Review Article

RADIATION BIOLOGY: CONCEPTS FOR RADIATION PROTECTION

R. Julian Preston*

Abstract—**The opportunity to write a historical review of the field of radiation biology allows for the viewing of the development and maturity of a field of study, thereby being able to provide the appropriate context for the earlier years of research and its findings. The pioneering work of Muller, Sax, and McClintock, and many others, has stood the test of time. The idea that x-rays could damage the genetic material and result in interactions that could lead to gene mutations and a range of chromosomal alterations is now interpretable in terms of induced DNA damage and errors of DNA repair. The expanded idea that such genetic alterations can be induced by DNA damage that is produced by one or two tracks of ionizing radiation remains the mainstay of radiation biology. The impact of the more recent molecular approaches to unraveling the mechanism behind this simple concept has confirmed this fundamental observation. The remarkable advances have allowed for a fairly complete understanding of the specific types of DNA damage induced by ionizing radiations and the pivotal role played by the errors of repair of double-strand breaks. Given our considerably enhanced knowledge of the details of the DNA repair processes involved, misrepair is a very unlikely event. The role of potential confounders of the concept of dose-response (e.g., bystander effects, genomic instability, and adaptive responses) is taking on a growing importance to the field. The evolving need is to begin to consider mechanistically-based dose-response models for cancer risk such that any potential impact of confounders on the response at low, environmental doses can be assessed. Thus, radiation biology research has always had a focus on how best to protect human health from radiation exposures and will continue to do so.**
Health Phys. 87(1):3–14; 2004

Key words: historical profiles; biokinetics; radiobiology; radiation protection

INTRODUCTION

ANNIVERSARIES PROVIDE an opportunity to reflect on the past, assess the present, and predict the future—perhaps without having to justify the exercise. Such an opportunity is provided by the upcoming 50th Anniversary of the Health Physics Society in 2005–2006. During this almost 50 years, the field of radiation biology has been through a number of clearly marked phases and is currently in another evolution given the advances made possible by the advent of the "new" molecular biology. It is pretty easy to predict that enormous advances will be made in the field, especially in the low dose area, over the next 5–10 years, given the progress of technical advances.

This review article is a personal view of the field of radiation biology by necessity, since selection has to be made from the vast array of information that has accumulated over the past 50 years. Such a selection process also means that there will be omissions, for which apologies are offered. Hopefully, the frequent use of review articles for reference to the published literature will minimize perceived or real omissions.

The overall aim of the review is to demonstrate the role that experimental radiation biology studies have played in the development of radiation protection standards. A recent review by Joel Bedford and Bill Dewey (Bedford and Dewey 2002) to recognize the 50th Anniversary of the Radiation Research Society provides an excellent source of information for a historical perspective of highlights to radiation genetics, radiation cytogenetics, radiation carcinogenesis, DNA repair and radiosensitivity, and cellular radiobiology. The authors asked the pertinent question, *Has Anything Important Been Learned by Irradiating Cells?* While, of course, the answer is yes, many important things have been learned as presented in their 40-page review. However, it remains appropriate to address the issue of "important" to what; my viewpoint will be important to risk assessment and radiation protection.

THE EARLY YEARS

The early years of radiation biology research were highlighted by several fundamental studies that provided sentinel information on the organization of genes and chromosomes. In 1927, H.J. Muller (Muller 1927) published his landmark research on the use of radiation to mutate genes of *Drosophila melanogaster*. The impact of this research was perhaps best described by the author:

* Environmental Carcinogenesis Division, NHEERL, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

For correspondence or reprints contact: the author at the above address, or email at preston.julian@epa.gov.

(*Manuscript received* 29 *February* 2004; *accepted* 2 *April* 2004)
0017-9078/04/0

"In conclusion, the attention of those working along classical genetic lines may be drawn to the opportunity, afforded them by the use of x-rays, of creating in their chosen organisms a series of artificial races for use in the study of genetic and 'phaelogenetic' phenomena. If, as seems likely on general considerations, the effect is common to most organisms, it should be possible to produce, 'to order,' enough mutations to furnish respectable genetic maps, in their selected species, and by the use of the mapped genes, to analyze the aberrant chromosome phenomena simultaneously obtained."

This research was quite clearly the harbinger of so much that followed over the next 70 plus years. In fact, the idea for broad genome mutagenesis and its use in functional genomics is identified by Muller.

Equally far-sighted predictions were made by Barbara McClintock (1931) based upon her studies of the behavior of maize chromosomes either under normal conditions or following x-irradiation. She associated specific chromosomal alterations with cellular or organism phenotypes, leading to the view that there existed a form of mutagenesis that was based on cytogenetic alterations as opposed to the previously described form that resulted from gene mutations.

These seminal research studies of Muller and McClintock were put into a dose-response framework by the pioneering work of Karl Sax (1938, 1939, 1940). Using *Tradescantia* microspores, it was shown that x rays could induce a variety of chromosome aberrations that were either the result of exchanges between chromosomes or deletions of ends (terminal) or interstitial regions of chromosomes. It was further shown that the exchanges increased as a nonlinear function of dose whereas the deletions increased linearly with dose. More recent studies (Revell 1966; Brewen and Brock 1968) showed that deletions were more likely to be the result of exchange events and that they also increased nonlinearly with dose. Sax also demonstrated that the effectiveness of a given dose was reduced if it was delivered over an extended period of time or split into two fractions separated by an hour or so. The effectiveness was much greater for neutrons than for x rays, even allowing for some dosimetric obstacles with neutrons. These data were extended by the elegant studies of Lea, Catcheside, and Thoday (reviewed in Lea 1962) to provide a formalism to the dose-response. The general shape of the dose-response curve for x-ray-induced chromosomal interchanges was defined as $Y = aD + bD^2$ (where Y is yield and D is dose). The aD and bD^2 terms of the curve were originally described as one-hit and two-hit processes. An elegant paper of Neary (1965) succinctly argued that these terms were more correctly defined as one-track and two-track processes. This latter definition is used throughout this review.

These early studies are highlighted because they form a basis of so much of the field of radiation biology that has been developed subsequently, including the more recent molecular aspects.

Another feature of the early years of the field was the development of assay systems that would allow for an enhancement of our understanding of the impact of ionizing radiation at the cellular and whole organism levels in a qualitative and a quantitative sense.

