transformation of human fibroblasts previously immortalized with ^{60}Co gamma rays. Int. J. Cancer 50: 639-643 (1992).

M41 Masse, R. Lung cancer in laboratory animals. Radiat. Environ. Biophys. 30: 233-237 (1991).

M42 Miyamoto, S., S. Sukumar, R.C. Guzman et al. Transforming c-Ki-ras mutation is a preneoplastic event in mouse mammary carcinogenesis induced in vitro by N-methyl-N-nitrosourea. Mol. Cell. Biol. 10: 1593-1599 (1990).

M43 Maurer, J., J.W.G. Janssen, E. Thiel et al. Detection of chimeric BCR-ABL genes in acute lymphoblastic leukaemia by the polymerase chain reaction. Lancet 337: 1055-1058 (1991).

M44 Maniatis, T. and H. Weintraub. Gene expression and differentiation. Curr. Opin. Genet. Dev. 2: 197-198 (1992).

M45 Mehta, P.P., J.S. Bertram and W.R. Lowenstein. The actions of retinoids on cellular growth correlate with their actions on gap junctional communication. J. Cell Biol. 108: 1053-1065 (1989).

M46 Miki, Y., I. Nishisho, A. Horii et al. Disruption of the APC gene by a retrotransposal insertion of L1 sequence in a colon cancer. Cancer Res. 52: 643-645 (1992).

M47 Murray, A.W. Creative blocks: cell cycle checkpoints and feedback controls. Nature 359: 599-604 (1992).

M48 Mules, E.H., J.R. Testa, G.H. Thomas et al. Cancer in relatives of leukaemic patients with chromosomal rearrangements at rare (heritable) fragile-site locations in their malignant cells. Am. J. Hum. Genet. 44: 811-819 (1989).

M49 Morris, T. and J. Thacker. Formation of large deletions by illegitimate recombination in the HPRT gene of primary human fibroblasts. Proc. Natl. Acad. Sci. (USA) 90: 1392-1396 (1993).

M50 Muto, M., E. Kubo and T. Sado. Cellular events during radiation induced thymic leukaemogenesis in mice: abnormal T cell differentiation in the thymus and defect of thymocyte precursors in the bone marrow after split dose irradiation. J. Immunol. 134: 2026-2031 (1985).

N1 Natarajan, A.T. and G. Obe. Molecular mechanisms involved in the production of chromosomal aberrations. Chromosoma 90: 120-127 (1984).

N2 Nusse, R. The activation of cellular oncogenes by retroviral insertion. Trends Genet. 2: 244-247 (1986).

N3 Nowell, P.C. Mechanisms of tumour progression. Cancer Res. 46: 2203-2207 (1986).

N4 Newcomb, E.W., R. Binari and E. Fleissner. A comparative analysis of radiation and virus induced leukaemias in BALB/C mice. Virology 140: 102-112 (1985).

N5 Nishizuka, Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature 308: 693-698 (1984).

N6 Nicholson, G. and L. Milas (eds.). Cancer Invasions and Metastasis: Biologic and Therapeutic Aspects. Raven Press, New York, 1984.

N7 Niwa, O., Y. Enoki and K. Yokoro. Overexpression and amplification of the c-myc gene in mouse tumours induced by chemicals and radiations. Jpn. J. Cancer Res. 80: 212-218 (1989).

N8 Namba, M., K. Nishitani, F. Fukushima et al. Multistep neoplastic transformation of normal human fibroblasts by Co-60 gamma rays and Ha-ras oncogenes. Mutat. Res. 199: 415-423 (1988).

N9 Nowell, P.C. Cytogenetics of tumour progression. Cancer 65: 2172-2177 (1990).

N10 National Council on Radiation Protection and Measurements. Comparative carcinogenicity of ionizing radiation and chemicals. NCRP Report No. 96 (1989).

N11 Namba, M., K. Nishitani, T. Kimoto et al. Multistep neoplastic transformation of normal human fibroblasts and its genetic aspects. p. 67-74 in: Cell Transformation Systems and Radiation Induced Cancer in Man. (K. Chadwick, B. Barnhart and C. Seymour, eds.) Adam Hilger, Bristol, 1989.

N12 Nurse, P. Universal control mechanisms regulating onset of M phase. Nature 344: 503-508 (1990).

N13 Nicolson, G.L., K.M. Dulski and J.E. Trosko. Loss of intercellular junctional communication correlates with metastatic potential in mammary adenocarcinoma cells. Proc. Natl. Acad. Sci. (USA) 85: 473-475 (1988).

N14 Newbold, R.F. Metabolic co-operation in tumour promotion and carcinogenesis. p. 301-317 in: Functional Integration of Cells in Animal Tissues. (J.D. Pitts and M.E. Finbow, eds.) Cambridge University Press, Cambridge, 1982.

N15 North, P., A. Ganesh and J. Thacker. The rejoining of double strand breaks in DNA by human cell extracts. Nucleic Acids Res. 18: 6205-6210 (1990).

N16 Nishisho, I., Y. Nakamura, Y. Miyoshi et al. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 253: 665-669 (1991).

N17 Neuman, W.L., M.L. Wasylyshyn, R. Jacoby et al. Evidence for a common molecular pathogenesis in colorectal, gastric and pancreatic cancer. Genes, Chrom. Cancer 3: 468-473 (1991).

N18 Nevins, J.R. E2F: A link between the Rb tumor suppressor protein and viral oncoproteins. Science 258: 424-429 (1992).

N19 Newcomb, E.W., M. Corominas, W. Bayona et al. Multistage carcinogenesis in murine thymocytes: involvement of oncogenes, chromosomal imbalances and T cell growth factor receptor. Anticancer Res. 9: 1407-1415 (1989).

