

Cellular and Tissue Response to Radiation Damage

1. DNA damage and its repair

Cells are under continuous onslaught not only from environmental stress such as radiation and toxic chemicals, but also internal metabolic stress, e. g., reactive oxygen species such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\bullet OH$), produced as by products of cellular, particularly mitochondrial, metabolism. Thus, very robust DNA repair systems have evolved to deal with DNA damage. These repair systems include nucleotide and base excision repair (Sancar, 1994; Hanawalt, 1994), mismatch repair (Modrich, 1994) and double strand break repair (see tables below).

<i>Damage to DNA</i>	<i>Effect</i>	<i>Repairability</i>	<i>Reference:</i>
<i>Altered Bases</i>	Primary	Easily Repaired	Ward, 1995
<i>SSB</i>	Primary and Secondary	Easily Repaired in most cases	Ward, 1985
<i>DSB</i>	Primary and Secondary	Not all accurately repaired	Lobrich, 1995 Ikpeme, 1995 Taucher-Scholz, 1995
<i>Fragmentation</i>	Primary and Secondary	Results from inability to repair primary damage	Holley and Chatterjee, 1996 Rydberg, 1996
<i>Deletions</i>	Secondary	Results from inability to repair primary damage	Kronenberg, 1995
<i>Chromosomal Rearrangements</i>	Secondary	Results from inability to repair primary damage	Nagasawa and Little, 1981 Morgan, 1996
<i>Protein-DNA Cross-link</i>	Secondary	Not repaired	Olinski, 1992

Table 1: Radiation Induced DNA Damage

Much of the early work on radiation damage has focused on DNA because of its unique role in storing and replicating the genetic information in the cell. The kinds of damage inflicted on DNA are summarized in Figure-1 and Table-1, along with several review references. Base damage by low-LET radiation occurs mainly through the generation of reactive species by the radiolysis of water, but most cells obtain robust systems to repair such damage with high fidelity. During this process of repair, transient single strand DNA breaks (SSB) are generated as intermediates in the repair pathway.

Double-stranded breaks (DSB) can be generated from clusters of oxidized bases induced by high doses of low-LET radiation (i.e. many damaged sites close together). High-LET radiation can also generate clusters of base damage, or can produce both SSB and DSB directly, i.e. independently without base damage (Ward, 1985). DSB are two orders of

magnitude less likely to be produced by ionizing radiation than are single base modifications (Cooper, 1996), but they are particularly lethal. Thus, the primary events due to ionizing radiation, in order of abundance, are thought to be: base damage > SSB >> DSB.

A single-base lesion in the DNA induced by isolated free radical attack usually neither kills cells nor leads to mutagenesis in mammals. This suggests that base excision repair, the path way primarily responsible for the repair of oxidatively damaged bases (Wallace, 1988) is both rapid and faithful. Consistent with Moron and Ebisuzaki (1987) showed that with sufficient ^{137}Cs γ -rays to kill 70% of the cells, were repaired within 30 minutes of irradiation, although they did not evaluate the fidelity of this repair. However, thiamine radicals formed in DNA as a result of gamma radiation of cells have been shown to react with tyrosine moieties in proteins, in which case repair of the resulting protein-DNA cross-link may not be possible.

All of these phenomena could cause mutations and/or cell killing if the damage is so extensive as to overwhelm the DNA repair system. The failure to repair DSB quickly and accurately is thought to lead to a variety of DNA rearrangements such as fragmentation and deletions, and a variety of chromosome aberrations (Morgan, 1996). Thus, the reparability of DNA damage is a critical issue with regard to whether ionizing radiation induces genomic instability and substantial changes in the genetic information. Our current knowledge about what we know about mechanisms of DNA repair is summarized below. Persistent and extensive damage is also thought to induce programmed cell death, although the exact mechanism of this induction is not known.