In 1941, Beadle and Tatum (1941) reported on the association between genetic alterations and defects in biochemical pathways that resulted in specific phenotypes. The mutations that they used for these studies were induced by x-irradiation. These types of study were extended to bacterial species and yeasts, thereby providing an ability to begin to address the underlying molecular basis of mutations. The study of x-ray-induced mutations was extended to mice through the efforts of William Russell, Liane Russell, and colleagues (Russell 1951) at Oak Ridge National Laboratory and Toby Carter and Mary Lyon and colleagues (Carter et al. 1956) at the Medical Research Council Radiobiology Unit in Harwell (UK). These groups each developed a recessive mutant tester mouse strain that could be used to detect mutations induced in germ cells at the seven selected loci (Oak Ridge) or five specific loci (Harwell). The collected data were broadly similar to those obtained previously for *Drosophila* and bacterial and fungal systems, namely that the frequency of mutations increased with increasing x-ray dose, that the frequency of mutations was decreased if the dose was fractionated or given over an extended period of time (i.e., chronically), and that high LET neutrons were more effective at inducing specific locus mutations compared to low LET x or gamma rays. It was later shown that the majority of the x-ray and neutron-induced mutations were the result of quite large deletions (Russell 1986). These types of radiation-induced mutation data formed the basis for the development of a genetic risk assessment (UNSCEAR 1977). In fact, the mouse data still are used for radiation-induced mutation in the genetic risk assessment process (UNSCEAR 2001). The human data on hereditary effects available from persons exposed as a result of the Japanese atomic bombs and the Chernobyl accident are quite limited and do not allow for a risk assessment to be determined. The recent expansion of data on the association between specific DNA alterations and disease phenotypes has meant that spontaneous mutations rates for humans can be used as part of the calculation for a genetic risk assessment (UNSCEAR 2001). The current risks, for a population exposed to radiation in one

generation only, to the progeny of the first post-radiation generation are estimated to be 3,000 to 4,700 cases per gray per one million progeny. This value constitutes 0.4 to 0.6% of the baseline frequency of the same disorders in the human population (UNSCEAR 2001).

A further significant technical advance enhanced the ability to assess cellular and molecular responses to radiation exposures. Puck and colleagues developed a single cell cloning technique in vitro that allowed cell survival curves to be constructed following radiation exposures (Puck and Marcus 1955). Initial studies were for cell killing by x rays, and the observation of a "shoulder" to the survival curve led to the idea that some form of cellular recovery occurred at low-medium doses. This general hypothesis was supported by the studies of Elkind and Sutton (1960) who showed that repair of x ray-induced cellular damage could occur in the period between doses given as two fractions. Of course, this concept of cellular repair was later to be interpreted in terms of specific DNA repair processes, as discussed later in this article.

An additional valuable experimental tool was developed from the single cell cloning approach, namely the ability to conduct somatic cell mutation analysis. The initial studies were performed using resistance to 8-azaguanine (8-AG) or 6-thioguanine (6-TG) as selectable markers for mutations induced by x rays (Knaap and Simons 1975). The resistant mutations selected represent alterations in the hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) gene that catalyzes the conversion of hypoxanthine or guanine into the purine nucleotide monophosphate. Mutant HGPRT cannot convert 8-AG or 6-TG into a toxic metabolite and hence the resistant phenotype. As a broad summary of data that have been developed for radiation-induced mutations at the HGPRT locus, frequencies of 20×10^{-6} mutants per locus per gray have been reported for exposures to high dose rate x rays or gamma rays (Thacker and Cox 1975; Thacker et al. 1977). These mutant frequencies are quite similar to those reported for the mouse specific locus tests and perhaps reflect a similar overall size to the gene and similar recoverability of the mutations. A number of other genetic loci have been utilized for in vitro and in vivo analysis of radiation-induced mutations. These include the thymidine kinase locus (trifluorothymidine resistance) (Evans et al. 1985; Liber and Thilly 1982; Liber et al. 1989) and the CD59 locus that is located on human chromosome 11, and is analyzed in a hybrid cell system that consists of a CHO cell that contains human chromosome 11 (A_L cell system). The x-ray-induced mutation frequency was reported to be much higher than for other systems (i.e., 1×10^{-3} Gy^{-1}), but since nearly the whole chromosome 11 could be lost without killing the cell, the mutational target is very large (Waldren et al. 1998).

In the area of cytogenetics, two serendipitous findings radically changed the field. These were (a) a hypotonic salt solution could be used to swell cells and make mammalian metaphase chromosomes much more readily visible (Hsu 1952) and (b) a mitogenic agent, phytohemagglutinin, could be used to induce peripheral lymphocytes to reenter the cell cycle and proceed to mitosis for observation (Moorhead et al. 1960). These two technical advances led to a range of studies designed to study the induction of chromosome aberrations in mammalian cells by ionizing radiations. In general, the basic set of responses were very similar to those reported a number of years previously for plant cells. The dose-response curve for exchange aberrations fit a linear-quadratic relationship, the yield of exchange aberrations was reduced when the dose was fractionated or delivered chronically, the dose-response curve for fission neutrons was linear and the effectiveness of fission neutrons was greater by a factor of 10 or more compared to x rays (reviewed by Bender 1995). The fact that these fundamental responses are similar across a wide range of species, including plants and animals, highlights that they have similar underlying mechanisms—the role of DNA double-strand break induction and DNA repair/misrepair are that link and will be discussed later.

Another significant development that resulted from the ability to analyze human chromosomes in stimulated peripheral lymphocytes was the use of chromosome aberrations as a biodosimeter for assessing dose received in radiation accidents (reviewed in Bender et al. 1988). There is an extensive literature on the application of this technique, and, in general, the estimated biological dose is a reliable match to the estimated or measured physical dose. An interesting aside to this approach is that the chromosome aberration calibration curve for human lymphocytes is obtained in vitro whereas, of course, the lymphocytes from accidentally exposed individuals are from in vivo exposures. The fact that biological and physical dose estimates are in very good agreement shows that lymphocytes respond similarly to in vitro and in vivo exposures. This is a useful concept that suggests that for radiation, in vitro cellular studies might well be predictive of in vivo cellular responses. Clearly, host factors will influence disease processes emanating from single cell or tissue responses. This general observation is in contrast to that observed for chemical exposures where activation and deactivation pathways that occur in vivo are difficult to replicate in vitro.

An additional observation from the use of peripheral lymphocytes as biological dosimeters is that the dose-response curve for chronic exposures is that defined by

the one-track component of the linear-quadratic in vitro (or in vivo) curve for chromosomal dicentrics (reviewed in NCRP 2001). Again, this provides evidence that the response to chronic exposures is predicted by the one-track process, leading to the important conclusion that at low doses there is little if any difference in the mutational response to acute or chronic exposures.

It appeared at this time that some new and quite different types of information were needed to move the field in new directions such that a more precise description of cellular damage and its processing could be derived.

THE MIDDLE YEARS

The required momentum was provided by a series of studies in the 1960's that markedly changed the concept of how cells respond to the radiation exposures. Rupert (1960), building on some earlier observations of Kelner (1949), showed that bacteria could repair UV-induced pyrimidine dimers in DNA by a process of photoreactivation, or enzymatic reversal by light. This repair led to a substantial increase in survival of UV-exposed bacteria. Shortly afterwards, Setlow and Carrier (1963) showed that bacteria could also effectively remove pyrimidine dimers from their DNA by a process called excision repair, so-called because the dimer and some adjacent nucleotides were enzymatically removed from the DNA and the excised product could be recovered as short DNA fragments (Boyce and Howard-Flanders 1964). The concept of DNA repair, developed from these early studies with ultraviolet light-induced DNA damage, has fundamentally changed our ability to interpret cellular responses to radiation exposures. It is interesting to note that the much earlier studies of the induction of mutations and chromosomal alterations by Muller, Sax, McClintock and others (discussed above) basically incorporated the concept of repair into the interpretation of their data; it required the more definitive experiments of Rupert, Setlow, and Carrier and others to substantiate the concept.

