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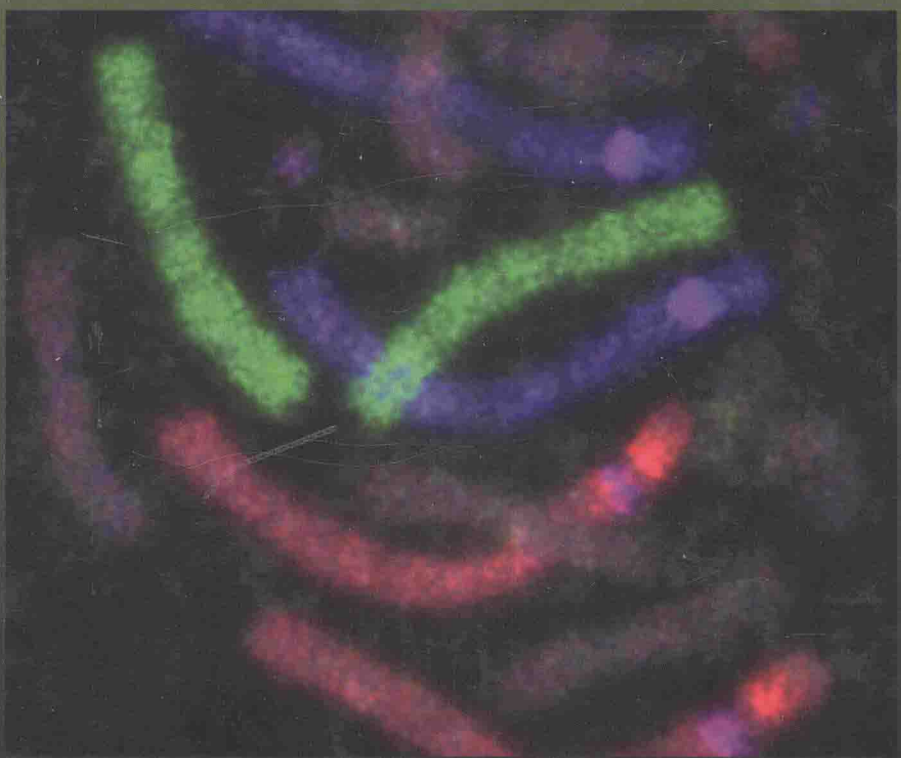
Current Topics in Developmental Biology



Volume 63

Edited by

Gerald P. Schatten

Current Topics in Developmental Biology

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Preface

This volume of Current Topics in Developmental Biology showcases an exciting array of topics in our field, from the protein response to DNA damage, to the controversy of mammalian taxonomy, to the role of antisense transcription in X inactivation, to the disposal of apoptotic cells, to actin filament formation, to longevity in dwarf mouse strains. For the developmental biology student seeking an exciting niche to study, this volume highlights a wealth of opportunity.

Early Events in the DNA Damage Response by Irene Ward and Junjie Chen of the Mayo Clinic explores the proteins that respond to double-strand breaks and replication arrest in DNA, and reveals what is becoming an increasingly complex picture of how these molecules identify and mediate DNA damage, which may lead to insights to and interventions for cancer.

In Afrotherian Origins and Interrelationships: New Views and Future Prospects by Terence Robinson of the University of Stellenbosch and Erik Seiffert of Duke University, the authors consider the controversial mammalian clade Afrotheria, a diverse collection including the mighty elephant, the sea-going manatee, and the aardvark. Do these mammals truly have the same ancestral phyla in common? While genetically it would appear so, the morphological data is confusing. The authors encourage both more sophisticated molecular testing and continued study of the fossil record to resolve this question.

The Role of Antisense Transcription in the Regulation of X-Inactivation by Claire Rougeulle and Philip Avner of Institut Pasteur is a sweeping review of our present understanding of how the group of *Tsix* antisense transcripts contribute to imprinted and random inactivation. As our knowledge of the function of non-coding RNAs increases, the authors counsel that we reconsider labeling such portions of the genome as "junk DNA."