A. Excision Repair Pathways

DNA repair systems include: DNA damage reversal, DNA excision repair, DNA mismatch repair and DNA recombinational repair. DNA damage reversal systems are photoreactivation, involving the separation of UV-induced pyrimidine dimers by a photolyase, and alkyl damage reversal by alkyl group acceptor proteins. DNA excision repair can be subdivided into two major variants: nucleotide excision repair (NER) and base excision repair (BER) (Sancar, 1994, 1995). NER is used to excise so-called bulky DNA adducts as part of the oligonucleotide. However, other forms of DNA damage, such as oxidative DNA damage, are also repaired by means of NER (Huang, 1994; Hanawalt, 1994).

NER is coupled to transcription in two ways. First, basal transcription and NER share components in the form of transcription factor TFIIH, two helicases which are identical to ERCC3/XPB (3'-5') and ERCC2/XPD (5'-3') (Schaeffer 1993, 1994; Frejter, 1992). ERCC and XP refer to genes required to repair UV-induced damage (Lehmann, 1994; Cleaver, 1968); these genes are called the ERCC for excision repair cross-complementing and XP for xeroderma pigmentosum. Second, a dedicated subpathway of NER acts on lesions in the transcribed strands of expressed genes when those lesions block

transcription (Bohr, 1985; Hanawalt, 1994). For some lesions this results in preferential repair of the transcribed strand, a feature that is lost in Cockayne's syndrome.

It is unlikely that the nucleotide excision repair pathway plays major role in repairing DNA damaged by ionizing radiation; unless something resembling a bulky adduct is formed, it probably does not. One exception is the XPG protein which is required for transcription-coupled repair of both X-ray and HZE particle induced base damage (Cooper, 1997), but none of the other XP gene products is known to be repaired for repair of damage induced by ionizing radiation. The XPF protein is an endonuclease homologous to ERCC (Sijbers, 1996), and complexes with ERCC1 to produce an endonuclease activity which is active on UV-damaged DNA; it is not known whether it also plays a role in repair of ionizing radiation-induced damage.

Base excision repair (BER) is the main system for removing small base damages, including oxidative DNA lesions (Wallace, 1994). In this respect it may be somewhat more relevant for heavy ion-induced DNA damage than NER. In contrast to NER, BER employs DNA lesion-specific glycosylases to recognize and bind to the damaged site, and remove the base. The resulting abasic site is cleaved to remove the 5'-deoxyribose phosphate and the one-nucleotide gap is closed by DNA polymerase β and sealed by DNA ligase (Wallace, 1994; Sancar, 1994). Defects in BER have been shown to result in hypersensitivity to both alkylating agents and ionizing radiation (Wallace, 1994). Table-IV.4 summarizes some of the enzymology thought to be required for base excision repair.

Other activities which play roles in DNA excision repair pathways, depending on the pathway and the exact nature of the DNA damage, include damage recognition proteins, helicases, DNA polymerases α and δ , replication factors, phosphatases (if phosphate is on 3' terminus of the nick), transcription factors, and DNA binding proteins (Sancar, 1994; Hanawalt, 1994). For example, it has recently been suggested that a gene mutated in subjects with Werner's syndrome, a premature aging syndrome, may code for a helicase activity (Yu *et al.*, 1996); such subjects are prone to genomic instability. Individuals with Bloom's syndrome, a DNA repair deficiency syndrome, also lack an apparent helicase activity (Ellis *et al.*, 1995).

The function of DNA mismatch repair (MMR) systems is to monitor the newly synthesized DNA strand for incorrect or mismatched bases which are removed and replaced by the correct equivalents (Modrich, 1994). In this process the correct strand is recognized on the basis of methylation signals. Heritable mutations in a MMR gene lead to a so-called mutator phenotype, that is, a very high susceptibility of the cell or organism to mutations (Loeb, 1994). An example is the characteristic microsatellite instability in patients with hereditary nonpolyposis colorectal cancer (HNPCC), a disease caused by germline mutations in MMR genes (Fishel *et al.*, 1994; Papadopoulos *et al.*, 1994). To some extent, DNA excision repair and MMR overlap. MMR is not only involved in the repair of mismatch bases, but also in the repair of chemical damage to DNA, processing of recombination intermediates, etc. (Modrich, 1994; Afzal *et al.*, 1995). Recent evidence indicates that mutations in MMR genes selectively abolish transcription-

coupled repair of UV -induced lesions in *Escherichia coli* and human-derived MMR-deficient cell lines (Mellon *et al.*, 1996).