This momentum for moving the field of radiation biology forward was sustained by the studies of McGrath and Williams (1966) who showed that ionizing radiation-induced DNA single-strand breaks could be measured in bacterial DNA using alkaline sucrose gradient velocity sedimentation. This method separated DNA fragments based on size, and the various size categories could be quantitated by scintillation counting of the incorporated radioactivity. Using this method, they could also show that the broken DNA fragments could rejoin. Similar approaches were used by Lett et al. (1967) to show that similar DNA breakage and rejoining occurred in mammalian cells following exposure to x-rays.

There was a rapid increase in the knowledge of what constituted radiation-induced DNA damage (reviewed in Ward 1988). It became apparent that there was a considerable complexity to the types of DNA damage that could be induced. These could be broadly categorized into single-strand DNA breaks (ssb); double-strand DNA breaks (dsb); and base damages (bd, over 100 different types). As a generalization, in mammalian cells 1 Gy of x rays induces 1,000 ssb, 1,000–2,000 bd, and 30 dsb. Thus, the yields of dsb are considerably lower than those for ssb and bd. In many ways this has been an obstacle to the study of dsb; detection methods have not generally been very sensitive for their quantitation and high radiation doses have had to be used to induce measurable frequencies. It is only more recently that sensitive methods have been developed that allow for quantitation at low doses (Blocher et al. 1989; Rothkamm and Lobrich 2003). This is of particular importance for the estimation of potential adverse outcomes at low (environmental) doses (NCRP 2001). It is an understanding of the molecular processes of DNA damage and repair that has aided in the development of sensitive methods, relying, for example, on the measurements of cellular responses at the histone H2AX level to provide information on dsb induction (Rothkamm and Lobrich 2003).

Along with the knowledge of the types and relative frequencies of DNA damage induced by ionizing radiations came the idea that mutations and chromosomal alterations could result from the misrepair (G_1 and G_2 of the cell cycle) or misreplication (S phase) of induced DNA lesions. This proposal was well-grounded in the much earlier studies of Sax and colleagues in their "breakage first" hypothesis (Sax 1940) as well as in the alternate (but not exclusive) one of Revell (1958) in his "exchange hypothesis." In the case of the breakage first approach, the first step in the process of formation of chromosome alterations is DNA breakage (generally regarded as dsb) followed by rejoining, either correctly to retain the integrity of a chromosome or incorrectly leading to a range of chromosomal alterations and deletion mutations. The exchange hypothesis simply regards all chromosomal aberration types and deletion mutations as resulting from exchanges at the site of DNA damage (generally regarded as dsb). The best evidence is that both apply (Duncan and Evans 1983) and indeed current details of the repair of dsb support this conclusion [reviewed in Jackson (2002) and discussed later]. It remains of relevance to note that within the framework of these two modes of formation of chromosomal alterations, the concept of one- and two-track aberration induction is maintained.

The identification of a role for misrepair of DNA damage in the formation of genetic alterations naturally led to discussions of the consequences of such misrepair in terms of adverse health outcomes, especially cancer. The answer was not very long in coming. Cleaver (1968) demonstrated that cells from individuals with *Xeroderma pigmentosum*, a syndrome that is characterized by a high degree of sensitivity to the induction of skin tumors by ultraviolet light (sunlight), were compromised in their ability to perform excision repair of cyclobutane pyrimidine dimers. The proposal was (and still is the case) that failure to adequately repair UV-induced DNA damage led to an increase in genetic alterations that could lead to an increased likelihood of skin tumor development. Not only did these seminal studies demonstrate the link between compromised DNA repair and cancer, but they also highlighted the concept of an underlying genetic control of radiosensitivity. This concept is expanded in the next section.

Thus, the middle years, covering the period from the 1960's through the 1980's, provided a more tangible interpretation of the data developed in the earlier years on the induction of chromosomal alterations and mutations. The identification of DNA damage types and the mode of their repair and misrepair was a significant step forward. It is appreciated that this short section is not at all reflective of all the detailed and informative studies conducted in this 20–30 year period. This aim is to develop the concepts underlying the progress that was made.

It was during the next 10 or 20 years that enormous progress in defining the details at the molecular level of cellular responses to ionizing radiations was made. This, as is usual in science, both confirmed and enhanced previous ideas and experimental data.

THE RECENT YEARS

The so-called "molecular revolution" completely changed our ability to probe into the functioning of cells, their interactions within tissues, and their response to external stressors, including ionizing radiation. The major advances made have been in large part the result of enormous and rapid technical advances. Our ability to manipulate DNA and control and assess gene function has been at the heart of this progress. The impact of these new techniques on the field of radiation biology has been reviewed in some detail by Bedford and Dewey (2002). The approach taken in this article is to extract the concepts from the detail and show how these point the way forward.

The idea presented in the previous section, that there was a genetic control of radiation sensitivity, led to an enhanced search for human diseases that could have DNA repair deficiencies as part of their etiology, and radiation sensitivity as a corollary. Over the period of 10 or 20 years, a number of such diseases were identified, and their radiation sensitivity investigated. An excellent summary table can be found in the ICRP Publication 79, *Genetic Susceptibility to Cancer* (1999). Of the 23 or so human disorders with a documented genetic susceptibility, the only ones that appear to have a demonstrated link between in vitro cellular sensitivity to ionizing radiations and heritable cancer susceptibility are ataxia-telangiectasia and Nijmegen breakage syndrome. The cellular defects underlying this sensitivity have been identified or indicated. The ATM (Ataxia-Telangiectasia Mutated) gene is mutated in patients with ataxia-telangiectasia (Savitsky et al. 1995). This gene encodes a cell cycle checkpoint protein that is a member of the phosphatidy1-3′ subgroup of kinases (Walworth and Bernards 1996). Other functions of this large gene in the context of DNA repair and cell cycle control have been identified (reviewed by Abraham 2003; Kastan et al. 2000). Nijmegen breakage syndrome (NBS) is a rare chromosome instability syndrome, characterized by radiosensitivity and radioresistant DNA synthesis S-phase checkpoint deficiency (Maser et al. 2001). NBS1 is part of a protein complex that contains MRE11 and RAD50, both of which are involved in DNA repair (Maser et al. 2001).

These types of studies and the information that they provide have certainly enhanced the basic knowledge of the underlying mechanisms of radiation sensitivity. However, it is fair to conclude that such information does not present a major impact on cancer risk assessments to the population since for any one of these syndromes the frequency is very low (e.g., ATM, 1 in 10^5 live births). However, in individual risk assessment, such as might be considered in the case of medical exposures, the presence of a genetic susceptibility could significantly impact the radiation regime.

The identification of radiation-sensitive syndromes in humans as well as the development of mouse models of radiosensitivity and cellular models has led to a much more complete understanding of the processes of DNA repair. Excellent reviews of the mechanisms of DNA repair can be found in Wood et al. (2001), Friedberg (2003), and Christmann et al. (2003) together with a fascinating review of the history of DNA repair by Friedberg (2002). The details of nucleotide excision repair, base excision repair, mismatch repair, and dsb repair can be found in these reviews. The concentration here is on the repair of dsb because, as discussed above, it appears that dsb play a pivotal role in cell killing and the formation of genetic alterations following exposure

to ionizing radiations (and other agents such as bleomycin and neocarzinostatin that can also induce DNA dsb by direct interaction with DNA). There appear to be two major pathways for the repair of dsb: homologous recombination and nonhomologous end-joining (NHEJ) (reviewed in UNSCEAR 2000a; Wood et al. 2001; Petrini and Stracker 2003). The relative roles for these two basic repair processes in mammalian cells have not been clearly established, although the generally held view is that NHEJ plays a more significant role. This is in contrast to yeast, for example, where homologous recombination is the majority repair pathway for dsb. However, the cell cycle dependence of repair pathway utilized, studied by Takata et al. (1998), suggests that NHEJ is dominant in G_1 and early-S, but that homologous recombination is dominant in late-S and G_2. The role of homologous recombination in the maintenance of chromosome stability in mammalian cells has been reviewed recently by Thompson and Schild (2001).