The Genetics of Hiding the Corpse: Engulfment and Degradation of Apoptotic Cells *in C. elegans* and *D. melanogaster* by Zheng Zhou, Paolo Manghas and Xiaomeng Yu of Baylor examines the proteins and receptors that make dying cells recognizable, and those responsible for initiating disposal by neighbor cells, with important implications regarding these processes in mammals, since phagocytosis impacts such mechanisms as inflammation and immune response.

In Beginning and Ending an Actin Filament: Control at the Barbed End by Sally Zigmond of the University of Pennsylvania describes the mechanisms whereby new filaments are formed and how they are elongated, and how filaments are capped. A suite of proteins acting as a complex are

responsible for this interplay, similar to the protein interplay inherent in cell migration and, probably, in other cellular dynamics.

Finally, in Life Extension in the Dwarf Mouse by Andrzej Bartke of Southern Illinois University and Holly Brown-Borg of the University of North Dakota, the authors consider the common factors contributing to longevity in several lines of dwarf mice. In many, the reduced synthesis of insulin-like growth factor seems to result in reduced cellular aging via oxidative stress, probably from reduced metabolic function. Intriguingly, animals subject to caloric restriction display a similar heightened response to oxidative stress, including a lower incidence of cancer.

This volume has benefited from the ongoing cooperation of a team of participants who are jointly responsible for the content and quality of its material. The authors deserve the full credit for their success in covering their subjects in depth yet with clarity, and for challenging the reader to think about these topics in new ways. The members of the Editorial Board are thanked for their suggestions of topics and authors. I also thank Leah Kauffman for her fabulous editorial insight and Anna Vacca for her exemplary administrative support. Finally, we are grateful to everyone at the Pittsburgh Development Center of Magee-Womens Research Institute here at the University of Pittsburgh School of Medicine for providing intellectual and infrastructural support for Current Topics in Developmental Biology.

Jerry Schatten
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Life Extension in the Dwarf Mouse

Andrzej Bartke and Holly Brown-Borg

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Early Events in the DNA Damage Response

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The ability to sense DNA damage and activate response pathways that coordinate cell cycle progression and DNA repair is essential for the maintenance of genomic integrity and the viability of organisms. During the last couple of years, several proteins have been identified that participate very early in the DNA damage response. Here we review the current understanding of the mechanisms by which mammalian cells detect DNA lesions, especially double-strand breaks, and mediate the signal to downstream transducers.

I. Introduction

DNA constantly encounters potentially deleterious assaults from both environmental and endogenous sources. To protect the integrity of their DNA, cells have evolved a variety of response pathways that initiate repair and carefully coordinate it with DNA transcription, replication, and cell-cycle progression. The main repair strategies are direct reversal of lesions, excision of damaged DNA, and rejoining of DNA breaks (Table I).

Direct repair of certain alkylation adducts or UV-induced photolesions by specialized single enzymes is the simplest and perhaps oldest repair

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Table I Overview of the Major DNA Repair Mechanisms

Main inducer	Type of damage	Repair pathway
Ultraviolet light	CPDs, 6-4PPs	Direct repair (photoreactivation)
Ultraviolet light certain chemotherapeutic drugs or environmental toxins (e.g., cisplatin or PAHs)	CPDs, 6-4PPs intrastrand adducts or other bulky adducts	Nucleotide excision repair (NER)
Oxygen radicals and other products from cellular metabolism (oxidation, hydrolysis, methylation)	Non-bulky base modifications	Base excision repair (BER)
Errant replication	Mispaired bases, insertions, deletions	Mismatch repair (MMR)
Bistranded BER-induced SSBs, recombination, replication fork collapse, ionizing radiation	Double-strand breaks (DSBs)	Non-homologous end-joining (NHEJ) and/or homologous recombination (HR)
Cisplatin	Interstrand crosslinks	

Abbreviations: CPDs, cyclobutane pyrimidine dimers; 6–4PPs, 6–4 photoproducts; PAHs, polycyclic aromatic hydrocarbons; SSBs, single-strand breaks.

