J. RADIAT. RES., 43: SUPPL., S13–S19 (2002)

Molecular Targets in Cellular Response to Ionizing Radiation and Implications in Space Radiation Protection

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DNA double strand breaks / DNA repair / Cell cycle checkpoints / Ionizing radiation / Space radiation

DNA repair systems and cell cycle checkpoints closely co-operate in the attempt of maintaining the genomic integrity of cells damaged by ionizing radiation. DNA double-strand breaks (DSB) are considered as the most biologically important radiation-induced damage. Their spatial distribution and association with other types of damage depend on radiation quality. It is believed these features affect damage reparability, thus explaining the higher efficiency for cellular effects of densely ionizing radiation with respect to γ-rays. DSB repair systems identified in mammalian cells are homologous recombination (HR), single-strand annealing (SSA) and non-homologous end-joining (NHEJ). Some enzymes may participate in more than one of these repair systems. DNA damage also triggers biochemical signals activating checkpoints responsible for delay in cell cycle progression that allows more time for repair. Those at G1/S and S phases prevent replication of damaged DNA and those at G2/M phase prevent segregation of changed chromosomes. Individuals with lack or alterations of genes involved in DNA DSB repair and cell cycle checkpoints exhibit syndromes characterized by genome instability and predisposition to cancer. Information reviewed in this paper on the basic mechanisms of cellular response to ionizing radiation indicates their importance for a number of issues relevant to protection of astronauts from space radiation.

INTRODUCTION

Genomic changes are the cellular effect of major concern after exposure to ionizing radiation. Mammalian cells cope with radiation-induced damage by means of a complex network of defense systems. Among them, DNA repair pathways have been particularly addressed since their discovery in the sixties. In recent years an increasing importance came into light of the role played by cell-cycle checkpoints (see ref. 1 for a review). These two mechanisms are triggered by DNA damage and closely co-operate in the attempt of maintaining genomic integrity of the cell. This picture implies the modification of target concept: besides the "classic" one, represented by the DNA molecule, "complementary" targets are now considered that affect the cell fate.

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"CLASSIC" RADIATION TARGETS

Ionizing radiation produces a wide spectrum of DNA lesions, such as: base damage, sugar damage, single strand breaks (SSB), double strand breaks (DSB), DNA-DNA and DNA-protein cross links.

Among these many types of lesions, emphasis is currently placed on DNA DSB as the most important for radiobiological effects such as chromosome aberration, cell killing and transformation. Numerous authors have related cell survival to the initial level of induced DSB[2–5]. However, evidence has been obtained in yeast[6] and in mammalian cells[7] for a more convincing correlation with residual DSB.

Both the spectrum of induced lesions and their spatial distribution are affected by radiation quality. It is expected that densely ionizing radiation produces clustered ionizations with relatively high efficiency. In addition to lesions correlated at very local scale (complex lesions), radiation quality may also affect production of lesions correlated at larger distances. This is determined by the interplay between the chromatin structure (considering its various levels of organization) and the radiation track structure[8–11]. As an example, Fig. 1 compares, at the nanometer scale, the