In the context of the role of DNA misrepair in the formation of chromosomal alterations following radiation exposures, both homologous recombination and NHEJ could be the pertinent DNA repair pathway for either a breakage first or an exchange model. However, there is one proviso to this. In the original recombination repair model of Szostak et al. (1983), modified from that of Resnick and Martin (1976), it was proposed that a single dsb could initiate recombination repair. However, the prevailing view is most, if not all, chromosomal alterations are produced via the induction of pairs of dsb (induced by one- or two-track processes). Thus, a model for homologous recombination repair of dsb needs to include such pairs of dsb. However, it should be noted that there is some evidence to suggest that a proportion of chromosomal alterations are initiated by a single dsb, followed by DNA strand invasion into an undamaged DNA molecule to initiate the recombination process (Griffin et al. 1998). The question of whether or not this is a common mechanism is still unanswered. The concept of homologous recombination repair being involved in aberration induction is attractive since it would help to explain the formation of specific chromosomal translocations that involve homologous DNA segments within or between chromosomes. Such specific translocations and inversions have been shown to be involved in the etiology of some tumor types, especially leukemias and lymphomas (Look 2002). It might well be that some form of specific chromosomal change is involved in the formation, or perhaps even the initiation, of tumors given the proposed role for genomic instability in tumor development (Lengauer et al. 1998). In a similar vein, the chromosomal alterations characteristic of specific leukemia or lymphoma classes frequently involve immunoglobulin loci (B-cells) or T-cell antigen receptor loci (T-cells). The significance of this is that NHEJ is involved in the rejoining of the dsb formed during the V(D)J recombination process in immune cells. V(D)J recombination is the process that generates variation in the antigen-binding pockets of B- and T-cell receptors through mixing by recombination of the variable (V), diversity (D) and joining (J) gene segments that constitute the V(D)J region of the receptor (reviewed in Market and Papavasiliou 2003). Thus, defects in NHEJ would be predicted to lead to increases in chromosomal radiosensitivity and to defects in V(D)J recombination. This has been shown to be the case (Jeggo et al. 1995). A proposed role of radiation in the cancer process would be to increase the frequency of dsb overall and the likelihood of a misrepair event in a critical region such as adjacent to an immunoglobulin locus.

Relatively recently, the question of the likelihood of a misrepair event has been considered in terms of the types of DNA damage induced. The prevailing view was that DNA damage consisted of singly damaged regions caused by individual dsb, ssb, or bd. The studies and calculations of Goodhead and colleagues (Dianov et al. 2001) and Ward (2000) led to the proposition that there were multiply damaged sites or sites of complex DNA lesions. Such localized regions could contain several classes of DNA damage and/or several lesions of one type. It was further proposed that such lesions could occur at relatively low doses and were somewhat more frequent following high-LET exposures. In addition, based on the complexity of the lesions, it was suggested that these would be more difficult to repair and more prone to misrepair with adjacent complex (or even single) lesions (Singleton et al. 2002). Further study is required to provide a more complete characterization of complex lesions and the efficiency of their repair. The nature of the dose-response curve for complex lesions, especially their induction kinetics at low doses (a few mGy) is not known and cannot as yet be readily predicted.

Some support for the role of complex lesions in the formation of chromosome alterations has been provided by recent studies using fluorescence in situ hybridization (FISH). In fact, the use of FISH has significantly changed the field of cytogenetics, including radiation cytogenetics, because of its ability to allow detection of transmissible alterations such as reciprocal translocations, inversions and insertions, that are rather refractory to analysis by the standard staining procedures (Liehr and Claussen 2002; deJong 2003). FISH utilizes DNA probes that can be hybridized to whole chromosome preparations or to

interphase nuclei and that can be complementary, for example, to a specific chromosome, a specific chromosomal region or a specific gene. Detection of hybridized regions is performed by a fluorescent antibody procedure and observation with a fluorescence microscope. A range of cytogenetic studies have been conducted for assessing responses to radiation. The basic tenet of the induction of chromosome aberrations by one- or two-track processes has been upheld for transmissible chromosome aberrations (e.g., reciprocal translocations and inversions) (Natarajan and Boei 2003). In addition, the utility of reciprocal translocations as a biomarker of exposure and as a dosimeter has been demonstrated, especially for assessment at quite long periods after exposure, for which dicentrics are a most inaccurate endpoint (Bender et al. 1988). An important corollary of these observations is that for chromosomal endpoints that are more likely to be predictive of tumor outcomes than their non-transmissible counterparts (e.g., dicentrics and deletions), the dose-response is linear over the low dose region (a few 10 s of mGy). This is predicted by the one-track component of the linear-quadratic dose-response curve that is observed. This simple statement and similar ones have led to much debate about the shape of the dose-response for tumors at low radiation doses. Cellular and molecular alterations support the hypothesis that the dose-response curve is linear for low-LET radiations over the low dose region (Preston 2003).

At the beginning of the previous paragraph, mention was made of the identification using FISH of a potential role for complex lesions in chromosome aberrations induction. This was the result of one of the more surprising observations made for radiation-induced chromosome aberrations. Even at relatively low doses so-called complex aberrations were observed (reviewed in Savage 2002). These involved the interactions of three or more chromosomal regions in the formation of exchanges and insertions. One viable explanation is that multiple interactions could involve high probability misrepair events such as those involving complex lesions. One important implication of these complex aberrations is that they tend to alter the generally-held view that chromosome alterations are randomly distributed among cells. Whether or not cells containing complex aberrations are viable is still an unanswered question, although the phenomenon of genomic instability, discussed later, perhaps opens the door to their sometimes being viable.

