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INSIGHTS FROM MODEL SYSTEMS
Cell-Cycle Regulation of Mammalian DNA Double-Strand-Break Repair

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The structural integrity of chromosomal DNA is of paramount importance to survival, and, consequently, all living cells have evolved mechanisms for the repair of DNA lesions. This paradigm recently has been made tragically clear, with the identification of a series of human cancer-predisposition syndromes that can arise from mutations in DNA-repair genes. Thus, defects in the nucleotide-excision repair pathway result in xeroderma pigmentosum, which presents with a greatly increased incidence of skin cancer (Wood 1996). Similarly, defects in the mismatch repair pathway predispose affected individuals to colon cancer and to other cancers (Kolodner 1995). Recently, it has become clear that defects in a third major DNA-repair pathway, DNA double-strand-break (DSB) repair, which have long been known to be associated with x-ray hypersensitivity and immune deficiency, probably underlie the familial predisposition to breast cancer and to ovarian cancer (Kinzler and Vogelstein 1997). This exciting revelation, coupled with the widespread clinical use of radiation exposure for cancer therapy, has prompted a sudden and intense interest in DNA DSB repair. This review will focus on the evidence that demonstrates that there are several different mechanisms of DNA DSB repair, which operate during distinct phases of the cell cycle.

It has been known for several years that, in mammals, defects in DNA DSB repair manifest themselves in two phenotypes, ionizing-radiation (IR) hypersensitivity and immune deficiencies. These two seemingly unrelated biological processes, in fact, are linked by the requirement of DNA DSBs as reaction intermediates. Thus, the exposure of mammalian cells to IR induces lesions, such as strand scissions, single-stranded breaks, DSBs, and base crosslinks (Friedberg et al. 1995), in chromosomal DNA. In particular, DNA DSBs appear to be the predominant cytotoxic lesions, since even a single unrepaired DNA DSB can be a lethal event (Frankenberg-Schwager and Frankenberg 1990). Similarly, the development of the mammalian immune system is dependent on a site-specific DNA-recombination process, called "lymphoid V(D)J recombination," that assembles the noncontiguous genomic segments (variable [V], diversity [D], and joining [J] elements) to create immunoglobulin and T-cell receptor genes (Lewis 1994). Analyses of V(D)J recombination products have proven that DNA DSBs are an essential intermediate in the V(D)J-reaction mechanism (Weaver and Alt 1997). Thus, the repair of DNA DSBs is an integral feature of IR sensitivity and of V(D)J recombination.

Intriguingly, a variety of pathways that act at different times during the cell cycle and that are likely to be very sensitive to the proliferative and ploidy states of the cell have evolved to ensure that such DSBs are repaired effectively. Recent work in mammalian and in yeast model systems suggests that at least two distinct pathways, mediated by as many as four discrete complexes, facilitate the repair of DSBs (Petrini et al. 1997; Kanaar and Hoeijmakers 1997). In one pathway, nonhomologous recombinational repair, DNA repair requires no or very little homology on the ends of the strands being rejoined. Two discrete complexes implicated in this pathway, the DNA-dependent protein kinase (DNA-PK) complex and the RAD50 complex, act primarily during the G1/early S phase of the cell cycle. In homologous recombinational repair, extensive homology is required between the region with the DSB and a template (usually a sister chromatid or a homologous chromosome) from which repair is directed. This type of repair is performed either by the RAD52 complex, which acts predominantly during the late S/G2 phase, or by the BRCA1/2 complex, which appears to act during the S phase. Other pathways for DSB repair (single-strand annealing [Lin et al. 1984], retrotransposon capping [Moore and Haber 1996*a*; Teng et al. 1996], and de novo telomere addition [Kramer and Haber 1993]) apparently are less active in mammals and will not be discussed here.