B. DSB Repair

The most relevant repair system for heavy ion-induced DNA damage is DSB repair; heavy ions as well as other forms of ionizing radiation and hydroxyl radicals induce DSB. In mammalian cells DSB are thought to be repaired by a form of illegitimate recombination which shares enzymatic activities used in V(D)J recombination, the mechanism by which variability is generated in genes encoding immunoglobulins and T - cell receptors (Taccioli and Alt, 1995; Chu, 1996) The rejoining reaction in V(D)J recombination does not require homology in the recombining DNA, but short stretches of homology are used if present. Several genes involved in both V(D)J recombination and DSB repair have been cloned (Table IV.5.). Several ionizing radiation-sensitive rodent cell mutants, defective in DSB rejoining, have been shown to be defective in components of DNA-dependent protein kinase, an abundant nuclear protein in human cells, which is also required for V(D)J recombination. They also exhibit defects in the rejoining steps in V(D)J recombination. Errors in this process, which can also catalyse recombination between sequences not normally involved in Ig or TCR gene rearrangements, may lead to the radiation-induced recombinations that cause cell transformation or cell death.

The genes identified so far as candidates for repair of ionizing radiation- induced damage to DNA are listed in Table IV.5. Thompson and Jeggo (1995) have recently catalogued the human genes as X-ray repair ~ross- ~omplementing (XRCC) genes because of their ability to substitute for rodent genes required for repair of X-ray-induced damage. Unfortunately, exact biochemical functions have not yet been established for most of the XRCC genes, except the XRCC1 gene which codes for the DNA-dependent protein kinase (Kirchgessner *et al.*, 1995). This enzyme interacts with the Ku70 (XRCC6) and Ku80 (XRCC5) proteins which form a complex and bind to the ends of DSB in DNA. Just as the exact activities of most of these XRCC gene products are unknown, the actual enzymatic pathway(s) for repair of DSB in mammalian DNA is unknown at this time. However, a reasonable model for how this might be accomplished in mammalian cells has been outlined by Chu (1996). In yeast, DSB repair occurs largely through homologous recombination (Game, 1993; Shinohara and Ogawa, 1995). The partial homology of some of the human XRCC genes with the yeast *rad* genes (Table IX.5), suggests overlap between gene products involved in DSB repair in yeast and mammalian cells.

One possible variable in reparability of damage involving DSB is whether the damage occurs in nucleosomal DNA or in linker DNA. Lobrich *et al.* (1995) have reported that most of the correct rejoining occurs within the first 2 hours following X-irradiation of cultured human fibroblasts, whereas incorrect rejoining occurs primarily between 2 and 20 hours. By 20 hours after irradiation essentially all of the double-strand ends were rejoined, and 70% of these were correctly joined. In this result, correct refers to the rejoining of pieces from a single chromosome, but does not imply that no bases have

been lost or added. It is tempting to hypothesize that DSB which are held together by histones can be rapidly and correctly joined, whereas DSB in linker DNA are more likely to join incorrectly leading to translocations. This hypothesis, even if correct, does not shed light on the actual joining mechanism.

C. SSB Repair

Repair of SSB may be trivial because of the need to accomplish this in almost all repair and replication pathways. SSB with 3' hydroxyl and 5' phosphoryl ends can thus be readily repaired by DNA ligase alone. Thus, while some "tailoring" of radiation-induced nicks in DNA by phosphatases, kinases and exonucleases may be required before ligation can occur, the enzymatic machinery for gap-filling and ligation is normally present. Tailoring of nicks may use the same enzymes involved in base excision repair. For a discussion of these pathways see Warner (1983).