Additional developments over the past few years have changed our views of how a cell protects itself against transmitting genetic damage to progeny cells, and thereby limiting the clonal expansion of cells that is required for tumor progression. Over the past few years, a vast amount of information has been collected that demonstrates the intricate and complex control of the passage of a cell through the cell cycle (reviewed by Clurman and Roberts 2002). It is noteworthy that this complex control process is broadly similar across an extensive range of species from yeast to humans. The pertinence of this control process to cellular radiation responses is exemplified by the importance of cell cycle checkpoint genes. In the past decade, it has become clear that specific points in a cell cycle are explicitly controlled and are linked such that successful passage through a proximate one (say G_1 to S) is necessary before passing through a more distal one (S to G_2). The most studied of the genes that control cellular checkpoints is p53, the so-called "guardian of the genome" (reviewed by Lane 1992 and Woods and Lane 2003). In simple terms, p53, for example, controls entry into S from G_1. In response to DNA damage, p53 is induced and itself serves as a transcription factor to initiate the expression of genes involved in the repair of the induced damage. At the same time it causes a block to the cell cycle, thereby preventing cells containing DNA damage from entering the S phase. This checking prevents a cell from replicating a damaged DNA template and thereby producing mutations. The checking also provides more time for a cell to repair its DNA before proceeding to replication. Clearly, this protective action is only partially preventive of mutation formation, since mutations do arise. The simple explanation is that in G_1, the misrepair of induced DNA damage leads to genetic alterations; this is not prevented entirely by p53 checking. Also, cells that are already in the S-phase have a finite probability of not repairing DNA damage prior to replication; the consequence is a DNA replication error that is manifest as a gene mutation. However, p53 provides a back up process, in concert with a collection of other control genes. Cells that contain excessive DNA damage or excessive genomic alterations can be removed from the replicative pool through the process of apoptosis or programmed cell death (reviewed by Kaina 2003). In this way, progression to tumorigenesis requires an optimal level of genomic damage for the selection process to operate effectively. For example, too little genomic damage or too much are not good substrates for positive selection pressures (Cahill et al. 1999). Much of the information developed on the mechanism of cellular protection afforded by p53 has been provided by radiation studies, to quite an extent because dsb are a very effective substrate for p53 induction. Clearly, the effectiveness of p53 function is of considerable importance to the likelihood of tumor outcomes following radiation exposures. The experimental need is to better understand the role of p53, and similar cell cycle control genes, at low exposure levels. Mutations of the p53 gene lead to a susceptibility

to cancer (e.g., Li-Fraumeni syndrome) and to a sensitivity to radiation-induced tumors in a heterozygous p53 knock out mouse model (Lee et al. 1994).

The molecular detail of the processes involved in DNA damage repair, misrepair of DNA damage, cell cycle control, apoptosis, the induction of genetic alterations and cancer formation have all allowed for a much improved understanding of how radiation might induce tumors. What is still required is to better understand how these types of information might be used in defining the nature of the dose-response curve for tumors at low radiation doses and how a quantitative estimate of cancer risk at low doses might be developed. The need for this type of approach is becoming more necessary as new and quite unexpected experimental data are being collected.

The current approach to cancer risk assessment for radiation exposures utilized by the ICRP and NCRP, for example, relies very heavily on human epidemiological data. These data have been collected from the Japanese atomic bomb survivors and groups exposed medically or occupationally. The risk coefficients that are applied for low dose risk estimates are obtained by linear extrapolation from the available tumor data. The approach is a pragmatic one, which uses tumor frequency as its basis, such that any possible confounders will be accounted for. Recent studies have led to extensive discussions about the need to reconsider this approach using, for example, biologically-based dose-response models for predicting tumor responses at low doses, thereby building confounders into the model (UNSCEAR 2000b).

What then are these new data that necessitate a discussion of current risk assessment practices? Three general observations have been made that perhaps challenge persistent dogma in the field of radiation biology. These are bystander effects, radiation-induced genomic instability, and adaptive responses.

It has been generally accepted that for a cell to sustain genetic damage, the nucleus has to be traversed by a track of high or low-LET radiation. Recent experimental studies have shown that, in fact, nearby cells (bystanders) can sustain genetic damage in the form of gene mutations and chromosomal alterations even though they have not received a dose directly. The various types of evidence for the existence of these bystander effects both in vitro and in vivo have been comprehensively reviewed by Morgan (2003a and b). These effects present a concern in a risk assessment framework, because if they occur at low doses, then a cellular response greater than that estimated on the basis of dose alone would be predicted—in essence the target cell population is increased. The mechanisms underlying the production of bystander effects are not well defined although they can involve cell-cell communication and cell signaling responses (Prise et al. 2003). It has also been proposed that such effects are mediated by oxygen radical species (Lorimore and Wright 2003; Morgan 2003c).

A second challenge to radiation biology law is provided by the observation of radiation-induced genomic instability (reviewed by Morgan 2003 and b). It has been generally accepted that maximal radiation-induced genetic damage is formed shortly (minutes to hours) after a radiation exposure. However, as originally reported by Kadhim et al. (1992), chromosomal alterations and gene mutations can occur many cell generations after the original exposure to either high- or low-LET radiations. There are a variety of reports of genomic instability being induced in in vitro cellular systems and to a lesser extent in in vivo animal systems. The underlying mechanism of induction of genomic instability is not known. It is, however, important to note that in the recent study of Dugan and Bedford (2003) no genomic instability was observed at long intervals following exposure to high- or low-LET radiations when low-passage *normal* human fibroblasts were used. Is some degree of instability that is already present required for the subsequent development of radiation-induced genetic instability? It is particularly important to note in terms of relevancy of these observations of genomic instability to cancer risk, that the level of instability observed at times after radiation exposure is considerably less than the extensive genomic instability that is the hallmark of tumor development. Perhaps the more significant observation is that the total genetic damage to a cell has to be integrated over time and is not simply represented by that observed shortly after radiation exposure. At the cellular level this could increase the slope of the dose-response curve at low exposures compared to the linear extrapolation from high exposures. This is predicated by the fact that radiation-induced genomic instability has not been reported to routinely occur at low exposure levels (a few mGy). In addition, these observations of radiation-induced genomic instability have not been related directly to tumor formation.

A third phenomenon that challenges the majority opinion is that of the adaptive response (reviewed by Wolff 1996; Leskov et al. 2001). The general observation is that a small priming dose of radiation (a few mGy) can reduce the effect of a large challenge dose of x rays. The phenomenon is not universal, varying among test systems and among individuals in human cellular studies. The underlying mechanism for an adaptive response has not been established, although a variety of inducible responses have been proposed to be involved. The outcome is that the cellular response under adaptive conditions is reduced not eliminated. This is in contrast

to the related phenomenon of hormesis, by which it is proposed a small amount of radiation is beneficial as regards cellular responses, leading to the generally described J-shape dose-response curve (reviewed by Upton 2001; Calabrese and Baldwin 2003). Again no mechanistic basis has been unequivocally established to explain hormesis. In addition, hormetic responses are not generalized ones but are assay system and endpoint specific. Clearly, further studies are needed to establish the overall relevance to cellular sensitivity of adaptive and hormetic responses. The impact of these two phenomena on cancer outcome will have to wait until the cellular relevance has been established.

The recent years have certainly enhanced our understanding of previously recognized radiation responses, challenged our previously firmly-held viewpoints, and provided the impetus for developing methods for incorporating mechanistic data into the risk assessment process. It is often said in science that what goes around comes around, confirmation rather than charting new paths. Maybe only some of what goes around will come around; some new paths seem to be set already.

THE UPCOMING YEARS

At this time, we have a clearer view of how radiations impact cells in a structural and functional sense. We also have enhanced our knowledge of how cells protect themselves from radiation-induced DNA damage through a suite of repair processes and cell cycle checkpoint controls. In addition, the process whereby a normal cell can progress to a metastatic tumor has been more clearly defined, especially in the generic sense by Hanahan and Weinberg (2000) with their concept of acquired characteristics. Of course, there are gaps to be filled and there is constant attention at the experimental level to address these gaps.