N20 Nicklas, J.A., J.P. O'Neill, T.C. Hunter et al. In vivo ionizing irradiations produce deletions in the hprt gene of human T-lymphocytes. Mutat. Res. 250: 383-396 (1991).

N21 Newton, J.A., A.K. Black, C.F. Arlett et al. Radiobiological studies in the naevoid basal cell carcinoma syndrome. Br. J. Dermatol. 123: 573-580 (1990).

N22 Nikjoo, H. and D.T. Goodhead. The relative biological effectiveness (RBE) achievable by high- and low-LET radiations. p. 491-502 in: Low Dose Radiation: Biological Bases of Risk Assessment. (K.F. Baverstock and J.W. Stather, eds.) Taylor and Francis, London, 1989.

N23 Norbury, C. and P. Nurse. Animal cell cycles and their control. Annu. Rev. Biochem. 61:441-470 (1992).

O1 Onions, D.E. and O. Jarrett (eds.). Naturally occurring tumours in animals as a model for human disease. Cancer Surv. 6: 1-181 (1987).

O2 Oberle, I., F. Rousseau, D. Heitz et al. Instability of a 550 base pair DNA segment and abnormal methylation in fragile X syndrome. Science 252: 1097-1102 (1991).

O3 Okumoto, M., R. Nishikawa, M. Iwai et al. Lack of evidence for the involvement of type-C and type-B retroviruses in radiation leukaemogenesis of NFS mice. Radiat. Res. 121: 267-273 (1990).

O4 Ozturk, M., B. Bressac, A. Puisieux et al. p53 mutation in hepatocellular carcinoma after aflatoxin exposure. Lancet 338: 1356-1359 (1991).

O5 Ootsuyama, A. and H. Tanooka. Threshold-like dose of local ß irradiation throughout the life-span of mice for induction of skin and bone tumours. Radiat. Res. 125: 98-101 (1991).

P1 Ponder, B. Gene losses in human tumours. Nature 335: 400-402 (1988).

P2 Pippard, E.C., A.J. Hall, D.J.P. Barker et al. Cancer in homozygotes and heterozygotes of ataxia-telangiectasia and xeroderma pigmentosum in Britain. Cancer Res. 48: 2929-2932 (1988).

P3 Perry, G.S., B.D. Spector, L.M. Schuman et al. The Wiskott Aldrich syndrome in the USA and Canada. J. Pediatr. 97: 72-79 (1980).

P4 Purtillo, D.T. and G. Klein. Introduction to Epstein-Barr virus and lymphoproliferative diseases in immunodeficient individuals. Cancer Res. 41: 4209 (1981).

P5 Priest, N.D. (ed.). Metals in Bone. MTP Press, Lancaster, 1984.

P6 Piper, J. and C. Lundsteen. Human chromosome analysis by machine. Trends Genet. 3: 309-313 (1987).

P7 Ponder, B.A.J. and M.M. Wilkinson. Direct examination of the clonality of carcinogen-induced colonic epithelial dysplasia in chimeric mice. J. Natl. Cancer Inst. 77: 967-976 (1986).

P8 Pantel, K., J. Boertman and A. Nakeff. Inhibition of haemopoietic recovery from radiation-induced myelosuppression by natural killer cells. Radiat. Res. 122: 168-171 (1990).

P9 Paterson, M.C., N.E. Gentner, M.V. Middlestadt et al. Hereditary and familial disorders linking cancer proneness with abnormal carcinogen response and faulty DNA metabolism. p. 235-267 in: Epidemiology and Quantification of Environmental Risk in Humans from Radiation and Other Agents. Volume 96. (A. Castellani, ed.) NATO ASI Series A, 1986.

P10 Pitts, J.D. and M.E. Finbow. The gap junction. J. Cell Sci. 4: 239-266 (1986).

P11 Petrusevska, R.T., G. Furstenburger, F. Marks et al. Cytogenetic effects caused by phorbol ester tumour promotors in primary mouse keratinocyte cultures: correlations with the convertogenic activity of TPA in multistage skin carcinogenesis. Carcinogenesis 9: 1207-1215 (1988).

P12 Ponder, B.A. Inherited predisposition to cancer. Trends Genet. 6: 213-218 (1990).

P13 Peters, G. Oncogenes at viral integration sites. Cell Growth Differ. 1: 503-510 (1990).

P14 Panozzo, J., D. Bertoncini, D. Miller et al. Modulation of expression of virus-like elements following exposure of mice to high- and low-LET radiations. Carcinogenesis 12: 801-804 (1991).

P15 Potten, C.S., Y.Q. Li, P.J. O'Connor et al. A possible explanation for the differential cancer incidence in the intestine based on distribution of the cytotoxic effects of carcinogens in the murine large bowel. Carcinogenesis 13(12): 2305-2312 (1992).

P16 Peltomaki, P., L.A. Aaltonen, P. Sistonen et al. Genetic mapping of a locus predisposing to human colorectal cancer. Science 260: 810-812 (1993).

R1 Radford, I.R., G.S. Hodgson and J.P. Matthews. Critical DNA target size model of ionizing radiation-induced mammalian cell death. Int. J. Radiat. Biol. 54: 63-79 (1988).

R2 Reznikoff, C.A., J.S. Bertram, D.W. Brankow et al. Quantitative and qualitative studies of chemical transformation of cloned C3H mouse embryo cells sensitive to post confluence inhibition of cell division. Cancer Res. 33: 3239-3249 (1973).

