REVIEW

Ionizing radiation-induced mutagenesis

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Ionizing radiation was the first environmental agent shown to be mutagenic (Muller, 1927) and is now recognized as a pancarcinogen (Upton, 1984; Shigematsu & Kagan, 1986). Due to background irradiation and modern medicine it is, apart from oxygen, probably the most common carcinogenic agent to which humans are exposed. Ionizing radiation can cause neoplastic transformation of human cells in culture, but such transformation of cells in culture is not due to a single event (Borek, 1980; Kennedy et al., 1980, 1984). Ionizing radiation exerts the bulk of its effects through the generation of oxygen-derived free radicals, in particular the hydroxyl radical (Teoule & Cadet, 1978; Hutchinson, 1985). Such reactive forms of oxygen also occur by chance as a byproduct of aerobic metabolism (Gerschman et al., 1954; Di Guiseppi & Fridovich, 1984). It has been suggested that many dietary mutagens and carcinogens, tobacco smoke and other cancer promoters, act through the production of such radicals (Ames, 1983; Joenje, 1983; Cerutti, 1985) and the deleterious free radical reactions involving oxygen have also been implicated in the genesis of spontaneous cancer in man (Totter, 1980).

Although the literature on the genetic effects of ionizing radiation is immense, the molecular mechanism(s) involved are for technical reasons not as clearly understood as in the case of ultraviolet (UV) irradiation or alkylating agents. One reason is the large number of radiation products induced in DNA, possibly more than 100 (Teoule & Cadet, 1978; Hutchinson, 1985), so that correlating mutagenesis with a specific lesion is a formidable task. Another reason is that the mutagenic effect relative to the cell killing effect is less for ionizing radiation than for UV light or alkylating agents, and therefore is more difficult and, as a corollary, less appealing to study. The topic has been the subject of several reviews in the past 10 years (Hutterman et al., 1978; Thacker & Cox, 1983; Hutchinson, 1985; Breimer & Lindahl, 1985b; Thacker 1985, 1986). Since Thacker's review (1986) was written, not only have the changes in DNA organization, as detected by Southern blotting, of mutants at several loci been reported, but also changes in DNA sequence. Here I review recent advances and attempt to evaluate ideas of a common mechanism of DNA damage and/or repair in spontaneous and ionizing radiation-induced mutation.

Studies on the effect of ionizing radiation at specific genetic loci

The comparative importance of the base alterations induced by ionizing radiation in mutagenesis has been indicated by genetic experiments in bacteria, bacteriophages and lower eukaryotes (Glickman et al., 1980; Levin et al., 1982; Conkling et al., 1976; Malling & de Serres, 1973; Das et al., 1986). Base substitutions are the most common type of mutational event caused by ionizing radiation, both transitions and transversions being found at A.T as well as G.C base pairs. These base substitutions appear to be random (Glickman et al., 1980; Kato et al., 1985). However,

these results have been obtained either by studying the reversion of nonsense mutations or by reverting radiation-induced mutants with point mutagens, and therefore there is some limitation to the types of events which can be detected (Schaaper et al., 1986).

Tindall et al., (1987) have studied the nature of a large number of ionizing radiation-induced forward mutations at the lambda cI locus. Irradiated phage was found to be mutated both when assayed in SOS (error prone repair) induced and non-induced host cells (recA deficient cells). This was in contrast to UV light which requires the induced SOS system, though the yield of mutants was higher than in the induced cells. The majority (~85%) of the mutants analysed were base-pair changes, the rest were frameshifts (though the analysis underestimates frameshifts). As mutations caused by double-strand breaks would not be detected in the phage experiments, they also studied the effect of irradiating lambda as prophage (i.e. already integrated in the E. Coli chromosome). The specificity of the base changes was lost though these still accounted for 78% of the mutation events. The proportion of frameshifts was unchanged but examples (6%) of internal rearrangements were also detected. The reason for these differences is not clear. The experimental conditions may favour base pair changes.

The role of base damage in ionizing radiation-induced mutagenesis in mammalian cells is unclear. A large volume of indirect evidence has been interpreted as suggesting that ionizing radiation does not induce simple base pair changes. This supposition is based on the observation that ionizing radiation does not increase the frequency of mutants at a locus coding for an essential gene where only point mutations are thought to be tolerated (ouabain resistance at the Na/K-ATPase locus) (Arlett et al., 1975; Thacker et al., 1978; Liber et al., 1983). The frequency at the non-essential hypoxanthine-guanine phosphoribosyl transferase (hprt) locus, on the other hand, is significantly increased (Arlett et al., 1975; Thacker & Cox, 1975; Liber et al., 1983).

On the other hand, in a Chinese hamster ovary (CHO) cell line especially susceptible to point mutations, X-ray induction of diptheria toxin resistance can be observed (Wood et al., 1983). Liber et al. (1986) have reported X-irradiation to induce mutations at two other essential, presumed 'point-mutation', loci. There has been one unconfirmed report of activation of a ras proto-oncogene by a radiation-induced single base alternation (G to A) (Guerrero et al., 1984). However, as the ras system will only tolerate base changes (i.e. it is equivalent to reversion at a nonsense codon) and as the change recorded is a common spontaneous change, the significance of this finding is not clear.

Conclusive tests have been, until recently, hindered by an inability to characterize mutations in mammalian cells at the molecular level. However, the cloning of the genomic and cDNA sequences for specific genes such as adenine phosphoribosyl transferase (aprt) (Lowy et al., 1980), hypoxanthine phosphorybosyl transferase (hprt) (Jolly et al., 1982; Konecki et al., 1982), dihydrofolate reductase (dhfr) (Chang et al., 1978) and thymidine kinase (tk) (Bradshaw & Deininger, 1984) has made it possible to study directly the effects of mutagens on endogenous and exogenous genes.

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Endogenous genes are genes which are part of the normal complement of the organism. Exogenous genes are foreign, often prokaryotic, DNA segments which have been introduced into the cell. Their position of integration and interaction with nuclear factors may affect their behaviour. The results of representative studies are summarized in Table I.

Endogenous genes

Most studies on the effect of ionizing radiation have been performed on the *hprt* locus in isolation. However, the study by Meuth's laboratory (Breimer *et al.*, 1986) characterized mutants at both *aprt* and *hprt* loci as well as determined changes in the DNA sequence. These studies show that there are locus specific effects influencing the type of mutation seen.

hprt locus The alterations in gene structure induced by ionizing radiation at the *hprt* locus have been determined at the level of restriction fragment length changes in Chinese hamster V79 cells (Vrieling *et al.*, 1985; Thacker, 1986; Fuscoe, *et al.*, 1986), CHO cells (Breimer *et al.*, 1986; Stankowski & Hsie, 1986; Gibbs *et al.*, 1987), and human lymphocytes (Skulimowski *et al.*, 1986; Liber *et al.*, 1987) (see Table I). The limit of resolution is ~500 bp. A major disadvantage of the *hprt* locus is that the gene is large (~39 kb) compared to the cDNA probe (1.3 kb), that is a probe of non-contiguous fragments is used, restricting the analysis.