D. Cell cycle checkpoints

In order to become permanent, a DNA lesion must be fixed in the genome of a cell in the form of a mutation, that is, a heritable alteration that can be corrected only by elimination of the cell. Genetic stability is controlled by a number of interrelated and overlapping cellular functions, the most important of which are the various DNA replication, repair and recombination pathways. A major component of cellular defense against DNA damage are cell cycle checkpoints, that is, monitoring systems for DNA damage that temporarily halt transcription and/ or replication until the lesions are repaired (Carr, 1996). Two important examples of cell cycle checkpoints are the tumor suppressor genes *p53* and *ATM*. The *p53* gene is thought to be activated by DNA damage, and to subsequently induce the p21 cdk inhibitor, which mediates growth arrest (Hartwell and Kastan, 1994). The *p53* gene product is also considered to play a key part in apoptosis pathways (Lowe *et al.*, 1993). When inactivated, as is the case in many tumors, the organism loses an important mechanism for eliminating unwanted cells, and regulating cell cycle progression.

Inactivation of the *ATM* gene product causes the recessive disease ataxia telangiectasia, which is characterized by greatly elevated cancer risk, hypersensitivity to ionizing radiation and failure to show normal damage-induced cell cycle arrest (Meyn, 1995a). The *ATM* protein might be an upstream component of a radiation-induced signal transduction pathway which is activated by DNA damage and involves the recruitment of *p53* (Savitsky *et al.*, 1995a), and perhaps other checkpoint proteins (Carr, 1996). The *ATM* protein shares the phosphoinositide 3-kinase (PI-3 kinase) domain with several large proteins in yeast, *Drosophila*, and mammals (Keith and Schreiber, 1995), including the catalytic subunit of DNA-dependent protein kinase (Hartley *et al.*, 1995). There is also significant homology between *ATM* and the yeast cell cycle checkpoint gene, *mecl*, which mediates transcription in response to DNA damage (Kiser and Weinert, 1996). Upon exposure to X-rays or radiomimetic drugs, cells from *AT* patients exhibit high rates of *p53*-dependent apoptosis (Meyn *et al.*, 1994). No information exists at this point about heavy ion-induced DNA damage and the *ATM/p53* response.

Review references:

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<i>Damage</i>	<i>Enzyme</i>	<i>Activity</i>	<i>Reference:</i>
<i>Base Excision Repair</i>	DNA Glycosylases	Remove damaged bases and create an AP site	
	AP endonuclease	Cleave DNA at AP site	Wallace, 1988 Wallace, 1994
	DNA deoxyribophosphodiesterase	Excise 5' deoxyribose phosphate group	Price and Lindahl, 1991
	DNA polymerase β	Replace missing nucleotide(s)	Warner, 1983
	DNA ligase	Re-seal the ss-break	Weindruch, 1993
<i>SSB</i>	DNA ligase	Re-seal the ss-break	
<i>DSB Protein Oxydation Lipid peroxidation</i>	XRCC gene products		
	Muticatalytic protease	Protein turnover	
	Phoospholipase A2	Remove damaged fatty acid	
	Acyl transferase	Replace damaged fatty acid	

Table 2: Enzymes involved in repair of radiation-induced damage in human cells

<i>Gene</i>	<i>Activity Function of Gene Product</i>	<i>Reference:</i>
<i>XRCC1</i>	Complexes with DNA ligase III	
<i>XRCC2, XRCC3</i>	Distant homologs of yeast <i>rad51</i> ; involved in recombinational repair and cross-link repair	
<i>XRCC4</i>	Distant homolog of yeast rad1 and XPF; may be endonuclease involved in nucleotide excision repair	Tompson and Jeggo, 1995 Kirchgessner, 1995 Jeggo, 1995 Chu, 1996
<i>XRCC5</i>	Ku80; complexes with Ku70 and binds to ds DNA ends; activates DNA-PK _{cs}	
<i>XRCC6</i>	Ku70; complexes to Ku80 and binds to ds DNA ends; activates DNA-PK _{cs} ; no cell line deficient in this gene has been isolated	
<i>XRCC7</i>	DNA-dependent protein kinase (DNA-PK _{cs}); deficient in V(D)J recombination	
<i>XRCC8</i>	??	
<i>XRCC9</i>	Correct mitomycin C sensitivity; involved in cross-link repair	