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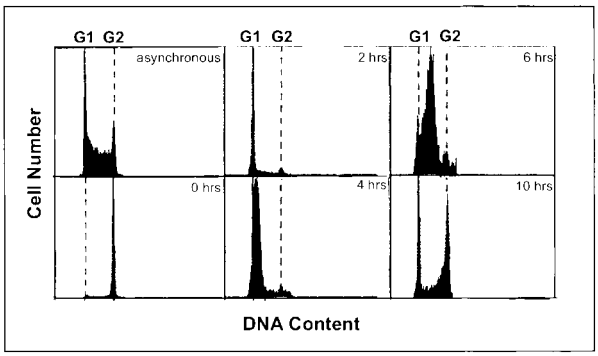
"And all together now . . ."

The normal, asynchronous growth of cells in culture obscures physiological differences between cells at different stages in the cell cycle. Cell synchronization provides the researcher with relatively homogeneous populations of cells that are restricted to a discrete phase of the cell cycle. Thus, serum deprivation of an asynchronous culture will result in a G0/G1 population; treatment with inhibitors of DNA synthesis blocks cells in the early S phase; and treatment with nocodozole, a microtubule polymerization inhibitor, creates a G2 population (Tobey et al. 1988). The addition back of serum or the removal of the inhibitors will yield relatively synchronized populations of cycling cells, at least for a single cell cycle. The distribution of the cells in the cell cycle can be quantified by fluorescence-activated cell sorting (FACS), after staining of the cells with propidium iodide; brighter cells have a higher DNA content.



The above figure illustrates a typical FACS profile for cells synchronized with nocodozole. The original population was heterogeneous in its G1/S/G2 populations, but, following treatment with nocodozole, >95% of the cells were arrested in the G2 phase (0 h). After removal of nocodozole, the cells synchronously moved into the G1 phase (2 h), the early S phase (4 h), the S phase (6 h), and then the G2 phase (10 h). After only 10 h, the culture was already noticeably asynchronous. We have analyzed DSB repair and DNA-PK activities in these timed cultures (Lee et al. 1997). By x-ray irradiating cells at different times following the nocodozole washout, we could identify stages in the cell cycle in which the cells were most sensitive to killing and stages in which induced DSB repair was highest. By comparing normal cells with those lacking DNA-PK activity, we could show that DSB repair during the G1 phase required the activity of DNA-PK.

Nonhomologous DNA DSB Repair

The DNA-PK Complex

The serine-threonine DNA-PK complex, which consists of at least three components, the 465-kDa catalytic subunit (DNA-PK_{cs}) and the heterodimeric Ku protein, is intimately involved in nonhomologous DNA DSB repair (Jackson 1996). DNA-PK_{cs} is the product of the severe combined immune deficiency (*scid*) gene (Hartley et al. 1995), and it has been long known that mice that are homozygously mutant at this locus are x-ray sensitive, defective in DNA DSB repair, and profoundly immune deficient (Hendrickson 1993). Ku, a heterodimeric protein of 70-kDa (Ku70) and 86-kDa (Ku86) subunits, complexes with DNA-PK_{cs} and binds tightly to double-stranded DNA ends (Jackson 1996). Several hamster cell lines (Zdzienicka 1995) that are x-ray sensitive and defective for DNA DSB repair carry mutations in the Ku86 gene (Singleton et al. 1997). These latter observations, as well as the anticipated immune defects, have been recapitulated in mice containing a targeted disruption in Ku86 (Nussenzweig et al. 1996; Zhu et al. 1996). Lastly, murine embryonic stem cells containing a targeted disruption of Ku70 are IR sensitive and V(D)J defective (Gu et al. 1997). Thus, the DNA-PK complex has been identified unequivocally as an important mammalian DNA-repair complex, and mutations in any of the DNA-PK subunits results in DNA DSB-repair defects and, hence, in profound x-ray sensitivity and immune deficiency.