About half of ionizing radiation-induced mutants in rodent cells had lost all detectable coding sequences. Another 20% were partial deletions. Breimer *et al.* (1986) also observed that the size of the *hprt* locus appeared to have increased, probably due to insertions, in the case of two mutants out of twelve examined. In these studies the vast majority (80–90%) of spontaneous mutations at the *hprt* locus showed no detectable change. A few partial deletions were observed; complete deletions were very rare (5%). The spectrum of changes induced by α-particles (Thacker, 1986) and those induced by decaying radioactive isotope (^{125}I) incorporated in the DNA (Gibbs *et al.*, 1987) were similar to those induced by γ-rays. Where both microscopic and molecular analysis have been performed together most of the chromosomal changes have been found to be unrelated to the location of the gene on the X-chromosome (Fuscoe *et al.*, 1986).

There were certain differences in the spectrum of changes observed in spontaneous and induced mutants of human cells in culture when compared with those seen in rodent cells (see Table I) (Skulimowski *et al.*, 1986; Liber *et al.*, 1987). In human cells there were relatively more radiation-induced mutants which showed no detectable change in gene organization and more spontaneous mutants which had totally lost the *hprt* gene than in rodent cells. The poor resolution of the *hprt* system and the lack of detailed description of the findings make it difficult to assess the relative importance of partial deletions, insertions and complex changes.

A molecular survey of *hprt* deficient patients showed that there was a marked genetic heterogeneity (~85% of cases appearing normal by DNA and RNA analysis) (Yang *et al.*, 1984; Wilson *et al.*, 1986).

Table I Frequency of changes in gene structure observed by Southern blot analysis in certain mutants in mammalian cells in culture arising spontaneously or induced by ionizing radiation (expressed as %)

Mutant collection	No detectable alteration[a]	Only altered sites[b]	Rearrangements			
			Partial deletions	Total deletions	Insertions	Complex
Endogenous						
aprt						
hamster[e]						
spontaneous (n=187)	88	7	4	—[c]	1	0
ionizing radiation (n=80)	81	1	8	—	1	9
hprt						
(i) hamster[f]						
spontaneous (n=52)	86	—	12	2	0	0
ionizing radiation (n=113)	31	—	27[d]	41	2	—[d]
(ii) human[g]						
spontaneous (n=28)	82	—	8	10	0	0
ionizing radiation (n=56)	43	—	7[d]	30	20[d]	
tk						
human[h]						
normal growth						
spontaneous (n=51)	20	—	—	70	10	
ionization radiation (n=56)	32	—	—	63	5	
slow growth						
spontaneous (n=120)	3	—	—	96	1	
ionization radiation (n=22)	4	—	—	91	4	
Exogenous						
gpt						
hamster[k]						
spontaneous (n=23)	40	0	43	17	0	0
ionizing radiation (n=25)	0	0	4	96	0	0

[a]Limit of resolution; *aprt* approx. 25 bp; *hprt* approx. 500 bp; *tk* approx. 200 bp; [b]Loss or gain of restriction endonuclease cleavage site; [c]—; not reported; [d]*hprt*: some complex changes associated with partial deletions, not clearly reported. Data compiled from: [e]Nalbantoglu *et al.* (1983), Breimer *et al.* (1986), Grosovsky *et al.* (1986); [f]Fuscoe *et al.* (1983), Vrieling *et al.* (1985), Breimer *et al.* (1986), Fuscoe *et al.* (1986), Thacker (1986), Gibbs *et al.* (1987); [g]Skulimowski *et al.* (1986), Liber *et al.* (1987); [h]Yandell *et al.* (1986, 1987). The significance of the classification in 'normal growth' and 'slow growth' is not clear; [k]Stankowski & Hsie (1986).

aprt *locus* The endogenous genetic locus coding for the non-essential enzyme adenine phosphoribosyl transferase (*aprt*) in cultured Chinese hamster ovary cells is particularly attractive for analysis of mutational events because it offers a much greater resolution than any other mammalian locus used so far (Meuth & Arrand, 1982; Nalbantoglu *et al.*, 1983). The locus is small (3.9 kb) and contains a large number of restriction endonuclease recognition sites closely spaced, allowing detection and mapping of alterations as small as 25 bp. It has as low a spontaneous mutational rate as that of *hprt*. While the locus is autosomal, strains having only a single copy of the gene have been identified (Nalbantoglu *et al.*, 1983) facilitating the collection and analysis of a large number of mutants. The genomic sequence has now been determined (Nalbantoglu *et al.*, 1986a). Of nearly 200 spontaneous mutants, deletions amounted to ~4% and insertions <1% (see Table I). Other types of mutation (changes of <25 bp) were mapped to restriction sites in another 7% (Nalbantoglu *et al.*, 1983; Grosovsky *et al.*, 1986) and these are predominantly single base changes (Nalbantoglu *et al.*, 1987; de Jong *et al.*, 1987).

Breimer *et al.* (1986) characterized 25 independent mutants induced by ionizing radiation. In contrast to those at the *hprt* locus the pattern of *aprt* gene fragments of mutant DNAs were predominantly (75%) indistinguishable from the wild type pattern, suggesting that base pair changes or deletions/insertions <25 bp were responsible. One mutant had a grossly rearranged gene structure. Four mutants were deletions. These were small in size (one of 20 bp, two of 3.2 kb and one of 4 kb) as compared to several deletions >10 kb found among spontaneous *aprt* mutants (Nalbantoglu *et al.*, 1983, 1986b) and to those at *hprt* locus which eliminated all coding sequence detectable with a cDNA probe. Insertion mutations are rare at the *aprt* locus but one spontaneous and one γ-radiation-induced mutant have been identified and characterized.

The structure of the 20 bp γ-ray-induced deletion mutant was similar to that of spontaneously induced deletions in that it appeared to involve a non-homologous recombination event between a short direct repeat sequence, one copy of which was retained in the mutant gene (Breimer *et al.*, 1986; Nalbantoglu *et al.*, 1986b, 1987). (The DNA sequence of the larger deletion mutants caused by γ-irradiation has yet to be reported.)