mechanism. It is conserved from bacteria to vertebrates, although humans seem to lack photolyases, the enzymes that reverse UV damage. UV lesions in humans are solely targeted by the nucleotide excision repair (NER) pathway. This versatile pathway also repairs various other bulky, helix-distorting lesions that arise, for instance, from exposure to genotoxic compounds such as polycyclic aromatic hydrocarbons (PAH). NER is a multistep process that comprises recognition of disrupted base pairing followed by unwinding of the DNA helix around the lesion and dual incision. The damaged oligonucleotide patch is subsequently excised, and the remaining gap is filled by regular DNA replication using the intact complementary strand as a template. A subpathway of NER, termed transcription-coupled repair (TCR) (versus global genomic repair [GGR]), targets damage that blocks DNA transcription and involves displacement of the stalled RNA polymerase (reviewed in Cleaver *et al.*, 2001). In addition, cells can use special polymerases to read through a lesion that blocks the normal replication machinery, although this aberrant translesion synthesis often comes at the expense of inserting point mutations (reviewed in Goodman and Tippin, 2000).

Another excision repair pathway, mismatch repair (MMR), targets mispaired bases and nucleotide insertion/deletion loops that arise during errant

DNA replication. Strand discrimination in eukaryotic cells is not yet fully understood but is thought to occur by contact of MMR proteins with the replication machinery (reviewed in Schofield and Hsieh, 2003).

Non-bulky base modifications, which are primarily caused by the normal cellular metabolism processes such as oxidation, hydrolysis, and nonenzymatic methylation as well as by the intrinsic molecular instability of the DNA itself, are mainly removed by the base excision repair pathway (BER). In BER, specific DNA glycosylases recognize and excise the modified base. The resulting abasic sugar is cleaved by an endonuclease. DNA polβ subsequently removes the 5′-terminal deoxyribose-phosphate residue and fills the single-nucleotide gap. The remaining nick is then sealed by a DNA ligase (reviewed in Memisoglu and Samson, 2000).

If single base lesions occur closely spaced on opposite strands, processing by BER can give raise to double-strand breaks (DSBs). Such bistranded damage clusters can form as a consequence of endogenous base damage or result from free radicals generated during radiolysis of water upon exposure of cells to ionizing radiation (IR) (Sutherland *et al.*, 2003; Wallace, 1998). IR can also introduce DSBs directly by depositing energy within the DNA and causing multiple breaks. Other important sources of DSB include HO endonuclease-induced DSBs that start mating type switch in yeast (Haber, 1992) and Spo11 transesterase-induced DSBs that initiate meiotic recombination in yeast and mammals (Mahadevaiah *et al.*, 2001; Sun *et al.*, 1989). DSBs are also introduced during the process of V(D)J recombination and class switch recombination (CSR), which is part of the normal development of the immune repertoire in B and T lymphocytes (Gellert *et al.*, 1992; Honjo *et al.*, 2002). Moreover, DSBs arise frequently during DNA replication when replication forks encounter single-strand breaks and collapse (Thompson and Schild, 2002).

DSBs are more challenging to repair than other DNA lesions and are considered the most toxic type of DNA damage. If left unrepaired or repaired improperly, they cause chromosomal aberrations such as translocations, amplifications, or deletions, which may be lethal or result in oncogenic transformation (Difilippantonio *et al.*, 2002; Zhu *et al.*, 2002). The two major pathways of DNA DSB repair are homologous recombination (HR), a highly accurate process that requires large regions of homologous sequence as a template, and nonhomologous DNA endjoining (NHEJ), which simply joins broken ends together, thereby often generating deletions, insertions, or base pair substitutions. If substantial regions of homology flank a DSB, cells can use a third repair pathway termed single-strand annealing (SSA), which involves the interaction of the two repeats and results in the loss of one flanking region plus the intervening DNA (Lin *et al.*, 1984). Similarly, very small, so-called microhomology regions can be used by a subpathway of NHEJ, which has also been designated

microhomology-driven SSA (Gottlich *et al.*, 1998), direct-repeat end-joining (Thacker *et al.*, 1999), and error-prone NHEJ (Pfeiffer *et al.*, 2000).