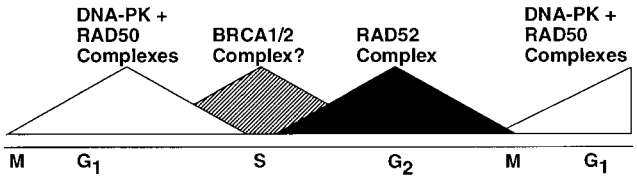


Figure 1 Linear illustration of the cell cycle. DNA DSB-repair activity fluctuates as a function of the cell cycle. The triangles above the line denote the activity of the four DNA DSB-repair complexes. The DNA-PK and RAD50 complexes are active during the G1/early S phase (unblackened triangle). The putative BRCA1/2 complex appears to be active predominantly during the S phase (hatched triangle), and the RAD52 complex is active during the late S phase and the G2 phase (blackened triangle).

The survival of mammalian mutant cells sensitive to IR depends on the cell-cycle position of the cell at the time of x-ray exposure, with maximal irradiation sensitivity occurring at the G1/S border (Weibezahn et al. 1985; Whitmore et al. 1989). Recently, we extended this observation by demonstrating that DNA-PK activity in wild-type cells—and sensitivity to IR in *scid* cells—fluctuated during the cell cycle (see sidebar) (Lee et al. 1997). Wild-type cells exhibited a peak of DNA-PK activity at G1/early S phase; in *scid* cells, the lack of this peak correlated precisely with hypersensitivity to x-ray irradiation and with a DNA DSB-repair deficit (Lee et al. 1997). These observations are consistent with data from studies of V(D)J recombination, a G1 event (Schlissel et al. 1993), and with data from studies of other cells lacking functional Ku86 or DNA-PK_{cs} (Weibezahn et al. 1985; Whitmore et al. 1989). Thus, DNA-PK is active predominantly during the G1/early S phase of the cell cycle (fig. 1). Interestingly, the activ-

ity of the complex is regulated by posttranslational phosphorylation (Chan and Lees-Miller 1996) and not by absolute protein levels, which basically remain constant throughout the cell cycle (Jongmans et al. 1996; Lee et al. 1997). DNA-PK may be a target of cyclin-dependent kinases (Cdk's), which promote the progression of cells, throughout the cell cycle, by the phosphorylation of other regulatory proteins. Alternatively, a fourth component of the DNA-PK complex, Ku86 autoantigen-related protein-1 (KARP-1) (Myung et al. 1997), might help restrict DNA-PK activity to the G1/early S phase. Although the activity of KARP-1 during the cell cycle has not yet been investigated rigorously, this gene has been shown to be regulated strongly by the p53 tumor suppressor gene (K. Myung, C. Braastad, D. M. He, and E. A. Hendrickson, unpublished data), which controls the G1-to-S phase transition following DNA damage (Kastan 1996), suggesting a G1-restricted KARP-1 expression pattern. Thus, DNA-PK may be regulated in the cell cycle not by Cdks but by the p53-inducible, G1-restricted expression of KARP-1 (K. Myung, C. Braastad, D. M. He, and E. A. Hendrickson, unpublished data). The product of a fifth gene, *XR-1,* does not appear to associate with the DNA-PK complex (Li et al. 1995), but mutation of *XR-1* results in phenotypes that are virtually indistinguishable from those of genes for the known DNA-PK complex subunits (Stamato et al. 1983; Li et al. 1995). The XR-1 protein associates with DNA ligase III (Nash et al. 1997) and with DNA ligase IV (Grawunder et al. 1997), which is implicated in DNA repair (Wilson et al. 1997). Thus, it is reasonable to assume that, although perhaps not physically associated with DNA-PK, XR-1 is involved crucially in the processing of DNA-PK repair intermediates (Weaver and Alt 1997).