The insertion mutations have also been sequenced (Breimer *et al.*, 1986; Meuth *et al.*, 1987). They are both small (58 bp in the case of the γ-ray-induced mutant, 285 bp for the spontaneous). They are accompanied by a deletion of 13 bp and 12 bp respectively at their sites of insertion. There is no duplication of flanking sequences. [Duplication of the flanking sequences at the position of insertion is a hallmark of insertion by transposable elements (Kleckner *et al.*, 1984; Weinert *et al.*, 1984; Grindley & Reed, 1985).] The target sites have some similarity but do not appear to be governed by homology with the inserted fragment. The sequences inserted in the spontaneous and radiation-induced mutants are very different. The insertion in the γ-irradiation-induced mutant has a sequence which is highly dispersed throughout the hamster genome, while the insertion in the spontaneous strain is unique. The hugely repetitive nature of the fragment inserted in the γ-radiation-induced mutant combined with the short direct repeats at one terminus and with the inverted repeats at the other end, makes it possible that it originally was longer and then was imprecisely excised.

Grosovsky *et al.* (1986) have also characterized genomic alterations in mutations at the *aprt* locus using the cell system developed by Meuth (see Table I). Likewise in their study only a few γ-ray induced and none of the spontaneously arising mutants had dectable genomic rearrangements. Sixteen of the ionizing radiation-induced mutants showing no detectable change by Southern blot analysis have recently been sequenced (Grosovsky *et al.*, 1987). Eleven were due to single base changes. Every possible substitution event was observed, though the numbers were too few to determine any bias. The rest were deletions of frame shifts. Of the four deletions, two were similar in structure to those described by Meuth's group (Breimer *et al.*, 1986; Nalbantoglu *et al.*, 1986b). Three γ-ray-induced mutants were reported to have intragenic deletions, which may have similar breakpoints, but the significance of this is not clear. This study shows that ionizing radiation can cause mutations in mammalian cells through single base-pair changes, and that these may account for as many as half the mutations induced at the hamster *aprt* locus.

dhfr *and* tk *loci* Studies of genetic alterations at the dihydrofolate reductase (*dhfr*) locus in CHO cells detected deletions and rearrangements, though several mutants showed no detectable alterations of gene structure (Graf & Chasin, 1982). Unfortunately the resolution of this system is limited; in the cell line studied one of the copies of the autosomal *dhfr* locus was inactivated by a presumed point mutation. Thus the analysis was performed in a background of normal sized fragments, which precluded the detection of complete deletions of the gene. A hemizygous strain has since been developed (Urlaub *et al.*, 1983). With overlapping probes they can monitor a 210 kb region. The resolution is ~100 bp. Using this system Chasin's group (Mitchell *et al.*, 1986; Urlaub *et al.*, 1986) have analysed the changes in spontaneously arising and γ- and UV-irradiation-induced mutants. Of 5 spontaneous mutants none showed deletions. Three of these had lost exon V from their mRNA through a different single base change in the sequences critical for splicing. This area could be a mutational hot spot. Eleven γ-ray-induced mutants were also analysed. All showed major changes: there were 8 large deletions (3 of the full 210 kb) and 3 large inversions. The two deletion mutants induced by UV-irradiation were also large deletions (>95 and 210 kb respectively). Thus in this system ionizing radiation causes mutations through major genetic events. Surprisingly the deletions induced by UV-irradiation were as large, suggesting that when deletions occur at this locus they are generally substantial. Only two of the γ-ray-induced deletion mutants showed microscopically detectable changes in the region of the chromosome where the *dhfr* gene is located, but two of the three inversions had altered chromosome banding.

Little's group (Yandell *et al.*, 1986, 1987) have analysed a large number of mutations at the thymidine kinase (*tk*) locus in a human B-lymphoblastoid cell line that is heterozygous at the *tk* locus, with one functional and one non-functional allele (see Table I). The heterozygosity is thought to be due to a single base change because of the restriction enzyme site alterations, which also make it possible to distinguish the inactive copy from the active copy of the gene. The gene is located on chr 17, and they used informative molecular markers linked to the locus to detect large scale events. Among both induced and spontaneous mutants allele loss was more common (>70%) than intragenic mutation; in many cases, loss extended beyond the locus under selection, but only in a small fraction showed detectable changes in chr 17. The limit of resolution in this system is ~200 bp.

These studies on endogenous loci in mammalian cell lines in culture demonstrate that a significant number of mutations are caused by minimal changes in gene structure, a substantial proportion of which are point mutations. Previous reports (Cox *et al.*, 1977; Cox & Mason, 1978) that ionizing radiation mutations were caused by loss of, or microscopically visible damage to, the chromosome carrying the gene do not seem consistent with more detailed molecular analysis. Indeed, where both cytogenic and molecular analysis have been performed together, most of the chromosomal changes have been found to be unrelated to the known localization of the genes investigated (Fuscoe *et al.*, 1986; Urlaub *et al.*, 1986; Yandell *et al.*, 1987). There are also locus specific effects. Large deletions were the most common alterations induced by ionizing radiation at the *hprt*

and *dhfr* loci, whereas at the *aprt* locus the majority of mutants had alterations in gene structure of <25 bp and the deletions observed were small. The CHO cell lines in which the *aprt* and *dhfr* loci were studied are hemizygous for the locus, the other allele being totally deleted. Thus recombinational repair using the other allele is not possible, though this should favour gross errors rather than point mutations. In the case of the *hprt* locus the other X-chromosome is absent from the cell lines used. However, the X-chromosome is an unusual chromosome (e.g. it can undergo inactivation) and it is not known whether this affects the types of mutations induced. Nevertheless the *hprt* locus is not significantly more prone to spontaneous mutational events than the autosomal *aprt* locus. Another feature is that no deletions extending downstream of *aprt* have been isolated (Nalbantoglu *et al.*, 1983; Breimer *et al.*, 1986). This suggests that there is some essential function encoded there which will limit the types of mutations that can occur.

The studies of human cells give a different picture to that seen in rodent cells. At the *hprt* locus in human cells there were apparently more spontaneous mutants that had suffered total deletion of the gene than in the rodent cells. Total deletions increased less in number on γ-irradiation in human cells than in rodent cells and there were more radiation-induced mutants that had no detectable change in gene structure. At the endogenous *tk* locus little difference was seen between the two types of mutants, complete loss of the functional allele being common. Data from a mouse lymphoma cell line suggest that multilocus lesions are less likely to be lethal at the heterozygous *tk* locus than at the hemizygous *hprt* locus (Evans *et al.*, 1986), and analyses of the *aprt* locus in rodent cells as well as studies of certain human tumours support the notion that complex genetic events play a role in expression of recessive mutations at heterozygous loci (Koufos *et al.*, 1985; Cavanee, 1986). On the other hand, the finding that ionizing radiation but not mitomycin-C-induced mutations at the *tk* locus showed similar genetic changes to those of spontaneous mutants is reminiscent of the findings at the *aprt* locus, and could be interpreted that a similar molecular process is involved in the generation of either mutant. How the presence of a second copy of an allele rendered inactive by a presumed point mutation affects the repair of lesions in the expressed copy is not known. There is evidence that DNA base lesions are removed much more efficiently from active than inactive genes (Bohr *et al.*, 1985; Smith, 1987), suggesting that the cell has powers to discriminate. It is not clear whether cells could also use part of the inactive copy in repair. The mutational response to ionizing radiation is different for human and rodent cells, particularly at low doses (Thacker & Cox, 1975; Grosovsky & Little, 1985), though it has been suggested that these differences may be more related to differences in cell killing than mutagenesis (Thacker & Cox, 1975). Some caution is needed in the comparison of mutations between species as well as at dissimilar loci.