In diploid yeast, DNA DSBs seem to be repaired almost exclusively through high-fidelity HR. Mammalian cells use recombinational repair as well (Liang *et al.*, 1998), although NHEJ makes an important contribution to DSB repair, especially during the G0 and G1 phases of the cell cycle when no sister chromatid is available (Lee *et al.*, 1997; Takata *et al.*, 1998). In addition, the relative contribution of HR and NHEJ appears to change with the developmental stage of a cell, with HR being the major repair pathway in embryonic cells, while NHEJ dominates in differentiated somatic cells (Essers *et al.*, 2000).

Repair of a DSB by HR involves $5' \rightarrow 3'$ resection of the broken DNA ends followed by identification and invasion of the homologous sequence at the sister chromatid or homologous chromosome. The 3′ overhangs of the invading strands then serve as primers for DNA synthesis, using the intact strand as a template. In contrast, NHEJ comprises simply the alignment of DSBs, which may have to be modified by nucleases and/or polymerases to obtain compatible ends that can then be ligated.

To allow time for repair of the various types of DNA lesions and to prevent damage from being passed onto daughter cells, cells activate so-called checkpoint signaling pathways that sense DNA lesions, amplify the signal, and transiently arrest or slow cell cycle progression. In addition, checkpoint pathways induce transcriptional programs and enhance DNA repair pathways. Although over the past decades much progress has been made in dissecting the different DNA damage response pathways, less is known about the initial events that trigger cell cycle checkpoints and stimulate DNA repair. In this chapter, we focus on the proteins that participate early in the response to DNA DSBs (e.g., ATM, DNA-PK, MRN complex, H2AX, MDC1, 53BP1, Chk2) and/or replication arrest (e.g., ATR, Rad17, 9-1-1 complex, Chk1) and discuss their role in safeguarding genome integrity.

II. Formation of Multiprotein Complexes

The dynamic formation of large multiprotein complexes at the region surrounding DNA lesions provided important insight into the early events in response to DNA damage. Among the first proteins that relocalize to these nuclear foci are MDC1/NFBD1, 53BP1, and the Mre11-Rad50-NBS1 (MRN) complex (Fig. 1). Induction of DNA DSBs in defined subnuclear volumes using ultrasoft X rays demonstrated that these foci indeed form at sites of DNA strand breaks (Nelms *et al.*, 1998). Moreover, immunofluorescence analyses showed that the proteins colocalize extensively with foci

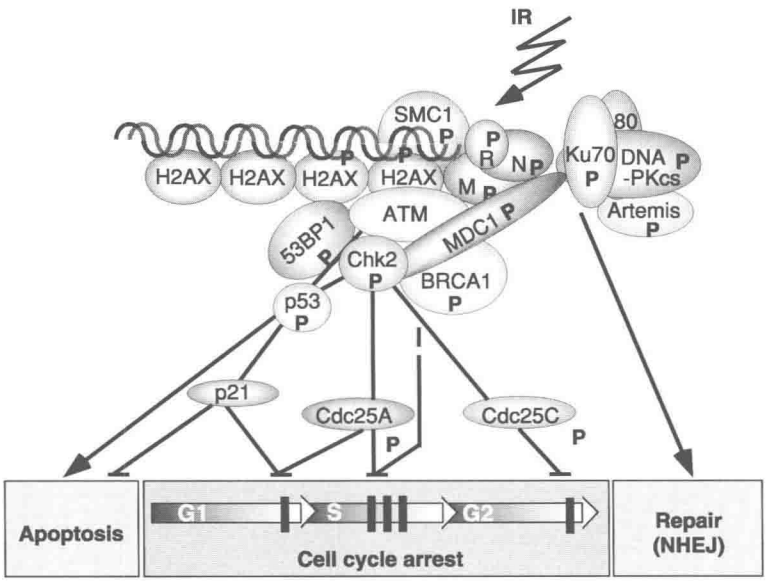

Figure 1 Formation of multiprotein complexes at the sites of DNA double-strand breaks. Exposure of cells to ionizing radiation results in the rapid recruitment of numerous proteins to the sites of DNA lesions. The ATM (ataxia telangiectasia mutated) kinase, which is central to this response, initiates a cascade of phosphorylation events (P) that activate cell cycle checkpoint pathways and, if necessary, apoptosis. How ATM participates in DNA repair is not well defined. In contrast, the related DNA-PK kinase, consisting of the DNA-PKcs and KU70/Ku80 subunits, attaches to DNA ends and is essential for nonhomologous DNA end-joining.