Table 3: Human DNA repair genes implicated in sensitivity to ionizing radiation

Proteins	Examples	Reference:
Transcription Factors	c-jun, junB, c-fos	Sherman, 1990 Mertz, 1992 Hallahan, 1993
	DNA-binding protein	Teale, 1992 Singh, 1990
	P53	Zhan, 1993 Hainut and Milner, 1993
Protein Kinases	Protein kinase C	Woloschak, 1990 Kim, 1992
Growth Factors	Basic fibroblast growth factor (bFGF)	Haimovitz-Friedman, 1991
	Transforming growth factor- β 1 (TGF- β 1)	Finkelstein, 1994 Langberg, 1994 Barcellos-Hoff, 1993 Barcellos-Hoff, 1994
	Epidermal growth factor (EGF)	Schmidt-Ullrich, 1996
	Interlukins	Hong, 1995
	Tumor necrosis factor α (TNF- α)	Chiang and McBride, 1991
Proto-oncogenes	<i>c-src</i> and <i>c-Ha-ras</i>	Anderson and Woloschak, 1992
Other proteins	<i>X-ray induced proteins</i>	Boothman, 1989 Boothman, 1993
	<i>Extracellular matrix proteins</i>	Finkelstein, 1994
	<i>Tissue-type plasminogen activator</i>	Boothman, 1991 Fukunaga, 1992

Table 4: examples of changes in gene expression induced by ionizing radiation

<i>Component</i>	<i>Radiation</i>	<i>Timing of Effect</i>	<i>Biological Effect</i>	<i>Reference:</i>
<i>TGF-β1</i>	γ-rays	1 hour – 7 days	Rapid activation predominately stromal	Barcellos-Hoff, 199 Barcellos-Hoff, 1994 Ehrhart, 1996
	Fe	12 hour – 14 days	Delayed activation, predominately epithelial	
<i>Collagen I</i>	γ-rays	1 day	Down regulation	
	Fe	--	No change	
<i>Collagen III</i>	γ-rays	1 – 3 days	Up-regulation	
	Fe	9 days	Up-regulation, delayed	
<i>Tenascin</i>	γ-rays	1 day	Up-regulation	
	Fe	5 – 9 days	Up-regulation, delayed	
<i>Fibronectin</i>	γ-rays	--	No change	
	Fe	--	No change	
<i>Collagen IV</i>	γ-rays	--	No change	
	Fe	--	No change	
<i>Laminin</i>	γ-rays	--	No change	
	Fe	9 days	disrupted	

Table 5: Radiation induced changes in the microenvironment (all these studies were done with mouse mammary glands)

2. CELL KILLING

Cell death in biological systems can be separated into two distinct forms: necrosis and programmed death or apoptosis (Kerr *et al.*, 1972; Arends *et al.*, 1994; Columbano, 1995). Necrosis results from massive cell injury, and often is accompanied by inflammation. This is more likely to occur when the dose to the cell or tissue is very high. Apoptosis is a more subtle process, and until recently it was generally assumed to be a biological process whose major function is to destroy unwanted cells during development. This perception began to change as roles emerged for apoptosis in the negative selection of thymocytes and lymphocytes (Nagata and Golstein, 1995) and as a balancing factor in maintaining proliferative homeostasis (Thompson, 1995; Williams, 1991). It is now believed that when cells become extensively damaged, apoptosis may become either a preferred or essential alternative to repair. This may be particularly important when using irradiation and drugs to damage and ultimately destroy cancer cells.

