7

The MRE11-RAD50 Complex

Diverse Functions in the Cellular DNA Damage Response

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1. INTRODUCTION

In recent years, progress toward understanding the mechanisms and molecules with which mammalian cells respond to DNA double-strand breaks (DSBs) has been dramatic. This is attributable in part to the analysis of DSB repair-deficient rodent cell lines, which led to the isolation and characterization of the DNA-PK complex, XRCC4, DNA ligase IV, and others *(3,43,49,59,60,79,83,100,103,112).* The gene products thus identified have provided many important insights regarding the functions that maintain genomic integrity in the face of genotoxic stress. These studies have also shed light on DNA recombination pathways that diversify genetic information in the establishment of the immune repertoire. Nonetheless, the bulk of our current understanding of DNA recombination pathways has come from genetic and biochemical studies in *Saccharomyces cerevisiae* and bacterial systems. As an alternative to phenotype-driven analysis, a number of investigators have pursued the identification of mammalian counterparts to bacterial and *S. cerevisiae* recombinational DNA repair proteins to examine the cellular DNA damage response in mammals *(46,80).* The potential of this comparative approach has been most impressively realized in the functional analysis of the yeast and mammalian Rad51 proteins, which are homologs of the bacterial RecA protein *(9,38,96).* More recently, the MRE11-RAD50 protein complex, with homologs in bacteria, *S. cerevisiae,* and mammals, has emerged as a central player in the DNA transactions that preserve genomic integrity in yeast and mammalian cells.

This chapter includes descriptions of *S. cerevisiae,* mouse, and human genes and their protein products. For the sake of consistency, the following nomenclature will be used throughout:

Wild-type gene or locus (human, mouse or yeast)……*hRAD50, mRad50,* or *ScRAD50*
Mutant gene or locus (human, mouse or yeast)……*hrad50, mrad50,* or *Scrad50*
Protein product (human, mouse or yeast)……hRAD50, mRAD50, or ScRad50p

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2. OVERVIEW OF THE *S. CEREVISIAE* AND MAMMALIAN MRE11-RAD50 PROTEIN COMPLEXES

The existence of the Mrellp-Rad50p protein complex was first suggested by the striking similarity of the *Scmrell, Scrad50,* and *xrs2* phenotypes *(1,2,40,42,80,84).* Subsequently, physical association of the *S. cerevisiae* proteins and of the human hMRE11 and hRAD50 gene products was established *(26,109). hMRE11* was first identified in a two-hybrid screen for proteins that interact with DNA ligase I, although the physiological relevance of this interaction is uncertain *(81).* The *hRAD50* cDNA is part of a collection of cDNAs originating from the chromosomal region spanning 5q23 to 5q31 that was isolated in an effort to identify a tumor-suppressor gene involved in myelodysplasia and myeloid leukemia that maps to that region. The *hRAD50* gene was identified among these cDNAs on the basis of its similarity to the *S. cerevisiae* gene as well as to a *Caenorhabditis elegans* EST *(26).* The *hRAD50* locus, which maps to 5q31, was subsequently ruled out as the locus of the AML tumor suppressor *(53).*

Phenotypic analyses implicate the *S. cerevisiae* Mre11p-Rad50p-Xrs2p complex in diverse aspects of both meiotic and mitotic recombination pathways. In meiosis, the complex is important prior to the formation of the initiating DSBs, apparently playing a role in modulating chromatin structure at DSB initiation sites *(75)* as well as in recruiting the protein(s) that mediate the actual cleavage event *(47).* Meiotic DSBs are formed by Spo11p, which becomes covalently attached via a phosphoester linkage to the 5′ ends at DSBs *(6,47).* This covalent association is normally transient, but is stabilized in certain *Scmre11* and *Scrad50* backgrounds (the *mre11S* and *rad50S* alleles) *(2,70,106).* This indicates that the complex is also important following DSB formation, and suggests that it mediates the endonucleolytic cleavage of Spo11p from the ends of initiating DSBs. The functions, if any, of the hMRE11-hRAD50 complex in mammalian meiosis remain to be established.

In mitotic cells, the ScMre11p-ScRad50p-Xrs2p complex functions in nonhomologous endjoining (NHEJ) *(35,46,80).* At first glance, homologous recombination seems to be normal in mutants of the complex; in fact *Scmre11, Scrad50,* and *xrs2* mutants exhibit increased rates of spontaneous heteroallelic recombination. However, the complex may play a global role in stabilizing or potentiating chromatid interactions during recombinational DNA repair, and thus function in homologous recombination as well as DNA endjoining *(14,68).* It is conceivable that this function is also relevant to the increased rate of chromosome loss observed in the corresponding mutants *(10,16,40,68).*

In mammalian cells, insights regarding the functions of the MRE11-RAD50 complex have come from genetic, biochemical, and cytological analyses. The mammalian complex consists of at least four members (Fig. 1). Whereas Mre11p and Rad50p are highly conserved, Xrs2p has been replaced in the mammalian complex by p95 (also known as NBS1 or Nibrin), with which it shares only limited similarity *(20,26).* Unlike MRE11 and RAD50, p95 is not essential for cellular survival *(61,116).* p95 deficiency forms the molecular basis of the rare chromosomal instability syndrome, Nijmegen breakage syndrome (NBS), also known as the ataxia telangiectasia (A-T) variant syndrome *(20,95).* The cellular phenotypic features of NBS suggest that p95 deficiency compromises the ability of cells to detect and signal the presence of DNA damage, thus implicating the hMRE11-hRAD50 complex in this function. Consistent with this role for the human complex, cytological analyses have unambiguously demonstrated that

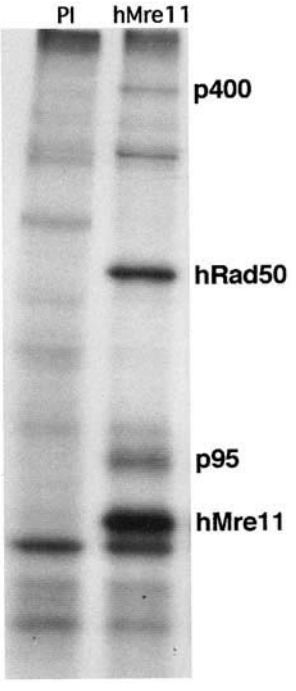

Fig. 1. The hMre11-hRad50-p95 complex. Human lymphoblast cells were metabolically labeled with ^{35}S-methionine, then immunoprecipitated with either preimmune serum (PI) or with hMre11 antiserum (hMre11). The immunoprecipitates were resolved by sodium dodecyl sulfate (SDS) gel electrophoresis, revealing the members of the hMre11-hRad50-p95 complex. Equivalent results are obtained using antisera derived against any of the known complex members. The identity of p400 remains unknown. A similar complex, comprised of at least ScMre11, ScRad50, and Xrs2p, exists in *S. cerevisiae (109).*

the complex associates with ionizing radiation (IR)-induced DNA DSBs early in the cellular DNA damage response *(64,71).*

3. IN VITRO ACTIVITIES

The Mre11p and Rad50p homolog exhibit impressive similarity to SbcD and SbcC, respectively, components of the bacterial exonuclease SbcCD *(94).* SbcCD is a large multimeric complex that exhibits ATP-dependent exonuclease activity, as well as ATP-independent endonuclease activity, both of which require Mn^{2+} as a cofactor *(22).* SbcD is the catalytic subunit of SbcCD, and exhibits strong similarity to the Mre11p homolog (Fig. 2). The sequence similarity among this phylogenetically diverse group of proteins primarily comprises four discrete domains, three of which are also found in protein phosphatases such as λ phosphatase *(55).* Structural studies of λ as well as the mammalian phosphatases demonstrate that residues within each of the three conserved domains are involved in metal-ion binding, which is in turn critical for enzymatic activity *(30,119,120).* The fourth domain is unique to the nucleases *(55).*

A number of laboratories have shown that both the yeast and human MRE11 proteins exhibit nuclease activity in vitro *(27,69,77,105,109).* The activities observed include 3′–5′ exonuclease activity on double-stranded DNA (dsDNA) substrates as well as

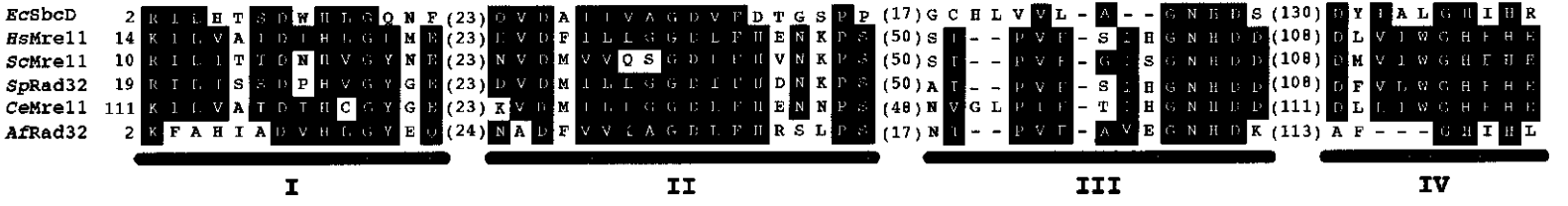

Fig. 2. Alignment of the amino acid sequences of the conserved N-terminal domains of the bacterial SbcD protein and the Mre11 homologs. Similar or identical amino acids are shaded in black. Domains are indicated by numerals I-IV below the sequence alignment. The number of the first residue in each species is indicated at the left. Numbers in parentheses indicate the number of amino acids between each domain. Organisms are as follows: *Ec, Escherichia coli; Hs, Homo sapiens; Sc, Saccharomyces cerevisiae; Sp, Schizosaccharomyces pombe; Ce, Caenorhabditis elegans;* and *Af, Archaeoglobus fulgudis.*

endonuclease activity on single-stranded DNA (ssDNA) and hairpin substrates. Hence the enzymatic activities of MRE11 are entirely consistent with the conservation between the Mre11p homologs and the bacterial nuclease SbcD. MRE11 nuclease activities similarly require Mn^{2+} as a cofactor, but unlike SbcD, MRE11 exonuclease activity does not appear to be ATP-dependent *(23,27,77,109).* 3′–5′ exonuclease activity has been observed on dsDNA templates with a 5′ overhang or blunt ends, but is inhibited by 3′ overhangs greater than 4 bp *(27,77,105,109).* Interestingly, ScMre11p exhibits a slight preference for binding to 3′ overhanging DNA ends, even though its nuclease activity on this substrate is extremely limited *(27,77).* On this basis, it has been speculated that the reduced rate of 5′–3′ resection at DSBs in mutants of the complex may reflect a role for ScMre11p (and by extension hMRE11) in recruiting the *bona fide* 5′–3′ exonuclease(s) to DSB termini in vivo *(77).*

The exonuclease activity of hMRE11 is slightly enhanced by hRAD50 *(77),* but neither the yeast nor the human MRE11 protein requires other complex members for nuclease activity. In contrast to most studies, Trujillo et al. purified the human complex from Raji cells under conditions that left the association of hMRE11, hRAD50, and p95 intact *(105).* They observed 3′–5′ exonuclease activity as with Mrell alone, indicating that hRAD50 and p95 do not alter the polarity of the MRE11 exonuclease.

The Mre11p homologs also exhibit endonuclease activity. This activity is of considerable interest in the context of the V(D)J recombination pathway because of the ability of hMRE11 to cleave a hairpin structure (*77;* Chapter 11). Hairpins are formed on DSBs induced at sites undergoing V(D)J recombination *(57).* Thus the MRE11-RAD50 complex may perform this function in vivo. Suitable mutant cell lines are under development, but a direct assessment of whether these proteins play any role in this process in vivo is not currently feasible. However, recent in vitro results suggest that the hairpin opening activity may be intrinsic to the RAG1 and RAG2 proteins *(7).*

The in vivo significance of the MRE11 nuclease activities is not clearly established, particularly in mitotic cells from yeast and humans. It is perhaps significant, however, that hMRE11 can facilitate NHEJ in vitro. Using linear dsDNA with mismatched 5′ overhanging termini, Paull et al. *(77)* showed that hMRE11-mediated 3′–5′ resection was required for joining of at least one strand of the mismatched termini by DNA ligase I or T4 DNA ligase. These in vitro data do not agree with the studies of Moreau et al. *(69),* who found that a nuclease-deficient *Scmre11* allele did not impair NHEJ in vivo. The extent to which this minimal in vitro reaction reflects the in vivo activity of the complex remains to be seen, but this result suggests a role for the MRE11 3′–5′ exonuclease in DNA endjoining.

4. THE *S. CEREVISIAE* MRE11p-RAD50p-XRS2p PROTEIN COMPLEX

The initial mutagenesis studies of *ScRAD50* revealed most functions of the ScMre11p-ScRad50p-Xrs2p complex *(2).* More recently, the characterization of ScMre11p has expanded our views about the functional significance of this complex in both meiotic and vegetatively growing cells. Mutagenesis of ScMre11p has distinguished particular functional domains of the protein and shed light on its distinct roles in meiotic and mitotic cells.

Null mutants of *ScMRE11, ScRAD50,* and *XRS2* confer essentially identical phenotypes, including IR sensitivity, elevated rates of mitotic interhomolog recombination,

and defects in the initiation of meiotic recombination *(1,2,29,40,62,91).* These data led Kleckner and others to suggest that ScMre11p, ScRad50p, and Xrs2p function in a protein complex *(1,40,84),* which was later confirmed through two-hybrid interaction testing as well as co-immunoprecipitation studies *(44,74,106,109).*

In addition to the three core members of the *S. cerevisiae* complex, genetic and physical evidence for additional members of the complex has come from meiotic cells. In GST "pull down" assays, the ScMre11p C-terminus appears to specifically bind three additional proteins of 40 kDa, 24 kDa, and 22 kDa present in meiotic, but not mitotic extracts *(109).* These proteins have not been identified. However, Usui and colleagues noted that molecular masses of the 24 kDa and 22 kDa species are similar to those (predicted) for Rec102 (23 kDa) and Rec104 (21 kDa), proteins that appear to function in the initiation of meiotic recombination *(8,17).* Further, Xrs2p is phosphorylated in mitotic cells, suggesting that the *S. cerevisiae* complex at least transiently associates with a protein kinase *(109).* In light of these observations as well as the diverse phenotypic outcomes of mutations in the core members of the complex, it is reasonable to assume that the functions of ScMre11p, ScRad50p, and Xrs2p require additional protein interactions. The identification of such interactions will certainly provide important insights regarding the diverse functions of this complex.

4.1. Meiotic Functions of the S. cerevisiae Mre11p-Rad50p-Xrs2p Protein Complex

ScMRE11, ScRAD50, and *XRS2* null mutants exhibit severe meiotic recombination defects. *Scmre11*Δ*, Scrad50*Δ*,* and *xrs2*Δ mutants are blocked at an early point in meiotic recombination, as the formation of viable spores in these mutants requires the *spo13* mutation, which causes the bypass of meiosis I and initiation of meiotic recombination. It was later established that ScMre11p, ScRad50p, and Xrs2p were each required for the formation of DSBs to initiate meiotic recombination *(1,2,19,40,44,51).* Although the formation of DSBs appears to be directly mediated by the Spo11p gene product *(47),* ScMre11p, ScRad50p, and Xrs2p are three of at least nine gene products aside from Spo11p that are required for this event (reviewed in *86*).

The mechanistic basis for the requirement of ScMre11p, ScRad50p, and Xrs2p in the formation of DSBs is not clear. However, certain observations suggest that the complex may influence the formation of "open" chromatin to facilitate cleavage at recombination hotspots. Ohta and colleagues observed that transitions in chromatin structure that normally occur prior to the initiation of meiotic recombination (reviewed in *58*) are affected in mutants of the *S. cerevisiae* Mre11p-Rad50p-Xrs2p complex *(75).* In meiotic cells, localized micrococcal nuclease (MNase) hypersensitive hotspots arise at sites that ultimately correspond to the sites of DSB formation *(58).* These sites are slightly less MNase sensitive in *Scmre11*Δ mutants than in wild-type cells, in contrast to *Scrad50*Δ and *xrs2*Δ mutants in which these sites are significantly more sensitive. In *Scmre11*Δ *Scrad50*Δ double mutants, the increased MNase sensitivity observed in the *Scrad50*Δ single mutant is suppressed to wild-type or slightly lower (i.e., *Scmre11*Δ) levels *(75).* Normal MNase sensitivity is observed in a strain expressing a *Scmre11* allele that lacks nuclease function, *Scmre11D16A* (Table 1). In contrast, reduced MNase sensitivity is conferred by the *Scmre11*Δ*C49* allele, which partially abrogates ScMre11p DNA binding (Table 1). These data suggest that the establishment of appro-

Table 1.
Compendium of *Scmre11* Mutant Alleles

Mutation	Allele name	Interaction with Rad50p	Nuclease activity	Mitotic phenotypes	Meiotic phenotypes	Reference
D16A	*mre11D16A*	ND	Null	Mild MMS sensitivity, short telomeres	DSBs formed but not processed	*(27)*
H18L	*mre11-11*	Weak	ND	Intermediate IR sensitivity	ND	*(15)*
D56N	*mre11D56N*	ND	Null	Weak IR sensitivity	DSBs formed but not processed	*(69)*
D56F	*mre11-2*	No	ND	Null	ND	*(15)*
F58S						
H125N	*mre11H125N*	ND	Null	Weak IR sensitivity	DSBs formed but not processed	*(69)*
H125L	*mre11-3*	Yes	ND	Weak IR sensitivity	Inviable spores	*(14,15)*
D126V						
H213Y	*mre11-58 (mre11S)*	Conflicting data (Subheading 4.5.)	Null	MMS sensitive	DSBs formed but not processed	*(70,106)*
H242L	*mre11-4*	No	ND	Null	ND	*(15)*
H243Y						
ΔAA410-420	*mre11-6*	Yes	Null	Intermediate MMS sensitivity	DSBs formed but not processed	*(109)*
Δ49 AA @ C-terminus	*mre11-ΔC49*	Yes	Wild-type	Wild-type, but does not bind dsDNA	No DSBs formed	*(27)*
Δ62 AA @ C-terminus	*mre11-T10*	Yes	Wild-type	ND	Inviable spores	*(70)*
Δ136 AA @ C-terminus	*mre11-5*	Yes	Wild-type	Wild-type, but does not bind dsDNA	No DSBs formed	*(109)*

ND, not determined.

priate chromatin structure at recombination hotspots requires the DNA binding, but not the nuclease activity of ScMre11p *(27)*.

The *Scmre11S* and *Scrad50S* alleles constitute a class of hypomorphic mutants *(2,19,27,69,70,106)*. Like null *Scmre11* and *Scrad50* mutants, the ***S*** mutants exhibit severe meiotic defects, but differ from the null mutants in two important respects. First, this mutant class is associated with very mild mitotic phenotypes. Second, these alleles do not block the formation of meiotic DSBs, but Spo11p protein remains covalently attached to the DSBs that do form in these strains. Because the protein-DNA adducts remaining at the DSB sites block any subsequent exonucleolytic processing required for DNA recombination, ***S*** mutants produce inviable spores even in *Scmre11S spo13* and *Scrad50S spo13* double mutants *(2,47,48,70)*. The persistent covalent attachment of Spo11p to DSBs formed in the *Scmre11S* and *Scrad50S* mutants suggests a complex of proteins including ScMre11p and ScRad50p cleaves Spo11p following DSB formation. As discussed in Subheadings 4, and 5.5, support for this interpretation comes from the observation that wild-type ScMre11p protein, but not the *Scmre11S* gene products, exhibits nuclease activity in vitro *(69,109)*.

4.2. Mitotic Functions of the S. cerevisiae Mre11p-Rad50p-Xrs2p Protein Complex

4.2.1. Illegitimate Recombination and NHEJ

The phenotypic features of mutants in the ScMre11p-ScRad50p-Xrs2p protein complex in mitotic cells provide a rather dramatic contrast to those in meiotic cells described earlier (Subheading 4.1.). Whereas DNA recombination is profoundly inhibited by mutation of complex members in meiotic cells, mitotic mutants exhibit a hyper-recombinational phenotype. That is, the frequency of spontaneous homologous recombination between heteroalleles in diploid yeast strains is dramatically increased in *Scmre11, Scrad50,* and *xrs2* mutants *(1,40,62)*. On this basis, the ability of mutants to carry out homologous recombination does not appear to be grossly impaired. Mutants of the ScMre11p-ScRad50p-Xrs2p complex are thus distinct from other *ScRAD52* epistasis group mutants such as *Scrad52* and *Scrad51* in which homologous recombination is effectively abolished *(28)*.

Initial clues that ScMre11p, ScRad50p, and Xrs2p function in illegitimate recombination pathways came from plasmid integration/transformation assays. Schiestl and Petes showed that linear DNA lacking any homology to the *S. cerevisiae* genome could nonetheless integrate into the chromosome via short (4 bp) stretches of homology *(90)*. This illegitimate recombination event is not affected by *Scrad52* mutations, but is profoundly impaired in *Scrad50* mutants *(91)*. The ScMre11p-ScRad50p-Xrs2p complex was further implicated in illegitimate recombination by characterization of the yeast NHEJ pathways. Plasmid reclosure assays to define the genetic requirements of NHEJ established the importance of the *S. cerevisiae* Ku70 and Ku80 homologs, DNA ligase IV, and the ScMre11p-ScRad50p-Xrs2p complex in this process *(12,13,67,107,108,115)*. Among those genes, only *Scmre11, Scrad50,* or *xrs2* mutations confer sensitivity to killing by IR or radiomimetic DNA damaging agents *(89,102,115)*. Because NHEJ is impaired to essentially the same extent in these mutants irrespective of their IR sensitivity, it appears that NHEJ per se does not contribute significantly to cellular survival after DSB induction. Further, the profound sensitivity to IR and DSB repair deficiency

observed in *Scmre11*Δ strains therefore argues that defects in the DNA damage response of ScMre11p complex mutants extend beyond NHEJ *(14,15).*

4.2.2. Mitotic Homologous Recombination

An important insight regarding the ScMre11p-ScRad50p-Xrs2p complex in DSB repair came from the observation that the NHEJ deficiency of *Scmre11*Δ mutants is cell-cycle phase-specific. Although the experiments in question specifically focused on NHEJ, the data obtained point to a role of the complex in homologous recombination as well. Moore and Haber found that the ability of *Scmre11*Δ and *Scrad50*Δ mutants to rejoin a chromosomal DSB by NHEJ was much less impaired if the DSB was induced during G1 (three-fold reduction in G1- vs 70-fold reduction in asynchronous cultures). Because overall the ability of the *Scmre11*Δ and *Scrad50*Δ mutants to repair the DSB was profoundly reduced, they inferred that the NHEJ functions of ScMre11p and ScRad50p were restricted to cells in the late S or G2 phase of the cell cycle *(68).* These investigators proposed that the NHEJ defects observed in *Scmre11*Δ and *Scrad50*Δ mutant strains reflect a failure to stabilize and protect the DSB ends from excessive degradation via association with the sister chromatid. Accordingly, their model suggests that the complex plays a role in establishing sister chromatid interactions. The increased rate of spontaneous allelic recombination observed in *Scmre11, Scrad50,* and *xrs2* mutants can also be accounted for by this model if, as a consequence of compromised sister chromatid association, spontaneous lesions normally repaired by sister recombination are repaired by allelic recombination *(68).*

The importance of the ScMre11p-ScRad50p-Xrs2p complex to sister chromatid association and recombination was first suggested by the dose response of *Scrad50* and *xrs2* strains in clonogenic survival assays. Asynchronous cultures of wild-type haploid cells exhibit a biphasic clonogenic survival curve upon irradiation with increasing dose, indicating that relatively sensitive and insensitive populations exist within the asynchronous culture. Because the relatively insensitive population generally corresponds in size to the fraction of cells in G2, its insensitivity is attributed to the presence of a sister chromatid for recombinational DNA repair. Survival curves of haploid *Scrad50* and *xrs2* mutants are not biphasic, suggesting that G2 cells are unable to utilize effectively the sister chromatid for DSB repair *(40,87).* Indeed, more recent analyses of sister chromatid recombination in our laboratory using synchronous cultures as well as chromosomal substrates strongly argue that facilitating the use of the sister chromatid as a template for DSB repair is the primary role of the ScMre11p-ScRad50p-Xrs2p complex in the cellular response to DSBs *(14).* Because sister chromatid interactions in mitotic cells and interhomologue interactions in meiotic cells may share some structural similarities *(52),* it is also noteworthy that *Scrad50* mutants exhibit decreased pairing of chromosomal homologs in meiosis *(113).* Collectively, these data suggest that, in addition to any enzymatic functions it may have, the ScMre11p-ScRad50p-Xrs2p complex plays an important structural role in facilitating chromatid interactions that are critical to DSB repair and the maintenance of chromosome stability.

4.3. Telomeres and Cell-Cycle Checkpoints

An additional manifestation of a structural role for the ScMre11p-ScRad50p-Xrs2p complex may take place at chromosome ends. Several yeast proteins involved in NHEJ are also important for the maintenance of telomeric DNA (*25;* Chapter 14). Abnormal

telomere shortening is observed in mutants of the ScMre11p-ScRad50p-Xrs2p complex, as well as the yeast Ku complex *(11,50,73,82).* In principle, telomere shortening can be attributed to decreased telomerase activity or to telomere degradation, reflecting the abrogation of telomere end protection. Although the specific mechanisms have not been established, analysis of the genetic interactions among telomerase component (*est1* and *est2*), *yku80, Scmre11,* and *Scrad50* mutants has provided some insight. Whereas *yku80* telomere shortening phenotypes are enhanced in *est1* and *est2* mutant backgrounds, this is not the case for *Scmre11* and *Scrad50* mutants. These data suggest that the ScMre11p-ScRad50p-Xrs2p complex is involved in telomere synthesis, and the Ku complex is involved in telomere end protection *(73).*

In light of cell-cycle checkpoint functions mediated by the hMRE11-hRAD50 complex described in Subheading 5.4.1., it is important to consider whether the *S. cerevisiae* complex fulfills a similar role in yeast. This question has not yet been explored in detail, but the evidence available is consistent with a role for the yeast complex in some aspects of cell-cycle checkpoint function. Using a strain in which constitutive expression of the HO endonuclease creates a DSB that must be repaired by NHEJ, Haber and colleagues showed that DSB induction leads to cell-cycle arrest, followed by cell death *(56).* In wild-type cells as well as *Scmre11* and *Scrad50* mutants, cell death was preceded by a limited number of cell divisions as evidenced by the formation of microcolonies. The formation of microcolonies is indicative of escape from, or adaptation to, the cell-cycle checkpoint that is activated in response to the DSB *(78).* Checkpoint adaptation is genetically distinct from checkpoint activation, as casein kinase II and *cdc5* mutants are checkpoint-proficient but adaptation-deficient *(104). yku70 (hdf1)* mutants also undergo cell-cycle arrest followed by cell death, but do not form microcolonies, indicating that these mutants are defective in their ability to adapt to the DSB-induced cell-cycle checkpoint. The adaptation defect of *yku70* mutants is suppressed by *Scmre11*Δ and *Scrad50*Δ mutations *(56),* indicating that the ScMre11p-ScRad50p-Xrs2p complex is required for this aspect of the cell-cycle checkpoint function. The mechanistic basis for this genetic interaction remains unclear. In addition, *Scrad50*Δ mutants are extremely sensitive to hydroxyurea in a manner suggestive of a role for the yeast complex in the activation of the S phase checkpoint *(50).* This facet of the ScMre11p-ScRad50p-Xrs2p complex's function is of great interest. Further examination of DNA damage-dependent cell-cycle checkpoint functions is underway in a number of laboratories. Time will tell whether the linkage of DSB repair to cell-cycle checkpoint functions seen in mammals is a conserved feature of this protein complex.