formed by phsophorylated H2AX (γ-H2AX), a variant of histone H2A that is randomly incorporated in approximately 20–30% of nucleosomes (Rogakou *et al.*, 1998). H2AX phosphorylation is damage dependent, and experiments using a pulsed microbeam laser to introduce DNA double-strand breaks into specific partial nuclear volumes of cells revealed that H2AX phosphorylation is confined to megabase areas surrounding strand breaks (Rogakou *et al.*, 1999). Phosphorylation of H2AX in response to IR is mediated by ATM (ataxia telangiectasia mutated) (Burma *et al.*, 2001), while the related ATR (ATM and Rad3-related) kinase phosphorylates H2AX in response to replication arrest (Ward and Chen, 2001). ATM and ATR have also been shown to phosphorylate numerous other proteins recruited to sites of DNA damage, including MDC1/NFBD1, 53BP1, NBS1 and members of the Rad9-Rad1-Hus1 (9-1-1) complex, and are thought to be key regulators in the DNA damage response.

A. ATM and ATR

ATM and ATR are conserved serine-threonine kinases characterized by a C-terminal catalytic motif containing a phosphatidylinositol 3-kinase domain. The gene that encodes ATM is mutated in the severe auotosomal recessive disorder ataxia telangiectasia (A-T). A-T patients suffer from progressive cerebellar degeneration, immunodeficiency, growth retardation, hypogonadism, chromosomal instability, and cancer predisposition (Gatti *et al.*, 2001). At the cellular level, A-T cells show hypersensitivity to IR, radio-resistant DNA synthesis (RDS), and a high frequency of chromosome aberrations (Abraham, 2001; Shiloh, 2003). ATR deficiency is even more severe, resulting in early embryonic lethality in mice (Brown and Baltimore, 2000). Partial loss of ATR activity has been associated with Seckel syndrome, a rare inherited disorder characterized by intrauterine growth retardation and microcephaly (O'Driscoll *et al.*, 2003).

ATM is primarily activated in response to DSBs, while ATR reacts to a wider range of lesions, including stalled replication forks. Both proteins are implicated in the sensing of DNA damage and/or the transducing of the damage signal and have been shown to associate with DNA *in vitro* (Smith *et al.*, 1999; Suzuki *et al.*, 1999; Unsal-Kacmaz *et al.*, 2002). Moreover, ATR undergoes dramatic relocation to sites of stalled replication forks in response to replication stress (Tibbetts *et al.*, 2000). Similarly, detergent extraction revealed rapid changes in the subcellular localization of ATM in response to radiomimetic agents, suggesting that a fraction of the ATM pool associates with sites of DNA DSBs (Andegeko *et al.*, 2001). It was therefore thought that both ATM and ATR might be activated through interaction with DNA or DNA-associated sensing units. However, a study by Bakkenist and Kastan (2003) showed that ATM exists as an inactive dimer or multimer in undamaged cells with the kinase domain of each molecule bound to the FAT (FRAP/ATM/TRRAP) domain of another ATM molecule. DSB-specific alterations in the higher order chromatin structure or exposure of cells to hypotonic stress or chromatin-modifying agents result in the dissociation of the ATM molecules. Dimer dissociation is induced independent of direct DNA binding by intermolecular autophosphorylation of ATM on Ser 1981 and results in monomers with accessible kinase domains that are free to migrate and phosphorylate substrates. Their finding is supported by an *in vitro* study showing that ATM can be activated by ATP in the absence of DNA by a mechanism involving autophosphorylation (Kozlov *et al.*, 2003).

It remains to be seen whether ATR becomes activated by a similar mechanism. The *in vitro* kinase activity of ATR seems not to increase after exposure of cells to various genotoxic agents (Abraham, 2001), although kinase-dead ATR failed to relocalize in response to DNA damage (Barr *et al.*, 2003). *In vitro* studies suggest that ATR-interacting protein (ATRIP),