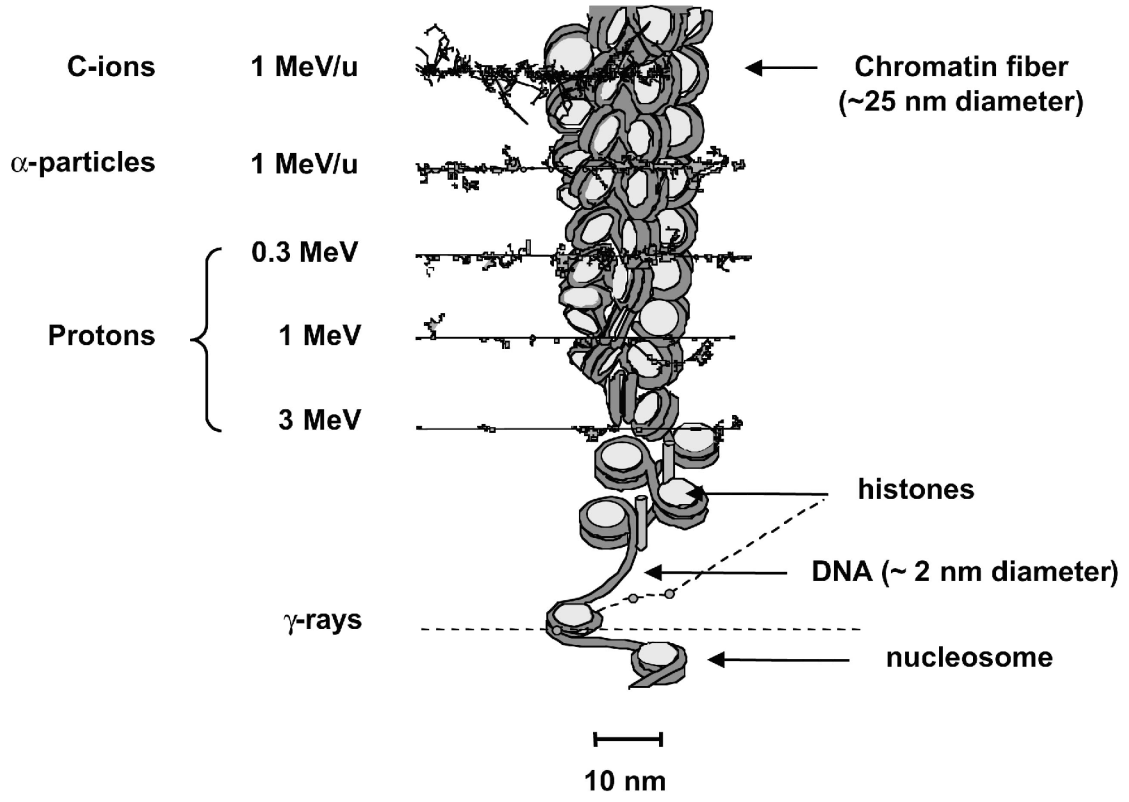

Fig. 1. Schematic representation of tracks of sparsely and densely ionizing radiations compared with relevant biological targets (chromatin fiber, nucleosomes and DNA double helix). Proton and alpha-particle tracks are based on the works of Paretzke[12] and carbon tracks on the works of Kramer and Kraft[13].

tracks of different types of radiation with the chromatin fiber, nucleosomes and DNA double helix.

For densely ionizing radiation the primary ionization sites occur in a correlated manner along the track of the particles and their typical pattern of energy deposition is expected to have an important role on their biological effects[14]. The current hypothesis is that reparability of DSB is affected by their spatial correlation and/or their association with other types of damage. This provides an explanation for the higher effectiveness of densely ionizing radiation with respect to sparsely ionizing radiation for a variety of biological end points. Experiments performed with light and heavy ions have shown that DSB rejoining ability strongly depends on particle type and energy (or LET)[15–17], giving evidence of a damage "quality" dependent on radiation quality. This issue is especially relevant for protection against space radiation, that mainly consists of protons and high charge and energy (HZE) ions.

"COMPLEMENTARY" RADIATION TARGETS

Among the targets that are relevant to the cell response to ionizing radiation consideration should be given, in addition to DNA, to the cellular defense systems. They are mainly represented by the DNA repair and the cell cycle checkpoint pathways, closely interacting to maintain genetic integrity in the events of DNA damage.

DNA DSB repair systems

Among the various repair systems known in mammalian cells, those acting on DSB are: homologous recombination (HR), single strand annealing (SSA, considered as a variant of HR because both are homology-driven), and non-homologous end-joining (NHEJ) (see refs. 18, 19 for reviews). Fig. 2 schematically shows the main steps of these pathways.

HR typically occurs in meiotic cells; in mitotic cells it is closely associated with the replication step. SSA takes place when repeat sequences flanks the two DNA ends and leads to loss of one of the two repeats and intervening DNA. NHEJ directly joins the two broken ends, and may involve limited termini degradation. HR may be regarded as error-free repair pathway, while SSA and NHEJ generally lead to loss of genetic integrity.