Homeostasis in many mammalian tissues may be a state of unstable equilibrium, with cells poised to either proliferate or die depending on the balance of factors impacting them. Most, if not all, of the cell death machinery may be present at all times, but whether it actually is used depends on a variety of both extracellular and intracellular signals. During apoptosis the cell activates this intrinsic suicide mechanism (Fraser and Evan, 1996), which fairly quickly kills the cell and disposes of the debris. Apoptosis is induced by a wide variety of stimuli such as oncoproteins, growth factors, metalloproteases, tumor necrosis factor, antibodies, ionizing radiation, DNA damage, etc. (Reed, 1994). This is particularly true in lymphocytes which have a "differentiated program" to die in response to certain signals (Nagata and Golstein, 1995).

The actual enzymatic machinery used in apoptotic death is becoming increasingly understood; this was greatly facilitated by the genetic analysis employed by Horwitz and others in *Caenorhabditis elegans* (summarized in Ellis *et al.*, 1991). The process involves activation of pre-existing proteases to effect cleavage of specific proteins such as poly (ADP-ribose) polymerase (Tewari *et al.*, 1995), and actin (Kayalar *et al.*, 1996). An endonuclease(s) is activated to degrade the nuclear DNA into double-stranded fragments which give the appearance of a "ladder" when separated by gel electrophoresis. The expression of *c-fos* and *c-jun* protooncogenes also appears to be required for apoptosis in some cells (Colotta *et al.*, 1992).

The mechanism by which ionizing radiation induces apoptosis is not known with any certainty, but until recently it was assumed the process might be initiated by a direct hit at the level of nuclear DNA. However, ionizing radiation, like other forms of environmental stress, induces a variety of biochemical responses and the actual trigger could be anyone of a number of lesions such as an oxidized protein or lipid, a disrupted membrane, or altered DNA structure. For example, irradiation has been shown to induce increases in the activity of protein kinase C (Woloschak *et al.*, 1990), a DNA binding protein (Teale *et al.*, 1992) and a variety of transcription factors (Sherman *et al.*, 1990; Brach *et al.*, 1991; Weichselbaum *et al.*, 1991).

Recently, more specific insights have been achieved in this fast developing field. The p53 gene product is known to be essential for radiation-induced apoptosis in thymocytes (Lowe *et al.*, 1993), and probably other tissues such as liver, kidney, brain, skin and the myocardium (Santana *et al.*, 1996); however, it may not be required in breast cells. It has also been shown that Bruton's tyrosine kinase is required for the radiation-induced apoptosis of lymphoma B cells (Uckun *et al.*, 1996), indicating that this protein kinase is involved in transduction of the signal, at least in B lymphocytes. Finally, a role for the transcription factor nuclear factor-kappa B (NF- κ B) in a fibrosarcoma cell line has now been established. (Wang *et al.*, 1996a) have shown that the activation of NF- κ B by ionizing radiation, as well as by tumor necrosis factor or chemotherapeutic agents, protects cells from apoptosis, apparently because NF- κ B plays a role in inducing anti-apoptotic genes. Thus, manipulation of I κ B, an inhibitor of NF- κ B, could affect the balance between the positive and negative intracellular factors regulating apoptosis.

Haimovitz-Friedman *et al.* (1994b) have shown that irradiation by ¹³⁷Cs γ -rays induces apoptosis of bovine aortic endothelial cells by activating acid sphingomyelinase, which then generates ceramide, a known inducer of apoptosis. Comparable levels of ceramide can even be induced by irradiating cell-free membranes, suggesting that DNA damage need play no role in this process. This observation does not preclude a separate DNA damage-dependent initiating event as well. Verheif *et al.* (1996) have shown that a wide variety of environmental stresses, including ionizing radiation, induce significant increases in intracellular ceramide levels in a dose-dependent manner, which is followed by apoptosis several hours later, suggesting that a common apoptotic pathway can be induced by different stimuli. In fact, in *Drosophila*, an ICE-protease appears to be required for induction of both apoptosis and ceramide production (Pronk *et al.*, 1996).