4.4. Nuclease Activity In Vivo

The complex's impact on checkpoint adaptation appears to correlate with its influence on the 5′-3′ resection at the DSB site *(56).* ScMre11p, ScRad50p, and Xrs2p deficiency also reduces the rate of 5′-3′ resection at the HO-induced DSB at the *MAT* locus during mating-type switching *(34,42,114).* In addition, 5′-3′ resection to facilitate single-strand annealing is slowed by ScRad50p and Xrs2p deficiency *(39,41).* The influence of Mre11p-Rad50p complex members on 5′-3′ end resection is paradoxical, because both yeast and human MRE11 proteins exhibit 3′-5′ exonuclease activity in vitro *(27,69,77,105,109).* It is conceivable that the complex regulates the activity of a *bona fide* 5′-3′ exonuclease or that the in vivo polarity is 5′-3′ as a result of cofactors not

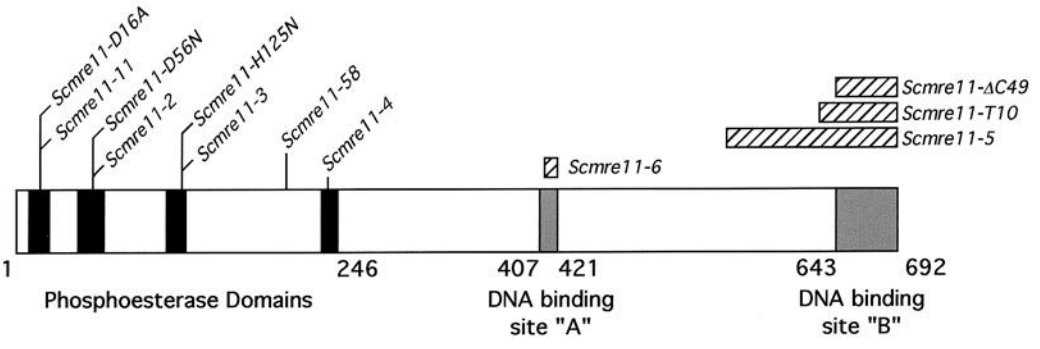

Fig. 3. Mutagenesis of functional domains of *S. cerevisiae* Mre11. Conserved phosphoesterase domains in the N-terminus of ScMre11 are represented by black vertical bars. DNA binding regions in the C-terminus are represented by gray bars. The numbers of the amino acids comprising each functional region are indicated below the diagram. The locations of point mutations in the phosphoesterase domain region are indicated by thin black lines above the diagram. The deletion and truncation mutations in the DNA binding regions are shown by hatched bars. Detailed descriptions of these mutations are given in Subheading 4.5. and Table 1.

present in the in vitro experiments. Alternatively, resection from the DSB site may proceed via the coordinated activities of a DNA helicase and the MRE11 endonuclease *(35,109).* However, on balance it appears very likely that the nuclease activity of MRE11 is not important for its mitotic functions in vivo. This idea is supported by recent data showing that a nuclease-deficient *Scmre11* allele has no impact on DSB resection during mating-type switching, DNA endjoining, or telomere maintenance *(69).*

4.5. Mutational Analyses

Mutagenesis of ScMre11p-ScRad50p-Xrs2p complex members has yielded phenotypic outcomes useful in defining functional domains of these proteins. To a great extent, the primary sequence of the proteins has guided these experiments. Initial mutagenesis targeted the N-terminal ATP binding domain of ScRad50p, generating two classes of point mutations *(2).* The first demonstrated that alteration of ATP binding domain consensus residues is tantamount to a null mutation. The second class, located within this domain but outside of the consensus region, gave rise to the *Scrad50S* mutants. As described earlier (Subheading 4.1.), these mutants exhibit relatively mild mitotic phenotypes and retain the ability to interact with ScMre11p *(16),* yet have severe defects in meiotic recombination *(2).* Mutational analysis to identify functional domains of Xrs2p has not been performed. However, such an investigation may be warranted given the potential analogy of Xrs2p to p95, described in Subheading 5.1.

More recently, mutagenesis of ScMre11p has revealed the protein domains that control nuclease activity, protein interactions, and DNA binding. Deletion and point mutations in the highly conserved N-terminal phosphoesterase domains support the hypothesis that this region of ScMre11p confers nuclease function (Fig. 3 and Table 1). Disruption of the nuclease activity of ScMre11p without affecting the protein's ability to interact with ScRad50p has little impact on mitotic cells, but confers severe meiotic defects, as illustrated by the *Scmre11H125N, Scmre11-3,* and *Scmre11-6* mutants, which

exhibit an ***S*** phenotype (Table 1) *(15,69,109).* As with *Scrad50S* alleles, the spore-inviability phenotype of these nuclease-deficient mutants reflects a defect in the processing of meiotic DSBs owing to the stable covalent attachment of Spo11p to DNA ends.

The heterogeneous phenotypic outcomes of mutations in the phosphoesterase domains of ScMre11p suggest that nuclease deficiency per se is not sufficient to account for the range of phenotypes observed. Because mutations in the first three phosphoesterase domains of ScMre11p *(15,27,69)* alter conserved residues shown to be important for metal-ion binding and catalysis in mammalian and bacteriophage serine/threonine phosphatases *(30,31,120),* one hypothesis is that mutations in these domains disrupt the secondary structure of the protein, thereby affecting other physical interactions required for function. Initial work by H. Ogawa's laboratory identified the N-terminus of ScMre11p as the ScRad50p interaction domain *(44),* and recent studies in our laboratory and others have identified point mutations in this region that disrupt the ScRad50p interaction *(15,70,106).* Impairing the interaction between ScMre11p and ScRad50p results in a null phenotype in mitotic cells, as demonstrated by the *Scmre11-2* and *Scmre11-4* mutants (Table 1) *(15).* The impact of complex disruption on meiotic recombination processes remains uncertain, although the similarities between the *Scmre11D56N* and *Scmre11-2* mutations suggests that the *Scmre11-2* mutant will exhibit a severe meiotic phenotype as well. In addition, there are conflicting data regarding the ability of the Scmre11-58p to interact with ScRad50p *(70,109).* The basis for this discrepancy is not clear, however, its mitotic phenotypes most closely resemble *Scmre11* alleles that lose the ability to interact with the complex.

Intragenic complementation is observed in diploid strains bearing the *Scmre11-5* and *Scmre11-58* mutations *(109)* and in strains bearing the *Scmre11S* and *Scmre11-T10* mutations *(70).* These observations demonstrate the importance of homotypic interactions in the function of the ScMre11p-ScRad50p-Xrs2p complex and also demonstrate that meiotic DSB formation and processing functions of ScMre11p reside in different domains of the protein. Further evidence for this comes from mutational analyses of two regions of ScMre11p important for DNA binding (Fig. 3). Deletion mutations of each of these regions confer marked defects in meiotic recombination (Table 1). Specifically, the *Scmre11-ΔC49* and *Scmre11-5* mutants are defective in meiotic DSB formation *(27,109),* whereas the *Scmre11-6* mutant is deficient in the processing of meiotic DSBs *(109).* Interestingly, the C-terminal region of ScMre11p bearing these DNA binding domains also contains the regions of interaction with meiosis-specific proteins described earlier (Subheading 4.1.), suggesting that ScMre11p may play a role in recruiting proteins to the sites of meiotic DSBs *(109).*

The ScMre11p-ScRad50p-Xrs2p complex has been shown to play a role in diverse aspects of meiotic and mitotic recombination processes. Mutational analyses in *S. cerevisiae* have been critical in the examination of null mutants of protein complex members, as well as in the identification of distinct functional domains of ScMre11p and ScRad50p. Based on the high degree of conservation of these proteins in mammalian cells, these findings may facilitate the design of mutations in mammalian MRE11-RAD50-p95 complex members.

5. THE MAMMALIAN MRE11-RAD50-p95 PROTEIN COMPLEX

The mammalian MRE11, RAD50, and p95 proteins were first identified in human cells as members of a complex that consists of at least four major components, one of which, p400, is currently unidentified (Fig. 1) *(20,26).* hMRE11, hRAD50, and p95 are abundant

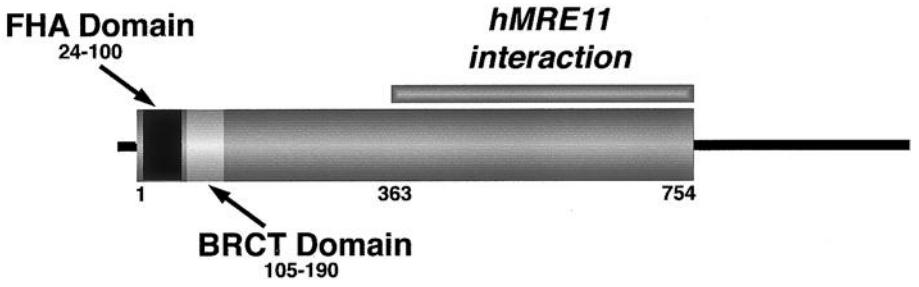

Fig. 4. Structure of the p95 coding sequence. The *NBS1* gene product, p95, was cloned by virtue of its association with the hMre11-hRad50 complex, both by direct protein sequencing from the purified complex and by yeast two-hybrid analyses *(20).* The FHA and BRCT domains, identified by sequence database comparisons, are highlighted; p95 currently is the only protein identified that contains both domains. The portion of p95 identified by yeast two-hybrid interaction with hMre11 is also shown. The thin black lines represent the 5′ and 3′ untranslated regions of the *NBS1* cDNA, and coding portions of the gene are indicated by the thick bar; the numbers below the diagram represent amino acids in the human p95 protein. *NBS1* was identified independently by positional cloning of the gene mutated in Nijmegen breakage syndrome (Subheading 5.4.) *(66,111).*

proteins in a variety of cell lines. Their physical association appears quite stable as all three proteins are co-immunoprecipitated using antisera directed against any one of the three, even in relatively harsh conditions *(20,26).* When purified from HeLa cells, hMRE11, hRAD50, and p95 co-elute in a complex of approx 1.5 MDa molecular mass, indicating that the stoichiometric relationships among members of the complex are not simply one to one. Further, as sizing column fractions corresponding to lower molecular mass do not contain appreciable amounts of hMRE11, hRAD50, or p95, the vast majority of these proteins in the cell are contained in the 1.5 MDa complex. The retention of p400 with the purified complex appears to depend on purification conditions, as this protein does not remain associated under certain chromatographic conditions *(105).* Experiments in vitro as well as in p95-deficient cells demonstrate that hMRE11 interacts directly with hRAD50 and p95, whereas hRAD50 and p95 do not appear to interact directly *(20,77).* Thus hMRE11 appears to be the central molecule in the complex.

5.1. p95: A Divergent Member of the Complex

Highly conserved homologs of *hMRE11* and *hRAD50* are found from yeast to mammals *(26,81).* The product of the *NBS1* gene, p95, appears to have replaced the *S. cerevisiae* Xrs2p protein in the mammalian complex. p95 and Xrs2p exhibit limited similarity in their N-termini, but there is otherwise little or no significant homology between the two. p95 appears to be conserved among mammals, but database searches do not identify strong similarities between p95 and proteins in nonmammalian species *(20).* Conversely, there are no obvious Xrs2p homologs outside of *S. cerevisiae.*

Insofar as both Xrs2p and p95 are stably associated with the Mre11p and Rad50p homologs in their respective species, it is conceivable that the two proteins are functional analogs. The lack of conservation between the two proteins argues against this idea. It is particularly telling that p95, but not Xrs2p, contains a BRCT (BRCA1 C-terminal) domain and a FHA (forkhead-associated) domain at its N-terminus (Fig. 4), both of which are found in proteins involved in DNA repair or cell-cycle control in yeast,

mammals, and other organisms *(10,18).* Interestingly, p95 is the first example of a protein that contains both domains. The phenotypic features of p95-deficient cells discussed later (Subheading 5.4.1.) support the suggestion that p95 function is relevant to DNA damage responses, lending circumstantial evidence for the importance of these conserved protein motifs in p95. Furthermore, *xrs2* and *nbs1* mutant cells in *S. cerevisiae* and humans, respectively, do not exhibit clear similarities in their phenotypic features. A more detailed assessment of Xrs2p and p95 functions is required to determine the extent to which these proteins, and the respective complexes in which they act, are indeed functional analogs.

5.2. Genetic Analyses of the Mammalian MRE11-RAD50-p95 Protein Complex

Genetic analysis of the mammalian MRE11-RAD50-p95 complex has been hampered by the fact that null mutants of *mMre11* and *mRad50* are inviable *(61,116).* Whereas heterozygous *mrad50*Δ/+ mice are normal in all respects, *mrad50*Δ/*mrad50*Δ embryos die at embryonic day 6.5 *(61).* Earlier embryos are not grossly affected, indicating that *mrad50*Δ cells are capable of limited growth. Histological analysis of mutant embryos at day 6.5 suggests that death reflects a gradual failure to proliferate rather than apoptosis *(61).* Day 6.5 of embryogenesis corresponds to the onset of very rapid cellular proliferation *(37),* suggesting that mRAD50 deficiency results in a failure to meet the increased replicative demand.

The bulk of spontaneously occurring DSBs is likely to arise during DNA replication, and available evidence suggests that such breaks are primarily repaired through homologous recombination with the sister chromatid *(24,54).* As described earlier (Subheading 4.2.2.), several lines of evidence indicate that *S. cerevisiae* Mre11p-Rad50p-Xrs2p complex mutants exhibit defects in their ability to utilize the sister chromatid as a template for DSB repair. If sister chromatid-based repair of such spontaneously arising DSBs were impaired by loss of mRAD50, *mrad50*Δ/*mrad50*Δ cells undergoing rapid proliferation would be disproportionately affected. Hence, the proliferative failure observed could be attributed to the rapid accumulation of unrepaired or misrepaired spontaneous DSBs during the normally rapid cell division that occurs at this stage. Experiments using a conditional *Gdmre11* mutant in the DT40 chicken cell line support this interpretation. Following inactivation of the *GdMRE11* gene, these cells proceed through several cell cycles before death. Karyotypic analysis during this process reveals that chromosomal aberrations begin to accumulate by 72 h after GdMRE11 depletion *(117).* These observations underscore the importance of the mammalian MRE11-RAD50-p95 complex specifically, and of DNA recombination functions generally, in normal cell growth.

Lethality of the *mmre11* and *mrad50* null mutations has precluded detailed analysis of recombinational DNA repair functions of those gene products. However, cultured *mrad50*Δ/*mrad50*Δ blastocysts are highly sensitive to killing by IR *(61).* Hence, it appears that mRAD50 deficiency leads to DSB repair deficiency, confirming that *mRad50* functions in the DNA damage response of murine cells as predicted from the phenotypic features of *Scrad50* mutant strains.

5.3. Cytology of the hMRE11-hRAD50-p95 Protein Complex

The hMRE11-hRAD50-p95 complex's role in the mammalian DSB repair response was revealed by cytological examination of its subcellular localization in human fibrob-

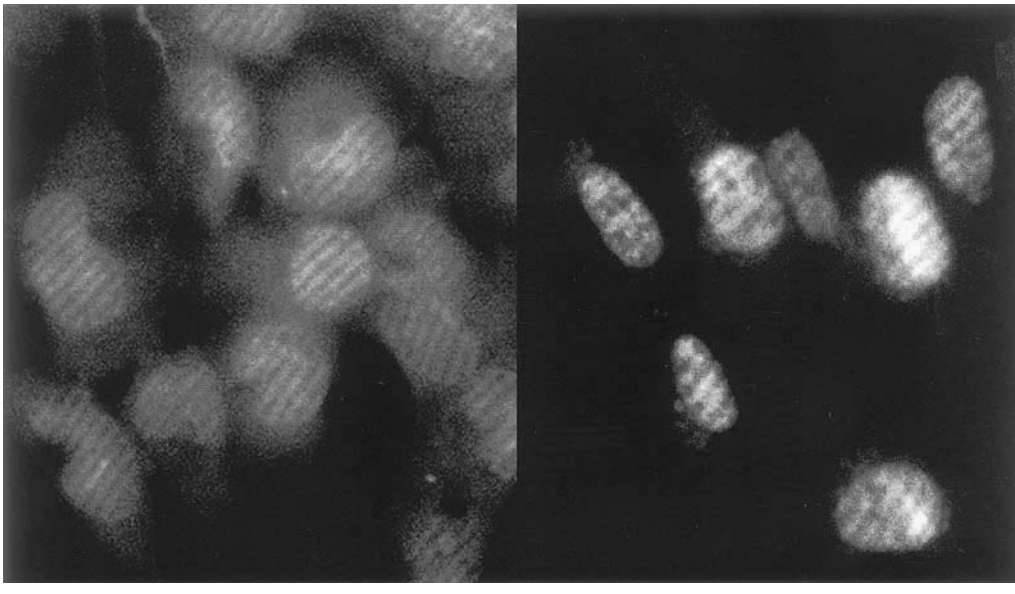

Fig. 5. The hMre11-hRad50-p95 protein complex localizes to DNA damage. Human fibroblasts were irradiated with ultrasoft X-rays through a gold grid *(71).* Left panel: At 30 min after irradiation, cells were labeled for DNA DSBs with BrdU. The DSBs are observed in the same stripe pattern as imposed by the presence of the gold grid. The DSBs persist in this pattern until they are repaired. Right panel: Identically irradiated cells were stained for the hMre11-hRad50-p95 protein complex with hMre11 antiserum. The complex redistributes into the same striped pattern as DNA DSBs, until DSB repair is complete. Independent determinations revealed that stripes of DSBs and hMre11 were identical, demonstrating that localization to DSBs is an important facet of the hMre11-hRad50-p95 complex's role in the cellular DNA damage response *(71).*

lasts. The hMRE11-hRAD50-p95 complex is homogeneously distributed in the nucleus, as shown by immunofluorescence with antisera directed against each of the complex members *(20,64).* However, upon the induction of DNA DSBs, the complex becomes associated with DSBs, and remains at DSB sites until the damage is repaired. These experiments have also yielded insight regarding the temporal and spatial behavior of the hMRE11-hRAD50-p95 complex with respect to other mammalian DSB repair proteins. Because p95 deficiency abrogates a specific cell-cycle checkpoint that functions in response to DNA damage (Subheading 5.4.1.), association of the hMRE11-hRAD50-p95 complex with DSBs suggests that the DNA-damage recognition functions of the complex are linked to the signal-transduction pathway(s) required to activate cell-cycle checkpoints.

5.3.1. Partial Volume Irradiation

Visualization of the hMRE11-hRAD50-p95 complex at DSBs required specialized techniques to induce and detect DNA DSBs in discrete subnuclear volumes. Taking advantage of the properties of ultrasoft X-rays to create DNA damage within a spatial range of 50 nm or less, Nelms et al. *(71)* induced DSBs within defined domains of fibroblast nuclei by irradiating cells through a special "striped" gold grid, a technique called partial volume irradiation. The DSBs, labeled fluorescently at dsDNA ends, were

subsequently observed in the striped pattern imposed by the grid mask soon after irradiation (20–30 min) (Fig. 5) *(71).* Whereas DSBs were no longer detectable by this method 90 min after irradiation in repair-proficient cells, the damage persisted in the striped pattern for up to 5 h after DSB induction in the human DSB repair-deficient cell line, 180BR (deficient in DNA ligase IV *[85]*). Independent determination of DSB repair kinetics show that the disappearance of stripes coincides with the completion (or lack) of DSB repair *(64,71).* These observations provided surprising but compelling evidence that the vast majority of DSBs remain stationary within the nucleus of irradiated cells, and thereby revealed an implicit requirement that cells possess a diffusible "sensor" of DNA damage to activate the cellular DNA damage response.

The hMRE11-hRAD50-p95 complex becomes associated with DSBs relatively early after their induction. Co-immunofluorescence with hMRE11 antiserum showed that the hMRE11-hRAD50-p95 complex had re-localized from its normal homogeneous nuclear distribution to the same "stripes" of DNA DSBs, and remained localized until the damage was repaired (Fig. 5) *(71).* In human DSB repair-deficient 180BR cells, hMRE11 remained associated with DSBs for the 5 h in which DSBs remained unrepaired and confined to stripes. This study thus demonstrated that an important spatial component of DSB repair is the localization of DSB repair proteins to the sites of DSBs, rather than the movement of DNA damage to fixed domains of repair complexes. The localization of hMRE11-hRAD50-p95 at DSBs is reflective of this complex's role in DSB repair and response pathways. Combined with the inability of p95-deficient cells to activate a DNA damage checkpoint pathway (Subheading 5.4.1.), these data suggest that the complex functions early in the mammalian DSB response and is situated to act either as a molecular sensor of DNA damage or in close conjunction with such sensors. However, neither the mechanism by which the hMRE11-hRAD50-p95 complex comes into contact with DNA DSBs nor the nature of the signal elicited by their association with DSBs has been defined.

5.3.2. Ionizing Radiation-Induced Foci

Experiments utilizing conventional "hard" X-rays, in which IR-induced DNA damage is uniformly distributed, have also proven useful in defining the role of the hMRE11-hRAD50-p95 complex in mammalian DSB responses. Upon γ-irradiation, the complex redistributes into focal nuclear structures, termed ionizing radiation-induced foci (IRIF) *(64).* Immunofluorescence co-localization experiments have demonstrated that IRIF contain each of the complex members *(20,64),* providing further evidence that hMRE11, hRAD50, and p95 act in a complex during the cellular response to DNA damage.

A number of observations support the idea that IRIF formation reflects the function(s) of the hMRE11-hRAD50-p95 complex in the response to DSB induction. First, hMRE11-hRAD50-p95 IRIF are dependent on the prior induction of DSBs, and form in a dose-dependent manner *(64);* IRIF are not induced by other types of DNA damage. Second, hMRE11-hRAD50-p95 IRIF formation is dependent on the genetic background of the cells examined. The DSB repair-deficient human cell line, 180BR, exhibits increased IRIF formation at equivalent X-ray doses compared to normal repair-proficient cells *(64),* consistent with the interpretation that IRIF multiplicity is a function of the number of DSBs. hMRE11 and hRAD50 IRIF are not detected in p95-deficient human cells (cells from patients with NBS; Subheading 5.4.), which

reveals that although p95 is not important for hMRE11-hRAD50 interaction *(20),* it is important for this aspect of the complex's function. hMRE11 and hRAD50 IRIF formation is also profoundly reduced in SV40-transformed A-T mutant cell lines when compared to normal SV40-transformed cells *(64).* The hMRE11-hRAD50 IRIF response is reduced to a lesser extent in primary (non-SV40-transformed) A-T cells *(63),* indicating that SV40 transformation affects the behavior of the complex. Conversely, the lack of DNA-PK_{cs} or p53 had no effect on IRIF formation *(64),* suggesting that although these proteins also have roles in cellular DSB responses, their functions are independent of hMRE11, hRAD50, and p95.

These cytological assays have proven useful in assessing the relative functions of the hMRE11-hRAD50-p95 complex and hRad51, a protein that mediates DNA strand exchange *(5,32).* hRad51 forms foci following DNA damage, and also during S phase in un-irradiated cells *(33,101).* Interestingly, hRad51 nuclear foci also appear to contain the BRCA1 and BRCA2 proteins, supporting a role for these tumor-suppressor proteins in recombinational DNA repair (*21,92;* Chapter 10). hMRE11-hRAD50-p95 IRIF do not co-localize with hRad51 foci, nor are they coincident within the same nucleus *(64).* SV40-transformed A-T cells, which demonstrated reduced hMRE11-hRAD50 IRIF formation, exhibited markedly increased numbers of cells with hRad51 foci *(64).* The formation of both hMRE11 (hRAD50) and hRad51 foci is induced by DNA damage. The exclusivity of hMRE11-hRAD50-p95 complex IRIF and those of hRad51 presumably reflects the differing spatial and temporal requirements for these protein complexes in DSB repair. Hence, these cytological data are consistent with the prevailing models of DSB repair that situate the functions of Rad51 and the MRE11-RAD50-p95 complex at distinct points in the DSB repair process. Further experiments using these cytological assays will be useful in the analysis of the DSB response of various mutants of the hMRE11-hRAD50-p95 complex.

5.4. Clinical Considerations

The genomic instability resulting from disruption of the ScMre11p-ScRad50p-Xrs2p complex bears some resemblance to the cellular features of chromosome instability syndromes in humans. Thus it had been suggested that congenital or somatically acquired deficiencies in hMRE11-hRAD50-p95 complex members might be causative of such instability in human cells *(80).* Unlike yeast, null mutations in *mMre11* and *mRad50* are lethal in the mouse *(61,116);* null mutations in either are presumably lethal in humans as well. However, the hypothesis that human genetic instability can be caused by disrupting the hMRE11-hRAD50-p95 complex has been validated by the discovery that *NBS1* mutations cause NBS, a hereditary chromosome instability disorder *(20,66,111).* This extremely rare disease is characterized by developmental defects and a predisposition to malignancy (reviewed in *110*). At the cellular level, peripheral lymphocytes from NBS patients harbor characteristic chromosomal rearrangements. NBS cell lines are sensitive to IR and exhibit radioresistant DNA synthesis (RDS). These cellular features are similar to those of cell lines derived from patients with A-T, another chromosome instability and cancer-prone syndrome *(95).* NBS had been described as an A-T variant syndrome until it became clear that they were genetically separable *(95).* Nearly all NBS patients described to date are homozygous for the same mutant allele of the *NBS1* gene, a frameshift mutation predicted to truncate greater than

two-thirds of the C-terminus from p95 *(111)*. The cellular and clinical sequelae of p95 deficiency underscore the importance of the hMRE11-hRAD50-p95 complex in the maintenance of genomic integrity. An additional demonstration of human genomic instability caused by Mre11-Rad50-p95 deficiency has recently been documented by the identification of *hMre11* mutations in another A-T like syndrome *(98)*.

5.4.1. Cellular Phenotypes Associated With NBS

The cellular defects associated with p95 deficiency have revealed much about how the hMRE11-hRAD50-p95 complex may integrate the DSB repair response with cell-cycle control. First, NBS cell lines are sensitive to IR and radiomimetics, and display highly elevated numbers of chromosome aberrations following IR *(4,72,99)*. In spite of p95's association with hMRE11 and hRAD50, physical analysis of DSB repair in NBS cells does not indicate a profound defect in DSB rejoining *(72)*. The most significant defect in the DNA damage response of p95-deficient cell lines is the failure to arrest DNA synthesis in response to IR. This defect in S-phase regulation is also a hallmark of A-T cells. However, unlike A-T cells, NBS cells appear to retain the ability to arrest in G1 and G2, and do not share the profound deficiency in p53 induction following IR *(45,65,118)*. Characterization of the S-phase checkpoint defects in A-T cells and *S. cerevisiae mec1* mutants suggests that this mode of cell-cycle regulation is manifest in the inhibition of replication origin firing *(76,88,97)*. Similar analyses have not been carried out in NBS cells. Understanding the molecular biology of this S-phase checkpoint will be crucial to discerning the role of the hMRE11-hRAD50-p95 complex in its activation.

The cellular defects that result from a lack of p95, in combination with the previously described cytological, genetic, and biochemical data, clearly demonstrate that the MRE11-RAD50-p95 complex is an important mediator of the cellular DNA DSB response. The hMRE11-hRAD50-p95 complex is uniquely situated in this DNA damage response by the linkage of DNA repair proteins to a molecule that functions in an S-phase checkpoint. The hMRE11-hRAD50-p95 complex localizes to DNA DSBs (Subheading 5.3.); however, in the absence of p95, cells fail to respond appropriately to the presence of DSBs. Thus, it is conceivable that the hMRE11-hRAD50-p95 complex functions in the recognition of DNA DSBs or in close concert with the sensors of such damage. The phenotypic features of NBS cells further indicate that the complex is important for the activation of the S-phase cell-cycle checkpoint. The molecular basis of cell-cycle checkpoint activation by the complex is not understood. This unresolved question remains one of the most critical issues in understanding the integration of the MRE11-RAD50-p95 complex's functions within the cellular DNA damage response.