Exogenous genes and shuttle vectors

As pointed out above among the difficulties of analysing changes at endogenous gene loci in mammalian cells are their size and complex structure. In addition there is now evidence that two genes can be encoded on opposite strands of the same DNA locus in mammalian cells (Adelman *et al.*, 1987) which could further limit the type of lesions tolerated. One way of circumventing these problems is to use a small, simple foreign gene either stably integrated in the gene line or as part of a 'shuttle-vector' system. In a shuttle-vector the gene studied is part of a construct which is able to replicate both in mammalian cells and another host, usually *E. coli*, so that the analysis is simplified (for technical details see Thacker, 1986). Early such vectors, especially those related to simian virus 40 (SV40), were prone to high levels of spontaneous mutagenesis (Razzaque *et al.*, 1983; Calos *et al.*,

1983; Hauser *et al.*, 1987). This has now been improved (Lebkowski *et al.*, 1985; Glazer *et al.*, 1986; MacGregor *et al.*, 1987). In particular the new vectors based on Epstein–Barr virus are likely to be of value in human cell systems (DuBridge *et al.*, 1987; Menck *et al.*, 1987). Unfortunately results of studies using shuttle vectors with ionizing radiation are not yet available.

Stankowski and Hsie (1986) have reported results of studies of mutations induced at the *E. coli gpt* gene in a CHO cell line (see Table I). The cell line was constructed from a *hprt* deletion mutant and contains a single, functional copy of the *gpt* stably integrated in its high molecular weight DNA. The gene is ∼700 bp long and mutants were selected by thioguanine resistance. Nearly all of the mutants induced by known point mutagens showed no detectable changes in gene structure, but all those induced by ionizing radiation were deletions (all but one total) of the *gpt* gene. However, of the spontaneously arising mutations, most were partial or total deletions of the *gpt* gene; only 40% were unchanged. The results they reported for analysis of the *hprt* locus of a different CHO cell line were in agreement with those of other workers (see above). Though these data may be interpreted as evidence for ionizing radiation causing pre-dominantly deletion mutations, the pattern of spontaneous mutations is different from that at *aprt* and *hprt* and closer to the situation at the endogenous *tk* locus. Whether this is due to some inherent property of the integrated prokaryotic DNA or the location of integration is not clear.

Goring *et al.* (1987) have analysed the spontaneous mutations in a chromosomally located single-copy Herpes simplex virus type I (HSV-I) thymidine kinase (*tk*) gene in a human TK⁻ cell line. The *neo* gene, which codes for resistance to the antibiotic G418, was placed next to the *tk* gene for the purpose of screening out gross cheomosomal alterations. TK deficient mutants were selected with suitable drugs either separately or in combination. Analysis of mutations by Southern blotting revealed that the majority of mutants had undetectable (<50 bp) alterations of gene structure. Inactivation of the gene was not due to extensive methylation. A high reversion frequency suggested that point mutation might be the cause. These findings contrast with those at the endogenous *tk* locus (Yandell *et al.*, 1986, 1987). However, the few complex changes in gene structure, including total deletions, reported here were much more common when two drugs had been used to select mutants, suggesting that some of the undetectable alteration mutants could have been leaky or that the two agents used interact differently with the thymidine kinase enzyme. The close proximity of the *neo* gene will screen out larger deletions, and selecting for G418 resistance may affect the functional state of the genome in the region of the *neo* gene (Roginski *et al.*, 1983).

DNA lesions and their repair

Ionizing radiation exerts the bulk of its damaging effects through oxygen derived free radicals, particularly hydroxyl radicals. It causes modification of the nitrogenous bases, single- and double-strand breaks and base-free (AP) sites (Ljungquist & Lindahl, 1974; Ward & Kuo, 1976; Cerutti, 1976; Teoule & Cadet, 1978; Hutchinson, 1985; Breimer & Lindahl, 1985a,b). For technical reasons most studies have been concerned with the formation and fate of strand breaks rather than base lesions. Indeed single-strand breaks have traditionally been regarded as the most frequent radiation lesion, but Hutchinson (1985) has estimated that base lesions are equally frequent, and Ward (1985) has calculated that base lesions may be at least twice as common as strand breaks.

Base damage

Pyrimidines Ionizing radiation causes ring saturation, contraction and fragmentation of pyrimidines (Teoule *et al.*,

1977; Teoule & Cadet, 1978; Breimer & Lindahl, 1985a; Hutchinson, 1985). The action is mediated by attack by hydroxyl radicals at the 5,6 double bond and, in the case of thymine in DNA, can be mimicked by the oxidizing agents potassium permanganate ($KMnO_4$) and osmium tetroxide (OsO_4) though the spectrum of lesions is different (Breimer & Lindahl, 1980, 1984, 1985a). Briefly, thymine glycol (5,6-dihydroxy-6-hydrothymine) is the common lesion following γ-irradiation and oxidation. 5-Hydroxy-5-methylhydantoin, a minor $KMnO_4$ product, is readily formed by γ-irradiation. Methyltartronylurea, a major $KMnO_4$ product at physiological pH, is a minor γ-irradiation product. 6-Hydroxy-5,6-dihydrothymine is generated by γ-irradiation but not by oxidation; it is also a minor UV-photo product (Fisher & Johns, 1976). Urea, which is a potential end product of all base damage in DNA, is readily generated by $KMnO_4$ treatment, though its total yield is γ-irradiated DNA has not been unequivocally determined. The main additional thymine lesion is 5-hydroxymethyluracil (α-hydroxythymine) (Teebor et al., 1984). This base can substitute for thymine in the DNA of at least one bacteriophage without obviously altering the coding specificity.