The organizational level at which observations can and are being made has moved from the single gene, single protein levels to whole cell and tissue levels. The use of microarray technologies for assessing the impact of environmental exposures, including ionizing radiation, on the gene expression levels for all the expressed genes in selected cells has greatly expanded the chances to establish whole genome responses. One of the aims is to be able to utilize these potentially sensitive techniques to provide information on low dose responses. Initial attempts have indicated that such an approach has merit (Amundson et al. 2003; Mercier et al. 2004). The clear need is to be able to relate changes in gene expression to adverse cellular outcomes that could subsequently lead to disease. This is, of course, no mean task. The overall approach will need to include parallel studies with proteins using protein arrays or mass spectrometry methods (Sreekumar et al. 2001; Aebersold 2003).

The ability to link gene expression changes to functional changes at the cellular level has been considerably enhanced by the use of interference RNA (siRNA) techniques (Kittler and Buchholz 2003). The use of gene region specific short RNA segments to destroy the homologous mRNA allows for transient inactivation of any selected gene. An advantage of this technique, and similar ones, is that the effect of loss of gene expression can be controlled, unlike the situation with knockout cell and animal models in which gene inactivation is complete and permanent. Initial studies to investigate the role of ionizing radiation in altering cellular processes at the transcription level have been quite promising (Yin et al. 2003). This is an area of research that will clearly have a significant impact over the next few years.

In the continuing quest to link molecular alterations to phenotypic responses, the use of sophisticated real-time imaging techniques will be most valuable. It is now possible to track changes at the protein level following exposure to ionizing radiation, for example, at the whole cell level using confocal microscopy and image analysis programs (Adams et al. 2003; Zimmermann et al. 2003). Such approaches can be utilized at the tissue level, which is of particular value when considering outcomes such as tumor development, since this is clearly a tissue response, requiring among other things interaction between normal cells and transformed cells (Hanahan and Weinberg 2000). Using real-time imaging techniques, it should be feasible to investigate the cellular responses that lead to bystander responses and perhaps adaptive responses. The ability to track specific proteins in cells has the potential to enhance our understanding of the time-related molecular changes associated with DNA repair. In particular, the recently recognized role of histones (previously considered to serve a structural function in chromatin) in DNA repair and the control of gene expression (Coleman et al. 2003) is ripe for further study by protein tracking approaches.

While there is a “desire to know” feature to the collection of the types of data described above and their interpretation, there is also a much-needed practical application. This need is the development of biologically-based dose-response (BBDR) models that can be used for radiation cancer risk assessment, as well as for the assessment of other adverse outcomes. The value is in the estimation of tumor responses at environmental doses that are pertinent for radiation protection standards. This need is predicated by the fact that tumor responses cannot be directly assessed at these low doses. In addition, the impact of possible confounders of tumor response can be adequately addressed by the

mechanistically-based BBDR models. It is noted that BBDR models have been utilized with some success for estimating low exposure level tumor responses for exposures to environmental chemicals (Conolly 2002). A comprehensive review of the use of modeling techniques for radiation exposures can be found in UNSCEAR (2001).

Progress in the area of the effects of ionizing radiation at the cellular and tissue levels over the next 5 to 10 years is set to outpace that over any other similar time period. However, any such progress is firmly set on the foundations provided over the past 50 or more years. The forward look has a requirement to take a backward look—and perhaps this is a potential value of the present review.

CONCLUSION

The research conducted in the early years of the field of radiation biology provided a firm foundation for the more recent developments. The role of ionizing radiation in the induction of gene mutations and chromosome alterations necessitating damage to the genetic material and some form of rejoining can now be interpreted in terms of specific types of DNA damage and the mechanisms by which such damage can be repaired or misrepaired. The introduction of the concept of one- and two-track processes for the induction of genetic alterations by low LET radiations remains the foundation of the dose-response curve for such alterations and provides the explanation for the responses following fractionated and chronic exposures—namely, linearity, even at low doses. The remarkable detail afforded by the more recent molecular biology techniques has served to confirm these foundations and to chart a path forward for providing the substrate for selecting biological indicators of response to go along with the current biological indicators of exposure (e.g., chromosomal alterations and gene mutations). The aim should be to begin to incorporate all the new types of information generated into the risk assessment process for ionizing radiations. This approach will help supplement the available human epidemiological data in setting radiation protection standards.

Acknowledgments—I am enormously grateful to a group of mentors who guided my way along some of the paths in this history of radiation biology. While I appreciate and respect so many, the following were perhaps the most influential with their guidance: Gerry Neary, John Evans, John Savage, David Scott, Grant Brewen, and Michael Fry. For this particular article I thank Drs. Kligerman and Wassom for their careful review and comments and special thanks to Carolyn Fowler for her preparation of the final manuscript. I also thank Mike Ryan for the invitation to write this review—it turned out to be an enjoyable experience.

This manuscript has been reviewed in accordance with U.S. EPA guidance but does not necessarily reflect EPA policy.

REFERENCES

Abraham RT. Checkpoint signaling: Epigenetic events sound the DNA strand-breaks alarm to the ATM protein kinase. Bioessays 25:627–630; 2003.

Adams MC, Salmon WC, Gupton SL, Cohan CS, Wittmann TK, Prigozhina N, Waterman-Storer CM. A high-speed multispectral spinning-disk confocal microscope system for fluorescent speckle microscopy of living cells. Methods 29:29–41; 2003.

Aebersold R. A mass spectrometric journey into protein and proteome research. J Am Soc Mass Spectrom 14:685–695; 2003.

Amundson SA, Lee RA, Koch-Paiz CA, Bittner ML, Meltzer P, Trent JM, Fornace AJ Jr. Differential responses of stress genes to low dose-rate gamma irradiation. Mol Cancer Res 1:445–452; 2003.

Beadle GW, Tatum EL. Genetic control of biochemical actions in Neurospora. Proc Natl Acad Sci 27:499–506; 1941.

Bedford JS, Dewey WC. Radiation Research Society. 1952–2002. Historical and current highlights in radiation biology: has anything important been learned by irradiating cells? Radiat Res 158:251–291; 2002.

Bender MA. Cytogenetics research in radiation biology. Stem Cells 13:(Suppl 1):172–182; 1995.

Bender MA, Awa AA, Brooks AL, Evans HJ, Groer PG, Littlefield LG, Pereira C, Preston RJ, Wachholz BW. Current status of cytogenetic procedures to detect and quantify previous exposures of radiation. Mutat Res 196:103–159; 1988.

Blocher D, Einspenner M, Zajackowski J. CHEF electrophoresis, a sensitive technique for the determination of DNA double-strand breaks. Int J Radiat Biol 56:437–448; 1989.

Boyce RP, Howard-Flanders P. Release of ultraviolet light-induced thymine dimers from DNA in E. coli K-12. Proc Natl Acad Sci 51:293–300; 1964.

Brewen JG, Brock RD. The exchange hypothesis and chromosome-type aberrations. Mutat Res 6:245–255; 1968.

Cahill DP, Kinzler KW, Vogelstein B, Lengauer C. Genetic instability and Darwinian selection in tumours. Trends Cell Biol 9:M57–M60; 1999.

Calabrese EJ, Baldwin LA. Hormesis: The dose-response revolution. Annu Rev Pharmacol Toxicol 43:175–197; 2003.

Carter TC, Lyon MF, Phillips RJ. Induction of mutations in mice by chronic gamma irradiation; Interim report. Br J Radiol 29:106–108; 1956.