5.4.2. Clinical Manifestations of p95 Deficiency

The clinical findings associated with p95 deficiency are represented by patients with NBS. However, the syndrome is extremely rare—fewer than 100 patients have been described to date—and patients exhibit some clinical variability. The diminutive size and microcephaly of the patients are the most common and prominent outward manifestations, and mental retardation has been noted for certain patients. NBS patients also display a strong predisposition to malignancy. Of 42 patients in the NBS registry as of 1996, 15 patients (between 1 and 22 yr of age) had developed malignant tumors; 80% of these were lymphomas *(110)*. Chromosome rearrangements in peripheral lymphocytes are common and typically involve regions on chromosomes 7 and 14 at which antigen recep-

tor loci undergo programmed gene rearrangement. Certain immunoglobulin isotype deficiencies have also been reported. Although NBS is a recessive disorder, heterozygotes may exhibit some of the phenotypes, especially predisposition to cancer *(93).* A more detailed review of clinical findings in NBS patients can be found elsewhere *(110).*

5.4.3. Clinical Manifestations of hMre11 Mutations

The recent identification of non-null mutations in *hMre11* that result in an A-T like disorder (termed ATLD) *(98)* further validates the hypothesis that mutations affecting the Mre11-Rad50-p95 complex lead to genomic instability. The phenotypes of these patients were puzzling in that they exhibited many of the clinical hallmarks of A-T, but mutations in the *ATM* gene were not detected *(36,98).* The finding of hypomorphic *hmre11* alleles was a suprising result because these patients do not share clinical phenotypes typically associated with NBS. However, cell lines derived from ATLD patients more closely resemble NBS cells with respect to radiosensitivity, RDS, and p53 induction, than classical A-T cells *(98).* The phenotypes represented by ATLD and NBS strongly suggest that the functions of the hMRE11-hRAD50-p95 complex and ATM overlap to some extent. However, a more complete analysis of these functions in mammals awaits the creation of genetically defined mouse models of MRE11, p95, and RAD50 deficiency.

6. TOWARD A MODEL FOR THE FUNCTION OF THE MRE11-RAD50-p95 PROTEIN COMPLEX IN THE CELLULAR RESPONSE TO DNA DAMAGE

The diverse phenotypic outcomes imparted by deficiency in members of the MRE11-RAD50-p95 (Xrs2p) complex preclude a simple unifying model for its function. Although our current picture of this complex has been assembled from data obtained in both yeast and mammals, the extraordinary conservation of MRE11 and RAD50 suggests that many functions of the complex are likely to be conserved. The pleiotropy of mutations affecting the complex situate it at the heart of the cellular response to DSBs (Fig. 6). From this perspective, elucidation of the genetic and physical interactions that link this complex to the network of functions that constitute the cellular response to DSBs is a crucial next step. A clear molecular definition of the in vivo enzymatic functions of the MRE11-RAD50-p95 (Xrs2p) complex will also provide important insight to its direct roles in the DSB response.

Its impact on sister chromatid recombination and chromatin structure suggest that the MRE11-RAD50-p95 (Xrs2p) complex plays an important structural, as opposed to an enzymatic role in aspects of DNA recombination and repair. Whereas this interpretation is supported by genetic analysis in *S. cerevisiae,* the abundance of the human MRE11-RAD50-p95 complex similarly argues against a purely enzymatic role in human cells *(20,26).* The telomere maintenance functions of the complex may also reflect such a structural role, because the complex does not appear to function in telomere end protection nor does it directly influence telomerase activity *(73).* Mechanistic information regarding the telomere-shortening phenotype of *S. cerevisiae* mutants and determination of whether the mammalian complex also functions at telomeres are important to this issue.

The abrogation of S-phase checkpoint activation in p95-deficient cells is one of several lines of evidence supporting the idea that the complex also mediates regulatory functions in the cellular response to DSBs. These regulatory functions presumably follow from the

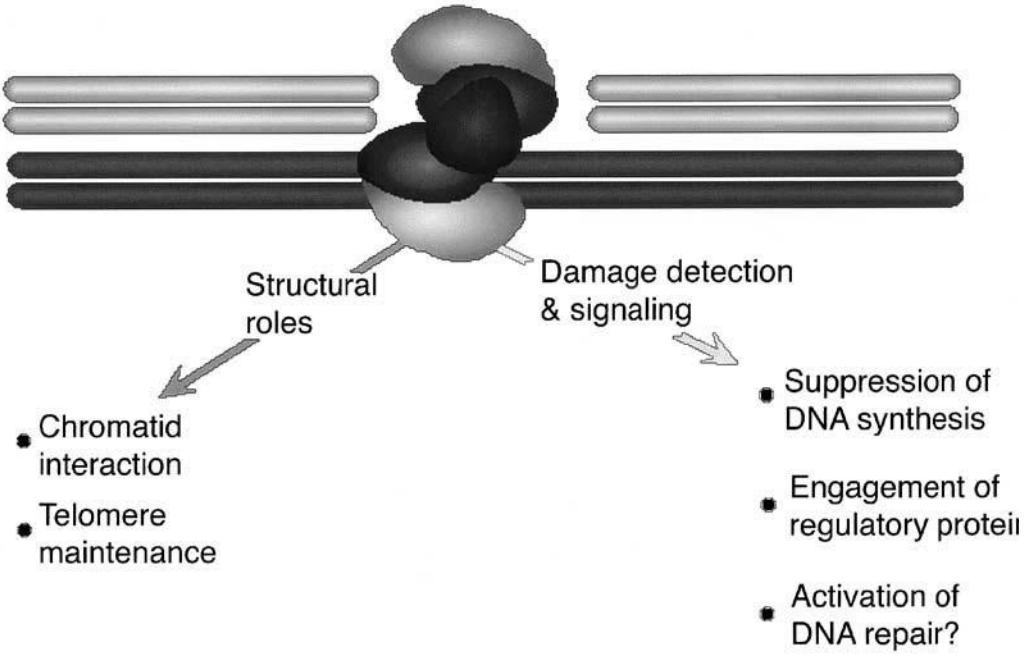

Fig. 6. Functions of the Mre11-Rad50-p95 (Xrs2p) protein complex in the DSB response. Based on cytological evidence, we hypothesize that the complex is situated at or near the site(s) of DSBs. Once at the site, the complex plays a structural role in facilitating the appropriate template utilization during the DSB repair process. Other structural roles for the complex may include its role in telomere maintenance. The phenotypic features of NBS cells indicate that once at the site of DNA damage, the complex is important for activating cell-cycle checkpoint responses, particularly those that lead to the suppression of DNA synthesis. Presumably, its role in cell-cycle checkpoint functions requires interaction between the complex and as yet unidentified regulatory proteins. Finally, we speculate that the signaling function of the complex may also lead to the activation of DNA repair, although evidence for this has not been established.

complex's DNA damage-recognition functions. What are the molecules downstream of the DNA damage recognition event? What is the molecular nature of the interaction between these molecules and the MRE11-RAD50-p95 complex? It is certainly clear that the complex interacts, either directly or indirectly, with proteins that mediate the S-phase checkpoint in human cells. Molecular characterization of this interaction will provide fundamental insight regarding the mechanisms of cell-cycle checkpoint activation.

Even in the absence of a comprehensive model for the complex's function, it is clear that MRE11, RAD50, and p95 (Xrs2p) play a fundamental role in the maintenance of genomic integrity. In the vast network of biochemical functions that must be integrated into the cell's response to genotoxic stress, the MRE11-RAD50-p95 (Xrs2p) complex occupies a critical hub at the interface of DNA repair pathways and the activation of cell-cycle checkpoints.

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REFERENCES

1. Ajimura, M., S. H. Leem, and H. Ogawa. 1993. Identification of new genes required for meiotic recombination in Saccharomyces cerevisiae. *Genetics* **133:** 51–66.
2. Alani, E., R. Padmore, and N. Kleckner. 1990. Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. *Cell* **61:** 419–436.
3. Albala, J. S., M. P. Thelen, C. Prange, W. Fan, M. Christensen, L. H. Thompson, and G. G. Lennon. 1997. Identification of a novel human RAD51 homolog, RAD51B. *Genomics* **46:** 476–479.
4. Antoccia, A., R. Ricordy, P. Maraschio, S. Prudente, and C. Tanzarella. 1997. Chromosomal sensitivity to clastogenic agents and cell cycle perturbations in Nijmegen breakage syndrome lymphoblastoid cell lines. *Int. J. Radiat. Biol.* **71:** 41–49.
5. Baumann, P., F. E. Benson, and S. C. West. 1996. Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. *Cell* **87:** 757–766.
6. Bergerat, A., B. de Massy, D. Gadelle, P. C. Varoutas, A. Nicolas, and P. Forterre. 1997. An atypical topoisomerase II from Archaea with implications for meiotic recombination [see comments]. *Nature* **386:** 414–417.
7. Besmer, E., J. Mansilla-Soto, S. Cassard, D. J. Sawchuk, G. Brown, M. Sadofsky, et al. 1998. Hairpin coding end opening is mediated by RAG1 and RAG2 proteins. *Mol. Cell* **2:** 817–828.
8. Bhargava, J., J. Engebrecht, and G. S. Roeder. 1992. The rec102 mutant of yeast is defective in meiotic recombination and chromosome synapsis. *Genetics* **130:** 59–69.
9. Bianco, P. R., R. B. Tracy, and S. C. Kowalczykowski. 1998. DNA strand exchange proteins: a biochemical and physical comparison. *Front. Biosci.* **3:** d570–603.
10. Bork, P., K. Hofmann, P. Bucher, A. F. Neuwald, S. F. Altschul, and E. V. Koonin. 1997. A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. *Faseb J.* **11:** 68–76.
11. Boulton, S. J., and S. P. Jackson. 1998. Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *EMBO J.* **17:** 1819–1828.
12. Boulton, S. J., and S. P. Jackson. 1996. Identification of a Saccharomyces cerevisiae Ku80 homologue: roles in DNA double strand break rejoining and in telomeric maintenance. *Nucleic Acids Res.* **24:** 4639–4648.
13. Boulton, S. J., and S. P. Jackson. 1996. Saccharomyces cerevisiae Ku70 potentiates illegitimate DNA double- strand break repair and serves as a barrier to error-prone DNA repair pathways. *Embo J.* **15:** 5093–5103.
14. Bressan, D. A., B. K. Baxter, and J. H. J. Petrini. 1999. The Mre11-Rad50-Xrs2 protein complex facilitates homologous recombination-based double-strand break repair in *Saccharomyces cerevisiae. Mol. Cell. Biol.* **19:** 7681–7687.
15. Bressan, D. A., H. A. Olivares, B. E. Nelms, and J. H. J. Petrini. 1998. Alteration of N-terminal phosphoesterase signature motifs inactivates *Saccharomyces cerevisiae* Mre11. *Genetics* **150:** 592–600.
16. Bressan, D. A., and J. H. J. Petrini. 1999. Unpublished results.
17. Bullard, S. A., S. Kim, A. M. Galbraith, and R. E. Malone. 1996. Double strand breaks at the HIS2 recombination hot spot in Saccharomyces cerevisiae. *Proc. Natl. Acad. Sci. USA* **93:** 13,054–13,059.
18. Callebaut, I., and J. P. Mornon. 1997. From BRCA1 to RAP1: a widespread BRCT module closely associated with DNA repair. *FEBS Lett.* **400:** 25–30.

19. Cao, L., E. Alani, and N. Kleckner. 1990. A pathway for generation and processing of double-strand breaks during meiotic recombination in S. cerevisiae. *Cell* **61:** 1089–1101.
20. Carney, J. P., R. S. Maser, H. Olivares, E. M. Davis, M. Le Beau, J. R. Yates, 3rd, et al. 1998. The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell* **93:** 477–486.
21. Chen, J., D. P. Silver, D. Walpita, S. B. Cantor, A. F. Gazdar, G. Tomlinson, et al. 1998. Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. *Mol. Cell* **2:** 317–328.
22. Connelly, J. C., E. S. de Leau, E. A. Okely, and D. R. Leach. 1997. Overexpression, purification, and characterization of the SbcCD protein from Escherichia coli. *J. Biol. Chem.* **272:** 19,819–19,826.
23. Connelly, J. C., and D. R. F. Leach. 1996. The sbcC and sbcD genes of Escherichia coli encode a nuclease involved in palindrome inviability and genetic recombination. *Genes Cells* **1:** 285–291.
24. Cox, M. M. 1998. A broadening view of recombinational DNA repair in bacteria. *Genes Cells* **3:** 65–78.
25. Critchlow, S. E., and S. P. Jackson. 1998. DNA end-joining: from yeast to man. *Trends Biochem. Sci.* **23:** 394–398.
26. Dolganov, G. M., R. S. Maser, A. Novikov, L. Tosto, S. Chong, D. A. Bressan, and J. H. J. Petrini. 1996. Human Rad50 is physically associated with hMrell: identification of a conserved multiprotein complex implicated in recombinational DNA repair. *Mol. Cell. Biol.* **16:** 4832–4841.
27. Furuse, M., Y. Nagase, H. Tsubouchi, K. Murakami-Murofushi, T. Shibata, and K. Ohta. 1998. Distinct roles of two separable in vitro activities of yeast Mrell in mitotic and meiotic recombination. *EMBO J.* **17:** 6412–6425.
28. Game, J. C. 1993. DNA double strand breaks and the *RAD50–RAD57* genes in *Saccharomyces. Cancer Biology* **4:** 73–83.
29. Game, J. C., and R. K. Mortimer. 1974. A genetic study of X-ray sensitive mutants in yeast. *Mutat. Res.* **24:** 281–292.
30. Goldberg, J., H. B. Huang, Y. G. Kwon, P. Greengard, A. C. Nairn, and J. Kuriyan. 1995. Three-dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1. *Nature* **376:** 745–753.
31. Griffith, J. P., J. L. Kim, E. E. Kim, M. D. Sintchak, J. A. Thomson, M. J. Fitzgibbon, et al. 1995. X-ray structure of calcineurin inhibited by the immunophilin-immunosuppressant FKBP12–FK506 complex. *Cell* **82:** 507–522.
32. Gupta, R. C., L. R. Bazemore, E. I. Golub, and C. M. Radding. 1997. Activities of human recombination protein Rad51. *Proc. Natl. Acad. Sci. USA* **94:** 463–468.
33. Haaf, T., E. I. Golub, G. Reddy, C. M. Radding, and D. C. Ward. 1995. Nuclear foci of mammalian Rad51 recombination protein in somatic cells after DNA damage and its localization in synaptonemal complexes. *Proc. Natl. Acad. Sci. USA* **92:** 2298–2302.
34. Haber, J. E. 1995. In vivo biochemistry: physical monitoring of recombination induced by site-specific endonucleases. *Bioessays* **17:** 609–620.
35. Haber, J. E. 1998. The many interfaces of Mre11. *Cell* **95:** 583–586.
36. Hernandez, D., C. M. McConville, M. Stacey, C. G. Woods, M. M. Brown, P. Shutt, et al. 1993. A family showing no evidence of linkage between the ataxia telangiectasia gene and chromosome 11q22–23. *J. Med. Genet.* **30:** 135–140.
37. Hogan, B. 1994. *Manipulating the Mouse Embryo: A Laboratory Manual,* 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
38. Ivanov, E. L., and J. E. Haber. 1997. DNA repair: RAD *alert. Curr. Biol.* **7:** R492–R495.
39. Ivanov, E. L., and J. E. Haber. 1995. RAD1 and RAD10, but not other excision repair genes, are required for double-strand break-induced recombination in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **15:** 2245–2251.

40. Ivanov, E. L., V. G. Korolev, and F. Fabre. 1992. *XRS2,* a DNA repair gene of *Saccharomyces cerevisiae,* is needed for meiotic recombination. *Genetics* **132:** 651–664.
41. Ivanov, E. L., N. Sugawara, J. Fishman-Lobell, and J. E. Haber. 1996. Genetic requirements for the single-strand annealing pathway of double-strand break repair in *Saccharomyces cerevisiae. Genetics* **142:** 693–704.
42. Ivanov, E. L., N. Sugawara, C. I. White, F. Fabre, and J. E. Haber. 1994. Mutations in XRS2 and RAD50 delay but do not prevent mating-type switching in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **14:** 3414–3425.
43. Jeggo, P. A., G. E. Taccioli, and S. P. Jackson. 1995. Menage a trois: double strand break repair, V(D)J recombination and DNA-PK. *Bioessays* **17:** 949–957.
44. Johzuka, K., and H. Ogawa. 1995. Interaction of Mre11 and Rad50: Two proteins required for DNA repair and Meiosis-specific double-strand break formation in *Saccharamoyces cerevisiae. Genetics* **139:** 1521–1532.
45. Jongmans, W., M. Vuillaume, K. Chrzanowska, D. Smeets, K. Sperling, and J. Hall. 1997. Nijmegen breakage syndrome cells fail to induce the p53-mediated DNA damage response following exposure to ionizing radiation. *Mol. Cell. Biol.* **17:** 5016–5022.
46. Kanaar, R., and J. H. J. Hoeijmakers. 1997. Recombination and joining: different means to the same ends. *Genes Function* **1:** 165–174.
47. Keeney, S., C. N. Giroux, and N. Kleckner. 1997. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* **88:** 375–384.
48. Keeney, S., and N. Kleckner. 1995. Covalent protein-DNA complexes at the 5′ strand termini of meiosis- specific double-strand breaks in yeast. *Proc. Natl. Acad. Sci. USA* **92:** 11,274–11,278.
49. Kirchgessner, C. U., C. K. Patil, J. W. Evans, C. A. Cuomo, L. M. Fried, T. Carter, et al. 1995. DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect. *Science* **267:** 1178–1183.
50. Kironmai, K. M., and K. Muniyappa. 1997. Alteration of telomeric sequences and senescence caused by mutations in RAD50 of Saccharomyces cerevisiae. *Genes Cells* **2:** 443–455.
51. Klapholz, S., and R. E. Esposito. 1980. Recombination and chromosome segregation in during the single division meosis in *spo12-1* and *spo13-1* diploids. *Genetics* **96:** 589–611.
52. Kleckner, N. 1996. Meiosis: how could it work? *Proc. Natl. Acad. Sci. USA* **93:** 8167–8174.
53. Le Beau, M. 1999. personal communication.
54. Leach, D. R. 1994. Long DNA palindromes, cruciform structures, genetic instability and secondary structure repair. *Bioessays* **16:** 893–900.
55. Leach, D. R. F., R. G. Lloyd, and A. F. W. Coulson. 1992. The SbcCD protein of Escherichia coli is related to two putative nucleases in the UvrA superfamily of nucleotide-binding proteins. *Genetica* **87:** 95–100.
56. Lee, S. E., J. K. Moore, A. Holmes, K. Umezu, R. D. Kolodner, and J. E. Haber. 1998. Saccharomyces Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* **94:** 399–409.
57. Lewis, S. M. 1994. P nucleotides, hairpin DNA and V (D) J joining: making the connection. *Semin. Immunol.* **6:** 131–141.
58. Lichten, M., and A. S. Goldman. 1995. Meiotic recombination hotspots. *Annu. Rev. Genet.* **29:** 423–444.
59. Liu, N., J. E. Lamerdin, R. S. Tebbs, D. Schild, J. D. Tucker, M. R. Shen, et al. 1998. XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol. Cell* **1:** 783–793.
60. Liu, N., J. E. Lamerdin, J. D. Tucker, Z. Q. Zhou, C. A. Walter, J. S. Albala, et al. 1997. The human XRCC9 gene corrects chromosomal instability and mutagen sensitivities in CHO UV40 cells. *Proc. Natl. Acad. Sci. USA* **94:** 9232–9237.
61. Luo, G., M. S. Yao, C. F. Bender, M. Mills, A. R. Bladl, A. Bradley, and J. H. Petrini. 1999. Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc. Natl. Acad. Sci. USA* **96:** 7376–7381.

62. Malone, R. E., T. Ward, S. Lin, and J. Waring. 1990. The *RAD50* gene, a member of the double strand break repair epistasis group, is not required for spontaneous mitotic recombination in yeast. *Curr. Genet.* **18:** 111–116.
63. Maser, R., and J. H. J. Petrini. 1999. Unpublished results.
64. Maser, R. S., K. J. Monsen, B. E. Nelms, and J. H. J. Petrini. 1997. hMre11 and hRad50 nuclear foci are induced during the normal cellular response to DNA double strand breaks. *Mol. Cell. Biol.* **17:** 6087–6096.
65. Matsuura, K., T. Balmukhanov, H. Tauchi, C. Weemaes, D. Smeets, K. Chrzanowska, et al. 1998. Radiation induction of p53 in cells from Nijmegen breakage syndrome is defective but not similar to ataxia-telangiectasia. *Biochem. Biophys. Res. Commun.* **242:** 602–607.
66. Matsuura, S., H. Tauchi, A. Nakamura, N. Kondo, S. Sakamoto, S. Endo, et al. 1998. Positional cloning of the gene for Nijmegen breakage syndrome. *Nature Genet.* **19:** 179–181.
67. Milne, G. T., S. Jin, K. B. Shannon, and D. T. Weaver. 1996. Mutations in two Ku homologs define a DNA end-joining repair pathway in *Saccharomyces cerevisiae. Mol. Cell. Biol.* **16:** 4189–4198.
68. Moore, J. K., and J. E. Haber. 1996. Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **16:** 2164–2173.
69. Moreau, S., J. R. Ferguson, and L. S. Symington. 1999. The nuclease activity of mre11 is required for meiosis but not for mating type switching, end joining, or telomere maintenance. *Mol. Cell. Biol.* **19:** 556–566.
70. Nairz, K., and F. Klein. 1997. mre11s: a yeast mutation that blocks double-strand-break processing and permits nonhomologous synapsis in meiosis. *Genes Dev.* **11:** 2272–2290.
71. Nelms, B. E., R. S. Maser, J. F. MacKay, M. G. Lagally, and J. H. J. Petrini. 1998. In situ visualization of DNA double-strand break repair in human fibroblasts. *Science* **280:** 590–592.
72. Nove, J., J. B. Little, P. J. Mayer, P. Troilo, and W. W. Nichols. 1986. Hypersensitivity of cells from a new chromosomal-breakage syndrome to DNA-damaging agents. *Mutat. Res.* **163:** 255–262.
73. Nugent, C. I., G. Bosco, L. O. Ross, S. K. Evans, A. P. Salinger, J. K. Moore, et al. 1998. Telomere maintenance is dependent on activities required for end repair of double-strand breaks. *Curr. Biol.* **8:** 657–660.
74. Ogawa, H., K. Johzuka, T. Nakagawa, S. Leem, and A. Hagihara. 1995. Functions of the yeast meiotic recombination genes, MRE11 and MRE2. *Adv. Biophys.* **31:** 67–76.
75. Ohta, K., A. Nicolas, M. Furuse, A. Nabetani, H. Ogawa, and T. Shibata. 1998. Mutations in the MRE11, RAD50, XRS2, and MRE2 genes alter chromatin configuration at meiotic DNA double-stranded break sites in premeiotic and meiotic cells. *Proc. Natl. Acad. Sci. USA* **95:** 646–651.
76. Painter, R. B. 1981. Radioresistant DNA synthesis: an intrinsic feature of ataxia telangiectasia. *Mutat. Res.* **84:** 183–190.
77. Paull, T. T., and M. Gellert. 1998. The 3′ to 5′ exonuclease activity of Mre 11 facilitates repair of DNA double-strand breaks. *Mol. Cell* **1:** 969–979.
78. Paulovich, A. G., D. P. Toczyski, and L. H. Hartwell. 1997. When checkpoints fail. *Cell* **88:** 315–321.
79. Peterson, S. R., A. Kurimasa, M. Oshimura, W. S. Dynan, E. M. Bradbury, and D. J. Chen. 1995. Loss of the catalytic subunit of the DNA-dependent protein kinase in DNA double-strand-break-repair mutant mammalian cells. *Proc. Natl. Acad. Sci. USA* **92:** 3171–3174.
80. Petrini, J. H., D. A. Bressan, and M. S. Yao. 1997. The RAD52 epistasis group in mammalian double strand break repair. *Semin. Immunol.* **9:** 181–188.
81. Petrini, J. H. J., M. E. Walsh, C. Di Mare, J. R. Korenberg, X. -N. Chen, and D. T. Weaver. 1995. Isolation and Characterization of the Human MRE11 homologue. *Genomics* **29:** 80–86.
82. Porter, S. E., P. W. Greenwell, K. B. Ritchie, and T. D. Petes. 1996. The DNA-binding protein Hdf1p (a putative Ku homologue) is required for maintaining normal telomere length in Saccharomyces cerevisiae. *Nucleic Acids Res.* **24:** 582–585.

83. Rathmell, W. K., and G. Chu. 1994. Involvement of the Ku autoantigen in the cellular response to DNA double-strand breaks. *Proc. Natl. Acad. Sci. USA* **91:** 7623–7627.
84. Raymond, W. E., and N. Kleckner. 1993. RAD50 protein of S. cerevisiae exhibits ATP-dependent DNA binding. *Nucleic Acids Res.* **21:** 3851–3856.
85. Riballo, E., S. E. Critchlow, S.-H. Teo, A. J. Doherty, A. Priestley, B. Broughton, et al. 1999. Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. *Curr. Biol.* **9:** 699–702.
86. Roeder, G. S. 1997. Meiotic chromosomes: it takes two to tango. *Genes Dev.* **11:** 2600–2621.
87. Saeki, T., I. Machida, and S. Nakai. 1980. Genetic control of diploid recovery after γ-irradiation in the yeast *Saccharomyces cerevisiae. Mutat. Res.* **73:** 251–265.
88. Santocanale, C., and J. F. Diffley. 1998. A Mec1- and Rad53-dependent checkpoint controls late-firing origins of DNA replication. *Nature* **395:** 615–618.
89. Schar, P., G. Herrmann, G. Daly, and T. Lindahl. 1997. A newly identified DNA ligase of Saccharomyces cerevisiae involved in RAD52-independent repair of DNA double-strand breaks. *Genes Dev.* **11:** 1912–1924.
90. Schiestl, R. H., and T. D. Petes. 1991. Integration of DNA fragments by illegitimate recombination in Saccharomyces cerevisiae. *Proc. Natl. Acad. Sci. USA* **88:** 7585–7589.
91. Schiestl, R. H., J. Zhu, and T. D. Petes. 1994. Effect of mutations in genes affecting homologous recombination on restriction enzyme-mediated and illegitimate recombination in *Saccharomyces cerevisae. Mol. Cell. Biol.* **14:** 4495–4500.
92. Scully, R., J. Chen, A. Plug, Y. Xiao, D. Weaver, J. Feunteun, et al. 1997. Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell* **88:** 265–275.
93. Seemanova, E. 1990. An increased risk for malignant neoplasms in heterozygotes for a syndrome of microcephaly, normal intelligence, growth retardation, remarkable facies, immunodeficiency and chromosomal instability. *Mutat. Res.* **238:** 321–324.
94. Sharples, G. J., and D. R. Leach. 1995. Structural and functional similarities between the SbcCD proteins of Escherichia coli and the RAD50 and MRE11 (RAD32) recombination and repair proteins of yeast. *Mol. Microbiol.* **17:** 1215–1217.
95. Shiloh, Y. 1997. Ataxia-telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart. *Annu. Rev. Genet.* **31:** 635–662.
96. Shinohara, A., and T. Ogawa. 1995. Homologous recombination and the roles of double-strand breaks. *Trends Biochem. Sci.* **20:** 387–391.
97. Shirahige, K., Y. Hori, K. Shiraishi, M. Yamashita, K. Takahashi, C. Obuse, et al. 1998. Regulation of DNA-replication origins during cell-cycle progression. *Nature* **395:** 618–621.
98. Stewart, G. S., R. S. Maser, T. Stankovic, D. A. Bressan, M. I. Kaplan, N. G. J. Jaspers, et al. 1999. The DNA double strand break repair gene *hMre11* is mutated in individuals with an ataxia telangiectasia-like disorder (ATLD). *Cell* **99:** 577–587.
99. Taalman, R. D., N. G. Jaspers, J. M. Scheres, J. de Wit, and T. W. Hustinx. 1983. Hypersensitivity to ionizing radiation, in vitro, in a new chromosomal breakage disorder, the Nijmegen Breakage Syndrome. *Mutat. Res.* **112:** 23–32.
100. Taccioli, G. E., T. M. Gottlieb, T. Blunt, A. Priestley, J. Demengeot, R. Mizuta, et al. 1994. Ku80: Product of the *XRCC5* gene and its role in DNA repair and V (D) J recombination. *Science* **265:** 1442–1445.
101. Tashiro, S., N. Kotomura, A. Shinohara, K. Tanaka, K. Ueda, and N. Kamada. 1996. S phase specific formation of the human Rad51 protein nuclear foci in lymphocytes. *Oncogene* **12:** 2165–2170.
102. Teo, S. H., and S. P. Jackson. 1997. Identification of Saccharomyces cerevisiae DNA ligase IV: involvement in DNA double-strand break repair. *EMBO J.* **16:** 4788–4795.
103. Thompson, L. H., K. W. Brookman, N. J. Jones, S. A. Allen, and A. V. Carrano. 1990. Molecular cloning of the human *XRCC1* gene, which corrects defective DNA strand break repair and sister chromatid exchange. *Mol. Cell. Biol.* **10:** 6160–6171.