Cytosine also undergoes ring reactions, but the glycol is unstable, and rupture is more common at the 4,5 bond. It is controversial whether ionizing radiation causes mutations through deamination of cytosine to uracil. Cytosine irradiated in solution can be deaminated to uracil, but this lesion has not been found in DNA (Teoule & Cadet, 1978; Hutchinson, 1985). Glickman et al. (1980) found no evidence that ionizing radiation increased mutations at 5-methylcytosine residues in E. coli. However, Tindall et al. (1987) report that two thirds of the mutations in irradiated lambda phage assayed in host cells not induced for the SOS system were G.C to A.T transitions and proposed that a cytosine product deaminated by irradiation was involved. An alternative explanation is that a non-deaminated product of cytosine, such as the ring contracted 1-carbamyl-imidazoldine-4,5-diol (Hahn et al., 1973), could miscode as thymine and would not be generated from 5-methylcytosine.

The hydroxyl radicals initially generate unstable hydroxy-hydroperoxidases of any base, which break down further into more stable derivatives. They may also be involved in forming protein DNA cross-links (Teebor et al., 1984; Simic & Dizdaroglu, 1985). These hydroperoxides may be reconstituted to the original base through the action of glutathione dependent enzymatic processes thus limiting the damage (Tan et al., 1986; Edgren, 1987).

The lesions induced in DNA are repaired by base excision through the action of DNA glycosylases, enzymes which cut the base-sugar bond of modified bases to release them in free form (Lindahl, 1982; Breimer & Lindahl, 1985b). In E. coli one single DNA glycosylase can remove several different forms of free radical altered thymine from DNA including thymine glycol, 5-hydroxy-5-methylhydantoin, methyl-tartronylurea and urea, and cleave the DNA at the resulting AP sites (Breimer & Lindahl, 1980, 1984). An analogous enzyme exists in mammalian, including human, cells. It has similar characteristics but is less active on thymine glycol than urea (Breimer, 1983; Hollstein et al., 1984; Higgins et al., 1987). It is also present in Drosophila (Breimer, 1986). 5-Hydroxymethyluracil-DNA glycosylase appears only to be present in differentiated mammalian cells (Hollstein et al., 1984; Boorstein et al., 1987). This is the first DNA glycosylase not to have a counterpart in lower organisms. Boorstein et al. (1987) have proposed that 5-hydroxymethyluracil in DNA may be weakly mutagenic because it slightly alters the physiochemical properties of DNA. To explain the phylogenetic difference they argue that unicellular organisms may tolerate a rare mutational event better than multicellular organisms, where supposedly even a very low mutagenic frequency could be disastrous. An alternative explanation would be that the altered base modifies coding by interacting with a mammalian protein or other factor not present or substantially different in lower organisms (i.e. that the presence of 5-hydroxymethyluracil in mammalian DNA causes mutation rather than these mutations being better tolerated in lower organisms). As even supplementing cell culture medium with thymidine can induce mutations through DNA precursor pool imbalance (Goncalves et al., 1984; Phear et al., 1987) this is unlikely to be a useful way of studying the properties of its 5-hydroxy derivative.

Several groups have now established that ring-saturated thymine lesions are non-coding rather than miscoding (Hariharan et al., 1977; Ide et al., 1985; Rouet & Essingmann, 1985; Clark & Beardsley, 1986; Hayes & Le Clerk, 1986). DNA templates have been oxidized to yield thymine glycols or further, unspecified, breakdown products (possibly urea). Essentially these experiments show that the thymine lesions strongly inhibit oxidized phage survival in vivo following transformation and DNA elongation by DNA polymerases in vitro. Sequence analysis showed that in vitro DNA synthesis terminated opposite thymine glycols but one nucleotide before putative urea residues. If the conditions of synthesis were relaxed by substitution of manganese for magnesium ions in the reaction mixture, dAMP was incorporated opposite the lesions. This is consistent with previous reports that DNA polymerases introduce dAMP residues opposite any non-informative lesion (Strauss et al., 1982; Boiteux & Laval, 1982; Schaaper et al., 1983; Kunkel, 1984) but it could also be due to thymine glycol coding as thymine (Ide et al., 1985). The processivity was dependent on the presence of a pyrimidine preceding the thymine glycol. Virtually all these experiments have been done with single polymerase subunits or proteolytic fragments of the subunits, so they probably have relaxed fidelity of action. Moreover eukaryotic DNA polymerases are able to copy past non-informative lesions more readily than are enzymes purified from prokaryotes (Kunkel et al., 1983).

No direct studies have been reported of the mutagenicity or repair of γ-irradiation-induced cytosine lesions. Though several are similar, they are not all equivalent to those of thymine.

E. coli mutants which are deficient in thymine glycol-DNA glycosylase (nth^-) are not more sensitive than wild type strains to the killing effects of ionizing radiation or hydrogen peroxide, though they show a weak mutator phenotype (Cunningham & Weiss, 1985). These results are not inconsistent with the DNA polymerase experiments. It has been reported that DNA exonuclease III (encoded by the xth gene) which accounts for 90% of total AP endonuclease activity in E. coli also can cut DNA at oxidized base residues other than thymine glycol (Kow & Wallace, 1985), which would enable the cell to deal with those lesions. However, xth defective mutants are extremely sensitive to hydrogen peroxide and ionizing radiation (Seeberg & Steinum, 1980; Demple et al., 1982). The phenotype of $nth^- xth^-$ double mutants is not known. They may not be viable aerobically as they may be even more sensitive to oxygen free radicals.

Purines Ionizing radiation also causes saturation and fragmentation of the imidazole ring of purines (Teoule & Cadet, 1978; Bonicel, et al., 1980; Breimer, 1984; Hutchinson, 1985; Cadet & Berger, 1985). Imidazole ring-opened adenine, 4,6-diamino-5-formamidopyrimidine, is structurally similar to imidazole ring-opened 7-methylguanine, which, if present in the template, blocks DNA chain elongation by E. coli DNA polymerase I in vitro (Boiteux & Laval, 1983). These ring-opened purines are excised from DNA by a DNA glycosylase, formamido-pyrimidine-DNA glycosylase (Chetsanga & Lindahl, 1979; Breimer, 1984) which is also present in mammalian cells (Breimer, 1983). The gene of the E. coli enzyme has now been cloned and sequenced (Boiteux et al., 1987). Thus its physiological role should soon be known.

The C-8 position of purine can also be hydroxylated. Nishimura's group has shown that 8-hydroxy-deoxy-

guanosine (8-OH-dG) can be produced in DNA *in vitro* by various oxygen radical producing mutagenic or carcinogenic agents including ionizing radiation (Kasai *et al.*, 1984). 8-OH-dG was detected in DNA isolated from HeLa cells after cells in tissue culture had been irradiated with X-rays and from the liver of mice after the whole animal had been irradiated with γ-rays (Kasai *et al.*, 1986). The amounts of 8-OH-dG in DNA after *in vivo* irradiation were much lower than those after *in vitro* irradiation. The 8-OH-dG produced in liver DNA by irradiation of mice decreased with time, suggesting active repair.