R3 Rabbitts, T.H., T. Boehm and L. Mengl-Gaw. Chromosomal abnormalities in lymphoid tumours: mechanisms and role in tumour pathogenesis. Trends Genet. 4: 300-304 (1988).

R4 Redpath, J.L., C. Sun, M. Coleman et al. The application of a human hybrid cell system to studies of radiation-induced neoplastic cell transformation: quantitative cellular and molecular aspects. p. 85-90 in: Cell Transformation Systems and Radiation Induced Cancer in Man. (K. Chadwick, B. Barnhart and C. Seymour, eds.) Adam Hilger, Bristol, 1989.

R5 Reznikoff, C.A., E.A. Bookland, A.J. Klingelhutz et al. SV-HUC-1: a human urinary tract epithelial cell line for multistep in vitro transformation studies. p. 57-66 in: Cell Transformation Systems and Radiation Induced Cancer in Man. (K. Chadwick, B. Barnhart and C. Seymour, eds.) Adam Hilger, Bristol, 1989.

R6 Robbins, P.D., J.M. Horowitz and R.C. Mulligan. Negative regulation of human c-fos expression by the retinoblastoma gene product. Nature 346: 668-671 (1990).

R7 Reik, W. and M.A. Surani. Genomic imprinting and embryonal tumours. Nature 338: 112-113 (1989).

R8 Reik, W. Genomic imprinting and genetic disorders in man. Trends Genet. 5: 331-336 (1989).

R9 Rowley, J.D., H.M. Golumb and J.W. Vardiman. Non-random chromosome abnormalities in acute leukaemia and dysmyelopoietic syndromes in patients with previously treated malignant disease. Blood 58: 759-763 (1981).

R10 Roifman, C.M. and E.W. Gelfand. Heterogeneity of the immunological deficiency in ataxia-telangiectasia. p. 273-285 in: Ataxia-Telangiectasia: Genetics, Neuropathology and Immunology of a Degenerative Disease of Childhood. (R.A. Gatti and M. Swift, eds.) A.R. Liss, New York, 1985.

R11 Rovera, G., B.A. Reichard, S. Hudson et al. Point mutations in both transforming and non-transforming codons of the N-ras proto-oncogenes of Ph+ leukaemias. Oncogene 4: 867-872 (1989).

R12 Robbins, P.D., J.M. Horowitz and R.C. Mulligan. Negative regulation of human c-fos expression by the retinoblastoma gene product. Nature 346: 668-671 (1990).

R13 Radler-Pohl, A., J. Pohl and V. Schirrmacher. Selective enhancement of metastatic capacity in mouse bladder carcinoma cells after transfection with DNA from liver metastases of human colon carcinoma. Int. J. Cancer 41: 840-846 (1988).

R14 Roe, D. (ed.). Diet, Nutrition and Cancer: From Basic Research to Policy Implications. A.R. Liss, New York, 1983.

R15 Rittling, S.R. and D.T. Denhardt. p53 mutations in spontaneously immortalized 3T12 but not 3T3 mouse embryo cells. Oncogene 7: 935-942 (1992).

R16 Reik, W. Imprinting in leukaemia. Nature 359: 362-363 (1992).

R17 Richards, R.I. and G.R. Sutherland. Heritable unstable DNA sequences. Nat. Genet. 1: 7-9 (1992).

R18 Rhim, J.S., J.H. Yoo, J.H. Park et al. Evidence for the multistep nature of in vitro epithelial cell carcinogenesis. Cancer Res. 50 (Suppl): 5653s-5657s (1990).

R19 Rhim, Y.S., J.H. Park and G. Jay. Neoplastic transformation of human keratinocytes by polybrene-induced DNA-mediated transfer of an activated oncogene. Oncogene 4: 1403-1409 (1989).

R20 Ruther, U., D. Komitowski, F.R. Schubert et al. c-fos expression induces bone tumours in transgenic mice. Oncogene 4: 861-865 (1989).

R21 Rotter, V., O. Foord and N. Navot. In search of the functions of normal p53. Trend, Cell Biol. 3: 46-49 (1993).

S1 Stehelin, D., H.E. Varmus, J.M. Bishop et al. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. Nature 260: 170-173 (1976).

S2 Streffer, C. and W. Muller. Radiation risk from combined exposures to ionizing radiations and chemicals. Adv. Radiat. Biol. 11: 173-210 (1984).

S3 Swift, M. and C. Chase. Cancer and cardiac deaths in obligatory ataxia-telangiectasia heterozygotes. Lancet I: 1049-1050 (1983).

S4 Swift, M., P.J. Reitnauer, D. Morrell et al. Breast and other cancers in families with ataxia-telangiectasia. N. Engl. J. Med. 316: 1289-1294 (1987).

S5 Strong, L. Genetic and environmental interactions. Cancer 40: 1861-1866 (1977).

S6 Sawey, M.J. and A.R. Kennedy. Activation of oncogenes in radiation induced malignant transformation. p. 433-438 in: Low Dose Radiation: Biological Bases of Risk Assessment. (K.F. Baverstock and J.W. Stather, eds.) Taylor and Francis, London, 1989.

S7 Sawey, M.J., A.T. Hood, F.J. Burns et al. Activation of c-myc and c-k ras oncogenes in primary rat tumours induced by ionizing radiation. Mol. Cell. Biol. 7: 932-935 (1987).

S8 Silver, A., G. Breckon, W.K. Masson et al. Studies on radiation myeloid leukaemogenesis in the mouse. p. 494-500 in: Radiation Research. Volume 2. (E.M. Fielden et al., eds.) Taylor and Francis, London, 1987.