In both HR and SSA pathways proteins of the Rad family are involved in damage recognition, while in NHEJ the

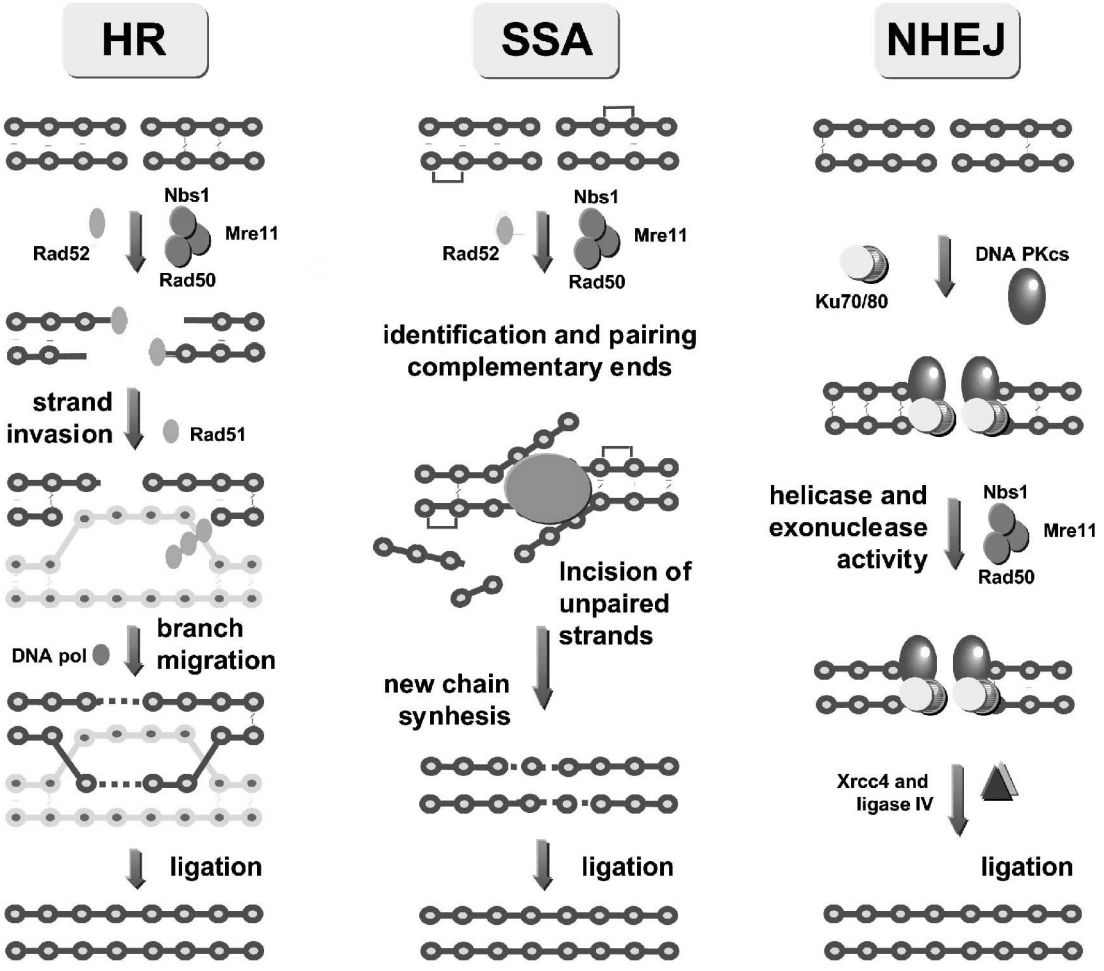

Fig. 2. Main steps of the DNA DSB repair systems acting in mammalian cells. HR: Homologous Recombination; SSA: Single Strand Annealing; NHEJ: Non-Homologous End-Joining

Ku70/Ku80 heterodimer exerts this function.