Thus, DNA-PK is involved primarily in nonhomologous recombinational repair, and this type of repair occurs during the G1/early S phase. This model fits well with the physical constraints of the cell cycle, because during G1 and early S phases the ploidy of the cell is merely 2*n,* and, with one of two alleles already damaged, repair via nonhomologous recombination might be more efficient. These data also agree with the results of studies performed on *Saccharomyces cerevisiae,* in which mutations in the genes for Ku homologues specifically affect nonhomologous DNA DSB repair (Barnes and Rio 1997). Interestingly, with the exception of the Ku heterodimer, none of the other three human genes (*DNA-PK*$_{cs}$, *KARP-1,* or *XR-1*) appear to be conserved in yeast. Thus, the DNA-PK complex may be the most recently evolved pathway for DSB repair. In view of the fact that nonhomologous DSB repair is inherently error prone, the increased percentage of noncoding DNA in mammals might have facilitated the evolution of this pathway.

The RAD50 Complex

Unlike the DNA-PK complex, for which all of the salient work was performed on mammals and then extended to yeast, the RAD50 complex (RAD50/MRE11/XRS2) initially was characterized exclusively in yeast. Only recently has it become clear that homologous proteins and, by extension, a complex exist in mammals. Mutations in any of the yeast RAD50-complex genes profoundly affect random integration of transfected DNA and strongly reduce nonhomologous DSB repair (Moore and Haber 1996*b*). Two mechanisms for nonhomologous DSB repair exist in yeast cells (Moore and Haber 1996*b*), one of which is affected by mutations in the Ku-homologue genes and the other by mutations in *RAD50.* The former pathway appears to predominate in the G1/early S phase, which is in agreement with the data observed for DNA-PK in mammalian cells. In contrast, the latter pathway is required in the late S/G2 phase (Moore and Haber 1996*b*). Thus, in yeast, Ku homologues and the RAD50 complex regulate two discrete pathways for the same type of repair events, with each complex restricted to different phases of the cell cycle. However, genetic experiments have shown that single mutations in the RAD50 complex and/or in the Ku homologues were no more defective than any double-mutant combination (Milne et al. 1996), suggesting that these components operate within a common pathway. Additional experiments will be necessary in order to elaborate on the degree of overlap between these complexes, in yeast.

In mammals, strikingly well conserved homologues of *RAD50* (h*RAD50;* Dolganov et al. 1996) and of *MRE11* (h*MRE11;* Petrini et al. 1995) recently have been cloned, and a homologue of *XRS2* also may exist (Dolganov et al. 1996). As in yeast, hRAD50 and hMRE11 have been shown to interact physically (Dolganov et al. 1996), and these proteins colocalize into foci, following x-ray irradiation (Maser et al., in press). Similar to the DNA-PK complex, the individual subunits of the RAD50 complex do not appear to fluctuate during the cell cycle, but the activity of the complex may be regulated by posttranslational modification and/or by cellular compartmentalization (Maser et al., in press). Recent work suggests that hRAD50 and hMRE11 act predominantly during the G1 phase (Maser et al., in press), but such a significant point of departure between fungi and mammals needs to be determined rigorously. The phenotypes of *MRE11* and *RAD50* knockout mice, which currently are under construction, should be extremely informative. Importantly, it remains to be determined whether the hRAD50 and the DNA-PK pathways overlap and/or whether they constitute independent mechanisms for the repair of similar lesions.

Homologous DNA DSB Repair

The RAD52 Complex

In yeast, the vast majority of DNA DSB repair proceeds by homologous recombination and is performed by the RAD52 complex. This complex consists of at least five proteins, RAD51, RAD52, RAD54, RAD55, and RAD57. Mammalian homologues for *RAD51, RAD52,* and *RAD54* already have been isolated, and it is anticipated that homologues for *RAD55* and *RAD57* exist (Petrini et al. 1997). hRAD51 is homologous to the bacterial *RecA* gene (Gupta et al. 1997), which encodes the archetypal recombinase for homologous recombinational repair, since it facilitates strand-exchange reactions between homologous pieces of DNA (West 1992). As expected, the hRAD51 protein is virtually undetectable in the G1 phase but is upregulated during S phase and G2 phase (Yamamoto et al. 1996), which is consistent with a role in homologous DNA DSB repair. Targeted disruption of the m*RAD51* gene results in early embryonic lethality, demonstrating that mRAD51 plays an essential role in mouse development, independent of its role in the repair of exogenously induced DNA damage (Lim and Hasty 1996; Tsuzuki et al. 1996). Targeted disruption of the mouse homologue of *RAD54* results in viable animals that are x-ray sensitive and that are impaired in homologous recombination (Essers et al. 1997). These studies unequivocally demonstrate a requirement for RAD54 in homologous recombination. The expression of hRAD54, like that of hRAD51, is very low in the G1 phase, increases during the late G1/S phase, and persists into the G2 phase (Kanaar et al. 1996).