Christmann M, Tomicic MT, Roos WP, Kaina B. Mechanisms of human DNA repair: An update. Toxicology 193:3–34; 2003.

Cleaver JE. Defective repair replications of DNA in xeroderma pigmentosum. Nature 218:642–656; 1968.

Clurman BE, Roberts JM. Cell cycle control: An overview. In: Vogelstein B, Kinzler KW, eds. The genetic basis of human cancer. New York: McGraw Hill; 2002: 145–161.

Coleman MA, Miller KA, Beernink PT, Yoshikawa DM, Albala JS. Identification of chromatin-related protein interactions using protein microarrays. Proteomics 3:2101–2107; 2003.

Conolly RB. The use of biologically based modeling in risk assessment. Toxicol 181–182:275–279; 2002.

deJong H. Visualizing DNA domains and sequences by microscopy: A fifty-year history of molecular cytogenetics. Genome 46:943–946; 2003.

Dianov GL, O'Neill P, Goodhead DT. Securing genomic stability by orchestrating DNA repair: Removal of radiation-induced clustered lesions in DNA. Bioessays 23:745–749; 2001.

Dugan LC, Bedford JS. Are chromosomal instabilities induced by exposure of cultured normal human cells to low- or high-LET radiation? Radiat Res 159:301–311; 2003.

Duncan AM, Evans HJ. The exchange hypothesis for the formation of chromatid aberrations: An experimental test using bleomycin. Mutat Res 107:307–313; 1983.

Elkind MM, Sutton H. Radiation response of mammalian cells grown in culture: I. Repair of x-ray damage in surviving Chinese hamster cells. Radiat Res 13:556–593; 1960.

Evans HH, Horng M-E, Mencl J, Glazier KG, Beer JZ. The influence of dose rate on the lethal and mutagenic effects of x-rays in proliferating L5178Y cells differing in radiation sensitivity. Int J Radiat Biol 47:553–562; 1985.

Friedberg EC. The intersection between the birth of molecular biology and the discovery of DNA repair. DNA Repair 1:855–867; 2002.

Friedberg EC. DNA damage and repair. Nature 421:436–440; 2003.

Griffin CS, Hill MA, Papworth DG, Townsend KM, Savage JR, Goodhead DT. Effectiveness of 0.28 keV carbon K ultrasoft x-rays at producing simple and complex chromosome exchanges in human fibroblast in vitro detected using FISH. Int J Radiat Biol 73:591–598; 1998.

Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 100:57–70; 2000.

Hsu TC. Mammalian chromosomes in vitro. The karyotype of man. F Hered 43:167–172; 1952.

International Commission on Radiological Protection. Genetic susceptibility to cancer. Oxford, UK: Elsevier; ICRP Publication 79; 1999.

Jackson SP. Sensing and repairing DNA double-strand breaks. Carcinogenesis 23:687–696; 2002.

Jeggo PA, Taccioli GE, Jackson SP. Menage a trois: Double strand break repair V(D)J recombination and DNA-PK. Bioessays 17:949–957; 1995.

Kadhim MA, Macdonald DA, Goodhead DT, Lorimore SA, Marsden SJ, Wright EG. Transmission of chromosomal instability after plutonium alpha particle irradiation. Nature 355:738–740; 1992.

Kaina B. DNA damage-triggered apoptosis: Critical role of DNA repair, double-strand breaks, cell proliferation and signaling. Biochem Pharmacol 66:1547–1554; 2003.

Kastan MB, Lim DS, Kim ST, Xu B, Canman C. Multiple signaling pathways involving ATM. Cold Spring Harb Symp Quant Biol 65:521–526; 2000.

Kelner A. Effect of visible light on the recovery of Streptomyces griseus conidia from ultra-violet irradiation injury. Proc Natl Acad Sci 35:73–79; 1949.

Kittler R, Buchholz F. RNA interference: Gene silencing in the fast lane. Semin Cancer Biol 13:259–265; 2003.

Knaap AG, Simons JW. A mutational assay system for L5178Y mouse lymphoma cells, using hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT)-deficiency as marker. The occurrence of long expression time for mutations induced by x-rays and EMS. Mutat Res 30:97–110; 1975.

Lane DP. Cancer. p53, guardian of the genome. Nature 358:15–16; 1992.

Lea DE. Action of radiations on living cells. Cambridge: Cambridge University Press; 1962.

Lee JM, Abrahamson JL, Kandel R, Donehower LA, Bernstein A. Susceptibility to radiation-carcinogenesis and accumulation of chromosomal breakage in p53 deficient mice. Oncogene 9:3731–3736; 1994.

Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 396:643–649; 1998.

Leskov KS, Criswell T, Antonio S, Li J, Yang CR, Kinsella TJ, Boothman DA. When x-ray-inducible proteins meet DNA double-strand break repair. Semin Radiat Oncol 11:352–372; 2001.

Lett JT, Caldwell I, Dean CJ, Alexander P. Rejoining of x-ray induced breaks in the DNA of leukaemia cells. Nature 214:790–792; 1967.

Liber HL, Thilly WG. Mutation assay at the thymidine kinase locus in diploid human lymphoblasts. Mutat Res 94:467–485; 1982.

Liber HL, Yandell DW, Little JB. A comparison of mutation induction at the tk and hprt loci in human lymphoblastoid cells; quantitative differences are due to an additional class of mutations at the autosomal tk locus. Mutat Res 216:9–17; 1989.

Liehr T, Claussen U. Current developments in human molecular cytogenetic techniques. Curr Mol Med 2:283–297; 2002.

Look AT. Genes altered by chromosomal translocations in leukemias and lymphomas. In: Vogelstein B, Kinzler KW, eds. The genetic basis of human cancer. New York: McGraw Hill; 2002: 57–92.

Lorimore SA, Wright EG. Radiation-induced genomic instability and bystander effects: Related inflammatory-type responses to radiation-induced stress and injury? A review. Int J Radiat Biol 79:15–25; 2003.

Market E, Papavasiliou FN. V(D)J recombination and the evolution of the adaptive immune system. PloS Biol 1:E16; 2003.

Maser RS, Zinkel R, Petrini JH. An alternative mode of translation permits production of a variant NBS1 protein from the common Nijmegen breakage syndrome allele. Nat Genet 27:417–421; 2001.

McClintock B. Cytological observations of deficiencies involving known genes, translocations and inversions in Zea mays. MO. Agric Exp Station Res Bull 163:1–30; 1931.

McGrath RA, Williams RW. Reconstruction in vivo of irradiated Escherichia coli deoxyribonucleic acid; The rejoining of broken pieces. Nature 212:534–535; 1966.

Mercier G, Berthault N, Mary J, Peyre J, Antoniadis A, Comet JP, Cornuejols A, Froidevaux C, Dutreix M. Biological detection of low radiation doses by combining results of two microarray analysis methods. Nucleic Acids Res 32:e12; 2004.

Moorhead PS, Nowell PW, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leucocytes cultured from human peripheral blood. Exptl Cell Res 20:613–616; 1960.

Morgan WF. Non-targeted and delayed effects of exposure to ionizing radiation: I. Radiation-induced genomic instability and bystander effects in vitro. Radiat Res 159:567–580; 2003a.