In NHEJ a central role is played by the DNA-dependent protein kinase, DNA-PK, which consists of the Ku heterodimer and a large component encoding the kinase catalytic domain, called DNA-PKcs (catalytic subunit)[20]. The latter is a member of the PIKK (phosphatidyl-inositol 3-kinase related kineses) family, which also includes ATM and ATR proteins[21]. Interaction of Ku heterodimer with DNA-PKcs activates the kinase activity and facilitates recruitment and activation of other NHEJ components. The ligation step is then performed by ligase IV/Xrcc4 heterodimer[22]. NHEJ is considered the predominant pathway for repairing DNA DSB in mammalian cells, although increasing evidence indicates a significant role of HR[23].

There are indications that some proteins may participate in more than one of the three repair systems. For instance, the Rad50/Mre11/Nbs1 complex, which contains helicase and exonuclease activities, is involved in HR and also in NHEJ particularly when DNA ends require processing before ligation.

Cell cycle checkpoints

DNA damage triggers biochemical signals that activate, in addition to DNA repair, checkpoints responsible for delay in cell cycle progression that allows more time for DNA repair. If DNA damage cannot be repaired, checkpoints aim to induce permanent cell cycle arrest or cell death in the attempt to eliminate such severely damaged cells. Checkpoints at G1/S and S-phases prevent replication of damaged DNA and those at G2/M phase prevent segregation of damaged chromosomes.

Components of this complex signaling pattern have been classified as sensors of DNA damage, signal transducers and effectors (Fig. 3). This pathway is based on protein phosphorilation cascade. A number of players have been identified or proposed as candidates (see for reviews refs.1,

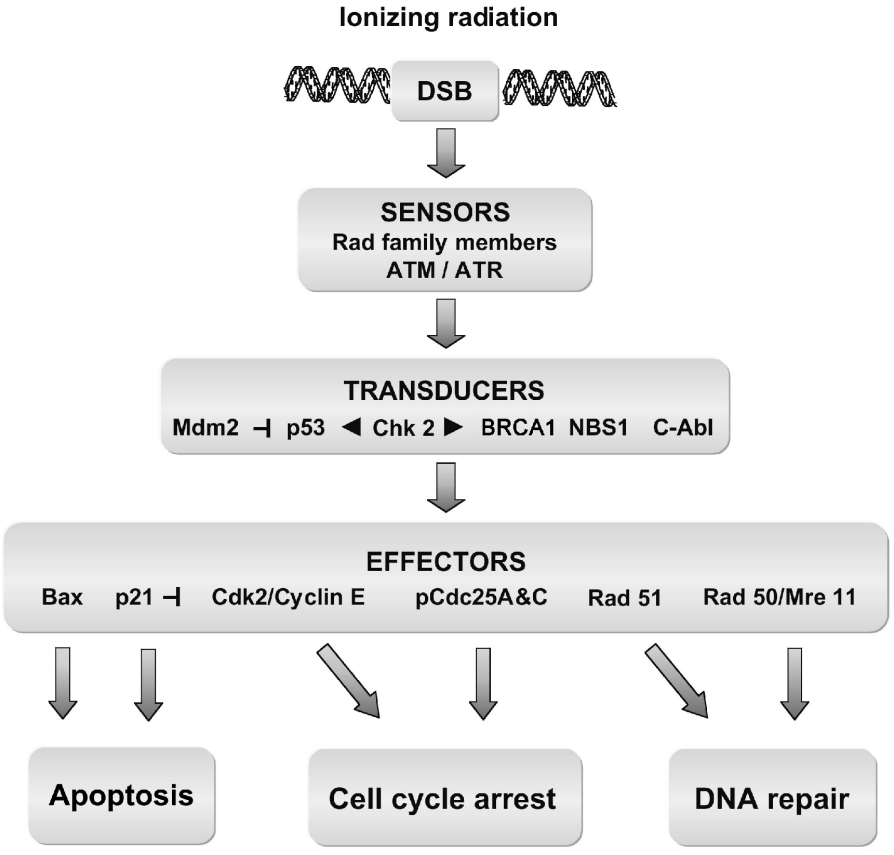

Fig. 3. Schematic representation of the signaling pattern activated by radiation-induced DNA DSB.