Mutation of any of the yeast *RAD52*-complex homologues likewise results in profound defects in homologous DNA DSB repair (Kanaar and Hoeijmakers 1997), demonstrating that the *RAD52* pathway is conserved throughout evolution. This model also fits well with the physical constraints of the cell cycle, in that, during the late S phase and the G2 phase, the ploidy of the cell increases to 4*n,* allowing homologous recombination to proceed off of an undamaged template. Thus, the RAD52 complex is involved primarily in homologous recombinational repair, and this type of repair appears to occur during the late S/G2 phase.

The BRCA1/2 Complex

A very exciting, but very preliminary, study implicates a role in DNA DSB repair for the tumor suppressor genes *BRCA1* and *BRCA2,* which are involved in familial predisposition to breast cancer and other cancers (Kinzler and Vogelstein 1997). In particular, it has been shown that *BRCA1* and *BRCA2* are expressed during the S and the G2 phases coordinately with RAD51 (Rajan et al. 1996; Yamamoto et al. 1996) and that hRAD51 physically interacts with both BRCA1 (Scully et al. 1997*b*) and BRCA2 (Mizuta et al. 1997; Sharan et al. 1997). *BRCA2* null embryos exhibit a profound x-ray hypersensitivity, strongly suggesting an integral and direct role in DNA repair. In addition, BRCA1 is phosphorylated, and its subnuclear localization altered in the S phase, following x-ray irradiation (Scully et al. 1997*a*). These reports suggest that hRAD51, BRCA1, and BRCA2 form a complex that repairs lesions associated with DNA replication. It remains to be determined whether BRCA1 and BRCA2 are involved specifically in DNA DSB repair; whether they are limited to homologous DSB repair, as RAD51 appears to be; whether they truly are active during the S phase; and, most importantly, whether they act as part of the RAD52 complex or in a distinct DNA-repair pathway.

Coordination of DNA DSB-Repair Pathways

DNA DSB repair is probably the least understood of the mammalian DNA-repair processes. In the past three years, however, an enormous amount of exciting data has invigorated the field and has provided ample reasons to be optimistic that progress in this area will continue at an exhilarating rate. It is interesting to speculate that the tumor suppressor gene *p53* may link these pathways together in a consistent fashion. Thus, it has been shown that p53 is required for the induced expression of KARP-1 and thus, presumably, for DNA-PK activity (K. Myung, C. Braastad, D. M. He, and E. A. Hendrickson, unpublished data). In addition, p53 has been shown to interact directly with, and to inhibit, hRAD51 (Sturzbecher et al. 1996). Thus, a working hypothesis may be that the exposure of cells to x-ray irradiation induces p53, which simultaneously causes a G1 cell-cycle arrest, activates nonhomologous DSB repair via DNA-PK, and inhibits homologous repair. After nonhomologous repair has taken place, p53 levels recede, inactivating DNA-PK and activating RAD51-dependent pathways in either the S phase (BRCA2 complex) or the G2 phase (RAD52 complex). Given the profound clinical relevance of x-ray exposure as a therapeutic tool and the prevalence of breast cancer in the human population, a better understanding of the phenomenon of DNA DSB repair clearly is warranted.

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