S9 Sado, T., H. Kamisaku, H. Kubo et al. Role of T-cell precursors in the bone marrow in thymic lymphomagenesis induced by split dose irradiation in B10 strain mice. p. 190 in: Proceedings of the 8th International Congress of Radiation Research. Volume 1 (1987).

S10 Silver, A., G. Breckon, J. Boultwood et al. Studies on putative initiating events for radiation-oncogenesis. p. 387-395 in: Low Dose Radiation: Biological Bases of Risk Assessment. (K.F. Baverstock and J.W. Stather, eds.) Taylor and Francis, London, 1989.

S11 Silver, A., J. Boultwood, G. Breckon et al. Interleukin-1ß gene deregulation associated with chromosomal rearrangement: a candidate initiating event for murine radiation-myeloid leukaemogenesis? Mol. Carcinog. 2: 226-233 (1989).

S12 Storer, J.B., T.J. Mitchell and R.J.M. Fry. Extrapolation of the relative risk of radiogenic neoplasms across mouse strains and to man. Radiat. Res. 114: 331-353 (1988).

S13 Sager, R. Genetic instability, suppression and human cancer. in: Gene Regulation in the Expression of Malignancy. Cancer Surv. 3: 321-334 (1984).

S14 Schirrmacher, V. Cancer metastasis. Adv. Cancer Res. 43: 1-74 (1985).

S15 Schmidt, J., E.I. Closs, E. Luz et al. Induction of osteogenic maturation and neoplastic transformation of in vitro differentiating skeletoblasts by C-type retroviruses from radiation-induced osteosarcomas. p. 239-250 in: Cell Transformation Systems and Radiation Induced Cancer in Man. (K. Chadwick, B. Barnhart and C. Seymour, eds.) A. Hilger, Bristol, 1989.

S16 Spiers, F.W. and J. Vaughan. The toxicity of the bone-seeking radionuclides. Leuk. Res. 13: 347-350 (1989).

S17 Sugarbaker, J.P., L.L. Gunderson and R.E. Wittes. Colorectal cancer. p. 800-803 in: Cancer: Principles and Practices of Oncology. (V.T. de Vita et al., eds.) Lippencott, Philadelphia, 1985.

S18 Sager, R. Tumour suppressor genes: the puzzle and the promise. Science 246: 1406-1412 (1989).

S19 Scrable, H.J., C. Sapienza and W.K. Cavanee. Genetic and epigenetic losses of heterozygosity in cancer predisposition and progression. Adv. Cancer Res. 54: 25-62 (1990).

S20 Sasaki, M., M. Okamoto, C. Sato et al. Loss of constitutional heterozygosity in colorectal tumours from patients with familial polyposis coli and those with non-polyposis colorectal carcinoma. Cancer Res. 49: 4402-4406 (1989).

S21 Sutherland, G.R. and R.N. Simmers. No statistical association between common fragile sites and non-random chromosome breakpoints in cancer cells. Cancer Genet. Cytogenet. 31: 9-16 (1988).

S22 Shippen-Lentz, D. and E.H. Blackburn. Functional evidence for an RNA template in telomerase. Science 247: 546-552 (1990).

S23 Sieweke, M.H., N.L. Thompson, M.B. Sporn et al. Mediation of wound-related Rouse sarcoma virus tumourigenesis by TGF-ß. Science 251: 1656-1663 (1990).

S24 Sandford, K.K. and R. Parshad. Detection of cancer prone individuals using cytogenetic response to X-rays. p. 113-129 in: Chromosome Aberrations: Basic and Applied Aspects. (G. Obe and A.T. Natarajan, eds.) Springer Verlag, Berlin, 1990.

S25 Seabright, M. High resolution studies on the pattern of induced exchanges in the human karyotype. Chromosoma 40: 333-346 (1973).

S26 Sabatier, L., M. Mulems, M. Prieur et al. Specific sites of chromosomal radiation-induced rearrangements. p. 211-224 in: New Trends in Genetic Risk Assessment. (G. Jolles and A. Cordier, eds.) Academic Press, New York, 1989.

S27 Sapienza, C. Genomic imprinting and dominance modification. Ann. N.Y. Acad. Sci. 564: 24-28 (1989).

S28 Stingl, G., S.I. Katz and I. Clement. Immunologic functions of Ia-bearing epidermal Langerhan cells. J. Immunol. 121: 2005-3013 (1978).

S29 Sankaranarayan, K. Ionizing radiation and genetic risks. III. Nature of spontaneous and radiation-induced mutations in mammalian in vitro systems and mechanisms of induction of mutations by radiation. Mutat. Res. 258: 75-97 (1991).

S30 Schrader, J.W. and R.M. Crapper. Autogenous production of a haemopoietic growth factor, persisting-cell-stimulating factor, as a mechanism for transformation of bone marrow derived cells. Proc. Natl. Acad. Sci. (USA) 80: 6892-6896 (1983).

S31 Sloan, S.R., E.W. Newcomb and A. Pellicer. Neutron radiation can activate k-ras via a point mutation in codon 146 and induces a different spectrum of ras mutations than does gamma radiation. Mol. Cell. Biol. 10: 405-408 (1990).

S32 Silver, A.R.J., W.K. Masson, A.M. George et al. The IL-1a and ß genes are closely linked (<70kb) on mouse chromosome 2. Somat. Cell Mol. Genet. 16: 549-556 (1990).

S33 Silver, A.R.J., A.M. George, W.K. Masson et al. DNA methylation changes in the IL-1(2F) chromosomal region of some radiation-induced acute myeloid leukaemias carrying chromosome 2 rearrangements. Genes, Chrom. Cancer 3: 376-881 (1991).