Analysis of the effects of the 8-OH-dG residues in DNA on the fidelity of DNA replication using a DNA synthesis system *in vitro* with *E. coli* DNA polymerase I (Klenow fragment) showed that this lesion does not inhibit synthesis but miscodes (Kuchino *et al.*, 1987). In addition to misreading of the 8-OH-dG residue itself, pyrimidines next to the 8-OH-dG residue were also misread. When placed between T and C, the 8-OH-dG residue directed the insertion of A, T, C or G with an almost equal frequency, indicating that it lacks specific base-pairing. In addition to misreading of the 8-OH-dG residue itself, pyrimidines next to the 8-OH-dG residue (G was not tested) were also misread. When the DNA synthesis was carried out *in vitro* without addition of the dideoxynucleosidetriphosphates (ddNTP) used in the Sanger sequencing method almost no termination was detected. This newly synthesized DNA was analysed by the sequencing method of Maxam and Gilbert and misreading in the region of 8-OH-dG (in the absence of ddNTPs) confirmed. The reactions were performed under standard conditions and not 'relaxed', but again a fragment of DNA polymerase was used. Thus it is likely that 8-OH-dG residues in DNA will be directly mutagenic. Depending on its location it could induce two amino acid changes and therefore lead to mutations that would be extremely unlikely to revert and hence not appear as a single base damage event in indirect analyses. Moreover, Kasai *et al.* (1986) calculated that the extent of formation of 8-OH-dG in DNA by ionizing radiation in their experiments was of the same order as that of thymine glycol formation, previously thought to be the most frequent DNA base lesion. Studies using modified plasmids are now required to establish that 8-OH-dG is mutagenic *in vivo*. Such experiments have to be carefully planned, for it is now clear that if lesions are only present in one strand of a DNA molecule, there is specific strand loss in *E. coli* so that the undamaged strand alone is replicated (Koffel-Schwartz *et al.*, 1987).

Hypoxanthine, which is read as guanine, has been reported as a minor radiation product of adenine (Ponnamperuma *et al.*, 1961). It is excised by a DNA glycosylase (Karran & Lindahl, 1978, 1980). However, the spectrum of base changes reported so far do not indicate that it is a major mutagenic lesion of ionizing radiation.

AP sites

AP sites in DNA arise spontaneously through depurination (Lindahl & Nyberg, 1972). Ionizing radiation induces AP sites in DNA directly and as a result of the action of DNA glycosylases on damaged bases. By analysing the DNA from γ-irradiated HeLa cells Moran and Ebisuzaki (1987) have reported not only that DNA strand breaks are rapidly repaired but that AP sites were generated and subsequently repaired. The transient nature of the AP sites, reaching a maximum by 2 min, suggests that they are an early intermediate in a DNA repair pathway and that their removal may be rate limiting.

There is now considerable evidence that AP sites are mutagenic *in vivo* (Schaaper *et al.*, 1983; Kunkel, 1984; Miller & Low, 1984; Gentil *et al.*, 1984; Loeb, 1985; Granger-Schnarr, 1986; Cunningham *et al.*, 1986; Loeb & Preston, 1986; Foster & Davis, 1987). Analysis of DNA synthesis on templates containing AP sites has shown that dAMP residues are preferentially introduced opposite these

lesions (Boiteux & Laval, 1982; Strauss *et al.*, 1982; Schaaper *et al.*, 1983; Kunkel, 1984). Such bypass can now also be achieved at AP sites under physiological conditions (Takeshita *et al.*, 1987). Thus ionizing radiation-induced AP sites will contribute to the mutational load. AP sites are subject to efficient repair by a number of DNA endo-nucleases (Ljungquist & Lindahl, 1974; Lindahl, 1979, 1982). Indeed the glycolase activity for radiation ring damaged thymine is also an endonuclease (Breimer & Lindahl, 1984 – see above). It has been reported that simultaneous presence of AP sites and base lesions in the same phage DNA interferes with the efficiency of their respective repair (Duker *et al.*, 1982). Tindall *et al.* (1987) reported a preferential substitution of A.T for any original base pair in irradiated lambda phage assayed in SOS induced hosts, and from Kunkel's work (1984), suggested that AP sites were the mutagenic intermediate. However, any non-informative lesion could in principle account for the result.

Strand breaks

DNA strand breaks are generated by a number of agents acting through oxygen derived free radicals, including ionizing radiation and radiomimetic drugs such as bleomycin. Single-strand breaks are generated directly by ionizing radiation, or as the result of the action of AP endonucleases with or without DNA glycosylases on lesions. Similarly double-strand breaks can be induced directly or as a result of enzymatic action at or near a single-strand break. Indeed, strand breaks are obligatory intermediates of excision repair, post replication repair and genetic recombination and any perturbation of their repair would be deleterious to the organism. The literature on ionizing radiation-induced strand-breaks and their physiological effects is enormous and confusing (for reviews see Cerutti, 1976; Hutterman *et al.*, 1978; Thacker & Cox, 1983; Hutchinson, 1985; Thacker, 1986). The initial number of strand-breaks is certainly a measure of the degree of insult (i.e. the total amount of energy deposited).

Briefly, γ-irradiation of DNA *in vitro* produces random single-strand breaks containing termini with 5′-phosphoryl-nucleotides on one side and on the other side either a normal nucleotide bearing a 3′-phosphoryl moiety or a 3′-phosphoryl glycolic acid ester formed from deoxyribose (Henner *et al.*, 1982, 1983; Hutchinson, 1985). Such single-strand interruptions are thus small gaps, resulting from the loss of at least one nucleoside. Bleomycin and neo-carzinostatin, radiomimetic chemotherapeutic drugs, cause oxygen radical dependent strand breakage at thymines and generate complex termini (Giloni *et al.*, 1981; Kappen *et al.*, 1982).

The nature of the double-strand break directly arising from ionizing radiation is less well characterized but likely to be complex. Double-strand breaks *in vivo* increase linearly with dose, rather than the square of the dose, indicating the formation of each by a single event (Hutchinson, 1985). They are also affected differently by radiation conditions than single-strand breaks (Hutchinson, 1985).

Radiation-induced single-strand and double-strand breaks are repaired very rapidly in both prokaryotes and eukaryotes (Kapp & Smith, 1970; Karran & Ormerod, 1973; Resnick & Martin, 1976; Krasin & Hutchinson, 1977; Game *et al.*, 1980; Brenner *et al.*, 1986; Ayarez *et al.*, 1987). The repair of the double-strand breaks and some of the single-strand breaks evidently occur through a recombination mechanism. Recombinational repair permits the recovery of coding information lost as a result of the injury. It involves the donation of an intact DNA strand into the damaged duplex from a homologous sister or daughter chromosome (Szostak *et al.*, 1983). The repair of single-strand breaks and double-strand breaks appear to be complete even in cells irradiated at doses causing up to 99% killing (Lehman & Stevens, 1977; Hariharan *et al.*, 1981).