21, 24).

Sensors scan chromatin for damage and translate it into biochemical signal that modulated the function of other proteins. Sensors may include Rad1/Rad9/Hus1 complex, Rad27. Also ATM is close to the start of the signaling pathway. Maybe it recognizes DNA damage through interaction with a component similar to the Ku heterodimer of DNA-PK.

Downstream of sensors, signal transmission is accomplished by transducers, such as Chk2, p53, Brca1, Nbs1, C-Abl. P53 induces genes such as p21 which is an inhibitor of Cdk-containing complexes, essential for entry in S-phase, therefore inducing G1 arrest. Another pathway for G1 block, p53-independent and faster than the previous one, has been recently identified[1]. It acts through degradation of Cdc25A with persistent inhibition of Cdk2 that leads to G1/S block.

Recently, the above mentioned Cdc25A degradation pathway has been proposed as a possible mechanism for slowing down the ongoing S-phase[25].

Control of G2/M checkpoints is accomplished through the axis ATM-Chk2 by phosphorilation of Cdc25C. The inactive form of this protein, bound to 14-3-3 protein, is not able to exert its phosphatase action on Cdc2, preventing entry into M phase. In addition, a p53-dependent mechanism acts through repression of Cdc2 and cyc-B promoters.

Although tranducers may be shared by different checkpoint pathways, specific effectors may be involved in G1/S, S or G2/M blocks[1].

Some evidence exists for a linkage between cell cycle arrest and DNA repair. A number of repair proteins appear to be regulated by checkpoint genes or to be substrates for checkpoint kinases. In particular, ATM protein has been reported to play a controlling role in HR[26].

There is also a linkage[27] with apoptosis (programmed cell death) which is considered, together with permanent cell cycle arrest, a mechanism used to eliminate severely damaged cells. For instance p53 induces, in addition to p21, other genes such as Bax, a member of the Bcl2 family, that promotes apoptosis[28]. On the other hand, significant suppression of p53 accumulation and of apoptosis induced by acute irradiation has been observed in human cells exposed to chronic pre-irradiation[29].

HUMAN SYNDROMES CORRELATED WITH DNA REPAIR AND CELL CYCLE CHECKPOINTS

In the last 30 years a number of human syndromes correlated with genetic disorders have been identified. Among them there is an heterogeneous group characterized by genome instability and predisposition to cancer. They are known collectively as "DNA repair syndromes" on the basis of hypersensitivity to one or more DNA damaging agents or mutagens, and are associated with lack or alteration of genes involved in DNA DSB repair and cell cycle checkpoint pathways.

Table 1 lists the most important of such syndromes, the related tumors and the genes/proteins involved. Well known syndromes such as Xeroderma Pigmentosum (XP) or Cockraine Syndromes (CS) are here not included because their hypersensitivity is related to UV radiation (the damage activating the nucleotide excision repair pathway is a lesion on a single chain, such as thymine dimers and adducts).

In Ataxia Talengectasia (AT) the pathways controlling the cell cycle progression are affected. In normal individuals, the ATM protein in the presence of DNA DSB induces a rapid reduction of the rate of DNA synthesis, facilitating damage repair. The lack of this key protein is characteristic of AT syndrome and has, as a consequence, a dramatic increase in radiation sensitivity.

It has been reported that some families, diagnosed as AT, have shown to have mutations in Mre11 gene, a component of the Rad50/Mre11/Nbs1 complex involved in both HR and NHEJ. Because of the similarity of clinical phenotypes, it has been suggested that up to 6% of diagnosed AT patients may actually harbor Mre11 mutations[30].