S34 Stanbridge, E.J. The evidence for human tumour suppressor genes. p. 3-13: in: Genetic Basis for Carcinogenesis. (A.G. Knudson et al, eds.) Taylor and Francis, London, 1990.

S35 Shippey, C.A., M. Layton and L.M. Secker-Walker. Leukaemia characterised by multiple sub-clones with unbalanced translocations involving different telomeric segments. Genes, Chrom. Cancer 2: 14-17 (1990).

S36 Starling, J.A., J. Maule, N.D. Hastie et al. Extensive telomere repeat arrays in mouse are hypervariable. Nucleic Acids Res. 18: 6881-6888 (1990).

S37 Sen, D. and W. Gilbert. Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis. Nature 334: 364-366 (1988).

S38 Schultz, R.M., S. Silberman, B. Persky et al. Inhibition by human recombinant tissue inhibitor of metalloproteinases of human amnion invasion and lung colonization by murine B16-F10 melanoma cells. Cancer Res. 48: 5539-5545 (1988).

S39 Steeg, P.S., G.B. Bevilacqua, L. Kopper et al. Evidence of a novel gene associated with low tumour metastatic potential. J. Natl. Cancer Inst. 80: 200-204 (1988).

S40 Sugahara, T., T. Aoyama, M. Ikebuchi et al. (eds). Proceedings of the International Conference on Risk Assessment of Energy Development and Modern Technology. Kyoto Health Research Foundation (1989).

S41 Srivastava, S., Z. Zou, K. Pirollo et al. Germ line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. Nature 348: 747-749 (1990).

S42 Sell, S. Basic Immunology: Immune Mechanisms in Health and Disease. Elsevier, New York, 1987.

S43 Silver, A. and R. Cox. Telomere-like DNA polymorphisms associated with genetic predisposition to acute myeloid leukaemia in irradiated CBA mice. Proc. Natl. Acad. Sci. (USA) 90: 1407-1410 (1993).

S44 Sidransky, D., A. von Eschenbach, Y.C. Tsai et al. Identification of p53 gene mutations in bladder cancers and urine samples. Science 252: 706-709 (1991).

S45 Sheer, D. Chromosomes and cancer. p. 269-295 in: Introduction to the Cellular and Molecular Biology of Cancer. (L.M. Franks and N.M. Teich, eds.) Oxford University Press, Oxford, 1990.

S46 Snipes, M.B. Long-term retention and clearance of particles inhaled by mammalian species. Crit. Rev. Toxicol. 20: 175-211 (1991).

S47 Sidransky, D., P. Frost, A. von Eschenbach et al. Clonal origin of bladder cancer. N. Engl. J. Med. 326: 737-740 (1992).

S48 Su, L.-K., K.W. Kinzler, B. Vogelstein et al. Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. Science 256: 668-670 (1992).

S49 Stallings, R.L., N.A. Doggett, K. Okamura et al. Chromosome 16 - specific DNA sequences that map to chromosomal regions known to undergo breakage/rearrangement in leukaemia cells. Genomics 13: 332-338 (1992).

S50 Sherman, M.L., R. Datta, D.E. Hallahan et al. Ionizing radiation regulates expression of the c-jun proto-oncogene. Proc. Natl. Acad. Sci. (USA) 87: 5663-5666 (1990).

S51 Shimoyama, Y. and S. Hirohashi. Cadherin intercellular adhesion molecule in hepatocellular carcinomas: loss of E-cadherin expression in an undifferentiated carcinoma. Cancer Lett. 57: 131-135 (1991).

S52 Smith, S.A., D.F. Easton, D.G.R. Evans et al. Allele losses in the region 17q12-21 in familial breast and ovarian cancer involve the wild-type chromosome. Nat. Genet. 2: 128-131 (1992).

S53 Sutherland, G.R., E.A. Haan, E. Kremer et al. Hereditary unstable DNA: a new explanation for some old genetic questions? Lancet 338: 289-292 (1991).

S54 Stamper, M.R. and J.C. Bartley. Induction of transformation and continuous cell lines from normal human mammary epithelial cells after exposure to benzo[α]pyrene. Proc. Natl. Acad. Sci. (USA) 82: 2394-2398 (1985).

S55 Schmidt, J., A. Luz and V. Erfle. Endogenous murine leukaemia viruses: frequency of radiation-activation and novel pathogenic effects of viral isolates. Leuk. Res. 12: 393-403 (1988).

S56 Sado, T. Experimental radiation carcinogenesis studies at NIRS. p. 36-42 in: Proceedings of the International Conference on Radiation Effects and Protection, March 18-29, 1992, Mito, Ibaraki, Japan (1992).

S57 Sado, T., H. Kamisaku and E. Kubo. Bone marrow-thymus interactions during thymic lymphomagenesis induced by fractionated radiation exposure in B10 mice: analysis using bone marrow transplantation between Thy 1 congenic mice. J. Radiat. Res. 32 (Suppl. 2): 168-180 (1991).

S58 Strauss, P.G., K. Mitreiter, H. Zitzelsberger et al. Elevated p53 RNA expression correlates with incomplete osteogenic differentiation of radiation-induced murine osteosarcomas. Int. J. Cancer 50: 252-258 (1992).

S59 Sturm, S.A., P.G. Strauss, S. Adolph et al. Amplification and rearrangement of c-myc in radiation-induced murine osteosarcomas. Cancer Res. 50: 4146-4153 (1990).

S60 Stegelmeier, B.L., N.A. Gillett, A.H. Rebar et al. The molecular progression of plutonium-239-induced rat lung carcinogenesis: Ki-ras expression and activation. Mol. Carcinog. 4: 43-51 (1991).