The impact of such cell death may be different for dividing and non-dividing cells. In tissues that can regenerate by proliferation of nearby undamaged cells, *e.g.*, the liver, direct cell killing may pose no special problem, and the effects of heavy ions, while quantitatively different, may not be qualitatively different than those induced by low-LET irradiation. In contrast, cell killing is liable to have a much larger impact on tissues containing a large number of non-proliferating cells, such as the central nervous system (CNS), where neuron-linked pathways may be disrupted. There are also negative regulators of apoptosis in mammalian cells, the best known of which is bcl-2. The *bcl-2* gene was originally identified as an oncogene because over-expression of this gene down-regulates apoptosis, and leads to altered growth (Williams, 1991). Whether or not a cell undergoes apoptosis, therefore depends both on the nature and extent of the damage inflicted, and on the status of the regulatory factors such as the bcl-2 protein.

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3. CHANGES IN GENE EXPRESSION

Radiation may alter the pattern of gene expression in such a way that cellular response to stimuli is also altered. Recent studies have demonstrated effects on intracellular signalling pathways, second messengers, and cell surface receptors. These factors determine whether a cell lives or dies, or gains neoplastic potential. In addition, radiation-induced gene expression may have extracellular ramifications such as induction of cytokines and growth factors, and alteration in extracellular matrix components. Such changes may also influence the response of other cells in the tissue, including transient residents like inflammatory cells and macrophages that may not have been irradiated themselves. Complex paracrine and endocrine cytokine cascades have been implicated in the generation of late effects following radiotherapeutic exposures {Anscher *et al.*, 1995). Interpreting the pattern of gene expression observed in cell cultures of a single cell type in terms of its relevance to tissue response is likely to be stymied by lack of appropriate context, as the pattern of gene expression may depend on the cell type, transformation status, and context. Changes in gene expression can both affect the response of cells to radiation exposure, and influence how cells respond to subsequent stimuli.

How cells sense radiation exposure is an area of active research. While it has been proposed that DNA damage is the trigger for changes in gene expression, it is also clear that other targets, such as the cell membrane, and even individual proteins, are important. Intracellular signalling, as well as additional events at the cell membrane, transduce signals to the nucleus in a coordinated, rapid and global mechanism of cellular response. Many transcription factor proteins are activated by pertinent signals *via* conformational change due to biochemical alterations such as phosphorylation, metal binding, or oxidation. Radiation exposure can cause dissociation of transcription factors from their inhibitor proteins (Brach *et al.*, 1991; Hallahan *et al.*, 1993a; Zhan *et al.*, 1993), and the mRNA for these transcription factors may then rapidly increase through autoactivation of a self-binding site in the promoter that is secondary to protein activation.

Cells are well-equipped to detect reactive oxygen species, which appear to be critical in a variety of tissue responses, including physiological events like inflammation, ischemia/reperfusion, and aging, and this may well be the fundamental stimulus in terms of radiation-induced gene expression. Transcription factor proteins contain redox-sensitive regions that affect their ability to bind to DNA sequences and thereby regulate gene expression. A specific example is *p53*, a tumor suppressor gene that encodes a nuclear phosphoprotein that can bind to DNA and negatively regulate gene expression. *p53* is induced by ionizing radiation (Zhan *et al.*, 1993) and was recently shown to be

redox-sensitive (Hainaut and Milner, 1993). Oxidation disrupts p53 conformation and DNA binding, whereas reduction favors folding into the active conformation. NF- κ B, OxyR and fos-jun transcription factors exhibit similar traits. Proteins that contain redox-sensitive elements perform a sensor function, and when such proteins are also part of a signal transduction pathway, they have the potential to amplify and elicit a concerted program of response.

Boothman *et al.* (1989, 1993) identified a set of unique radiation-induced transcripts and proteins that are elevated in cultured melanoma cells and fibroblasts soon after exposure to relatively low doses (< 6 Cy) of X-irradiation, but the identity and function of these proteins are not known. These, and some of the other changes in gene expression induced by ionizing radiation discussed above, are summarized in Table IV .6.

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