Loss of functional Nbs1 is typical in patients affected by Nijmegen breakage syndrome[31], while helicase activity is defective in both Bloom and Werner syndromes[32]. BRCA1 and 2, involved in different types of DSB repair, encode multifunctional proteins whose mutant phenotypes predispose both to breast and to ovarian cancer[33].

In general, all the major G1/S checkpoint transducers and effectors qualify as either tumor suppressors or proto-oncogenes and their loss of function or over-expression have been identified in many types of human malignancies. Examples of tumor suppressor genes are Chk2 and p53, lacking in Li-Fraumeni syndrome, or RB, that is defective in familiar retinoblastoma. Among proto-oncogenes, examples (not reported in Table I) are Cdc25A and Cyclin D and E, whose expressions have been found altered in many types of cancer, although until now there is no evidence of association with any hereditary syndrome (see ref.1 and references therein).

SOME ASPECTS AND QUESTIONS RELEVANT TO SPACE RADIATION

A deeper understanding of the basic mechanisms of cellular response to radiation is important for a number of aspects relevant to protection of astronauts from space radiation, such as the following.

Table 1. Human syndromes associated with cancer proneness and with lack or alteration of genes involved in DNA DSB repair and cell cycle checkpoints

Syndrome	Disorders	Gene/protein
Alaxia Telangectasia	breast carcinoma	ATM
Alaxia Telangectasia-like disorders	breast carcinoma, lymphoid tumors	Mre11
Njimegen breakage Syndrome	many types of cancer	Nbs1
Bloom Syndrome	many types of cancer	helicases
Werner Syndrome	many types of cancer	helicases
Familiar breast and ovary cancer	breast carcinoma, ovary cancer	BRCA
Li-Fraumeni Syndrome	breast carcinoma, lung, colon, urinary, bladder, testicular tumors	Chk2
Li- Fraumeni Syndrome	many types of cancer	p53
Familiar melanoma	many types of cancer	p16
Familiar retinoblastoma	many types of cancer	RB

Understanding of the molecular and cellular mechanisms involved in radiation-induced late effects

Studies on neural precursor cells have shown that energetic Fe ions induce apoptosis and over expression of p53 more effectively that γ-rays[34]. This observation could have important consequences on risk assessment for astronauts during interplanetary missions. Using a model of human lens epithelial cells, it has recently been found[35] that X-rays or protons induce dramatic changes in the expression of cyclin-dependent kinase inhibitors, leading to a disruption of normal cell differentiation. It is interesting to determine whether the changes induced by HZE particles are qualitatively and quantitatively similar or different.

Elucidation of the action of protons and HZE particles

The biological effects of these particles are quantitatively and qualitatively different from those caused by other types of ionizing radiation, especially γ-rays, for which many epidemiological and experimental data exist. Maybe different repair pathways are exploited by the cell, depending on the type of damage. For instance, it has been proposed that NHEJ generates genomic rearrangements through DSB misrejoining if DSB are close in time and space[36]. It can be expected that these conditions apply to exposure to densely ionizing particle tracks. Moreover, observation of peculiar effects of densely ionizing particles related to cell cycle control[37] rises the question about whether some check-point pathways can be specifically triggered by the correlated DNA damage induced by protons or HZE particles.

Guidance to plan animal experiments

Consideration of cellular and molecular mechanisms can help in selecting representative animal tumorigenesis systems so as to aid interspecies extrapolation of risk.

Help in selecting genetically radioresistant individuals or in recognizing radiosensitive ones

A better understanding of the genetic bases of cellular response could also help in assessing specific susceptibility to protons and/or HZE particles.

Developing means to increase astronauts' radioresistance

Specific diets has been proposed for astronauts in order to minimize oxidative stress, and a more complete knowledge of the molecular mechanisms able to cope with this stress could offer a very useful guidance to develop such countermeasures.

ACKNOWLEDGEMENTS

The authors thank F. Grisanti and M. Sabatini for their assistance in the manuscript preparation. This work was supported by the Italian Space Agency (ASI) and by the UE Contract FIGH-CT-1999-0012.

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