S61 Sakai, H. and K. Ogawa. Mutational activation of c-Ha-ras genes in intraductal proliferation induced by N-nitroso-N-methylurea in rat mammary glands. Int. J. Cancer 49: 140-144 (1991).

S62 Saint, R. and P.L. Wigley. Developmental regulation of the cell cycle. Curr. Opin. Genet. Dev. 2: 614-620 (1992).

S63 Solomon, E., J. Borrow and A.D. Goddard. Chromosome aberrations and cancer. Science 254: 1153-1160 (1991).

S64 Swift, M., D. Morrell, R.B. Massey et al. Incidence of cancer in 161 families affected by ataxia-telangiectasia. N. Engl. J. Med. 325: 1831-1836 (1991). See also Correspondence, N. Engl. J. Med. 326: 1357-1361 (1991).

S65 Sakamoto, H., M. Oyamada, K. Enomoto et al. Differential changes in expression of gap function proteins connexin 26 and 23 during hepatocarcinogenesis in rats. Jpn. J. Cancer Res. 83: 1210-1215 (1992).

S66 Saul, R.L. and B.N. Ames. Background levels of DNA damage in the population. Basic Life Sci. 38: 529-535 (1986).

T1 Tanooka, H. Monoclonal growth of cancer cells: experimental evidence. GANN 79: 657-665 (1988).

T2 Thacker, J. The nature of mutants induced by ionizing radiation in cultured hamster cells. Mutat. Res. 160: 267-275 (1986).

T3 Trent, J.M., E.J. Stanbridge, H.L. McBride et al. Tumourigenicity in human melanoma cell lines controlled by introduction of human chromosome 6. Science 247: 568-571 (1990).

T4 Trakhtenbrot, L., R. Krauthgamer, P. Resnitzky et al. Deletion of chromosome 2 is an early event in the development of radiation-induced myeloid leukaemia in SLJ/J mice. Leukaemia 2: 545-550 (1988).

T5 Toguchida, J., K. Ishizaki, M.S. Sasaski et al. Preferential mutation of paternally derived RB gene as the initial event in sporadic osteosarcoma. Nature 338: 156-158 (1989).

T6 Taylor, A.M.R. Cytogenetics of ataxia-telangiectasia. p. 53-81 in: Ataxia-Telangiectasia. (B.A. Bridges and D.G. Harnden, eds.) John Wiley and Sons, Chichester, 1982.

T7 Taalman, R.D.F.M., N.E.G. Jaspers, J.M.J. Scheres et al. Hypersensitivity to ionizing radiation in vitro in a new chromosomal breakage disorder, the Nijmegen Breakage Syndrome. Mutat. Res. 112: 23-32 (1983).

T8 Thacker, J. Radiation-induced mutation in mammalian cells at low doses and dose rates. Adv. Radiat. Biol. 16: 77-117 (1992).

T9 Terzaghi-Howe, M. Induction of pre-neoplastic alterations by X-rays and neutrons in exposed rat tracheas and isolated tracheal epithelial cells. Radiat. Res. 120: 352-363 (1989).

T10 Terzaghi-Howe, M. Interactions between cell populations influence expression of the transformed phenotype in irradiated rat tracheal epithelial cells. Radiat. Res. 121: 242-247 (1990).

T11 Trosko, J.E., C.L. Chang and B.V. Madhukar. Modulation of intercellular communication during radiation and chemical carcinogenesis. Radiat. Res. 123: 241-251 (1990).

T12 Trosko, J.E., C.L. Chang, B.V. Madhukar et al. Intercellular communication: a paradigm for the interpretation of the initiation/promotion/progression model of carcinogenesis. in: Chemical Carcinogenesis: Modulation and Combination Effects. (J.C. Arcos, ed.) Academic Press, New York, 1992.

T13 Tanaka, K., I. Satokata, Z. Ogita et al. Molecular cloning of a mouse DNA repair gene that complements the defect of group A xeroderma pigmentosum. Proc. Natl. Acad. Sci. (USA) 86: 5512-5516 (1989).

T14 Trosko, J.E., V.M. Riccardi, C.C. Chang et al. Genetic predisposition to initiation or promotion phases in human carcinogenesis. p. 13 in: Biomarkers, Genetics and Cancer. (H. Anton-Guirgis and H.T. Lynch, eds.) Van Nostrand Reinhold, New York, 1985.

T15 Thompson, L.H., K.W. Brookman, N.J. Jones et al. Molecular cloning of the human XRCC1 gene, which corrects defective DNA strand break repair and sister chromatid exchange. Mol. Cell. Biol. 10: 6160-6171 (1990).

T16 Tomatis, L., C. Agthe, H. Bartsch et al. Evaluation of the carcinogenicity of chemicals: a review of the monograph program of the International Agency for Research on Cancer (1971 to 1977). Cancer Res. 38: 877-885 (1978).

T17 Toguchida, J., T. Yamaguchi, S.H. Drayton et al. Prevalence and spectrum of germline mutations of the p53 gene among patients with sarcoma. N. Engl. J. Med. 326: 1301-1308 (1992).

T18 Tveito, G., I.L. Hansteen, H. Dalen et al. Immortalization of normal human kidney epithelial cells by nickel(II). Cancer Res. 49: 1829-1835 (1989).

T19 Takahashi, R., T. Hashimoto, H.J. Xu et al. The retinoblastoma gene functions as a growth and tumor suppressor in human bladder carcinoma cells. Proc. Natl. Acad. Sci. (USA) 88: 5257-5261 (1991).