104. Toczyski, D. P., D. J. Galgoczy, and L. H. Hartwell. 1997. CDC5 and CKII control adaptation to the yeast DNA damage checkpoint. *Cell* **90:** 1097–1106.
105. Trujillo, K. M., S. S. F. Yuan, E. Lee, and P. Sung. 1998. Nuclease activities in a complex of human recombination and DNA repair factors rad50, mre11, and p95 [In Process Citation]. *J. Biol. Chem.* **273:** 21,447–21,450.
106. Tsubouchi, H., and H. Ogawa. 1998. A novel mre11 mutation impairs processing of double-strand breaks of DNA during both mitosis and meiosis. *Mol. Cell. Biol.* **18:** 260–268.
107. Tsukamoto, Y., and H. Ikeda. 1998. Double-strand break repair mediated by DNA end-joining. *Genes Cells* **3:** 135–144.
108. Tsukamoto, Y., J. Kato, and H. Ikeda. 1997. Budding yeast Rad50, Mre11, Xrs2, and Hdf1, but not Rad52, are involved in the formation of deletions on a dicentric plasmid. *Mol. Gen. Genet.* **255:** 543–547.
109. Usui, T., T. Ohta, H. Oshiumi, J. Tomizawa, H. Ogawa, and T. Ogawa. 1998. Complex formation and functional versatility of Mre11 of budding yeast in recombination. *Cell* **95:** 705–716.
110. van der Burgt, I., K. H. Chrzanowska, D. Smeets, and C. Weemaes. 1996. Nijmegen breakage syndrome. *J. Med. Genet.* **33:** 153–156.
111. Varon, R., C. Vissinga, M. Platzer, K. M. Cerosaletti, K. H. Chrzanowska, K. Saar, et al. 1998. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell* **93:** 467–476.
112. Weaver, D. T. 1995. What to do at an end: DNA double-strand break repair. *Trends Genet.* **11:** 388–392.
113. Weiner, B. M., and N. Kleckner. 1994. Chromosome pairing via multiple interstitial interactions before and during meiosis in yeast. *Cell* **77:** 977–991.
114. White, C. I., and J. E. Haber. 1990. Intermediates of recombination during mating type switching in Saccharomyces cerevisiae. *EMBO J.* **9:** 663–673.
115. Wilson, T. E., U. Grawunder, and M. R. Lieber. 1997. Yeast DNA ligase IV mediates non-homologous DNA end joining. *Nature* **388:** 495–498.
116. Xiao, Y., and D. T. Weaver. 1997. Conditional gene targeted deletion by Cre recombinase demonstrates the requirement for the double-strand break repair Mre11 protein in murine embryonic stem cells. *Nucleic Acids Res.* **25:** 2985–2991.
117. Yamaguchi-Iwai, Y., E. Sonoda, M. S. Sasaki, C. Morrison, T. Haraguchi, Y. Hiraoka, et al. 1999. Mre11 is essential for the maintenance of chromosomal DNA in vertebrate cells. *EMBO J.* **18:** 6619–6629.
118. Yamazaki, V., R. D. Wegner, and C. U. Kirchgessner. 1998. Characterization of cell cycle checkpoint responses after ionizing radiation in Nijmegen breakage syndrome cells. *Cancer Res.* **58:** 2316–2322.
119. Zhuo, S., J. C. Clemens, D. J. Hakes, D. Barford, and J. E. Dixon. 1993. Expression, purification, crystallization, and biochemical characterization of a recombinant protein phosphatase. *J. Biol. Chem.* **268:** 17,754–17,761.
120. Zhuo, S., J. C. Clemens, R. L. Stone, and J. E. Dixon. 1994. Mutational analysis of a Ser/Thr phosphatase. *J. Biol. Chem.* **269:** 26,234–26,238.

8

Repair of DNA Double-Strand Breaks and Mismatches in *Drosophila*

Carlos C. Flores

1. SCOPE

This review focuses on recent progress in the study of long-patch mismatch repair (MMR) and double-strand break repair (DSBR) in *Drosophila melanogaster.* Some excellent reviews that overlap these subjects have been published recently *(52,64,92,154).* A web site summarizing the DNA repair genes of *Drosophila* has been assembled *(24).* Also, FlyBase *(45)* and the Berkeley *Drosophila* Genome Project (BDGP) *(6)* are superb resources for information on most aspects of Drosophila research. For comprehensive reviews on how meiotic recombination is intertwined with the progression of oocyte development, *see* Morris and Lehmann *(116)* and Gonzalez-Reyes *(56).* Insightful reviews of other types of DNA repair in Drosophila have also been published *(39,64,92,124,154).*

2. OVERVIEW

Historically, *Drosophila* research has greatly increased our knowledge of DNA repair and recombination. For example, pioneering studies of the effects of X-rays on chromosomes *(118)* and the detailed cytogenetics of meiotic recombination *(26)* were performed in fruitflies. *Drosophila* research in general, and DNA repair studies in particular, are poised for a renaissance. The publicly funded genome projects (primarily BDGP), in conjunction with Celera, announced that the genome sequence is nearly complete *(1).* This project also produced a wealth of accompanying resources: thousands of cDNAs, genomic clones, and P element insertion lines. A promising new gene-targeting method was developed, which not only may make it easier to mutate genes at will, but also may become a valuable additional tool to investigate recombinational repair *(141).* Techniques to study the repair of DNA injected into embryos have been refined. And with the accumulation of nearly a century of data, mutants, and genetic tools, such as deletions and balancer chromosomes, along with the ease of mutational screens, and methods for producing somatic clones, *Drosophila* remains an unparalleled genetic powerhouse.

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In the past decade studies in yeast and mice have dominated in the accelerating field of DNA repair. So what has *Drosophila* contributed to this juggernaut? Flies have furnished several components including some strange surprises. For example, *Drosophila* has fueled the re-evaluation of homologous recombination (HR) mechanisms that do not rely on Holliday-junction cleavage *(120).* Studies in *Drosophila* also produced a curious mystery: during repair of a DSB, there can be a cis-bias in the use of repair templates that extends over many megabases *(41).* It is puzzling how co-linearity can be evaluated over such a long linear distance within the relatively small nucleus. Flies were also first to furnish an interesting link between nucleotide-excision repair (NER), MMR, and meiotic recombination, by analysis of the *mei-9* gene *(155).* Other Drosophila studies have uncovered an association between regulation of oocyte development and meiotic recombination involving spindle-class genes with roles in DSBR *(49).*

3. MISMATCH REPAIR IN DROSOPHILA

3.1. Early Steps of Mismatch Repair

Mismatched bases can arise in the DNA duplex by several mechanisms, though probably they most often occur as a result of DNA replication errors. In all organisms a major pathway to repair these errors is the long-patch MMR system. MMR is studied in humans and several model organisms but is most thoroughly understood in *Escherichia coli* and secondly *Saccharomyces cerevisiae.* In *E. coli,* base-pair mismatches and small insertions or deletions are recognized by a homodimer of MutS protein. MutS binds to these structures and recruits other proteins, including MutL, needed to resolve the contradiction. The complex can identify which strand to repair by sensing the methylation state of both strands. A region of up to a few kilobases is removed from the "mutant" (under-methylated) strand by exonuclease activity. DNA polymerase then replaces the sequence, thereby resolving the mismatch (reviewed in *22,140*). In all organisms this process employs homologs of the *E. coli mutS* and *mutL* genes *(22,32,85,100).*

In eukaryotes the method of strand discrimination is still largely a mystery. The most popular explanation is that a pre-existing nick is usually present and that the broken strand is targeted for degradation and correction. The most commonly considered sites of MMR are newly replicated DNA and HR intermediates. Pre-existing nicks would be present near the replication fork and are strongly predicted in Holliday structures and newly resolved Holliday structures of recombination intermediates. But mismatches can also arise through chemical damage to DNA, and it is not clear whether there is a nick-independent method of strand discrimination for long-patch MMR.

Replication can give rise to mismatches either by misincorporation or by polymerase slippage in regions of sequence repeats. Extensive repeats of very short DNA sequences (microsatellites) are highly vulnerable to mutation in the absence of MMR *(158,163).* Changes in the number of repeats are easily detected by analyzing the size of polymerase chain reaction (PCR) products. Through this hallmark of repeat instability, MMR was discovered to be important in preventing human cancer. People who inherit a mutation in the *mutS* or *mutL* homologs *hMSH2, hMLH1,* (and to a lesser degree, *hMSH6, hPMS1,* or *hPMS2*) have a greatly increased risk of certain cancers, especially hereditary nonpolyposis colon cancer (HNPCC) *(42,95,123,132).* Simple DNA repeats are extremely unstable in the cells of these cancers and in some sporadic cancers (reviewed in *22,103,139).*

Table 1
MutS and MutH Homologs Identified in Yeast, Humans, and Drosophila

S. cerevisiae[a]	Function[b]	H. sapiens[c]	*D. melanogaster*[c]
MSH1	9	?	None
MSH2	1, 2, 3, 6, 7, 8, 10	*hMSH2* 40% (966 a.a.)	*spel1* 35% (909 a.a.)
MSH3	3, 6, 7, 8, 11	*Dupl (hMSH3)* 34% (993 a.a.)	None
MSH4	6, 7, 8, 12	*hMSH4* 32% (776 a.a.)	None
MSH5	6, 7, 12	*hMSH5* 29% (650 a.a.)	None
MSH6	1, 2, 6, 11	*hMSH6* 32% (1172 a.a.)	*Dmmsh6* 25% (1044 a.a.)
MLH1	1, 2, 5, 8, 13	*hMLH1* 36% (787 a.a.)	*Dmmlh1* 35% (771 a.a.)
PMS1	1, 2, 3, 6, 14	*hPMS1* 30% (336a.a)	None
?	?	*hPMS2* 33% (904 a.a.)	*Dmpms2* 33% (929 a.a.)
MLH2	8, 14	?	None
MLH3	3, 4, 5, 14	*hMLH3* 24% (374a.a.)	None

[a] Name of *S. cerevisiae* gene.

[b] Function of characterized yeast and/or human protein. 1, Repair of base:base mismatches; 2, Repair of small-loop mismatches; 3, Repair of larger-loop mismatches; 4, Required for normal levels of meiotic recombination, 5, Required for normal levels of meiotic reciprocal exchange; 6, Required for heteroduplex rejection; 7, Required for trimming nonhomologous single-stranded ends; 8, Required for resistance to certain DNA damaging agents; 9, Mitochondrial DNA MMR; 10, Forms dimer with either MSH3 or MSH6; 11, Forms dimer with MSH2; 12, Forms an MSH4/MSH5 dimer; 13, Forms dimers with PMS1, MLH2, and MLH3; 14, Forms dimer with MLH1.

[c] Name of homologous gene/percent amino acid identity (length of aligned region). ?: no homolog identified; None: apparently no homolog exists. Alignments were performed by the algorithm of Altschul et al. *(2a)* with the BLOSUM62 identity matrix and gap penalty of 11, gap extension penalty of 1.

3.2. Drosophila MMR Genes

The MMR system of Drosophila appears less complex than either the human or yeast system. Certainly, the contingent of recognizable MMR genes is smaller in flies. Although *S. cerevisiae* has at least six MutS homologs *(MSH1-6),* and at least five exist in humans, only two MSH genes have been discovered in flies. Also, *Drosophila* seems to have only two MutL homologs (MLH) compared to four in yeast, and four or more in humans (Table 1). This is somewhat similar to the case in the nematode, *Caenorhabditis elegans,* but it is curious that the yeast system correlates more closely to humans. Even more surprising is the apparent absence of the meiosis-specific MSH genes in Drosophila (*MSH4* and *MSH5*) that are present in yeast, nematode, and human. Although some MSH and MLH genes of yeast and humans appear to be partially redundant for particular phenotypes, none are completely redundant. The absence of MSH1, MSH3, MSH4, MSH5, and MLH2 and MLH3 in flies can be rationalized in at least three ways: (1) Drosophila does not require the specialized functions of the missing genes; (2) unrelated proteins provide those specialized functions; or (3) the genes extant in Drosophila produce broader activities that encompass the specialized functions (the production of multiple proteins from a single gene may also play a role). Some combination of the latter two possibilities may be the most likely scenario.

Three Drosophila MMR genes have been cloned using sequence similarity: two MLH genes, *Dmlh1* and *Dpms2,* and the MSH gene *spellchecker1 (spel1).* More recently, an *msh6* gene has been identified within the sequence produced by the BDGP.

These genes that participate in the earliest steps of MMR are highly conserved and easy to identify by sequence similarity alone. The other genes involved in MMR can be less obvious because they include more generally employed DNA helicases, SS-exonucleases, DNA polymerase, and DNA ligase.

3.2.1. spel1

The *spellchecker1* gene was isolated by virtue of its similarity to other MSH genes using degenerate PCR. Its sequence indicates that it is a member of the MSH2 branch of the family. In yeast and humans, *msh2* is required for virtually all MMR in the nucleus. Null *spel1* mutant Drosophila were assembled using a pair of chromosomes bearing unique, large deletions. The deletions only overlap in a small region containing *spel1* and an adjacent gene known as *lethal(2)35Aa.* Because the adjacent gene is required for survival, a cloned copy was supplied as a transgene *(43).*

The *spel1* mutants created in this way are viable and fertile. DSBR does not appear grossly hindered in *spel1* mutants because they are not significantly more sensitive to γ-irradiation. Neither are they affected in their sensitivity to methylmethane sulfonate (MMS) *(43).* In contrast, *msh2* mutant human cancer cell lines and mouse cells have an increased tolerance to simple methylating agents *(19,23).* Presumably, in the mammalian case, the intact MMR system recognizes the damaged DNA but cannot complete repair. Instead it converts the methylated bases into a lesion that is more lethal and/or transmits a signal leading to cell-cycle arrest and apoptosis.

In *E. coli,* yeast and humans, short DNA repeats (microsatellites) are highly vulnerable to mutation in the absence of MMR *(42,158,163).* Microsatellites are also very unstable in *spel1* mutant flies. Chromosomes were analyzed after passage through 12 fly generations without SPEL1. One of the most unstable microsatellite loci analyzed had detectable mutations in over 25% of the tested chromosomes. No mutations were detected at that same locus in a hemizygous $spel1^{+/-}$ background (0 out of 192 chromosomes) *(43).* Loss of SPEL1 also destabilizes microsatellite repeats during the process of gene conversion. In this experiment, gene conversion was triggered by induction of a DSB in the parental germline. The products of conversion were recovered in the progeny and their structure was analyzed. The fidelity of copying a dinucleotide repeat from the homolog was fivefold higher in controls than in *spel1* mutants *(43).*

3.2.2. Dmmlh1 and Dmpms2

Drosophila homologs of *MLH1* and *PMS2* genes have been cloned and sequenced. Their genomic locations have also been identified, but as yet no mutants are known to exist. *Dmlh1* mRNA is abundant in ovaries and embryos and is present at lower levels in later stages. *Dpms2* mRNA appears to be expressed more uniformly throughout development. Curiously, *Dpms2* appears to have sex-specific transcriptional start-points or differential splicing such that a 5′ segment is found in transcripts from male larvae and male adults but not from females *(18,108,109,160).*

3.2.3. Tosca

The *tosca (tos)* gene encodes a putative exonuclease in flies *(34).* Its amino acid sequence establishes it as a member the XPG/Rad2 superfamily of endo/exonucleases. It is sufficiently related to *S. pombe,* mouse, and human EXO1 that it is probably the Drosophila *exo1* homolog. However, it appears that the expression of *tos* is completely

restricted to the female germline and early embryo. Early in oogenesis, *tos* mRNA is enriched in the pro-oocyte at a time that coincides with pachytene, implying that TOS may play a role in meiotic recombination. (Note that meiotic crossing-over occurs only in female Drosophila, not in males.) Later, *tos* mRNA is very abundant during the extremely rapid nuclear divisions of the early embryo, suggesting an additional role during this extraordinary DNA replication. MMR has been proposed as a possible role of TOS at this stage *(34).* This notion is supported by evidence that *exo1* of *S. pombe* has a role in MMR as demonstrated by genetic analysis *(146),* and the human EXO1 protein binds to MSH2 *(150).* Murine *exo1* is highly expressed in the testes and is especially abundant during the stage at which meiotic recombination occurs *(96).* Levels of EXO1 are also high in the lymphoid tissues of the mouse, especially the spleen. If *tosca* is the main exonuclease of MMR in flies, it is hard to explain why its expression is undetectable after early embryonic development and absent in males. It is possible that TOS has a partially redundant role in MMR.

3.2.4. mei-9

On the surface, MMR in flies may seem to fit the simple paradigm worked out in *E. coli,* with a few minor modifications, e.g., SPEL1/MSH6 heterodimer replacing MutS, and a MLH1/PMS2 heterodimer replacing MutL. However, it is likely to be more complex, as illustrated by *mei-9.* The *mei-9* gene was first identified almost 30 years ago in a screen for mutants defective in meiotic recombination. It was originally found that mutations in *mei-9* reduced meiotic crossing-over to less than 10% of the normal level *(3).* Later, *mei-9* mutants were isolated in screens for mutagen sensitivity. Through these studies it was discovered that they were very hypersensitive to ionizing radiation, MMS, nitrogen mustard, 2-acetylaminofluorence (AAF), and ultraviolet (UV) irradiation *(15,151).* Furthermore, *mei-9* mutants were found to be deficient in the repair of UV-induced pyrimidine dimers *(38).* This block in NER proved to be at some stage before strand cleavage *(4,15).*

Meanwhile, evidence that *mei-9* might also be involved in MMR surfaced when it was discovered that even though the frequency of meiotic gene conversion was unaffected, *mei-9* mutants exhibited postmeiotic segregation (PMS) *(28).* During meiotic recombination, heteroduplex is formed at the sites of exchange. Normally it is repaired before mature gametes are formed, but any heteroduplex that fails to be corrected will generate mosaic offspring. With PMS, the two maternal (or two paternal) alleles represented in the heteroduplex begin segregating at the first zygotic division to create an individual that is a patchwork of the two genotypes. Elevated levels of PMS indicate a defect in MMR during meiotic recombination.

No other genes were known to serve such central roles in NER, MMR, and HR. When the *mei-9* gene was cloned and analyzed, it became clear that it was related to the NER incision enzymes Rad1p of yeast and XPF of humans *(155).* These proteins are part of a nuclease that cleaves one DNA strand 5′ of bulky lesions. Rad1p interacts with Rad10p, (as does XPF with ERCC1) to comprise a structure-specific endonuclease. The structure that is recognized and cleaved is the single-strand to double-strand transition at the 5′ end of the excision-repair bubble. A similar structure is presumed to occur in intermediates of reciprocal exchange in the form of Holliday junctions. Like several MMR genes, *RAD1* was recently discovered to play a role in regulating recom-

bination between closely related sequences containing heterologies *(33,122).* How this might relate to the role(s) of *mei-9* in meiotic crossing-over makes an engaging discussion *(154).*

3.3. MMR in Drosophila Extracts

In 1990, Holmes, Clark, and Modrich demonstrated a heteroduplex repairing activity in extracts of cultured Drosophila cells *(72).* For correction to be efficient, one stand of the substrate plasmid had to be nicked, and repair was heavily biased to the nicked strand. They found that G-T mismatches were more efficiently repaired than G-G or A-C. C-C mismatches were repaired less frequently than any of those above. This repair was associated with DNA synthesis in the region between the mismatch and the nick, consistent with the MMR model from *E. coli (72).*

More recently, Bhui-Kaur et al. *(10)* established that base:base mismatches can also be repaired very efficiently by extracts prepared from Drosophila embryos or adult cells. Of all the mismatches tested, only G-G, T-G, and to some degree C-C were shown to be repaired in a nick-dependent way. For these three mismatches, the efficiency of repair was higher when one strand was nicked, and the nicked strand was preferentially repaired. In contrast, repair of G-A, C-A, A-A, C-T, and T-T mismatches was not specific to the nicked strand. The authors suggested that G-A, C-A, and A-A, may be repaired predominantly by a mechanism involving an adenine-glycosylase rather than MMR. Similarly, the C-T, and T-T mismatches might be repaired by a thymine-glycosylase. Indeed, A-glycosylases and T-glycosylases have been found in other organisms *(10).* However, damaged bases rather than mismatches are thought to be the substrates of primary relevance for these repair enzymes. Recently it was found that mouse cells (and to a lesser degree human cells) devoid of long-patch MMR are able to efficiently and specifically repair A-C mismatches *(127).* In this case, only the A is replaced and no nick is required. These cells were also able to repair A-G mismatches with a low efficiency but no significant repair of other mismatches occurred. Drosophila has a gene similar to *8-oxoguanine DNA glycosylase (OGG1),* and a putative thymine glycosylase. Enigmatically this glycosylase matched *TDG,* a G:T specific thymine-DNA glycosylase that primarily repairs deaminated 5-methylcytosines by "base-excision repair." Until recently it was thought that there was no methylated DNA in *Drosophila.* It has now been shown that 5-methycystine is produced, but only during the early embryonic period *(102a).*

The nick-directed, long-patch MMR activity may be inducible by X-rays because specific activities of extracts were found to increase five to six-fold after X-irradiation *(10).* Also, the *mei-9* gene is required specifically for the nick-directed MMR in this *in vitro* system. Extracts from *mei-9* mutants were 5- to 12-fold less efficient at repairing G-G and T-G mismatches than those from wild-type flies, yet their activity on A-G, G-A, and C-C mismatches was not affected *(10).*

4. DOUBLE-STRAND BREAK REPAIR

4.1. Introduction

The DNA in living cells sustains spontaneous double-strand breaks (DSBs). Replication-fork disintegration is probably the most common route by which breaks arise, and through attempts to replicate damaged DNA *(31,143).* DNA breaks are also induced by

ionizing radiation and certain chemicals. In addition, there are special circumstances under which breaks are formed as part of a developmental program (e.g., meiotic recombination and V(D)J recombination). In order to study DSBR, it is very useful to be able to direct when and precisely where the breaks are made. Several methods have been developed to achieve this goal.

Traditionally, the repair of DSBs has been categorized into two classes: 1) HR, which requires that the broken end(s) pair with an intact homolog (or other homologous sequence) and use it as a template for DNA synthesis; and 2) nonhomologous end-joining (NHEJ), in which ends are rejoined without consulting a homologous sequence. It is now clear that even within these two categories multiple pathways are used to accomplish repair. Many DSBR studies have been performed in *S. cerevisiae,* but yeast apparently has a different relative preference for HR and NHEJ than higher eukaryotes. This is one of the reasons why studies of DSBR in *Drosophila* should be valuable.

4.2. Analysis of DSBR In Vivo: Germline Events

4.2.1. P Element DSBR System

For the last 10 years, the premier tool for analyzing repair of DSBs in Drosophila has been the transposon known as the P element. This tool was honed after the discovery that P elements move by a cut and paste mechanism. When they jump, they leave behind a DSB at the "donor" site. These breaks are usually repaired by HR, i.e., gene conversion (Fig. 1). The initial clue that implicated gene conversion was the discovery that a P element in the *white* gene underwent precise loss much more frequently when a homolog was present *(40).* Gloor et al. *(54)* proved that homologous sequences could direct the repair of the donor site by following the transfer of sequence polymorphisms from an engineered template to the site of P element excision. This demonstrated that the phenomenon could be used for targeted gene replacement as well as to study DSBR.

Examining the fate of such donor sites has yielded many insights into how DNA breaks are repaired. In the most prolific version of this system, P-induced breaks are created by crossing flies that contain a nonautonomous element (lacking transposase) to flies that contain a stable transposase source. Typically, the repair products are not analyzed in the flies that suffer the breaks, but in their progeny. This is desirable because the progeny are usually homogeneous and depict the result of a single repair event. Even though this strategy examines products that arise in the germ-line, it is known that the majority are from pre-meiotic repair because a pronounced clustering of conversion events is detected. Engineered-sequence polymorphisms also allowed properties of the conversion tracts to be scrutinized. The average length of these conversion tracts is about 1.4 kb. Most tracts are contiguous, i.e., not interrupted by unconverted sites, and most extend bi-directionally from the breaksite *(54).*

4.2.2. Template Preference

Conversion frequencies reveal a hierarchy of preference for template utilization. The order of preference is: sister chromatid > allelic site containing a P element at the identical position > allelic site (without P) > allelic site (without P) on a multiply inverted homolog > ectopic site in cis > ectopic site on a homolog > ectopic site on another chromosome. This order can be partially explained by proximity and by the extent of identity between the broken ends and the potential template. Of course sister chromatids are

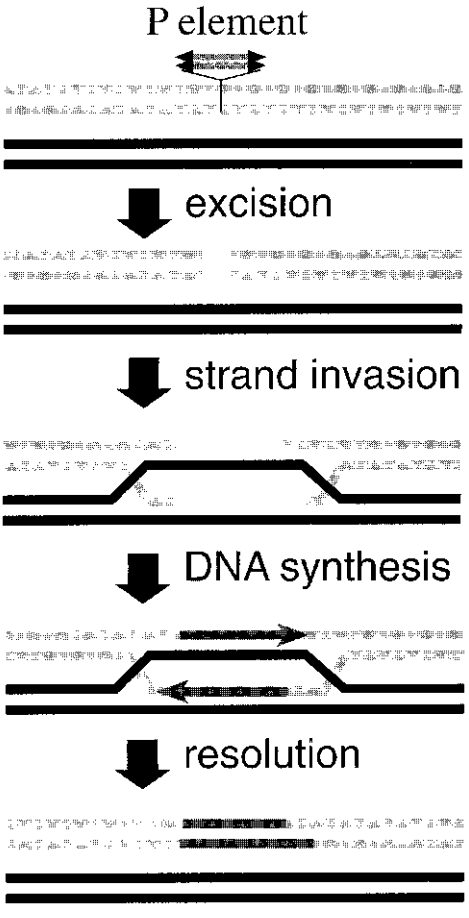

Fig. 1. P element-induced gene conversion. When a P element is mobilized, it leaves behind a DSB. Frequently the broken chromosome is repaired by gene conversion, which can be guided by the corresponding sequence from its homolog. In this depiction, two Holliday junctions are formed and then resolved to yield noncrossover products.

identical before the P element excises, whereas a homolog, though very similar, will often contain polymorphisms in the vicinity of the break. If the entire P element sequence is excised during transposition, it should not matter whether or not a potential template carries a P element at the same site. However, cleavage by P transposase generates 17-base, 3′ overhangs that consist of the terminal 17 bases of the element *(9)*. Junctions with these 17 bases only match a homolog with a P element at the same site (Fig. 2). Indeed, the presence of 17 bases of terminal heterology has a major effect. Compared to a homologous chromosome without a P element, homologs that have a small P element fragment containing at least the 17 bases from both termini, greatly increased conversion *(77)*. The quality of the sequence match is also very important. In one study using homologous chromosomes as templates, the number of single bp heterologies was varied within a ~3.5 kbp region. The frequency of gene conversion dropped from 19% when no mismatches were present to only 5% when the template had 15 mismatches *(119)*.