Morgan WF. Non-targeted and delayed effects of exposure to ionizing radiation: II. Radiation-induced genomic instability and bystander effects in vivo, clastogenic factors and transgenerational effects. Radiat Res 159:581–596; 2003b.

Morgan WF. Is there a common mechanism underlying genomic instability, bystander effects and other nontargeted effects of exposure of ionizing radiation? Oncogene 22:7094–7099; 2003c.

Muller HJ. Artificial transmutation of the gene. Science 66:84–87; 1927.

Natarajan AT, Boei JJ. Formation of chromosome aberrations: Insights from FISH. Mutat Res 544:299–304; 2003.

National Council for Radiological Protection. Evaluation of the linear-nonthreshold dose-response model for ionizing radiation. Bethesda, MD: NCRP; NCRP Report No. 136; 2001.

Neary GJ. The relation between the exponent of dose response for chromosome aberrations and the relative contribution of "two-track" and "one-track" processes. Mutat Res 2:242–246; 1965.

Petrini JH, Stracker TH. The cellular response to DNA double-strand breaks: Defining the sensors and mediators. Trends Cell Biol 13:458–462; 2003.

Preston RJ. The LNT model is the best we can do—today. J Radiol Prot 23:263–268; 2003.

Prise KM, Folkard M, Michael BD. A review of the bystander effect and its implications for low-dose exposure. Radiat Prot Dosim 104:347–355; 2003.

Puck TT, Marcus PI. Rapid method of viable cell titration and clone production with HeLa cells in tissue culture: The use of x irradiated cells to supply conditioning factors. Proc Natl Acad Sci 41:561–568; 1955.

Resnick MA, Martin P. The repair of double-strand breaks in the nuclear DNA of Saccharomyces cerevisiae and its genetic control. Mol Gen Genet 143:119–129; 1976.

Revell SH. A new hypothesis for the interpretation of chromatid aberrations and its relevance to theories for the mode of action of chemical agents. Ann N Y Acad Sci 68:802–807; discussion 808–810; 1958.

Revell SH. Evidence for a dose-squared term in the dose-response curve for real chromatid discontinuities induced by x-rays, and some theoretical consequences thereof. Mutat Res 3:34–53; 1966.

Rothkamm K, Lobrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. Proc Natl Acad Sci 100:5057–5062; 2003.

Rupert CS. Photoreactivation of transforming DNA by an enzyme from baker's yeast. J Gen Physiol 43:573–595; 1960.

Russell LB. Information from specific-locus mutants on the nature of induced and spontaneous mutations in the mouse. Prog Clin Biol Res 209B:437–447; 1986.

Russell WL. X-ray induced mutations in mice. Cold Spring Habor Symp Quant Biol 16:327–336; 1951.

Savage JR. Reflections and meditations upon complex chromosomal exchanges. Mutat Res 512:93–109; 2002.

Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkenazi M, Pecker I, Frydman M, Harnik R, Patanjali SR, Simmons A, Clines GA, Sartiel A, Gatti RA, Chessa L, Sanal O, Lavin MF, Jaspers NGJ, Taylor MR, Arlett CF, Miki T, Weissman SM, Lovett M, Collins FS, Shiloh Y. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 268:1749–1753; 1995.

Sax K. Chromosome aberrations induced by x-rays. Genetics 23:494–516; 1938.

Sax K. An analysis of x-ray induced chromosomal aberrations in Tradescantia. Genetics 25:41–68; 1940.

Sax K. The time factor in x-ray production of chromosomal aberration. Proc Natl Acad Sci 25:225–233; 1939.

Setlow RB, Carrier WL. The disappearance of thymine dimers from DNA: An error-correcting mechanism. Proc Natl Acad Sci 51:226–231; 1963.

Singleton BK, Griffin CS, Thacker J. Clustered DNA damage leads to complex genetic changes in irradiated human cells. Cancer Res 62:6263–6269; 2002.

Sreekumar A, Nyati MK, Varambally S, Barrette TR, Ghosh D, Lawrence TS, Chinnaiyan AM. Profiling of cancer cells using protein microarrays: Discovery of novel radiation-regulated proteins. Cancer Res 61:7585–7593; 2001.

Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW. The double-strand-break repair model for recombination. Cell 33:25–35; 1983.

Takata M, Sasaki MS, Sonoda E, Morrison C, Hashimoto M, Utsumi H, Yamaguchi-Iwai Y, Shinohara A, Takeda S. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. EMBO J 17:5497–5508; 1998.

Thacker J, Cox R. Mutation induction and inactivation in mammalian cells exposed to ionising radiation. Nature 258:429–431; 1975.

Thacker J, Stretch A, Stephens MA. The induction of thioguanine-resistant mutants of Chinese hamster cells by gamma-rays. Mutat Res 42:313–326; 1977.

Thompson LH, Schild D. Homologous recombinational repair of DNA ensures mammalian chromosome stability. Mutat Res 477:131–153; 2001.

United Nations Scientific Committee on the Effects of Atomic Radiation. Sources and effects of ionizing radiation. Report to the General Assembly with Scientific Annexes. New York: United Nations; 1977.

United Nations Scientific Committee on the Effects of Atomic Radiation. DNA repair and mutagenesis, Annex F. Report to the General Assembly with Scientific Annexes. New York: United Nations; 2000a.

United Nations Scientific Committee on the Effects of Atomic Radiation. Biological effects at low radiation doses, Annex G. Report to the General Assembly with Scientific Annexes. New York: United Nations; 2000b.

United Nations Scientific Committee on the Effects of Atomic Radiation. Hereditary effects of radiation. Report to the General Assembly with Scientific Annex. New York: United Nations; 2001.

Upton AC. Radiation hormesis: Data and interpretations. Crit Rev Toxicol 31:681–695; 2001.

Waldren C, Vannais D, Drabek R, Gustafson D, Kraemer S, Lenarczyk M, Kronenberg A, Hei T, Ueno A. Analysis of mutant quantity and quality in human-hamster hybrid AL and AL-179 cells exposed to 137Cs-gamma or HZE-Fe ions. Adv Space Res 22:579–585; 1998.

Walworth NC, Bernards R. Rad-dependent response of the chk1-encoded protein kinase at the DNA damage checkpoint. Science 271:353–356; 1996.

Ward JF. DNA damage produced by ionizing radiation in mammalian cells: Identities, mechanisms of formation and reparability. Prog Nucleic Acid Res Mol Biol 35:95–125; 1988.

Ward JF. Complexity of damage produced by ionizing radiation. Cold Spring Harb Symp Quant Biol 65:377–382; 2000.

Wolff S. Aspects of the adaptive response to very low doses of radiation and other agents. Mutat Res 358:135–142; 1996.

Wood RD, Mitchell M, Sgouros J, Lindahl T. Human DNA repair genes. Science 291:1284–1289; 2001.

Woods YL, Lane DP. Exploiting the p53 pathway for cancer diagnosis and therapy. Hematol J 4:233–247; 2003.

Yin E, Nelson DO, Coleman MA, Peterson LE, Wyrobek AJ. Gene expression changes in mouse brain after exposure to low-dose ionizing radiation. Int J Radiat Biol 79:759–775; 2003.

Zimmermann T, Rietdorf J, Pepperkok R. Spectral imaging and its applications in live cell microscopy. FEBS Lett 546:87–92; 2003.

■ ■