Substantial work has been undertaken to establish the

effect of strand-breaks and their repair in mammalian cells (for review of techniques see Thacker, 1986). The results are generally inconclusive. To extrapolate from the results obtained by introducing into mammalian cells comparatively small (a few kb) segments of prokaryotic DNA which have been subjected to restriction enzyme digestion, to general models for *in vivo* repair of the highly organized mammalian cell DNA, is fraught with difficulties. Also, many of the vectors commonly used in these experiments, are prone to high levels of spontaneous mutagenesis in mammalian cells (see above). Also, some conclusions are based on introducing prokaryotic DNA endonucleases into eukaryotic cells. As the specificity of such enzymes can be affected by the reaction conditions, such data must be interpreted with caution.

There is indirect evidence that double-strand breaks may be the lesion responsible for chromosome aberration induced by ionizing radiation (for review see Thacker, 1986), though other groups have concluded that aberrations arise during the repair of induced DNA base damage (Preston, 1982). Recently a series of Chinese hamster ovary cell lines derived mutants (*xrs*) defective in the repair of double-strand breaks but containing normal levels of thymineglycol-DNA glycosylase have been isolated and characterized (Kemp *et al.*, 1984). The increased number of double-strand breaks remaining in these strains 20 min after irradiation, correlated well with the increase in chromosome breaks (Kemp & Jeggo, 1986).

Mammalian cells contain all the enzymes needed to mediate homologous recombination (Wake & Wilson, 1979; de Saint Vincent & Wahl, 1983). In prokaryotes and lower eukaryotes analysis of the mechanism and enzymology of recombination has been facilitated by the availability of mutants defective in this process often isolated because of radiation sensitivity (e.g. *rec A⁻* of *E. coli*). The *rad-52* mutants of yeast repair single-strand breaks but not double-strand breaks (Resnick & Martin, 1976) and are extremely deficient in both mitotic and meiotic recombination (Game *et al.*, 1980). Moore *et al.* (1986) have studied the recombination proficiency of the most extremely deficient double-strand break repair (*xrs*) mutant isolated by Kemp and Jeggo (1986). In an *in vivo* assay the mutants showed a 6-fold reduced homologous recombination frequency when compared to the parental cell line. However, *in vitro* assay of cell free extracts did not show any significant difference in proficiency. Hamilton and Thacker (1987) have further studied the *xrs* mutants and reported that while homologous recombination of plasmid molecules may not be substantially reduced in these *xrs* mutants, processes involved in the stable integration of plasmid DNA into genomic DNA are significantly impaired. Hoy *et al.* (1987) have reported that a CHO mutant which, though only slightly more sensitive to the effects of γ-irradiation, is considerably sensitive to some but not all alkylating agents and has a high level of sister chromatid exchange and decreased ability to rejoin strand breaks, is deficient in homologous recombination. Recently Jones *et al.* (1987) have reported the isolation of new X-ray sensitive mutants of V79-4 hamster cells. The availability of these mutants should help in characterizing DNA repair mechanisms.

Cancer prone inherited disorders

A number of rare inherited disorders associated with increased sensitivity to ionizing radiation and risk of malignancy have been described, including ataxia telangiectasia (AT), Fanconi's anaemia (FA), Bloom's syndrome (BS) and inherited retinoblastoma. These syndromes could be regarded as naturally occurring mutants. There is a wealth of conflicting literature concerning these disorders partly because of extrapolation from data obtained with one or two cell lines to the whole syndrome. Cell free extracts of fibroblasts grown in culture failed to reveal deficiencies for AP endonucleases and urea-DNA glycosylase in the strains

tested (Teebor & Duker, 1975; Breimer, 1983). Reports that AT fibroblasts were defective in excision repair of γ-ray damaged DNA (Paterson *et al.*, 1976) and that cell extracts of AT cells were deficient in an enzyme which enhanced the priming activity of γ-irradiated DNA for DNA polymerase I (Inoue *et al.*, 1977; Edwards *et al.*, 1980) have not been confirmed (van der Schans *et al.*, 1981; Shiloh *et al.*, 1981). The repair proficiency of these fibroblasts generally decrease with increasing passage (Sognier & Hittleman, 1983) making deficiency data difficult to interpret. Painter and Young (1980) have suggested that the cause of the increased radiation sensitivity in AT cells may be a defect in their ability to inhibit DNA synthesis following irradiation, thus giving less time for repair to take place. A single-strand break induced by ionizing radiation appears to be the stimulus for this effect; a break introduced by a repair activity has no effect (Painter & Young, 1987). There is evidence that AT cells are specifically sensitive to agents that break the deoxyribose moiety of DNA via a targetted free radical mechanism (Shiloh *et al.*, 1983; Joenje *et al.*, 1987). Cox *et al.* (1984, 1986) have proposed a model for the deficiency in AT. Normally there is a competition between ligation and exonuclease digestion of broken DNA. In AT cells a factor which modulates exonuclease activity is altered so that the balance is shifted from ligation to excessive digestion of DNA at the point of strand-breakage. The model is based on data derived from introducing vectors which are prone to spontaneous modification (see above) and the work awaits independent confirmation. There has been one unconfirmed report that the AT defect can be reversed by DNA transfection (Green *et al.*, 1987).

In whatever way DNA strand breaks arise and associated gaps are reconstituted, ultimately the DNA has to be joined together again, a reaction catalysed by DNA ligase (for review see Söderhall & Lindahl, 1976). Mammalian cells, in contrast to bacteria, have two different DNA ligases, designated I and II, both present in cell nuclei. They do not cross-react serologically. DNA ligase I, which is larger, is induced during cell proliferation and may be active during chromosomal replication, but ligase II is present in similar amounts in growing and nongrowing cells. Only ligase II can catalyse the joining of DNA–RNA hybrids (Arrand *et al.*, 1986). Using this differential assay Willis and Lindahl (1987) have shown that DNA ligase I is deficient in Bloom's syndrome. Independent data are consistent with this interpretation (Chan *et al.*, 1987).

In the case of inherited retinoblastoma it now appears that there is a somatic alteration of the normal allele such that it unmasks the mutant allele (Cavanee, 1986). In the sporadic form of the disease the tumorigenic version of the allele has to be acquired (a rare event), but in the familial form it is inherited.