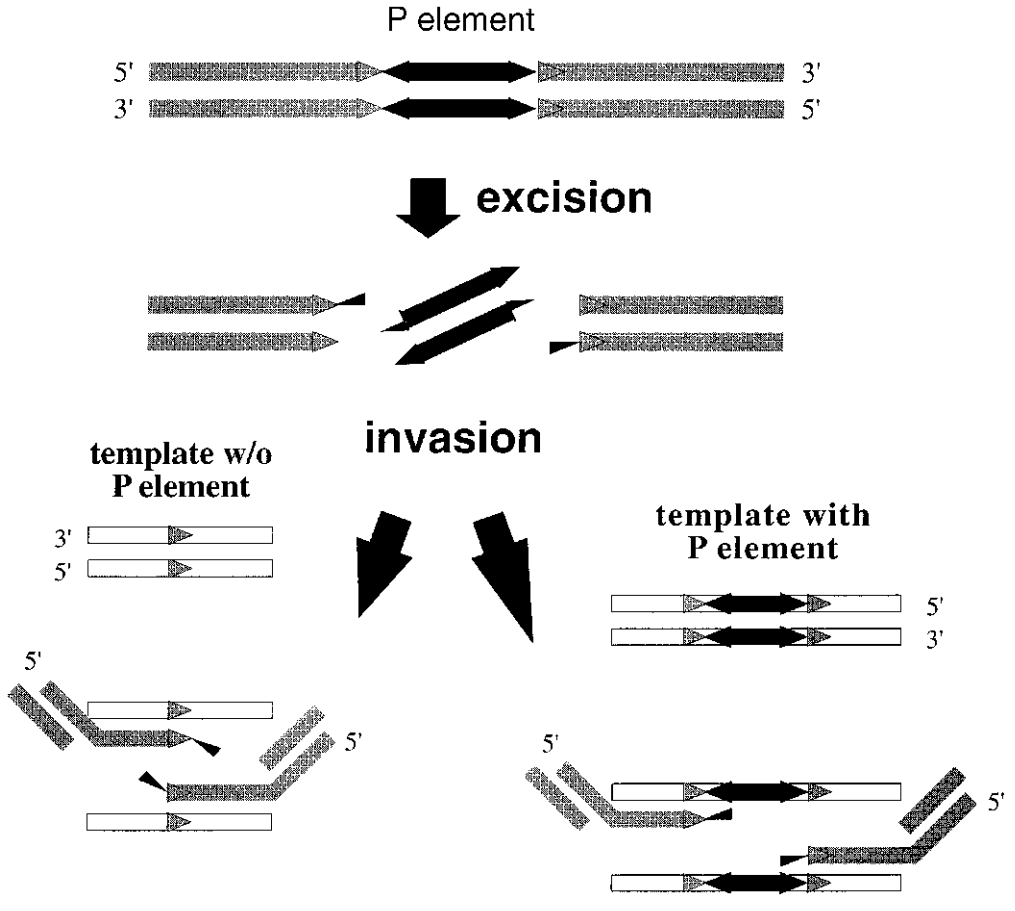

Fig. 2. P element excision produces unusual breaks with 3′ overhangs of 17 bases. Repair of the "donor" chromosome is influenced by the presence of 17 bases of P element sequence. Templates that contain a P element at the same site support a much higher frequency of conversion than templates without P. Left, The 17 base extensions must be removed before repair synthesis can occur on the template lacking a P element. Right, An invading 3′ end can anneal perfectly to the P-containing template and is ready to be extended by repair synthesis.

Another plausible component of the template hierarchy might be the average physical distance between template and breaksite. Sites on sister chromatids could be expected to be close, especially soon after replication. Homologs may be partially paired throughout the cell cycle, but multiple inversions may disrupt pairing *(47,55,70).* It is unclear how a template located several Mbp distant can be recognized as co-linear and utilized preferentially, considering how the nucleus often resembles a mass of spaghetti *(41).* A linear-tracking model seems untenable but alternative models, such as one where each chromosome occupies a compact nuclear domain, seem equally unfeasible.

Extrachromosomal circular plasmids can also be used as templates for P-induced conversion. For multiple reasons it is difficult to compare the efficiency of plasmid and chromosomal templates. Plasmids are injected into the embryos at a many-fold excess to the number of chromosome copies. Also plasmids persist for a fraction of the fly's lifespan, and the copy number probably varies greatly from cell to cell. Still the fre-

quency is high enough that plasmids can be used for gene targeting and transformation of Drosophila by DSBR *(81)*.

4.2.3. Homology Requirements

Gene-targeting studies demonstrated that both insertions and deletions can be efficiently copied into a P element-induced breaksite if they are encompassed within homologous sequence. As mentioned earlier, mismatches and terminal homology affect template efficiency during gene conversion *(77,119)*. Although different types of heterologies affect the overall rate of conversion differently, among conversion events the frequency of inserting 8 kbp, deleting 136 bp, or creating a single-base change is the same *(120)*. Several other experiments have addressed the minimum amount of homologous sequence required for efficient conversion. One study used a break at w^{hd} (a P element insertion in the eye-color gene known as *white*) and a set of ectopic templates containing ~3 kbp of homology to the left of the breakpoint and varying amounts to the right. On the right of the breakpoint all templates began with 238 bp of matching sequence followed by an 8 kb heterologous insertion then resumed with either 0, 25, 51, 375, or 493 bp of additional matching sequence. The rate of conversion of the heterologous insertion was very low when the outer homology was 51 bp or less. Conversion rates were moderate with 375 bp of homology and high when 493 bp of homology was present *(35)*.

In most experiments that use ectopic templates, an interesting class of aberrant events is recovered. These peculiar conversions all retain one P element end at a position that corresponds to a P terminus in the donor. They also contain a sequence duplication beginning at that same point. These aberrant products, dubbed "conversion duplications," can be explained if the terminal 17 bp are sufficient to direct recombination at one end *(120)*. The 17-base tail may either prime synthesis from a P end in the template or serve as the site of alignment when the two sides of the break come together after template-directed synthesis (Fig. 3). The view that 17 bases is sufficient is also supported by the transposon swap phenomenon *(48,58,69,82,107,156)*. In a stock that has two distinguishable P elements, one can precisely replace the other when mobilized. Presumably, gene conversion allows the 17-base tails to find the ends of the other P element anywhere in the genome and copy the sequence of that element into the break. It is a formal possibility that more than 17 bases are required for these events if they occur in two steps. In that case, initial repair of the break could use the sister chromatid to extend the P element sequence to 31 bases or more (note that the terminal 31 bases of P elements are perfect inverted repeats). The extended tails could use the additional homology to pair with the second P element. However, Preston and Engels succeeded in converting a DS oligo into a P-induced break guided only by the 17 bases of P on both sides *(137)*. Current data suggest that although 17 bases of matching sequence is too short to produce the highest conversion frequency, it can suffice.

4.2.4. Synthesis-Dependent Strand Annealing

Conversion duplication products were instrumental in the development of a new model of HR called synthesis-dependent strand annealing (SDSA) *(120)*. In SDSA, after a DSB is formed, the two 3′ ends independently invade and copy homologous template(s). This creates long single-stranded tails that anneal at complementary regions. The annealed strands are processed until fully double-stranded by any necessary synthesis and/or trimming and repair is completed by ligation (Fig. 4). The SDSA model is distinct in that it does not

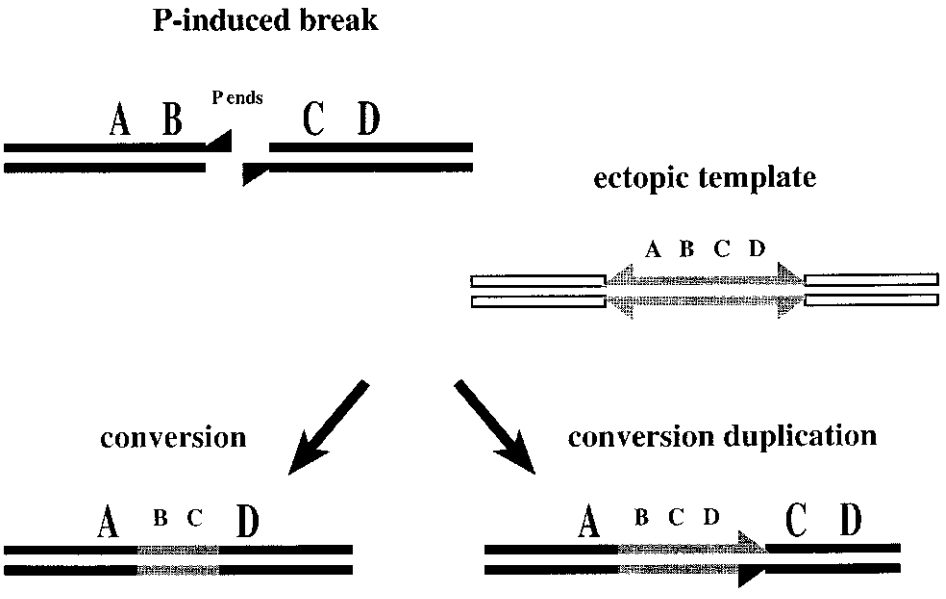

Fig. 3. An example of a "conversion-duplication" structure. When P-induced DSBR utilizes a template that resides ectopically within another P element, a few percent of the events recovered are of the "conversion-duplication" class. The structure suggests that alignment on one side of the break has been directed by the termini of the two transposons.

invoke Holliday junctions but rather migrating repair "bubbles," and it entails the association of long single-strands as in single-strand annealing *(166).* These features can easily explain the origin of the conversion duplications and are in accord with the fact that conversion is only infrequently associated with crossing-over. In contrast, models that include resolution of Holliday junctions predict frequent crossing-over. SDSA may be a widely used mechanism because this model best explains results from a variety of settings including alterations in sequence repeats in yeast and rye, intron homing in bacteriophage T4, and formation of defective transposons in Drosophila and maize *(11,91,117,120,133,145).* The strongest evidence for SDSA comes from recombination products that include sequence information from two separate templates *(76,133,135).* No other model can easily explain such "bitemplate" events.

4.2.5. Effects of Heterologous Insertions

Despite the fact that large insertions are included within successful conversion tracts as frequently as single-base differences, they do not affect conversion in the same way. The amount of flanking homology required is greater for large heterologies *(36).* Also the overall frequency of conversion is strongly affected by large heterologies (~8 kb) located 238 bp from the breaksite *(35).* The frequency is less affected by shorter heterologies (242 bp to 4 kbp) and unaffected by short (25 bp) insertions *(35).* It seems that the proximity to the break is a crucial factor, because a large insertion 2 kbp from a breaksite had little effect on conversion frequency *(40).*

4.2.6. Possible Chromatin Effects

The fact that homologous templates can be found at all is astonishing when the true state of chromosomal DNA is considered. The DNA in cells is compacted by several

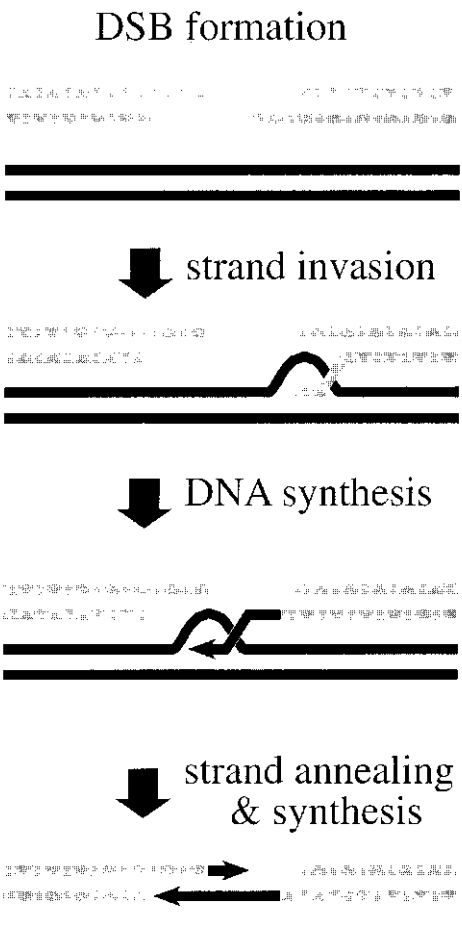

Fig. 4. Synthesis-dependent strand annealing. *See* text for details. For simplicity, this depiction shows invasion and synthesis initiating only with the broken right end. Apparently both ends can invade and extend independently, sometimes involving separate homologous templates.

levels of organization into chromatin. Evidence is mounting that chromatin remodeling is a very important aspect of nuclear activity that is capable of regulating processes such as transcription and DNA repair *(115).* The absence of one chromatin "insulator" protein known as *suppressor of Hairy wing (su(Hw)),* leads to an approx fourfold increase in conversion rates when an ectopic template is used *(93).*

4.2.7. Other DSB Systems

Other methods have been used to make DSBs in Drosophila chromosomes and these have been used to test which aspects are general to DSBR, and which are specifically influenced by P element idiosyncrasies. Repair of chromosome breaks caused by the rare-cutting endonucleases HO and I-*Sce*I have been analyzed *(78,137).* So far, the general conclusion is that most of the characteristics of P-induced DSBR are shared with these other systems. For example, when the recognition site for HO endonuclease is placed in the *white* gene on the X chromosome, conversion-tract distributions are remarkably similar to those recovered by P mobilization *(78).* When conversion uses the homologous X chromosome as a template, there is a similar, low level of associated

crossing-over. Ectopic templates located in *cis* to the break are used preferentially just as is the case with P elements. Also, examples of repair utilizing two different templates, so-called bi-template events, have been recovered, suggesting that both ends produced by HO cleavage can independently invade templates, again similar to the case with P elements *(78)*.

I-*SceI* endonuclease cuts Drosophila chromosomes very efficiently, but the relative frequency of the alternative outcomes (end-joining, conversion with or without crossing-over) is still under investigation *(137)*. Conversions can be recovered at a rate comparable to P-induced DSBR and again, as with P, crossovers are much rarer than conversions *(137)*. When I-*SceI* cuts are made between direct repeats of homologous sequence, which provides an opportunity for single-strand annealing, very high rates of recombination are seen *(141)*.

Another transposon known as the mariner element also induces homolog-directed gene conversion when it excises in Drosophila *(101)*. Mariner is a member of the extremely widespread mariner/Tc1 transposon superfamily—the genomes of many plant and animal species are replete with these elements. Faulty DSB repair after mariner excision also appears to be the source of defective copies of mariner *(101)*. Similar to the scenario with P elements, aborted or inaccurate gene conversion replaces an internally deleted element into the site of the excised transposon.

4.2.8. Gene Targeting

Until recently, targeted-gene replacement in Drosophila was limited to the immediate vicinity of P element insertions or other engineered sites of DSB formation. The first successful targeted disruption of an unmodified gene was reported by Rong and Golic *(141)*. In most targeting systems, DNA ends are required for high levels of recombination, but all attempts to provoke gene replacement by injecting linear substrates into fly embryos have failed. The trick seems to be to create linear, extrachromosomal recombination donors in vivo *(141)*. A donor cassette was engineered and inserted randomly into the Drosophila genome by P element mediated transformation. The cassette contained a segment of the targeted gene with an I-*SceI* cleavage site in the middle. This was flanked by two FRT sites, (substrates for FLP recombinase), arranged as direct repeats. The action of FLP recombinase on these sites loops out the intervening sequence creating an extrachromosomal circle and at the same time reseals the chromosome that is now deleted for this sequence. Cleavage by I-*SceI* linearizes the circle creating the (presumed) active donor with recombinogenic ends.

Recombinant products were recovered by selecting for homology-directed reversion of a mutation at the target locus. The homologous sequences at the ends were oriented to form an "ends-in" donor, so the expected outcome was integration at the targeted site producing a tandem duplication *(63)*. About one-third of the selected products had the expected configuration *(141)*.

The other two-thirds of the products were composed of three structural classes that can be explained by a mechanism in which dimers of the circular donor arise prior to integration. Indeed, about 7% of the products appeared to be simple integrations of a dimerized donor, thus creating tandem triplications *(141)*. This arrangement should be unstable if FLP recombinase or I-*Sce*I is still present. The central copy of the triplication would be efficiently excised by FLP because it is flanked by FRT sites (excision would produce a configuration identical to the simple "ends-in" integration, therefore some in the expected

class could originate through donor dimers). The central copy of the triplication would contain an I-*Sce*I cleavage site, so if any I-*Sce*I endonuclease remains, it would provoke a second round of DSBR. One of the likely products of such secondary repair would be simple "allelic replacement" of the targeted mutant allele with the donor allele. This outcome could ensue from a cleaved triplication by gene conversion, single-strand annealing, or sister chromatid exchange. Fully one third of the recombinants recovered were "allelic substitution" products, yet they are not easily explained without assuming donor dimerization *(141).* DSBR of the triplication is also likely to produce some events that collapse the structure to duplications identical to the simple integration products. The final class (23% of the total) has a structure similar to the expected tandem arrangement except that one copy of the duplication has a small deletion or insertion near the position of the I-*Sce*I site *(141).* These scars are reminiscent of NHEJ, yet homology undoubtedly directed integration. A donor dimer could suffer inactivation of one of its I-*Sce*I sites by NHEJ and still be targeted through cleavage at the other site. Integration after extensive degradation of one end by exonuclease could result in the observed structures. But how could dimers form when there is only one copy of the cassette in the genome in the first place? The simplest theory is that dimers arise through replication. If the circles could replicate, multimers would be formed readily by FLP mediated co-integration or I-*Sce*I mediated DSBR. Alternatively dimers could arise (intra- or extrachromosomally) in G2 cells by interaction of copies from the sister chromatids. However, it is unclear why dimers appear to be the favored substrates. What key recombinational advantage might dimers provide? Because of the excitement over this new gene-targeting scheme, details of the mechanism (whether dimers are involved or not) should emerge quickly.

4.3 Analysis of Somatic DSBR

There is evidence that the relative efficiency of gene conversion and NHEJ is different in Drosophila somatic cells vs premeiotic germ cells. Also the size of the most frequent deletions created by NHEJ in somatic cells seems to differ from those arising in premeiotic germ cells *(8,44,53,77,78,125,161,169).* To a large degree the apparent disparity can be explained by differences in experimental designs. Often a phenotypic screen was used to select the product before analysis. In some experiments, a large number of copies of linearized plasmids were injected into embryos *(8,125),* whereas in others, repair of a single break at a chromosomal site was studied *(44,53,77,78,161,169).* However, even when similar conditions are used, there are persistent differences in somatic and germline repair *(53).* Unfortunately it is not possible to control for variability that results from the different life histories of diverse tissues. Factors such as the number of cell divisions, rate of DNA synthesis, and duration of cell-cycle phases may affect the ratio of the types of products recovered and obscure whether certain repair pathways are truly modulated in somatic verses germline cells. There is clear evidence of cell-type-specific regulation of DNA repair in yeast (*see* Chapter 5).

4.4. Analysis of DSBR in Embryos

4.4.1. P Element Loss, Reversion Assays, and NHEJ

Assays for DNA repair after transposon excision have been performed in Drosophila embryos *(126).* Plasmids bearing a transposon were injected into preblastoderm embryos. Transposase was produced either from a co-injected plasmid or a chromosomal source. In most of these assays, repair was analyzed phenotypically after plasmids

were re-isolated from embryos and transformed into *E. coli.* This type of assay selects for a small subset of repair events that restore the function of a bacterial gene that the transposon has disrupted. Because there is no opportunity for homologous repair, NHEJ events that produce small in-frame insertions or deletions are recovered. Despite the severe bias inherent in such assays, they have been very useful. For example, it was shown that γ-irradiation can enhance the recovery of these repair products *(61),* whereas mutations in *mus309* diminish recovery *(8).*

4.4.2. Homologous Recombination (HR) and Nonhomologous End-Joining in Embryos

The repair of plasmids that have been linearized in vitro can also be assayed in Drosophila embryos *(37,59).* Linear DNA substrates are injected into the early embryo prior to cellularization. Intramolecular repair and some HR events lead to plasmid recircularization, which can be followed by PCR or analyzed after transformation of *E. coli.*

One study of DSB repair in embryos analyzed the sequence of 122 NHEJ junctions *(37).* Most of these, (117 events) had deletions of plasmid sequence, but a quarter of the junctions had lost nucleotides from only one side of the break. Slightly more than half of the deletions were <10 bp, 41% were 10–100 bp, and 7% longer than 100 bp. The addition of bases that are not present in the original plasmid was also a common feature, but most of the insertions were less than 20 bases. As seen in other studies *(88,134,142,144,149),* almost all of the products that did not have insertions of extra bases had been joined at regions of microhomology. These junctions contained one to three bases that could have come from either side, suggesting that repair was influenced by short regions of complementarity *(37).*

Intermolecular HR could be detected when a homologous fragment was co-injected with the plasmid. The homologous fragment was designed to span the site of plasmid cleavage and contained 18 additional basepairs at the exact position of the break. Thus homologous repair using the fragment as a template is revealed by transfer of these 18 bp into the plasmid. This type of repair product was readily detected by PCR even though NHEJ appears to predominate. Irradiation of the embryos with γ-rays prior to substrate injection was found to stimulate HR, especially the production of mature recombinant circles capable of transforming *E. coli (37).*

Another study used embryos in a similar way to investigate both intramolecular and intermolecular HR as well as NHEJ *(59).* The substrate for intramolecular HR and NHEJ was a linearized plasmid that contained two copies of a short sequence. HR within the duplication would recircularize the plasmid and reconstruct a tetracycline resistance gene. NHEJ was much more frequent than intramolecular HR. As in other studies, deletions were common at these NHEJ junctions. The average length of deletion was 14 bp. Intermolecular HR was also assayed with a linearized plasmid and a homologous fragment. In this case, recombination was detected by (homology-directed) reversion of a mutation near the site of plasmid cleavage. Once again, HR was easily detected although it was not very efficient compared to NHEJ *(59).*

4.5. Genes Involved in DSBR

4.5.1. spindle Genes

Recently the studies of oocyte development and DSBR converged with the surprising discovery that several mutants defective in embryonic axis formation had lost DSBR functions. These so-called spindle-class genes were isolated by selecting mutations that

produced ventralized embryos lacking dorsal structure. Such embryos are narrower, more pointy, and more symmetrical than wild-type (hence the name spindle). This phenotype is caused by the inability to establish dorsal/ventral polarity that is the direct result of a failure to accumulate GURKEN protein in the anterior-dorsal region of the oocyte *(49,57).* Remarkably, three of the spindle-class genes that have been identified appear to have a primary role in DSBR/meiotic recombination. These include a *RAD54* homolog, a *RAD51/DMC1* homolog, and a third DNA repair gene, *mus301.*

How do mutations in repair genes lead to developmental defects of the oocyte? These mutants cannot complete repair of meiotically induced DSBs that initiate crossing-over. This block in DSBR activates a meiotic checkpoint so that the oocytes do not complete prophase I. As part of the response to this checkpoint, VASA protein is modified, which in turn inhibits *gurken* mRNA translation. Without sufficient GURKEN, the dorsal-vertral axis cannot be formed. In a *mei-W68* mutant background, no meiotic breaks are made, and the DSBR-deficient spindle class mutants are able to progress through prophase I. Alternatively, if the oocyte has a faulty meiotic checkpoint, as in a *mei-41* mutant, the DSBR-deficient spindle class mutants are again able to proceed through prophase I *(50)* (Fig. 5).

4.5.1.1. *SPN-B*

The SPN-B protein is a member of the RecA/RAD51 family *(49).* Several proteins in this family have been shown to catalyze strand-transfer in vitro, to play roles in DSBR, and to form specific complexes during meiotic recombination. The *spn-B* message is expressed throughout oogenesis. It has not been reported whether it is expressed in other stages and tissues *(49).* Mutations in *spn-B* are not known to affect mitotic DNA repair. For example, they are not hypersensitive to MMS *(49),* but they do have several defects in meiotic recombination. *spn-B* mutant females are nearly sterile. In the rare offspring, meiotic crossovers are decreased 4- to 10-fold and X-chromosome nondisjunction (NDJ) is increased about 100-fold. The oocyte chromosomes of *spn-B* mutants often fail to form into a compact karyosome but remain diffuse and thread-like *(57).*

Among RAD51 family members, SPN-B is most closely related to human XRCC3 and RAD51C (about 35% identical, 49% similar amino acids). It also shares about 27% identity/43% similarity to human DMC1 and 30% identity/44% similarity to human RAD51. Owing to its conspicuous meiotic phenotypes, it has been compared to DMC1, which is exclusively expressed during meiosis in yeast and mammals and is indispensable for meiotic recombination. It is possible that the full spectrum of *spn-B* function has not been uncovered yet.