T20 Tauchi, H. and S. Sawada. Suppression of gamma and neutron induced neoplastic transformation by ascorbic acid in Balb/c3T3 cells. Int. J. Radiat. Biol. 63: 1-6 (1993).

T21 Tatsumi, K., A. Fujimori, A. Tachibana et al. Concurrent analysis of mutagenesis by ionizing radiation at the APRT and HPRT loci in human lymphoblastoid cells. in: Low Dose Irradiation and Biological Defense Mechanisms. (T. Sugahara et al., eds.) Elsevier Science Publishers, Amsterdam, 1992.

U1 United Nations. Sources, Effects and Risks of Ionizing Radiation. United Nations Scientific Committee on the Effects of Atomic Radiation, 1988 Report to the General Assembly, with annexes. United Nations sales publication E.88.IX.7. United Nations, New York, 1988.

U2 United Nations. Genetic and Somatic Effects of Ionizing Radiation. United Nations Scientific Committee on the Effects of Atomic Radiation, 1986

Report to the General Assembly, with annexes. United Nations sales publication E.86.IX.9. United Nations, New York, 1986.

U3 United Nations. Ionizing Radiation: Sources and Biological Effects. United Nations Scientific Committee on the Effects of Atomic Radiation, 1982 Report to the General Assembly, with annexes. United Nations sales publication E.82.IX.8. United Nations, New York, 1982.

U4 United Nations. Sources and Effects of Ionizing Radiation. United Nations Scientific Committee on the Effects of Atomic Radiation, 1977 report to the General Assembly, with annexes. United Nations sales publication E.77.IX.1. United Nations, New York, 1977.

U11 Urlaub, G., P. Mitchell, E. Kas et al. Effects of γ-rays at the dihydroifolate reductase locus: deletions and inversions. Somat. Cell Mol. Genet. 12: 555-566 (1986).

U12 Ullrich, R.L. Cellular and molecular changes in mammary epithelial cells following irradiation. Radiat. Res. 128: 5136-5140 (1991).

U13 Umeki, S., S. Kyoizumi, Y. Kusunoki et al. Flow cytometric measurement of somatic cell mutations in Thorotrast patients. Jpn. J. Cancer Res. 82: 1349-1353 (1991).

V1 Varmus, H. and J.M. Bishop (eds.). Biochemical mechanisms of oncogene activity: proteins encoded by oncogenes. Cancer Surveys 5: (1986).

V2 Vousden, K.H. and C.J. Marshall. Three different activated ras genes in mouse tumours: evidence for oncogene activation during progression of a mouse lymphoma. EMBO J. 4: 913-917 (1984).

V3 Van der Bliek, A.M. and P. Borst. Multidrug resistance. Adv. Cancer Res. 52: 165-204 (1989).

V4 Van der Hoof, A. Stromal involvement in malignant growth. Adv. Cancer Res. 50: 159-196 (1988).

V5 Van den Berghe, H., K. Vermaelen, C. Mecucci et al. The 5q-anomaly. Cancer Genet. Cytogenet. 17: 189-255 (1985).

V6 Van der Mey, A.G.L., P.D. Maaswinkel-Mooy, C.J. Cornellisse et al. Genomic imprinting in hereditary glomus tumours: evidence for new genetic theory. Lancet 2: 1291-1294 (1989).

V7 Van Duin, M., J. deWit, H. Odijk et al. Molecular characterisation of the human excision repair gene ERCC-1: cDNA cloning and amino acid homology with the yeast DNA repair gene RAD 1O. Cell 44: 13-23 (1986).

V8 Van der Rauwelaert, E., J.R. Maisin and J. Merregaert. Provirus integration and myc amplification in ^{90}Sr induced osteosarcomas of CF1 mice. Oncogene 2: 215-222 (1988).

V9 Verbeek, S., M. van Lohuizen, M. van der Valk et al. Mice bearing the E mu-myc and E mu-pim-1 transgenes develop pre-B-cell leukaemia prenatally. Mol. Cell. Biol. 11: 1176-1179 (1991).

V10 Vahakangas, K.H., J.M. Samet, R.A. Metcalf et al. Mutations of p53 and ras genes in radon-associated lung cancer from uranium miners. Lancet 339: 576-580 (1992).

W1 Watson, J.D. (ed.). The Molecular Biology of the Gene. Benjamin Commings, New York, 1988.

W2 Waxman, J. and K. Sikora (eds.). The Molecular Biology of Cancer. Blackwells, Oxford, 1989.

W3 Woodruff, M. Tumour clonality and its biological consequences. Adv. Cancer Res. 50: 197-229 (1988).

W4 Weinstein, I.B. Protein kinase, phospholipid and control of growth. Nature 302: 750-751 (1983).

W5 Weinberg, R.A. Oncogenes and the Molecular Origins of Cancer. Cold Spring Harbour Laboratory, 1989.

W6 Wallace, M.R., D.A. Marchuk, L.B. Andersen et al. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. Science 249: 181-186 (1990).

W7 Woloschak, G.E., C.L. Chin-Mei and P. Shearin-Jones. Regulation of protein kinese C by ionizing radiation. Cancer Res. 50: 3963-3967 (1990).

W8 Wheelock, E.F. and M.K. Robinson. Endogenous control of the neoplastic process. Lab. Invest. 48: 120-139 (1983).

W9 Werness, B.A., A.J. Levine and P.M. Howley. Association of human papilloma virus type 16 and 18E6 proteins with p53. Science 248: 76-79 (1990).

W10 Ward, J.F. DNA damage and repair. p. 403-421 in: Physical and Chemical Mechanisms in Molecular Radiation Biology. (W.A. Glass and M.N. Varma, eds.) Plenum Press, New York, 1991.