Possible future directions

In the last few years great advances have been made in analysing the molecular mechanisms of mutagenesis and carcinogenesis. It appears that locus specific effects are important in determining the types of mutations tolerated. There is now considerable evidence that ionizing radiation can induce mutations in mammalian cells through small genetic events, a substantial proportion of which are single base pair changes, rather than cytogenetically and microscopically observable events. However, the only way of ascertaining base changes in a gene (or the exact nature of any point mutation) is to determine the DNA sequence. For an endogenous gene this involves considerable labour of isolating, cloning and subcloning. The efficiency of the cloning procedure can be optimized by biological selection (Maniatis *et al.*, 1982; Grosovsky *et al.*, 1987). Nevertheless, even the *aprt* gene is too large to be sequenced conveniently and routinely.

A number of methods have been developed to increase the

resolution of mapping the site of point mutations so that the DNA segment required to be sequenced can be limited. By careful optimization of the conditions of hybridization of the probe, chromosomal DNA from hundreds of bacterial mutants can potentially be examined for small changes including single base-pair changes without any DNA cloning (Miller & Barnes, 1986). The sensitivity of this method might be further increased by including tetramethylammonium chloride in the hybridization buffers, which abolishes the preferential melting at A.T v G.C base-pairs, thus making the procedure independent of the base composition of the probe (Wood et al., 1985). Another method is the ribonuclease A cleavage procedure pioneered by Maniatis (Myers et al., 1985). It is based on the property that some single base mismatches in RNA hybrids with RNA or DNA will be cleaved by RNase A, though the efficiency varies depending on the exact nature of the mismatch, certain base-pairs being cleaved poorly or not at all. Indeed mismatches resulting from deletions, insertions or rearrangements offer greater potential for RNase cleavage because of more extensive single-stranded regions within the hybrids. The method has been used by Gibbs and Caskey (1987) to identify and locate mutations at the hprt locus in Lesch-Nyhan patients not uncovered by other means. However, no such or any other mRNA analyses have been reported on ionizing radiation-induced mutants. Single base-pair mismatches can be detected in DNA by chemical modification which alters the migration of the DNA fragments during gel electrophoresis (Novack et al., 1986). Single-stranded regions within a duplex fragment are accessible to carbodiimide, which reacts with unpaired G and T residues. Intact linear duplex DNA molecules do not react, whereas molecules containing single-base mismatches react quantitatively. After carbodiimide reaction the DNA molecules are electrophoresed in high percentage polyacrylamide gels to resolve modified and unmodified fragments. The resolution is best with small (<600 bp) fragments containing short single-stranded regions. However, the method could be made general by utilizing the repair enzyme UvrABC. It can cleave DNA at carbodiimide modified bases (E. Seeberg, personal communication; Thomas et al., 1986). The enzyme cuts phosphodiester bonds four residues upstream and eight downstream of the lesion. Heteroduplexes are made between the wild type and mutated gene and treated with carbodiimide. The DNA is then cut on either side of the modified base by UvrABC. The resultant 12–13mer is released and the single-stranded region is cut with S1 nuclease. Thus two new fragments are generated. The fragments are then separated by electrophoresis on an agarose gel and detected and analysed in the usual manner. In principle there is no limit to the size of fragments that could be investigated.

However, the methods outlined above are still comparatively labour intensive. It is not clear whether the 'genomic sequencing' procedure described by Church and Gilbert (1984) can be used in mutation studies. An alternative would be to amplify the region of interest (Saiki et al., 1985). Briefly, two oligonucleotide primers are synthesized that hybridize just beyond each end of the segment of interest, one to each DNA strand. The primers are annealed to denatured genomic DNA and then extended by DNA polymerase. Since the newly synthesized DNA strands are themselves templates for the primers, repeated cycles of denaturation, primer annealing, and extension results in exponential accumulation of the region defined by the primers (220,000-fold amplification has been described). It is not known how big a fragment can be amplified, nor whether the process is sufficiently specific, but the method could potentially dispense with the need of cloning and possibly allow analysis of mutants grown in microtitre plate wells.

A potentially powerful system would be to raise transgenic mice that contain a copy of a suitable shuttle vector in all cells of the animal. Such a strain could be used to perform mutagenesis experiments in the whole animal and could be of general use to approach physiologically relevant questions of mutagenesis.

The similarities observed between spontaneously arising and ionizing radiation-induced mutations at certain loci suggest that they may arise through common mechanisms of DNA change and/or repair. Ionizing radiation and other agents acting through oxygen free radicals may induce base changes through the generation of 8-hydroxylpurines in DNA, which miscode. Alternatively they may cause AP sites, either directly or as intermediates of repair. Likewise, the more complex changes of deletions and insertions could be due to similar initial lesions or compromised repair. Indeed, there is considerable evidence that the ability of cells to limit the effect of mutagenic insults may be of critical importance and that inadequate repair of lesions increases the rate of induction of mutations (Lindahl, 1982; Cunningham et al., 1986; Smith, 1987; Foster & Davis, 1987; Yatagai et al., 1987).

Some of the DNA-repair systems involved in the correction of alkylation damage and complex lesions are present in very small amounts intracellularly, but are inducible in E. coli (Witkin, 1976; Lindahl, 1982; Walker, 1984). Treatment with hydrogen peroxide induces an apparently analogous DNA repair pathway (Demple & Holbrook, 1983), and there is some overlap between functions induced and those induced through heat shock (Christman et al., 1985; Privalle & Fridowitch, 1987). Paraquat, probably acting through oxygen-derived free radicals, can also induce DNA repair (Chan & Weiss, 1987). The exact mechanisms involved have yet to be established. Wolff's group has described an analogous mammalian response, in that incorporation of radioactive isotope into DNA or irradiation with a low dose induces resistance to a challenge dose of ionizing radiation (Oliveri et al., 1984; Shadley & Wolff, 1987). Such induced cells could be sources of new repair enzymes, and defective mutants would establish their physiological importance.

Conclusions

Data accumulated over the past two years demonstrate that ionizing radiation can induce mutations in mammalian cells through single base-pair changes at least at one locus. At other loci large scale events dominate. Microscopically visible chromosome changes play an insignificant part. The base-pair changes are probably mediated through miscoding base lesions such as 8-hydroxyguanine or AP sites. The similarity between some ionizing radiation-induced and spontaneously arising mutants is probably due to a common pathway of DNA damage (through oxygen-derived free radicals) and/or repair. However, the ultimate pattern of mutations will be established by analysing additional, different loci and by determining the DNA sequence of an adequate number of representative mutants. At present this is a formidable task, but with the development of more powerful methods of analysis and automated sequencing technology it is entirely feasible. The understanding of the cellular systems involved in limiting the effect of ionizing radiation will help in the design of more powerful radiosensitizers for clinical use and also in radioprotection.

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