4.5.1.2. *SPN-D*

The molecular identity of *spn-D* has not been revealed, but it produces a mutant phenotype very similar to that of *spn-B.*

4.5.1.3. *OKR (DMRAD54)*

Another spindle-class gene was named *okra (okr).* Female *okr* mutants, are also sterile, producing ventralized embryos. Unlike *spn-B* and *spn-D* mutants, *okr* mutants are hypersensitive to MMS. The *okr* gene encodes the Drosophila homolog of *RAD54.* It shares 54% identical amino acids with human RAD54 and 48% with yeast Rad54p *(49,87).* Hypersensitivity to MMS is also seen in yeast *rad54* mutants. Yeast Rad54p

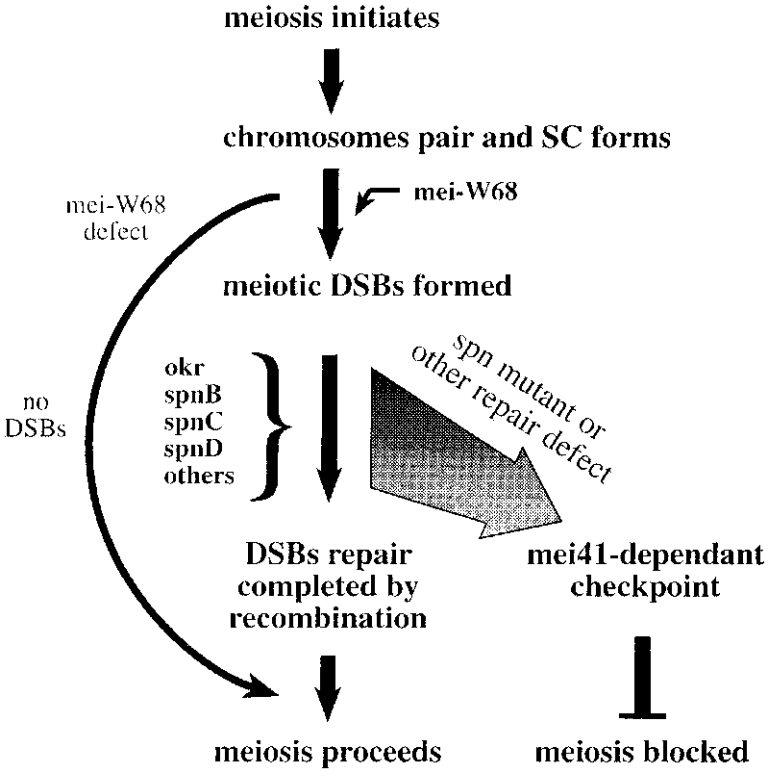

Fig. 5. A DSB-induced meiotic checkpoint. MEI-W68 appears to be required for the formation of meiotic DSBs while some of the spindle-class genes are directly involved in the repair of these meiotic breaks. If the breaks fail to be repaired, as in a *spn* mutant, a *mei41* -dependant checkpoint arrests the progress of meiosis. Oocytes mutant for *mei-W68* and a *spn* gene circumvent this checkpoint because no DSBs are produced.

participates in DSBR and HR. It can stimulate RAD51p-directed homologous pairing, and appears to have a particularly important role in DSBR utilizing the sister chromatid. Consistent with a DSBR function, both yeast and Drosophila *rad54/okr* mutants are hypersensitive to X-rays *(87).* In addition, flies with *okr* mutations are hypersensitive to breaks made by P element excision and to the crosslinking agents, mitomycin C (MMC) and cisplatin *(86).* Mutations in *okr* and *mus309* (Subheading 4.5.1.4) are synergistic with respect to X-ray sensitivity, but roughly additive with respect to MMS sensitivity *(86).*

In an assay for induced loss of heterozygosity and HR in somatic cells, *okr* mutations had little affect when the inducing agent was MMS, MMC, or cisplatin. In contrast, *okr* mutations abolished the recovery of X-ray-induced loss of heterozygosity events *(86).* Transcription of *okr* occurs at all developmental stages but is highest in ovaries and early embryos *(87).* Yeast and mice mutant for *rad54* have little or no defect in meiosis. Strong *okr* mutants, on the contrary, are completely sterile in females, apparently unable to repair meiotic DSBs. Meiotic recombination and NDJ have been measured in weak (nonsterile) *okr* mutants. These mutants produce about 50% of the normal level of recombinants and about 20-fold more NDJ events *(49).*

4.5.1.4 *spn-C (mus301)*

Mutant *spindle-C* flies also lay ventralized embryos and are sensitive to MMS. The *spn-C* gene is allelic to the mutagen sensitive gene, *mus301*. A mutant P element-insertion line failed to complement both *mus301* and *spn-C* and *spn-C* mutants do not complement mutant *mus301* alleles for MMS sensitivity *(50,152).* There are five mutant alleles of *mus301* and these yield varying degrees of sterility when homozygous. As in *spn-B* and *okr,* females with partially fertile alleles of *mus301* produce increased frequency of NDJ *(16).* Hypersensitivity to X-rays is also seen in *mus301* mutants *(129).* Intriguingly, the *mus301* gene maps to a region that contains a homolog of *recQ4 (89)* (*see* Subheading 4.5.6.2.).

4.5.2. mei-41(ATR, MEC1)

MEI-41 is a member of a protein family whose C-termini are related to phosphatidylinositol 3-kinases *(62).* Many members of this family are DNA damage-inducible checkpoint signaling proteins including yeast Mec1p and human ATM and ATR. The *mei-41* gene derives its name from the defect in meiotic recombination that results from "weakly" mutated alleles *(27).* Such alleles produce reduced levels of meiotic exchange with concomitant increase in NDJ. Strong mutations cause female sterility through maternal-effect embryonic lethality *(159).* Mutations also cause severe sensitivity to DNA damaging agents *(17,105)* and P element-induced breaks *(5).* MEI-41 has an essential role in embryonic development in a unique DNA replication/DNA damage checkpoint that is necessary for the transition from the extremely rapid, maternally programmed, early cell divisions to the zygotically controlled divisions after the midblastula stage *(159).* The Drosophila *CHK1* homolog, *grapes,* and Drosophila *wee1* are also components of this pathway *(138,159).*

MEI-41 has another intriguing role in female meiosis. In wild-type oocytes the presence of chiasmata leads to arrest in metaphase I. Certain mutants defective in meiotic crossovers but not repair by gene conversion (i.e. *mei-9* and *mei-218*) show early entry into anaphase. This precocious entry into anaphase requires MEI-41 *(113).* One possibility is that MEI-41 may be required to extinguish a signal that normally arrests meiotic progression until DSBR is complete *(154).*

4.5.3. Ku70

Mammalian cells possess several protein complexes that sense the presence of DSBs and alert the cell to elicit the appropriate response. One such complex is DNA-dependent protein kinase (DNA-PK). DNA-PK is made up of a large subunit that encodes the protein kinase activity and a heterodimer of two smaller subunits, Ku80 (or Ku86) and Ku70, that constitute a DNA end-binding activity. The Ku86/Ku70 dimer must be bound to DNA in order to stimulate the catalytic subunit of DNA-PK. Activated DNA-PK phosphorylates several DNA binding proteins including p53 and is required for p53-mediated apoptosis. Besides its role in signaling and cell-cycle checkpoints, the Ku heterodimer has been implicated in the repair process itself. Evidence suggests that Ku protects DNA ends from excessive nucleolytic degradation and may in fact tether the two ends together to facilitate repair *(13,14,29,97,131).* Ku is also involved in protecting and maintaining telomeres.

Drosophila Ku70 was originally isolated during a search for inverted repeat binding protein (Irbp), a factor that binds to the outer half of P element terminal repeats *(7).* The bound proteins were purified and antibodies were raised against peptide fragments.

This led to the isolation of a *Ku70* cDNA. Although they are likely to be a true homologs, the human and Drosophila Ku70s have diverged significantly, retaining only 27% identical amino acids. Drosophila *Ku70* was independently cloned as yolk protein factor 1b, *(Ypbf1b)* by virtue of its apparent sequence-specific DNA-binding within the *Yp1* gene *(74).* When the *Ku70* gene was mapped cytogenetically, it was found to reside in the vicinity of a gene called *mus309.* Beall and Rio *(8)* showed that a genomic fragment containing the *Ku70* gene partially rescued female sterility and MMS sensitivity of *mus309* mutants. Therefore, *Ku70* and *mus309* were considered the same gene despite discrepancies in map positions and predicted phenotypes. A gene encoding Ku86 was recently identified *(51).*

4.5.4. mus309 (blm)

Several of the phenotypes of *mus309* mutants could conceivably be attributed to defects in DSBR including reduced fertility, reduced viability (especially in males), altered frequency of meiotic recombination, increased NDJ and chromosome loss, and increased mitotic exchange. In addition, more specific studies have shown that mutations in *mus309* affect the repair of DSBs induced by P element transposition, HO endonuclease, and X-rays *(78,86).*

The *mus309* gene was identified in 1981 *(16).* Three mutations, *mus309*^{D1}, *mus309*^{D2}, and *mus309*^{D3}, were determined to be allelic because they failed to complement each other for recessive sensitivity to MMS and nitrogen mustard. However, the D1 allele was recovered with a linked recessive lethal, and the D3 allele was associated with a second (unidentified) mutagen-sensitive mutation. The D2 allele has a recessive female-sterile phenotype. The heteroallelic combination *mus309*^{D1}/*mus309*^{D2} causes sterility in both females and males *(16),* and both *mus309*^{D2} and *mus309*^{D3} are female sterile over a deletion *(167).* The D1 allele has been lost and thus, most analyses have been performed with a combination of the D2 and D3 alleles even though these flies are only weakly fertile.

Preliminary results from *mus309*^{D2}/*mus309*^{D3} mothers show that their sons survive ~five-fold less often than daughters. This sex bias is absent in the progeny of *mus309* mutant fathers *(78).* About 4% of the total progeny, (over 25% of the males) from *mus309* mothers are XO (null Y). These flies could derive from maternal chromosome loss or meiotic NDJ. The frequency of XO males is at least 75-fold higher than in wild-type controls. The occurrence of XXY females, which is indicative of NDJ, is elevated to a lesser degree (~10-fold), implying that chromosome loss is contributing the major effect. Paternal chromosome loss and NDJ are also elevated in *mus309* mutant fathers *(78,89).*

mus309 mutations also affect meiotic recombination, but in a complex way. The recombination rate on the 2nd chromosome is reduced over-all by two-fold, but the effect is not uniform across the chromosome length. Exchange in the left and right arms is reduced two- to three-fold and increased ~three-fold in the central region *(78).*

As mentioned previously, there is no meiotic recombination in male Drosophila, so any recombinant chromosomes arising in the male germ line derive from nonmeiotic events. Therefore the rate of spontaneous recombination in the male germline is very low in a wild-type background, (less than 10^{-5} per chromosome per generation). In a *mus309* mutant the rate is increased over 2000-fold *(78).* Two possible explanations for this increase are (1) more DNA damage occurs (or persists) in *mus309* mutants, which leads to a higher recombination rate, or (2) the same amount of DNA damage occurs

but it more often results in crossing-over. Given the evidence for increased chromosome loss, the first scenario seems more likely.

Beall and Rio *(8)* showed that *mus309*^{D2}/*mus309*^{D3} flies are hypersensitive to P mobilization. The sensitivity was more severe in males, (~6-fold) than in females (~1.8-fold). This sensitivity is presumed to be caused by inefficient repair of DSBs. Because the mobilized P elements were inserted in the sex-specific X chromosome, the higher sensitivity in males may be owing to the absence of a homolog to serve as a repair template. Alternatively, the sex bias might be explained if recessive lethals are produced in both male and female *mus309* mutants but they are complemented by the homolog in females.

When P elements are mobilized from the X chromosome in the germline of wild-type males, deletions are found near the break site in about 30% of the offspring. In a *mus309* mutant, the proportion jumps to about 90% *(78).* In this assay, only one X homolog is present, so repair by HR can only take place when the sister chromatid is available. Nonetheless, one subclass representing 22% of the progeny have deletions within the body of the P element, indicating that repair synthesis using the sister as a template was initiated but failed to complete or resolve properly. This type of event is observed five times more often in the descendants of *mus309* fathers than those from wild-type fathers. This suggests that *mus309* is involved in HR.

In a similar assay, breaks were induced on the X chromosome in males that had an ectopic template for homologous repair. In this experiment, P element mobilization led to a "reversion" rate that was 1.5- to 3-fold lower in *mus309* mutants than wild-type controls. These "reversion" events represent successful repair from the ectopic template. In contrast, the rate of "marker loss," which is caused by internal deletions and flanking deletions, increased 2.5- to 5-fold in *mus309* mutants. Very similar results were obtained when the DNA breaks were produced by HO endonuclease cleavage rather than P transposition *(78).*

The idea that *mus309* mutants are defective in DSBR is supported by the fact that *mus309*^{D2}/*mus309*^{D3} mutants are three-fold more sensitive to X-rays than wild-type flies *(87).* A synergistic effect of *mus309* with *DmRad54* was also observed. Whereas *DmRad54*$^{-/-}$ mutants are seven-fold more sensitive, the *mus309*$^{-/-}$ *DmRad54*$^{-/-}$ double mutants are 40-fold more sensitive to X-rays than wild-type. The same double mutant combination does not show synergy in MMS sensitivity *(86).*

The *mus309* gene was recently shown to be the *Drosophila* homolog of the human Bloom syndrome gene, BLM. Deletion mapping, rescue by transgene, and the sequence of mutants all confirm that *mus309* is *Dmblm* rather thatn *Ku70 (see Subheading 4.5.6.1.; 89).*

4.5.5. mus209 (PCNA)

The *mus209* gene encodes the proliferating cell nuclear antigen (PCNA) homolog of Drosophila *(66).* The existing mutations cause recessive lethality or recessive temperature sensitive (ts) lethality. PCNA is involved in DNA replication, DNA repair, recombination, and cell-cycle regulation. It is a component of both DNA polymerase δ and polymerase ε. FEN-1 and p21$^{WAF1/Cip1}$ (called DACAPO in Drosophila), bind to the same site on PCNA. FEN-1 is also an essential replication factor; it is an endo/exonuclease required for processing Okazaki fragments during lagging strand DNA synthesis. Binding of p21 to PCNA is thought to inhibit replication by displacing FEN-1 *(173).* The ts-alleles of *mus309* cause recessive sensitivity to MMS, ionizing radiation, and

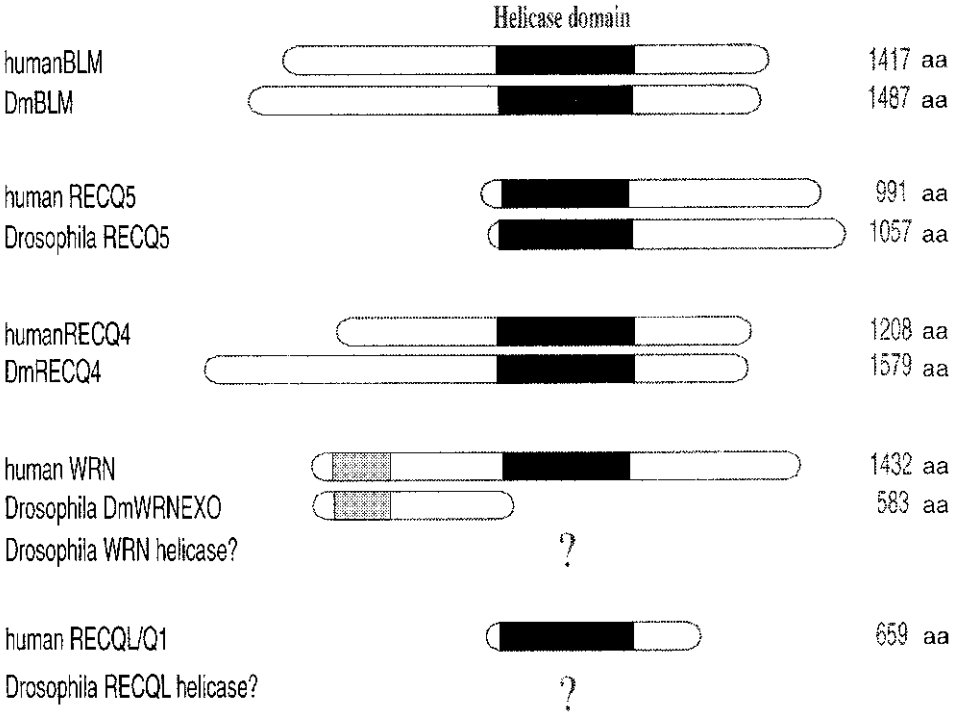

Fig. 6. Drosophila and human RECQs. The overall structure of the known RECQ proteins of Drosophila and human are compared. The locations of the conserved helicase domains are shown in black. The total number of amino acids predicted for each protein is indicated (note that RECQ5 and others may produce more than one protein by alternative splicing). A putative exonuclease, related to the WRN exonuclease domain, occurs in Drosophila within a smaller ORF that does not encode a helicase. Genes closely related to the WRN helicase and human RECQL have not been found in Drosophila.

bleomycin at the permissive temperature *(65–67).* PCNA is also required to repair P element-induced DSBs *(67).* Partial complementation of several of the mutant defects can be achieved with a combination of heteroalleles *(68).*

4.5.6. The RECQ family

The RECQ family is composed of a group of related proteins that contain the seven canonical helicase motifs and share sequence similarity both within and around these motifs. Several members have been confirmed as DNA helicases, being able to unwind the two strands of the double helix. RECQ members have roles in repair of DNA by HR and in genome stabilization *(30,79).* The RECQ family consists of at least four subfamilies. Eukaryotes frequently retain four or more *recQ* homologs, often including representatives from three of the subfamilies. This suggests that each group provides distinct functions *(90).*

4.5.6.1. *DmBlm*

Drosophila *Dmblm* gene is a RecQ family member that is closely related to the human *BLM* gene *(90)* (Fig. 6). Mutations in *BLM* cause Bloom syndrome, an autosomal recessive disorder characterized by growth deficiency, immunodeficiency, cancer

susceptibility, and chromosome instability. The DMBLM protein is also similar to *S. cerevisiae* Sgs1p, although it is more distantly related to Sgs1p than it is to BLM. Despite this divergence, *Dmblm* can partially rescue MMS hypersensitivity of *sgs1* yeast *(90),* indicating that functional similarities persist.

4.5.6.2. *RECQ4*

Another RECQ homolog of *D. melanogaster,* DMRECQ4, is very similar to human RECQ4 within and around the helicase domain. Mutations in human RECQ4 cause Rothmund-Thomson syndrome *(84).* This syndrome manifests in abnormalities of the skin and skeleton, signs of premature aging, chromosome instability, and a predisposition to cancer *(83,99,172).* The *DmrecQ4* transcript is about 4.9 kb. The gene maps to 66B10-66C1 close to *spn-C/mus301.*

4.5.6.3. *RECQ5*

Drosophila has at least one other *recQ*-like gene that is most similar to human *RECQ5.* It appears to be produced in two isoforms. One set of mRNAs would generate a protein of about 54 kDa that consists of little more than the helicase core region. Another set of mRNAs encodes a protein more than double this size with an protracted C-terminus abundant in charged residues, as seen in many other *recQ* members *(153)* (Fig. 6). The human *RECQ5* transcript also exists in multiple forms capable of producing short or extended proteins *(153,157).* Drosophila RECQ5 is concentrated in the nucleus, compatible with a DNA helicase activity.

4.5.7. Drosophila Homologs of MRE11, RAD50, and NBS1

In *S. cerevisiae,* Mre11p, Rad50p, and Xrs2p proteins form a complex involved in the repair of DSBs. An analogous complex is formed in humans between hMRE11, hRAD50, and hNBS1 *(25)* (*see* Chapter 7). This complex localizes to foci at the site of DNA breaks where it may be involved in both enzymatic (end-processing) and damage-signaling functions *(104,121,170).* Null mutations of mouse *mMre11* and *mRad50* are lethal *(102,174).* Non-null mutations in human *nbs1* and *mre11* cause the severe chromosome-instability disorders Nijmegen breakage syndrome and Ataxia telangiectasia-like disorder, respectively *(25,106,162,171).*

Drosophila has *MRE11, RAD50,* and *NBS1* homologs, but no mutations in these genes have been reported. The sequences of Drosophila, human, and yeast MRE11 and RAD50 proteins are very similar. In contrast, the Drosophila NBS and hNBS1 proteins are rather diverged and they share very little resemblance to yeast Xrs2p. The similarity that they do share is limited to their N-termini in a region that constitutes a forked head-associated (FHA) domain (Fig. 7). The FHA domain is an amino acid sequence motif found in many proteins of diverse functions. A few FHA-containing proteins are involved in DNA damage-inducible cell-cycle checkpoints (e.g., Dun1p, Spk1p, Mek1p), but many have no apparent connection to DNA repair. The FHA domain is assumed to play a role in nuclear signaling *(71).* The Drosophila and human NBS proteins share an additional domain in common bordering the FHA domain. It includes a BRCA1 C-terminal (BRCT) domain, originally identified in the breast cancer-susceptibility gene BRCA1. Many DNA repair proteins and some cell-cycle checkpoint proteins contain BRCT domains (e.g., DNA ligases). This domain is thought to form an interface for protein-protein interactions *(12).* Human NBS1 is phosphorylated in response to ionizing radiation. This phosphorylation is ATM-dependant and is required

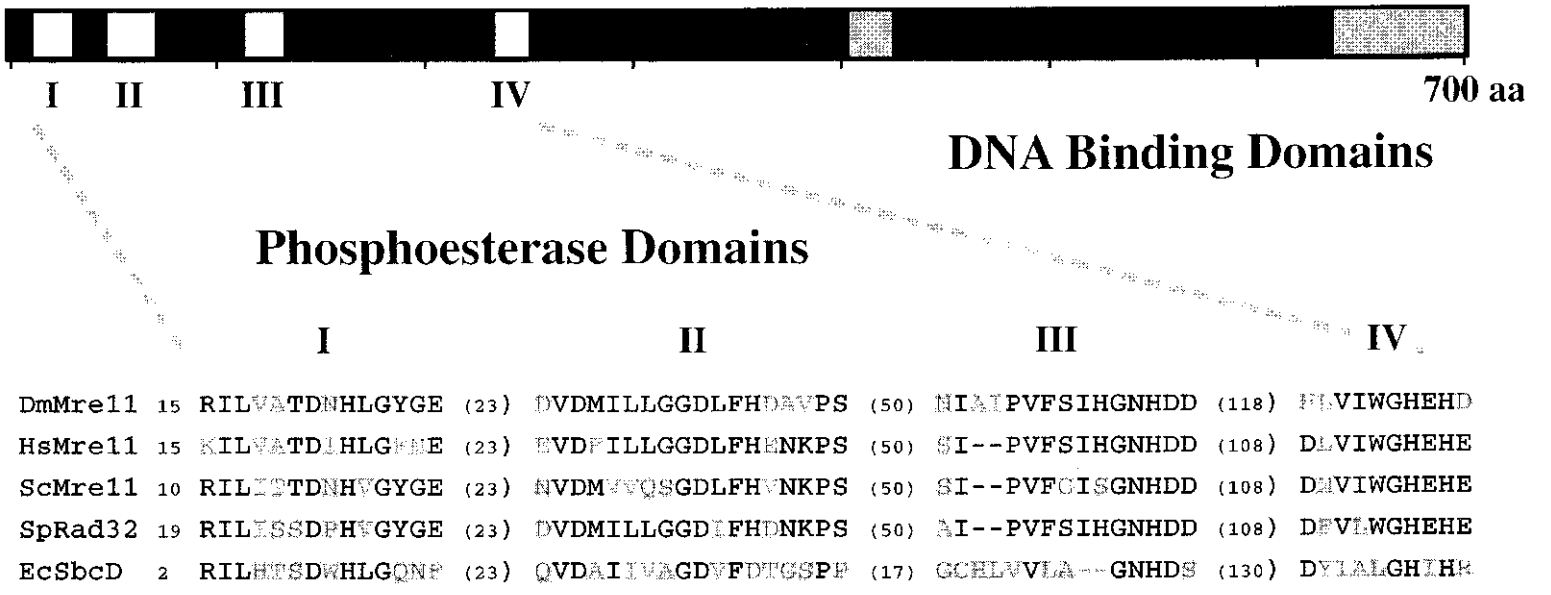

Fig. 7. Domain structure of MRE11 proteins. MRE11 proteins contain four conserved domains near the amino termini that are common to many proteins with phosphoesterase activity. Two apparent DNA binding domains are in the carboxy terminal halves. Also illustrated is an alignment of the amino acid sequence in the phosphoesterase domains from Drosophila, human, and yeast MRE11, as well as the more distantly related SbcD protein from *E. coli.*

for normal induction of an S-phase checkpoint *(98).* Drosophila *nbs* mRNA is found in both males and females and during most developmental stages, but it is most abundant in early embryos, pupae, and the adult head *(44).*

The Drosophila *mre11* gene was isolated using degenerate PCR. The MRE11 amino acid sequence is 36% and 29% identical to human and yeast homologs, respectively *(44).* MRE11 contains several distinct functional domains. Two domains involved in binding to DNA are highlighted in Fig. 8. Another domain contains phosphoesterase sequence motifs, which are required for MRE11 exonuclease activity and some but not all of the in vivo phenotypes of yeast and human *mre11* mutants.

The presumed sequence of Drosophila RAD50 is 29% identical to human RAD50 and 27% identical to yeast Rad50p. These proteins contain a large region of diverged sequence in their centers that may adopt a coiled coil structure, whereas the N- and C-termini are much more highly conserved. The N-terminal half of human RAD50 is able to bind to the BRCA1 protein *(176).* The structure of the Rad50 catalytic domain from a thermophilic bacterium suggests a mechanism that is driven by cycles of ATP-dependant dimerization and dissociation upon hydrolysis *(73).* Drosophila rad50 is expressed most highly in 0–8-hr embryos and moderately in late larval to adult stages *(44).*

4.5.8. p53 (Dmp53)

There have been rumors of a fly gene similar to human *p53* for several years *(39),* but it was only recently isolated *(20,75,130).* The fact that Drosophila possessed an intact signaling pathway capable of responding to human p53 was demonstrated earlier *(175).* Human *p53* expressed in Drosophila developing eye tissue was shown to cause severe apoptosis *(175).* The Drosophila homolog of p53 can bind to the same DNA targets as human p53 *(20,75,130).* Overexpression of *Dmp53* also induces apoptosis in flies *(130).* Dominant-negative mutant forms of DMP53 inhibit transactivation *(20,75)* and radiation-induced apoptosis *(20,130)* but do not appear to affect X-ray-induced cell-cycle arrest *(130).* Neither does overexpression of *Dmp53* seem to induce G1 arrest *(130).* Studies in the genetically tractable Drosophila model should provide new insight into the complexities of p53 function in cell-cycle control, DNA repair, and apoptosis. Some transcriptional targets of Drosophila p53 have been discovered *(20).*

4.5.9. Poly(ADP-Ribose)Polymerase

Poly(ADP-ribose)polymerase (PARP) plays an important regulatory role in DSBR in eukaryotes. Once a chromosome is broken, PARP quickly binds to the ends and begins to modify itself and other specific proteins in the vicinity by adding ADP-ribose residues until long, branched chains are attached. Many of the known PARP targets are DNA-binding proteins. Modification by PARP is believed to cause the dissociation of proteins from the DNA, clearing the region and thus facilitating DSBR. Activation of PARP causes rapid redistribution of many proteins within the nucleus. In Drosophila, PARP transcripts are highly abundant through the first half of embryogenesis and are distributed homogeneously except for the pole cells *(60).* The mRNA is also found at moderate levels in pupae and adults *(60).* Two classes of transcripts have been detected *(80,114).* Form I produces a full-length, active PARP enzyme, whereas form II lacks exon 5 encoding the auto-modification domain and appears to have no enzyme activity *(114).* The biological significance of form II is unknown though it can interfere with growth and development in cultured rat cells *(80).* Although no consensus cleavage site for a CED-3 like protease is found in fly PARP,

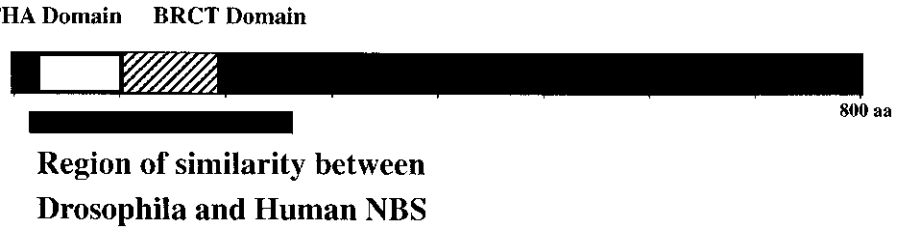

Fig. 8. Domain structure of NBS proteins. Only the first third of the human and Drosophila NBS proteins resemble each other. Within this region, both proteins share amino acid sequences matching the FHA domain consensus and the BRCT domain consensus.

it does appear to be processed during induced apoptosis *(136).* In Drosophila testes, PARP is activated in response to α-irradiation *(94).* PARP often forms foci or localizes to the nuclear rim in irradiated premeiotic and postmeiotic cells. In primary spermatocytes, PARP staining co-localizes with the discrete chromosomes at the periphery of the nuclei *(94).* Deletions of the *parp* gene lead to lethality late in embryogenesis *(114).* More subtle manipulations of PARP will be required to dissect its role in DSBR.