W11 Windle, J.J., D.M. Albert, J.M. O'Brien et al. Retinoblastoma in transgenic mice. Nature 343: 665-669 (1990).

W12 Ward, J.F. The yield of DNA double stand breaks produced intracellularly by ionizing radiation: a review. Int. J. Radiat. Biol. 57: 1141-1150 (1990).

W13 Wagner, S. and M.R. Green. A transcriptional tryst. Nature 352: 189-190 (1991).

W14 Weinberg, R.A. Tumour suppressor genes. Science 254: 1138-1146 (1991).

W15 Weiss, L. Principles of Metastasis. Academic Press, Orlando, 1985.

W16 Wyke, J.A. Viruses and cancer. p. 203-229 in: Introduction to the Cellular and Molecular Biology of Cancer. (L.M. Franks and N.M. Teich, eds.) Oxford University Press, Oxford, 1990.

W17 Warner, J.F. and G. Dennert. Effects of a cloned cell line with NK activity on bone marrow transplants, tumour development and metastasis in vivo. Nature 300: 31-34 (1982).

W18 Wigley, C. and A. Balmain. Chemical carcinogenesis and precancer. p. 148-174 in: Introduction to the Cellular and Molecular Biology of Cancer. (L.M. Franks and N.M. Teich, eds.) Oxford University Press, Oxford, 1990.

W19 Wolf, C.R. Cytochrome P-450: Polymorphic multigene families involved in carcinogen activation. Trends Genet. 2: 209-214 (1986).

W20 Weinstein, B. Growth factors, oncogenes and multistage carcinogenesis. J. Cell Biochem. 33: 213-224 (1987).

W21 Weichselbaum R.R., D.E. Hallahan, V. Sukhatme et al. Biological consequences of gene regulation after ionizing radiation exposure. J. Natl. Cancer Inst. 83: 480-484 (1991).

W22 Wright, P.A., E.D. Williams, N.R. Lemoine et al. Radiation-associated and "spontaneous" human thyroid carcinomas show a different pattern of ras oncogene mutation. Oncogene 6: 471-473 (1991).

W23 Walker, C., L.T.L. Goldsworthy, D.C. Wolf et al. Predisposition to renal cell carcinoma due to alteration of a cancer susceptibility gene. Science 255: 1693-1695 (1992).

W24 Willman, C.L., C.E. Sever, M.G. Pallavicini et al. Deletion of IRF-1, mapping to chromosome 5q31.1, in human leukaemia and pre-neoplastic myelodysplasia. Science 259: 968-971 (1993).

W25 Williams, A.C., S. Harper and C. Paraskeva. Neoplastic transformation of a human colonic epithelial cell line: experimental evidence for the adenoma to carcinoma sequence. Cancer Res. 50: 4724-4730 (1990).

W26 Wallin, H., H. Andersson, L.L. Nielsen et al. p53 mutations in human liver tumours induced by alpha-radiation. Cancer Detect. Prev. 17: 91 (1993).

W27 Ward, J.F. Response to commentary by D. Billen. Radiat. Res. 126: 385-387 (1991).

Y1 Yunis, J.J., A.L. Soreng and A.E. Bowe. Fragile sites are targets of diverse mutagens and carcinogens. Oncogene 1: 59-70 (1987).

Y2 Yuspa, S.H. and M.C. Poirier. Chemical carcinogenesis: from animal models to molecular models in one decade. Adv. Cancer Res. 50: 25-70 (1988).

Y3 Young, J.D.E. and Z.A. Cohn. How killer cells kill. Sci. Am. 258: 28-36 (1988).

Y4 Young, R.A. and T.J. Elliott. Stress proteins, infection and immune surveillance. Cell 59: 5-8 (1989).

Y5 Yasukawa, M., T. Terasima and M. Seki. Radiation-induced neoplastic transformation of C3H10T1/2 cells is suppressed by ascorbic acid. Radiat. Res. 120: 456-467 (1989).

Y6 Yonish-Rouach, E., D. Resnitzky, J. Lotem et al. Wild type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature 352: 345-347 (1991).

Y7 Yamasaki, H., M. Hollstein, M. Mesnil et al. Selective lack of intercellular communication between transformed and non-transformed cells as a common property of chemical and oncogene transformation of BALB/c 3T3 cells. Cancer Res. 47: 5658-5662 (1987).

Y8 Yotti, L.P., C.L. Chang and J.E. Trosko. Elimination of metabolic co-operation in Chinese hamster cells by a tumour promotor. Science 208: 1089-1091 (1979).

Y9 Yu, S., M. Pritchard, E. Kremer et al. Fragile X genotype characterised by an unstable region of DNA. Science 252: 1179-1181 (1991).

Y10 Yasukawa, M., T. Terasima and M. Seki. Radiation-induced neoplastic transformation of C3H10T½ cells is suppressed by ascorbic acid. Radiat. Res. 120: 456-467 (1989).

Z1 Zhang, R., J.D. Haag and M.N. Gould. Reduction in the frequency of activated ras oncogenes in rat mammary carcinomas with increasing N-methyl-N-nitrosourea doses or increasing prolactin levels. Cancer Res. 50: 4286-4290 (1990).

Z2 Zhang, R., J.D. Haag and M.N. Gould. Quantitating the frequency of initiation and cH-ras mutation in in situ N-methyl-N-nitrosourea-exposed rat mammary gland. Cell Growth Differ. 2: 1-6 (1991).

Z3 Zur Hausen, H. Viruses in human cancers. Science 254: 1167-1173 (1991).

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