4.5.10. Other Repair Genes

Many studies have probed the function of Drosophila DSBR genes. A sample of these includes: the *mei-9* gene, a homolog of the yeast *RAD1* and human *XPF* genes *(154); mei-W68,* a homolog of the yeast meiotic endonuclease gene *SPO11 (111,112);* a *RAD51* homolog that has elevated expression in the ovaries *(2,110); Rrp1,* a gene encoding a combination AP endonuclease/3′ exonuclease similar to mammalian *APEX* genes *(147,148,168);* the *grapes* gene, which is homologous to human *CHK1 (46,159,164,165);* and *loki,* a gene encoding a serine/threonine kinase that is expressed in the ovary and early embryo and is similar to human *CHK2* and *S. cerevisiae DUN1 (128). mus304* confers mutagen sensitivity and is another checkpoint gene, but as yet is only known in flies *(21).* Dozens of other repair genes have been identified in flies but await experimental analysis. These include *FEN1, KU86, XRCC1, RAD21, RAD17, RAD1,* a *HUS1*-like gene, *RAD9,* DNA ligase I, DNA ligase III, DNA ligase IV, and genes with weaker matches to *XRCC2, RAD51, ATM, ATXR, FRAP,* and many others. Among DNA repair genes that are conspicuously absent from Drosophila homologs of *RAD52, RAD57,* and *RAD59,* and the MMR genes *MSH3, MSH4, MSH5, MLH2,* and *MLH3.* This explosion of enticing new leads to explore has ushered in a new era for research of DNA repair in Drosophila.

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REFERENCES

1. Adams, M. D. et al. 2000. The genome sequence of Drosophila melanogaster. *Science* **287:** 2185–2195.

2. Akaboshi, E., Y. Inoue, and H. Ryo. 1994. Cloning of the cDNA and genomic DNA that correspond to the recA-like gene of Drosophila melanogaster. *Jpn. J. Genet.* **69:** 663–670.
2a. Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, D. J. Lipman, (1997). "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." *Nucleic Acids Res* **25:** 3389–3402.
3. Baker, B. S., and A. T. C. Carpenter. 1972. Genetic analysis of sex chromosomal meiotic mutants in *Drosophila melanogaster. Genetics* **71:** 255–286.
4. Banga, S. S., J. B. Boyd, K. Valerie, P. V. Harris, E. M. Kurz, and J. K., de Riel. 1989. denV gene of bacteriophage T4 restores DNA excision repair to mei-9 and mus201 mutants of Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA* **86:** 3227–3231.
5. Banga, S. S., A. Velazquez, and J. B. Boyd. 1991. P transposition in Drosophila provides a new tool for analyzing postreplication repair and double-strand break repair. *Mutat. Res.* **255:** 79–88.
6. The Berkeley Drosophila Genome Project (BDGP). http://www.fruitfly.org/.
7. Beall, E. L., A. Admon, and D. C. Rio. 1994. A Drosophila protein homologous to the human p70 Ku autoimmune antigen interacts with the P transposable element inverted repeats. *Proc. Natl. Acad. Sci. USA* **91:** 12,681–12,685.
8. Beall, E. L., and D. C. Rio. 1996. Drosophila IRBP/Ku p70 corresponds to the mutagen-sensitive mus309 gene and is involved in P-element excision in vivo. *Genes Dev.* **10:** 921–933.
9. Beall, E. L., and D. C. Rio. 1997. Drosophila P-element transposase is a novel site-specific endonuclease. *Genes Dev.* **11:** 2137–2151.
10. Bhui-Kaur, A., M. F. Goodman, and J. Tower. 1998. DNA mismatch repair catalyzed by extracts of mitotic, postmitotic, and senescent Drosophila tissues and involvement of mei-9 gene function for full activity. *Mol. Cell. Biol.* **18:** 1436–1443.
11. Biriukova, T., I. Biriukova, and P. Georgiev. 1999. [Mechanisms of excising the P-element in a model system at the yellow locu of Drosophila melanogaster]. *Genetika* **35:** 28–36.
12. Bork, P., K. Hofmann, P. Bucher, A. F. Neuwald, S. F. Altschul, and E. V. Koonin. 1997. A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. *FASEB J.* **11:** 68–76.
13. Boulton, S. J., and S. P. Jackson. 1996. Identification of a Saccharomyces cerevisiae Ku80 homologue: roles in DNA double strand break rejoining and in telomeric maintenance. *Nucleic Acids Res.* **24:** 4639–4648.
14. Boulton, S. J., and S. P. Jackson. 1996. Saccharomyces cerevisiae Ku70 potentiates illegitimate DNA double-strand break repair and serves as a barrier to error-prone DNA repair pathways. *EMBO J.* **15:** 5093–5103.
15. Boyd, J. B., M. D. Golino, and R. B. Setlow. 1976. The mei-9 alpha mutant of Drosophila melanogaster increases mutagen sensitivity and decreases excision repair. *Genetics* **84:** 527–544.
16. Boyd, J. B., M. D. Golino, K. E. Shaw, C. J. Osgood, and M. M. Green. 1981. Third-chromosome mutagen-sensitive mutants of Drosophila melanogaster. *Genetics* **97:** 607–623.
17. Boyd, J. B., M. D. J. Golino, T. D. Nguyen, and M. M. Green. 1976. Isolation and characterization of X-linked mutations in *Drosophila melanogaster* which are sensitive to mutagens. *Genetics* **84:** 485–506.
18. Bradberry, W., C. S. Hong, X. R. Ren, D. Borromeo, R. Brooks, and B. McKee. 1995. Molecular analysis of *Drosophila* homologs (*dmlh-1* and *dmlh-2*) of the *mutL, hexB,* and *PMS1* mismatch repair genes. Presented at the 36th Annual Drosophila Research Conference.
19. Branch, P., G. Aquilina, M. Bignami, and P. Karran. 1993. Defective mismatch binding and a mutator phenotype in cells tolerant to DNA damage. *Nature* **362:** 652–654.
20. Brodsky, M. H., W. Nordstrom, G. Tsang, E. Kwan, G. M. Rubin, and J. M. Abrams. 2000. Drosophila p53 binds a damage response element at the reaper locus. *Cell* **101:** 103–113.
21. Brodsky, M. H., J. J. Sekelsky, G. Tsang, R. S. Hawley, and G. M. Rubin. 2000. mus304 encodes a novel DNA damage checkpoint protein required during Drosophila development. *Genes Dev.* **14:** 666–678.

22. Buermeyer, A. B., S. M. Deschenes, S. M. Baker, and R. M. Liskay. 1999. Mammalian DNA mismatch repair. *Annu. Rev. Genet.* **33:** 533–564.
23. Buermeyer, A. B., C. Wilson-Van Patten, S. M. Baker, and R. M. Liskay. 1999. The human MLH1 cDNA complements DNA mismatch repair defects in Mlh1-deficient mouse embryonic fibroblasts. *Cancer Res.* **59:** 538–541.
24. Burtis, K., R. S. Hawley, and G. M. Rubin. 1999. Survey of DNA repair in Drosophila melanogaster. http://www.dmrepair.ucdavis.edu/dmrepair/Findings.htm.
25. Carney, J. P., R. S. Maser, H. Olivares, E. M. Davis, M. Le Beau, J. R. Yates, 3rd, et al. 1998. The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell* **93:** 477–486.
26. Carpenter, A. T. 1975. Electron microscopy of meiosis in Drosophila melanogaster females: II. The recombination nodule: a recombination-associated structure at pachytene? *Proc. Natl. Acad. Sci. USA* **72:** 3186–3189.
27. Carpenter, A. T. 1979. Recombination nodules and synaptonemal complex in recombination-defective females of Drosophila melanogaster. *Chromosoma* **75:** 259–292.
28. Carpenter, A. T. C. 1982. Mismatch repair, gene conversion, and crossing-over in two recombination-defective mutants of *Drosophila melanogaster. Proc. Nat. Acad. Sci. USA* **79:** 5961–5965.
29. Cary, R. B., S. R. Peterson, J. Wang, D. G. Bear, E. M. Bradbury, and D. J. Chen. 1997. DNA looping by Ku and the DNA-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* **94:** 4267–4272.
30. Chakraverty, R. K., and I. D. Hickson. 1999. Defending genome integrity during DNA replication: a proposed role for RecQ family helicases. *Bioessays* **21:** 286–294.
31. Cox, M. M., M. F. Goodman, K. N. Kreuzer, D. J. Sherratt, S. J. Sandler, and K. J. Marians. 2000. The importance of repairing stalled replication forks. *Nature* **404:** 37–41.
32. Crouse, G. F. 1998. Mismatch repair systems in *Saccharomyces cerevisiae,* in *DNA Damage and Repair,* vol. I: *DNA Repair in Prokaryotes and Lower Eukaryotes* (Nickoloff, J. A. and M. F. Hoekstra, eds.), Humana Press, Totowa, NJ, pp. 411–448.
33. Datta, A., A. Adjiri, L. New, G. F. Crouse, and S. Jinks Robertson. 1996. Mitotic crossovers between diverged sequences are regulated by mismatch repair proteins in *Saccharomyces cerevisiae. Mol. Cell. Biol.* **16:** 1085–1093.
34. Digilio, F. A., A. Pannuti, J. C. Lucchesi, M. Furia, and L. C. Polito. 1996. Tosca: a Drosophila gene encoding a nuclease specifically expressed in the female germline. *Dev. Biol.* **178:** 90–100.
35. Dray, T., A. Coveney, and G. B. Gloor. Unpublished results.
36. Dray, T., and G. B. Gloor. 1997. Homology requirements for targeting heterologous sequences during P-induced gap repair in Drosophila melanogaster. *Genetics* **147:** 689–699.
37. Ducau, J., J.-C. Bregliano, and C. de La Roche Saint-Andre. 2000. Gamma-irradiation stimulates homology-directed DNA double-strand break repair in Drosophila embryo. *Mutat. Res.* **460:** 69–80.
38. Dusenbery, R. L., S. C. McCormick, and P. D. Smith. 1983. Drosophila mutations at the *mei-9* and *mus(2)201* loci which block excision of thymine dimers also block induction of unscheduled DNA synthesis by methyl methanesulfonate, ethyl methanesulfonate, N-methyl-N-nitrosourea, UV light and X-rays. *Mutat. Res.* **112:** 215–230.
39. Dusenbery, R. L., and P. D. Smith. 1996. Cellular responses to DNA damage in Drosophila melanogaster. *Mutat. Res.* **364:** 133–145.
40. Engels, W. R., D. M. Johnson-Schlitz, W. B. Eggleston, and J. Sved. 1990. High-frequency P element loss in Drosophila is homolog-dependent. *Cell* **62:** 515–525.
41. Engels, W. R., C. R. Preston, and D. M. Johnson-Schlitz. 1994. Long-range *cis* preference in DNA homology search extending over the length of a Drosophila chromosome. *Science* **263:** 1623–1625.
42. Fishel, R., M. Lescoe, M. Rao, N. Copeland, N. Jenkins, J. Garber, M. Kane, and M. Kolodner. 1993. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. *Cell* **75:** 1027–1038.

43. Flores, C., and W. Engels. 1999. Microsatellite instability in Drosophila spellchecker1 (MutS homolog) mutants. *Proc. Natl. Acad. Sci. USA* **96:** 2964–2969.
44. Flores, C., and W. Engels. Unpublished results.
45. FlyBase. 1999. The FlyBase database of the Drosophila Genome Projects and community literature (http://flybase.bio.indiana.edu:7083/). *The FlyBase Consortium Nucleic Acids Res.* **27:** 85–88.
46. Fogarty, P., S. D. Campbell, R. Abu-Shumays, B. S. Phalle, K. R. Yu, G. L. Uy, M. L. Goldberg, and W. Sullivan. 1997. The Drosophila grapes gene is related to checkpoint gene chk1/rad27 and is required for late syncytial division fidelity. *Curr. Biol.* **7:** 418–426.
47. Fung, J. C., W. F. Marshall, A. Dernburg, D. A. Agard, and J. W. Sedat. 1998. Homologous chromosome pairing in Drosophila melanogaster proceeds through multiple independent initiations. *J. Cell Biol.* **141:** 5–20.
48. Geyer, P. K., K. L. Richardson, V. G. Corces, and M. M. Green. 1988. Genetic instability in Drosophila melanogaster: P-element mutagenesis by gene conversion. *Proc. Natl. Acad. Sci. USA* **85:** 6455–6459.
49. Ghabrial, A., R. P. Ray, and T. Schupbach. 1998. okra and spindle-B encode components of the RAD52 DNA repair pathway and affect meiosis and patterning in Drosophila oogenesis. *Genes Dev.* **12:** 2711–2723.
50. Ghabrial, A., and T. Schupbach. 1999. Activation of a meiotic checkpoint regulates translation of Gurken during Drosophila oogenesis. *Nature Cell Biol.* **1:** 354–357.
51. Gloor, G. Personal communication.
52. Gloor, G. B., and D. H. Lankenau. 1998. Gene conversion in mitotically dividing cells: a view from Drosophila. *Trends Genet.* **14:** 43–46.
53. Gloor, G. B., J. Moretti, J. Mouyal, and K. J. Keeler. 2000. Distinct P element excision products in somatic and germline cells of *Drosophila melanogaster. Genetics* **155:** 1821–1830.
54. Gloor, G. B., N. A. Nassif, D. M. Johnson-Schlitz, C. R. Preston, and W. R. Engels. 1991. Targeted gene replacement in Drosophila via P element-induced gap repair. *Science* **253:** 1110–1117.
55. Golic, M. M., and K. G. Golic. 1996. A quantitative measure of the mitotic pairing of alleles in Drosophila melanogaster and the influence of structural heterozygosity. *Genetics* **143:** 385–400.
56. Gonzalez-Reyes, A. 1999. DNA repair and pattern formation come together. *Nature Cell Biol.* **1:**E150–E152.
57. Gonzalez-Reyes, A., H. Elliott, and D. St Johnston. 1997. Oocyte determination and the origin of polarity in Drosophila: the role of the spindle genes. *Development* **124:** 4927–4937.
58. Gonzy-Treboul, G., J. A. Lepesant, and J. Deutsch. 1995. Enhancer-trap targeting at the Broad-Complex locus of Drosophila melanogaster. *Genes Dev.* **9:** 1137–1148.
59. Hagmann, M., R. Bruggmann, L. Xue, O. Georgiev, W. Schaffner, D. Rungger, P. Spaniol, and T. Gerster. 1998. Homologous recombination and DNA-end joining reactions in zygotes and early embryos of zebrafish (Danio rerio) and Drosophila melanogaster [see comments]. *Biol. Chem.* **379:** 673–681.
60. Hanai, S., M. Uchida, S. Kobayashi, M. Miwa, and K. Uchida. 1998. Genomic organization of Drosophila poly(ADP-ribose) polymerase and distribution of its mRNA during development. *J. Biol. Chem.* **273:** 11,881–11,886.
61. Handler, A. M., and S. P. Gomez. 1997. P element excision in Drosophila is stimulated by gamma-irradiation in transient embryonic assays. *Genet. Res.* **70:** 75–78.
62. Hari, K. L., A. Santerre, J. J. Sekelsky, K. S. McKim, J. B. Boyd, and R. S. Hawley. 1995. The mei-41 gene of D. melanogaster is a structural and functional homolog of the human ataxia telangiectasia gene. *Cell* **82:** 815–821.
63. Hastings, P. J., C. McGill, B. Shafer, and J. N. Strathern. 1993. Ends-in vs. ends-out recombination in yeast. *Genetics* **135:** 973–980.

64. Henderson, D. S. 1999. DNA repair defects and other (mus)takes in Drosophila melanogaster. *Methods* **18:** 377–400.
65. Henderson, D. S., D. A. Bailey, D. A. Sinclair, and T. A. Grigliatti. 1987. Isolation and characterization of second chromosome mutagen-sensitive mutations in Drosophila melanogaster. *Mutat. Res.* **177:** 83–93.
66. Henderson, D. S., S. S. Banga, T. A. Grigliatti, and J. B. Boyd. 1994. Mutagen sensitivity and suppression of position-effect variegation result from mutations in mus209, the Drosophila gene encoding PCNA. *EMBO J.* **13:** 1450–1459.
67. Henderson, D. S., and D. M. Glover. 1998. Chromosome fragmentation resulting from an inability to repair transposase-induced DNA double-strand breaks in PCNA mutants of Drosophila. *Mutagenesis* **13:** 57–60.
68. Henderson, D. S., U. K. Wiegand, D. G. Norman, and D. M. Glover. 2000. Mutual correction of faulty PCNA subunits in temperature-sensitive lethal mus209 mutants of Drosophila melanogaster. *Genetics* **154:** 1721–1733.
69. Heslip, T. R., and R. B. Hodgetts. 1994. Targeted transposition at the vestigial locus of Drosophila melanogaster. *Genetics* **138:** 1127–1135.
70. Hiraoka, Y., A. F. Dernburg, S. J. Parmelee, M. C. Rykowski, D. A. Agard, and J. W. Sedat. 1993. The onset of homologous chromosome pairing during Drosophila melanogaster embryogenesis. *J. Cell Biol.* **120:** 591–600.
71. Hofmann, K., and P. Bucher. 1995. The FHA domain: a putative nuclear signalling domain found in protein kinases and transcription factors. *Trends Biochem. Sci.* **20:** 347–349.
72. Holmes J., Jr., S. Clark, and P. Modrich. 1990. Strand-specific mismatch correction in nuclear extracts of human and Drosophila melanogaster cell lines. *Proc. Natl. Acad. Sci. USA* **87:** 5837–5841.
73. Hopfner, K.-P., A. Karcher, D. S. Shin, L. Craig, L. M. Arthur, J. P. Carney, and J. A. Tainer. 2000. Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. *Cell* **101:** 789–800.
74. Jacoby, D. B., and P. C. Wensink. 1994. Yolk protein factor 1 is a Drosophila homolog of Ku, the DNA-binding subunit of a DNA-dependent protein kinase from humans. *J. Biol. Chem.* **269:** 11484–11491.
75. Jin, S., S. Martinek, W. S. Joo, J. R. Wortman, N. Mirkovic, A. Sali, M. D. Yandell, N. P. Pavletich, M. W. Young, and A. J. Levine. 2000. Identification and characterization of a p53 homologue in Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA* **97:** 7301–7306.
76. Johnson-Schlitz, D. M., and W. R. Engels. Unpublished results.
77. Johnson-Schlitz, D. M., and W. R. Engels. 1993. P element-induced interallelic gene conversion of insertions and deletions in Drosophila. *Mol. Cell. Biol.* **13:** 7006–7018.
78. Johnson-Schlitz, D. M., and W. R. Engels. Unpublished results.
79. Karow, J. K., L. Wu, and I. D. Hickson. 2000. RecQ family helicases: roles in cancer and aging. *Curr. Opin. Genet. Dev.* **10:** 32–38.
80. Kawamura, T., S. Hanai, T. Yokota, T. Hayashi, P. Poltronieri, M. Miwa, and K. Uchida. 1998. An alternative form of poly(ADP-ribose) polymerase in Drosophila melanogaster and its ectopic expression in rat-1 cells. *Biochem. Biophys. Res. Commun.* **251:** 35–40.
81. Keeler, K. J., T. Dray, J. E. Penney, and G. B. Gloor. 1996. Gene targeting of a plasmid-borne sequence to a double-strand DNA break in Drosophila melanogaster. *Mol. Cell. Biol.* **16:** 522–528.
82. Keeler, K. J., and G. B. Gloor. 1997. Efficient gap repair in Drosophila melanogaster requires a maximum of 31 nucleotides of homologous sequence at the searching ends. *Mol. Cell. Biol.* **17:** 627–634.
83. Kitao, S., N. M. Lindor, M. Shiratori, Y. Furuichi, and A. Shimamoto. 1999. Rothmund-thomson syndrome responsible gene, RECQL4: genomic structure and products. *Genomics* **61:** 268–276.

84. Kitao, S., A. Shimamoto, M. Goto, R. W. Miller, W. A. Smithson, N. M. Lindor, and Y. Furuichi. 1999. Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. *Nature Genet.* **22:** 82–4.
85. Kolodner, R. D., and G. T. Marsischky. 1999. Eukaryotic DNA mismatch repair. *Curr. Opin. Genet. Dev.* **9:** 89–96.
86. Kooistra, R., A. Pastink, J. B. Zonneveld, P. H. Lohman, and J. C. Eeken. 1999. The Drosophila melanogaster DmRAD54 gene plays a crucial role in double-strand break repair after P-element excision and acts synergistically with Ku70 in the repair of X-ray damage. *Mol. Cell. Biol.* **19:** 6269–6275.
87. Kooistra, R., K. Vreeken, J. B. Zonneveld, A. de Jong, J. C. Eeken, C. J. Osgood, et al. 1997. The Drosophila melanogaster RAD54 homolog, DmRAD54, is involved in the repair of radiation damage and recombination. *Mol. Cell. Biol.* **17:** 6097–6104.
88. Kramer, K. M., J. A. Brock, K. Bloom, J. K. Moore, and J. E. Haber. 1994. Two different types of double-strand breaks in Saccharomyces cerevisiae are repaired by similar RAD52-independent, nonhomologous recombination events. *Mol. Cell. Biol.* **14:** 1293–1301.
89. Kusano, K. Personal communication.
90. Kusano, K., M. E. Berres, and W. R. Engels. 1999. Evolution of the RECQ family of helicases: A drosophila homolog, Dmblm, is similar to the human bloom syndrome gene. *Genetics* **151:** 1027–1039.
91. Langdon, T., C. Seago, R. Jones, H. Ougham, H. Thomas, J. Forster, and G. Jenkins. 2000. De novo evolution of satellite DNA on the rye B chromosome. *Genetics* **154:** 869–884.
92. Lankenau, D. H., and G. B. Gloor. 1998. In vivo gap repair in Drosophila: a one-way street with many destinations. *Bioessays* **20:** 317–327.
93. Lankenau, D. H., M. V. Peluso, and S. Lankenau. 2000. The Su(Hw) chromatin insulator protein alters double-strand break repair frequencies in the Drosophila germ line. *Chromosoma* **109:** 148–160.
94. Lankenau, S., A. Burkle, and D. H. Lankenau. 1999. Detection of poly(ADP-ribose) synthesis in Drosophila testes upon gamma-irradiation. *Chromosoma* **108:** 44–51.
95. Leach, F., et al. 1993. Mutations of a *mutS* homolog in hereditary nonpolyposis colorectal cancer. *Cell* **75:** 1215–1225.
96. Lee, B. I., M. Shannon, L. Stubbs, and D. M. Wilson, 3rd. 1999. Expression specificity of the mouse exonuclease 1 (mExol) gene. *Nucleic Acids Res.* **27:** 4114–2410.
97. Liang, F., and M. Jasin. 1996. Ku80-deficient cells exhibit excess degradation of extrachromosomal *DNA. J. Biol. Chem.* **271:** 14,405–14,411.
98. Lim, D. S., S. T. Kim, B. Xu, R. S. Maser, J. Lin, J. H. Petrini, and M. B. Kastan. 2000. ATM phosphorylates p95/nbsl in an S-phase checkpoint pathway. *Nature* **404:** 613–617.
99. Lindor, N. M., Y. Furuichi, S. Kitao, A. Shimamoto, C. Arndt, and S. Jalal. 2000. Rothmund-Thomson syndrome due to RECQ4 helicase mutations: report and clinical and molecular comparisons with Bloom syndrome and Werner syndrome. *Am. J. Med. Genet.* **90:** 223–228.
100. Liu, A.-L. 1998. Biochemistry of mammalian DNA mismatch repair, in *DNA Damage and Repair,* vol. II: *DNA Repair in Higher Eukaryotes,* (Nickoloff, J. A. and M. F. Hoekstra, eds.), Humana Press, Totowa, NJ, pp. 95–118.
101. Lohe, A. R., C. Timmons, I. Beerman, E. R. Lozovskaya, and D. L. Hartl. 2000. Self-inflicted wounds, template-directed gap repair and a recombination hotspot. Effects of the mariner transposase. *Genetics* **154:** 647–656.
102. Luo, G., M. S. Yao, C. F. Bender, M. Mills, A. R. Bladl, A. Bradley, and J. H. Petrini. 1999. Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc. Natl. Acad. Sci. USA* **96:** 7376–7381.
102a. Frank, L., Ramsahoye, B. H., Jaenisch, R. 2000. Development: DNA methylation in Drosophila melanogaster. *Nature* **408:** 538–540.

103. Lynch, H. T., and A. de la Chapelle. 1999. Genetic susceptibility to non-polyposis colorectal cancer. *J. Med. Genet.* **36:** 801–818.
104. Maser, R. S., K. J. Monsen, B. E. Nelms, and J. H. Petrini. 1997. hMre11 and hRad50 nuclear foci are induced during the normal cellular response to DNA double-strand breaks. *Mol. Cell. Biol.* **17:** 6087–6096.
105. Mason, J. M., M. M. Green, K. E. Shaw, and J. B. Boyd. 1981. Genetic analysis of X-linked mutagen-sensitive mutants of Drosophila melanogaster. *Mutat. Res.* **81:** 329–343.
106. Matsuura, S., H. Tauchi, A. Nakamura, N. Kondo, S. Sakamoto, S. Endo, D. et al. 1998. Positional cloning of the gene for Nijmegen breakage syndrome. *Nature Genet.* **19:** 179–181.
107. McCall, K., and W. Bender. 1996. Probes of chromatin accessibility in the Drosophila bithorax complex respond differently to Polycomb-mediated repression. *EMBO J.* **15:** 569–580.
108. McKee, B. 2000. Personal communication.
109. McKee, B. 1994. Cloning and molecular characterization of a *Drosophila* homolog *(dmlh-1)* of the bacterial mutL and *hexB* and the yeast *PMS1* mismatch repair genes. Presented at the 35th Annual Drosophila Research Conference.
110. McKee, B. D., X. Ren, and C. Hong. 1996. A recA-like gene in Drosophila melanogaster that is expressed at high levels in female but not male meiotic tissues. *Chromosoma* **104:** 479–488.
111. McKim, K. S., B. L. Green-Marroquin, J. J. Sekelsky, G. Chin, C. Steinberg, R. Khodosh, and R. S. Hawley. 1998. Meiotic synapsis in the absence of recombination [see comments]. *Science* **279:** 876–878.
112. McKim, K. S., and A. Hayashi-Hagihara. 1998. mei-W68 in Drosophila melanogaster encodes a Spo11 homolog: evidence that the mechanism for initiating meiotic recombination is conserved. *Genes Dev.* **12:** 2932–2942.
113. McKim, K. S., J. K. Jang, J. J. Sekelsky, A. Laurencon, and R. S. Hawley. 2000. mei-41 is required for precocious anaphase in Drosophila females. *Chromosoma* **109:** 44–49.
114. Miwa, M., S. Hanai, P. Poltronieri, M. Uchida, and K. Uchida. 1999. Functional analysis of poly(ADP-ribose) polymerase in Drosophila melanogaster. *Mol. Cell. Biochem.* **193:** 103–107.
115. Moggs, J. G., P. Grandi, J. P. Quivy, Z. O. Jonsson, U. Hubscher, P. B. Becker, and G. Almouzni. 2000. A CAF-1-PCNA-mediated chromatin assembly pathway triggered by sensing DNA damage. *Mol. Cell. Biol.* **20:** 1206–1218.
116. Morris, J., and R. Lehmann. 1999. Drosophila oogenesis: versatile spn doctors. *Curr. Biol.* **9:** R55–R58.
117. Mueller, J., J. Clyman, Y. Huang, M. Parker, and M. Belfort. 1996. Intron mobility in phage T4 occurs in the context of recombination-dependent DNA replication by way of multiple pathways. *Genes Dev.* **10:** 351–364.
118. Muller, H. J. 1927. Artificial transmutation of the gene. *Science* **66:** 84–87.
119. Nassif, N. A., and W. R. Engels. 1993. DNA homology requirements for mitotic gap repair in Drosophila. *Proc. Nat. Acad. Sci. USA* **90:** 1262–1266.
120. Nassif, N. A., J. Penney, S. Pal, W. R. Engels, and G. B. Gloor. 1994. Efficient copying of nonhomologous sequences from ectopic sites via P element-induced gap repair. *Mol. Cell. Biol.* **14:** 1613–1625.
121. Nelms, B. E., R. S. Maser, J. F. MacKay, M. G. Lagally, and J. H. Petrini. 1998. In situ visualization of DNA double-strand break repair in human fibroblasts. *Science* **280:** 590–592.
122. Nicholson, A., M. Hendrix, S. Jinks-Robertson, and G. F. Crouse. 2000. Regulation of mitotic homeologous recombination in yeast. Functions of mismatch repair and nucleotide excision repair genes. *Genetics* **154:** 133–146.
123. Nicolaides, N. C. et al. 1994. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature* **371:** 75–80.
124. Nivard, M. J., J. Wijen, and E. W. Vogel. 1998. Germ cell mutagenesis in Drosophila: multiple endpoint analysis. *Acta Biochim. Pol.* **45:** 545–559.

125. O'Brochta, D. A., S. P. Gomez, and A. M. Handler. 1991. P element excision in Drosophila melanogaster and related drosophilids. *Mol. Gen. Genet.* **225:** 387–394.
126. O'Brochta, D. A., and A. M. Handler. 1988. Mobility of P elements in drosophilids and non-drosophilids. *Proc. Nat. Acad. Sci. USA* **85:** 6052–6056.
127. Oda, S., O. Humbert, S. Fiumicino, M. Bignami, and P. Karran. 2000. Efficient repair of A/C mismatches in mouse cells deficient in long-patch mismatch repair. *EMBO J.* **19:** 1711–1718.
128. Oishi, I., S. Sugiyama, H. Otani, H. Yamamura, Y. Nishida, and Y. Minami. 1998. A novel Drosophila nuclear protein serine/threonine kinase expressed in the germline during its establishment. *Mech. Dev.* **71:** 49–63.
129. Oliveri, D. R., P. V. Harris, and J. B. Boyd. 1990. X-ray sensitivity and single-strand DNA break repair in mutagen- sensitive mutants of Drosophila melanogaster. *Mutat. Res.* **235:** 25–31.
130. Ollmann, M., L. M. Young, C. J. Di Como, F. Karim, M. Belvin, S. Robertson, et al. 2000. Drosophila p53 is a structural and functional homolog of the tumor suppressor p53. *Cell* **101:** 91–101.
131. Pang, D., S. Yoo, W. S. Dynan, M. Jung, and A. Dritschilo. 1997. Ku proteins join DNA fragments as shown by atomic force microscopy. *Cancer Res.* **57:** 1412–1415.
132. Papadopoulos, N. et al. 1994. Mutation of a mutL homolog in hereditary colon cancer [see comments]. *Science* **263:** 1625–1629.
133. Paques, F., W. Y. Leung, and J. E. Haber. 1998. Expansions and contractions in a tandem repeat induced by double-strand break repair. *Mol. Cell. Biol.* **18:** 2045–2054.
134. Paull, T. T., and M. Gellert. 2000. A mechanistic basis for Mre11-directed DNA joining at microhomologies. *Proc. Natl. Acad. Sci. USA* **97:** 6409–6414.
135. Peronnet, F., F. San Giorgio, J. A. Lepesant, J. S. Deutsch, and G. Gonzy-Treboul. 2000. Three-partner conversion induced by the P-element transposase in Drosophila melanogaster. *Mol. Gen. Genet.* **262:** 1123–1131.
136. Poltronieri, P., T. Yokota, Y. Koyama, S. Hanai, K. Uchida, and M. Miwa. 1997. PARP cleavage in the apoptotic pathway in S2 cells from Drosophila melanogaster. *Biochem. Cell Biol.* **75:** 445–449.
137. Preston, C. R., and W. R. Engels. unpublished results.
138. Price, D., S. Rabinovitch, P. H. O'Farrell, and S. D. Campbell. 2000. Drosophila wee 1 has an essential role in the nuclear divisions of early embryogenesis. *Genetics* **155:** 159–166.
139. Prolla, T. A., S. Baker, and M. R. Liskay. 1998. Genetics of DNA mismatch repair, microsatellite instability, and cancer, In *DNA Damage and Repair,* vol. II: *DNA Repair in Higher Eukaryotes,* (Nickoloff, J. A. and M. F. Hoekstra, eds.), Humana Press, Totowa, NJ, pp. 443–464.
140. Rasmussen, L. J., L. Samson, and M. G. Marinas. 1998. Dam-directed DNA mismatch repair., In *DNA Damage and Repair,* vol. I: *DNA Repair in Prokaryotes and Lower Eukaryotes,* (Nickoloff, J. A. and M. F. Hoekstra, eds.), Humana Press, Totowa, NJ, pp. 205–228.
141. Rong, Y. S., and K. G. Golic. 2000. Gene targeting by homologous recombination in Drosophila. *Science* **288:** 2013–2918.
142. Roth, D. B., and J. H. Wilson. 1986. Nonhomologous recombination in mammalian cells: role for short sequence homologies in the joining reaction. *Mol. Cell. Biol.* **6:** 4295–4304.
143. Rothstein, R., B. Michel, and S. Gangloff. 2000. Replication fork pausing and recombination or "gimme a break". *Genes Dev.* **14:** 1–10.
144. Rouet, P., F. Smih, and M. Jasin. 1994. Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. *Mol. Cell. Biol.* **14:** 8095–8106.
145. Rubin, E., and A. Levy. 1997. Abortive gap repair: underlying mechanism for Ds element formation. *Mol. Cell. Biol.* **17:** 6294–3602.
146. Rudolph, C., O. Fleck, and J. Kohli. 1998. Schizosaccharomyces pombe exol is involved in the same mismatch repair pathway as msh2 and pms 1. *Curr. Genet.* **34:** 343–350.
147. Sander, M., K. Lowenhaupt, W. S. Lane, and A. Rich. 1991. Cloning and characterization of Rrp 1, the gene encoding Drosophila strand transferase: carboxy-terminal homology to DNA repair endo/exonucleases. *Nucleic Acids Res.* **19:** 4523–4529.

148. Sander, M., K. Lowenhaupt, and A. Rich. 1991. Drosophila Rrp 1 protein: an apurinic endonuclease with homologous recombination activities. *Proc. Natl. Acad. Sci. USA* **88:** 6780–6784.
149. Schiestl, R. H., J. Zhu, and T. D. Petes. 1994. Effect of mutations in genes affecting homologous recombination on restriction enzyme-mediated and illegitimate recombination in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **14:** 4493–4500.
150. Schmutte, C., R. C. Marinescu, M. M. Sadoff, S. Guerrette, J. Overhauser, and R. Fishel. 1998. Human exonuclease I interacts with the mismatch repair protein hMSH2. *Cancer Res.* **58:** 4537–4542.
151. Schweizer, P. M. 1989. A cell-cycle stage-related chromosomal X-ray hypersensitivity in larval neuroblasts of Drosophila mei-9 and mei-41 mutants suggesting defective DNA double-strand break repair. *Mutat. Res.* **211:** 111–124.
152. Sekelsky, J. personal communication.
153. Sekelsky, J. J., M. H. Brodsky, G. M. Rubin, and R. S. Hawley. 1999. Drosophila and human RecQ5 exist in different isoforms generated by alternative splicing. *Nucleic Acids Res.* **27:** 3762–3769.
154. Sekelsky, J. J., K. C. Burtis, and R. S. Hawley. 1998. Damage control: the pleiotropy of DNA repair genes in Drosophila melanogaster. *Genetics* **148:** 1587–1598.
155. Sekelsky, J. J., K. S. McKim, G. M. Chin, and R. S. Hawley. 1995. The Drosophila meiotic recombination gene mei-9 encodes a homologue of the yeast excision repair protein Rad1. *Genetics* **141:** 619–627.
156. Sepp, K. J., and V. J. Auld. 1999. Conversion of lacZ enhancer trap lines to GAL4 lines using targeted transposition in Drosophila melanogaster. *Genetics* **151:** 1093–1101.
157. Shimamoto, A., K. Nishikawa, S. Kitao, and Y. Furuichi. 2000. Human RecQ5β, a large isomer of RecQ5 DNA helicase, localizes in the nucleoplasm and interacts with topoisomerases 3α and 3β. *Nucleic Acids Res.* **28:** 1647–1655.
158. Sia, E. A., R. J. Kokoska, M. Dominska, P. Greenwell, and T. D. Petes. 1997. Microsatellite instability in yeast: dependence on repeat unit size and DNA mismatch repair genes. *Mol. Cell. Biol.* **17:** 2851–2858.
159. Sibon, O. C., A. Laurencon, R. Hawley, and W. E. Theurkauf. 1999. The Drosophila ATM homologue Mei-41 has an essential checkpoint function at the midblastula transition. *Curr. Biol.* **9:** 302–312.
160. Stapleton, W., C. Hong, X. Ren, D. Borromeo, R. Brooks, and B. McKee. 1998. Presented at the 39th Annual Drosophila Research Conference.
161. Staveley, B. E., T. R. Heslip, R. B. Hodgetts, and J. B. Bell. 1995. Protected P-element termini suggest a role for inverted-repeat- binding protein in transposase-induced gap repair in Drosophila melanogaster. *Genetics* **139:** 1321–1329.
162. Stewart, G. S., R. S. Maser, T. Stankovic, D. A. Bressan, M. I. Kaplan, N. G. Jaspers, et al. 1999. The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell* **99:** 577–587.
163. Strand, M., T. A. Prolla, R. M. Liskay, and T. Petes. 1993. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* **365:** 274–276.
164. Su, T. T., S. D. Campbell, and P. H. O'Farrell. 1998. The cell cycle program in germ cells of the Drosophila embryo. *Dev. Biol.* **196:** 160–710.
165. Su, T. T., S. D. Campbell, and P. H. O'Farrell. 1999. Drosophila grapes/CHK1 mutants are defective in cyclin proteolysis and coordination of mitotic events. *Curr. Biol.* **9:** 919–922.
166. Sugawara, N., and J. E. Haber. 1992. Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. *Mol. Cell. Biol.* **12:** 563–575.
167. Szabad, J. Personal communication to FlyBase.
168. Szakmary, A., S. M. Huang, D. T. Chang, P. A. Beachy, and M. Sander. 1996. Overexpression of a Rrp1 transgene reduces the somatic mutation and recombination frequency induced by oxidative DNA damage in Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA* **93:** 1607–1612.

169. Takasu-Ishikawa, E., M. Yoshihara, and Y. Hotta. 1992. Extra sequences found at P element excision sites in Drosophila melanogaster. *Mol. Gen. Genet.* **232:** 17–23.
170. Trujillo, K. M., S. S. Yuan, E. Y. Lee, and P. Sung. 1998. Nuclease activities in a complex of human recombination and DNA repair factors Rad50, Mre11, and p95. *J. Biol. Chem.* **273:** 21,447–21,450.
171. Varon, R., C. Vissinga, M. Platzer, K. M. Cerosaletti, K. H. Chrzanowska, K. Saar, et al. 1998. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell* **93:** 467–476.
172. Vennos, E. M., M. Collins, and W. D. James. 1992. Rothmund-Thomson syndrome: review of the world literature. *J. Am. Acad. Dermatol.* **27:** 750–762.
173. Warbrick, E., D. P. Lane, D. M. Glover, and L. S. Cox. 1997. Homologous regions of Fen1 and p21/Cip1 compete for binding to the same site on PCNA: a potential mechanism to co-ordinate DNA replication and repair. *Oncogene* **14:** 2313–2321.
174. Xiao, Y., and D. T. Weaver. 1997. Conditional gene targeted deletion by Cre recombinase demonstrates the requirement for the double-strand break repair Mre11 protein in murine embryonic stem cells. *Nucleic Acids Res.* **25:** 2985–2991.
175. Yamaguchi, M., F. Hirose, Y. H. Inoue, M. Shiraki, Y. Hayashi, Y. Nishi, and A. Matsukage. 1999. Ectopic expression of human p53 inhibits entry into S phase and induces apoptosis in the Drosophila eye imaginal disc. *Oncogene* **18:** 6767–6775.
176. Zhong, Q., C. F. Chen, S. Li, Y. Chen, C. C. Wang, J. Xiao, P. L. Chen, Z. D. Sharp, and W. H. Lee. 1999. Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. *Science* **285:** 747–750.

9

Double-Strand Break Repair and Homologous Recombination in Mammalian Cells

Maria Jasin

1. INTRODUCTION

A paradigm shift has occurred over the last few years in the understanding of homologous recombination. It has long been known that DNA double-strand breaks (DSBs) in yeast are potent inducers of homologous recombination and that homologous recombination is the major pathway in yeast to repair DSBs (Chapter 16, Vol. 1). Compared with nonhomologous repair, homologous recombination has generally been considered to be inconsequential as a DSB repair pathway in mammalian cells. However, homologous repair can precisely restore the damaged DNA to its original sequence, suggesting that it should be a preferred pathway for repair, at least under some circumstances. Recently, direct examination of repair products in mammalian cells has demonstrated the importance of homologous recombination during the repair of DSBs. Supporting this conclusion has been the identification of DNA repair defects in mutant cell lines and the construction of mouse knockouts of genes implicated in homologous recombination. This chapter discusses basic parameters of DSB repair by homologous recombination in mammalian cells and emerging evidence for the involvement of various proteins in the repair process.

2. TOOLS TO STUDY CHROMOSOMAL DSB REPAIR

Experiments that address the mechanism of DSB repair generally begin with the introduction of one or more DSB(s) into a target molecule. DSBs can be introduced into either plasmids or chromosomal DNA by a number of techniques. Although the focus of this chapter is on the introduction of a DSB at a defined site in mammalian chromosomes, it is important at the outset to contrast this approach with other experimental approaches.

2.1. Chromosomal vs Plasmid DSB Repair

There is an extensive literature on the analysis of DSB repair in mammalian cells using plasmid substrates *(134).* These substrates are cleaved in vitro by restriction enzymes, introduced into cells, and then recovered either as plasmids or integrated into genomic DNA to determine the mode of repair. However, the relevance of plasmid DSB repair to

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chromosomal DSB repair is uncertain because plasmid substrates are introduced without chromatin proteins and are rapidly degraded after transfection *(80).* Not surprisingly, substantial differences between plasmid and chromosomal homologous recombination products have been reported *(150).* This contrasts with studies of V(D)J recombination in which plasmid substrates appear to faithfully recapitulate aspects of DSB repair during endogenous antigen-receptor gene rearrangement *(77).* The plasmid substrates in this case replicate and become chromatinized before DSBs are introduced into them by the recombinase proteins. For most other studies, especially for homologous recombination, direct examination of chromosome DSB repair is more biologically relevant.

2.2. Nonspecific Agents for Introducing DSBs into Genomic DNA

Various nonspecific agents have been used to introduce DSBs into mammalian genomes, including ionizing radiation (IR) and radiomimetic drugs. Although IR causes a variety of lesions, e.g., single-strand breaks and base damage, DSBs have been deduced to be the toxic lesions (see Chapter 25, Vol. 2). IR and radiomimetic drugs are useful for understanding the global response of a cell to DNA damage and for the identification of repair mutants. However, the molecular analysis of the repair of such breaks is difficult, because the position of the break site is unknown and multiple lesions are introduced. Alternatively, restriction enzymes provide sequence specificity, as well as a defined type of DSB, i.e., a 5′ or 3′ overhang or blunt end. Restriction enzymes are introduced by electroporation or streptolysin O poration of cells *(14).* Although the potential number of cleavage sites for restriction enzymes is numerous (e.g., 1.5×10^6 sites per genome for a 6 bp recognition site), the enzymes can be titrated to introduce a limited number of breaks to allow cell survival. As a result of the cleavage specificity, it is possible in some cases to infer the chromosomal location of the DSB and to determine the molecular mechanism of repair. For example, mutations in the *aprt* gene have been selected after restriction-enzyme electroporation and then mapped to determine if their location corresponds to the location of a cleavage site for the introduced restriction enzyme *(108).* This approach has been useful in studies of mutagenic nonhomologous repair because loss of function mutations can be selected at a gene such as *aprt.* However, the utility of this approach is limited to a few such selectable markers and the sites of cleavage are difficult to predict. As with IR and radiomimetic drugs, multiple DSBs are introduced per cell, making unclear what effect the introduction of global damage has on the repair of one particular DSB. A large number of DSBs may induce a DNA damage response that would not normally be found in cells with one or a few breaks in the genome.

2.3. The Rare-Cutting I-SceI Endonuclease

To overcome the limitations of more general DNA damaging agents, a broadly useful system to study DSB repair has been developed that relies on the expression of an endonuclease with few (or no) endogenous sites in mammalian genomes *(63).* The endonuclease, I-*Sce*I, is derived from *Saccharomyces cerevisiae* and studies with it parallel those in yeast that use the HO endonuclease (Vol. 1, Chapter 16). I-*Sce*I is from a class of endonucleases involved in intron homing *(6),* its normal role being to initiate a gene conversion event by the introduction of a DSB into the mitochondrial rDNA locus *(34).* I-*Sce*I has been used in mammalian cells to introduce DSBs into genomic DNA,

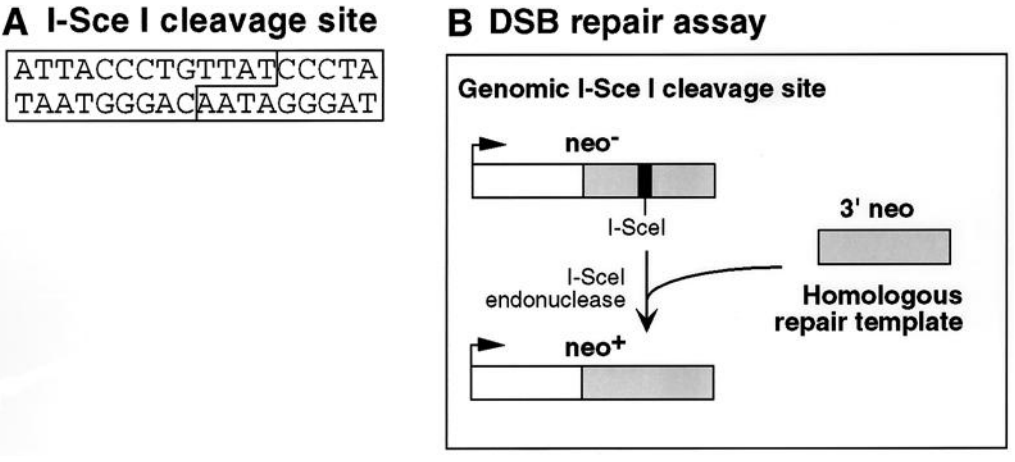

Fig. 1. DSB repair assay system for mammalian cells. **(A)** The 18 bp I-*Sce*I cleavage site *(25).* I-*Sce*I endonuclease cleavage produces a 4 base 3′ overhang. **(B)** The I-*Sce*I DSB repair-assay system. This system can be used to assay homologous or nonhomologous repair *(117).* The I-*Sce*I site is cloned into a selectable marker gene, in this case the *neo* gene, disrupting its function. The *neo*⁻ gene is integrated into the genome of mammalian cells. I-*Sce*I endonuclease is expressed in vivo from a transfected I-*Sce*I expression vector and cells that contain a *neo*⁺ gene can be selected after DSB repair. In this case, DSB repair occurs from a homologous *neo* fragment that contains the 3′ end of the gene (3′ *neo*) and can correct the I-*Sce*I site mutation. To analyze different types of recombination, the homologous fragment can be transfected (gene targeting), linked to the *neo*⁻ gene (intrachromosomal recombination), or unlinked on another chromosome (interchromosomal recombination). NHEJ can be assayed in nonselected cells as those that have lost an intact I-*Sce*I site.

and, like its natural role in the mitochondrial gene conversion event, it is not believed to play any role in the subsequent repair process after the initial introduction of the DSB.

The scarcity I-*Sce*I cleavage sites in complex genomes is owing to the length of its recognition site. The site is 18 bp and is nonpalindromic, having a 4 base 3′ overhang following cleavage (Fig. 1A). Mutagenesis studies of the recognition site have shown that the identities of most nucleotides within the 18 bp are essential for efficient cleavage activity *(25).* Not surprisingly then, the yeast nuclear genome contains no I-*Sce*I cleavage sites *(143).* For mammalian cells, an 18 bp site would statistically be expected to occur once every 20 mammalian genomes, although the small amount of degeneracy in the site makes conclusive determination of the number of sites difficult. The coding region for I-*Sce*I has been modified to a universal code in which mitochondria-specific codons are replaced with more standard codons, allowing the endonuclease to be expressed in *E. coli* and other cell types *(24).* The enzyme has been purified *(95)* and biochemically charactacterized *(105),* and it is sold commercially (Boehringer Mannheim). When I-*Sce*I has been used in yeast, similar kinetics and products of repair have been obtained as when DSBs are introduced by HO endonuclease *(38,112).*

2.4. I-SceI Expression in Mammalian Cells: In Vivo DSBs

A number of I-*Sce*I expression vectors have been constructed for use in mammalian cells. In some vectors, the I-*Sce*I coding region (universal code version) has been fused

at the N-terminus to three copies of a nuclear localization signal (nls) and an HA epitope tag *(see 32).* The nls may assist in translocation of the endonuclease to the nucleus although it is clearly not essential *(22).* Without an nls, the 235 amino acid protein is presumably small enough to diffuse passively through nuclear pores. Several promoters have been used to express I-*Sce*I, including human cytomegalovirus *(22,122),* mouse phosphoglycerate kinase *(32,131),* and chicken β-actin *(118),* as well as meiosis specific promoters (P.J. Romanienko and M. Jasin, unpublished results). Consistent with a lack of I-*Sce*I sites in the mouse genome (or at least a small enough number of sites that I-*Sce*I-generated DSBs are efficiently repaired), constitutive expression of I-*Sce*I is not toxic to mouse cells *(121)* and mice expressing I-*Sce*I during meiosis are fertile (P.J. Romanienko and M. Jasin, unpublished results).

DSBs were first detected in vivo using plasmid substrates, where it was found that I-*Sce*I cleavage would stimulate recombination between adjacent repeats, similar to when plasmids were pre-cleaved in vitro by restriction enzymes *(121).* Recombination between plasmid repeats is believed to occur by the single-strand annealing pathway *(83).* In this pathway, the DSB at opposite ends of each repeat provides an entry site for an exonuclease, so that resultant single-strands are able to anneal at exposed regions of homology. This is considered a nonconservative pathway because sequence information between the annealed homology regions is lost. Although this pathway is relevant to some forms of chromosomal recombination (*see* Subheading 4.), it probably predominates in plasmid recombination because plasmid DNA is readily degraded by cellular nucleases upon transfection.

3. CHROMOSOMAL DSBs AND RECOMBINATIONAL REPAIR DURING GENE TARGETING

3.1. Chromosomal DSBs Generated by I-SceI

Plasmid-recombination experiments demonstrated that I-*Sce*I was functional within mammalian cells, anticipating the advance in the field that came when I-*Sce*I was applied to chromosomal DSB repair studies *(22,122).* One approach for studying chromosomal DSB repair is illustrated in Fig. 1B. The I-*Sce*I cleavage site is integrated into the genome by standard transfection protocols, typically in the context of a defective selectable marker such as the neomycin phosphotransferase *(neo)* gene. An endonuclease expression vector is subsequently transfected into the cells to transiently express I-*Sce*I and a DSB is introduced into the chromosomal site within the first 24 h or so after transfection. Repair presumably occurs rapidly thereafter. Thus far, synchronous cleavage in 100% of the cells has not been achieved, limiting direct analysis of processing of the broken chromosome ends. However, DSB repair products have been readily recovered by selecting for restoration of the previously defective selectable marker after a particular type of repair *(22,32,81,99,118,122,123,131,140).* Individual repair events have also been examined after random cloning after I-*Sce*I cleavage to study repair without bias to the particular pathway that is used *(79).* Alternatively, analysis of unselected repair products has been performed on populations of cells using PCR *(79).*

3.2. DSBs Induce Recombination 100- to 1000-Fold

If homologous recombination mechanisms in mammalian cells are similar to those in *S. cerevisiae,* a single DSB in genomic DNA would be expected to induce recombination.

This was first tested in gene-targeting experiments, which allow the introduction of mutations into genomes *(15).* Gene targeting in mammalian cells is inefficient both in absolute frequency and in comparison to random integration of DNA. Early studies demonstrated that a DSB in a transfected plasmid would significantly increase homologous recombination with the chromosome *(57,65,66).* However, in recombination models, the recombining partner that contains the DSB is normally the recipient of genetic information (e.g., *49,137*), suggesting that a more substantial induction of gene targeting would occur if the DSB were in the chromosomal target rather than the transfected plasmid.

This was tested by introducing a DSB into the genome with I-*Sce*I during the transfection of a homologous repair template (Fig. 1B). The frequency of gene targeting was at least two to three orders of magnitude higher with a DSB at the target locus than without *(22,122),* conclusively demonstrating that a DSB in a mammalian genome is highly recombinogenic. Because the chromosome was converted at the DSB site to the sequence of the incoming plasmid DNA, the mechanism of recombination appeared to be different from the nonconservative single-strand annealing mechanism discussed for plasmid recombination (*see* Subheading 4).

The length of homology shared by the plasmid and chromsome in these experiments was relatively short. In one case, it was only 700 bp, short enough that spontaneous recombinants were extremely rare *(122).* More recent experiments have demonstrated that DSB-induced recombinants can be detected with as little as 69 bp of homology, despite the fact that the 18 bp I-*Sce*I site interrupts this very short length of homology (C. Richardson, J. Winderbaum, and M. Jasin, unpublished results). The I-*Sce*I cleavage site is cleanly removed from the chromosome during recombination and converted to the sequence of the incoming DNA. By modifying the targeting fragment, it has been possible to introduce single bp changes into the chromosome at frequencies approaching 0.1% of transfected cells *(35,122).* Expression cassettes for foreign genes have also been introduced into the genome in this manner *(22).* Some of these experiments were performed in embryonic stem (ES) cell lines *(35,131),* which are used for creating mutant mice *(15).* Targeting has been performed with linear or circular DNA. With linear fragments, targeting events have been recovered in which homologous recombination occurred at both ends of the fragment whereas other events had a homologous event at one end and a nonhomologous rejoining event the other end *(122).* The proportion of these two types of events suggested that homologous recombination was nearly as frequent as nonhomologous repair of DSBs (*see* Subheading 5). Interestingly, the proportion of targeting events with coupled nonhomologous events was much lower in ES cells *(131).*

3.3. DSB-Induced Gene Targeting at Endogenous Loci

The recombinogenicity of DSBs in ES cells suggests that DSB-promoted gene targeting may be useful for efficiently altering the mouse genome. One round of gene targeting by conventional methods is necessary to introduce the I-*Sce*I site at the locus to be modified. If this first round of targeting brings in a marker that can be selected against, subsequent targeting events should be readily selectable and occur at high frequency upon break induction *(63).* The utility of this approach requires that the recombinogenicity of DSBs is not limited to randomly integrated I-*Sce*I sites, but that it is also found at I-*Sce*I sites targeted to specific chromosomal loci. This has indeed been

found at the *pim*-1 locus on chromosome 17 *(118)* and the *hprt* locus on the X chromosome *(32)*. At the *hprt* locus, gene targeting frequencies of 1% have been achieved using efficient calcium phosphate transfection protocols. In this case, the targeting fragment was 5.5 kb and included small palindromic insertions *(32)*. One case of a "gene exchange" has been performed at an endogenous locus in ES cells in which an ezrin gene mutant was integrated at the villin locus at high efficiency using DSB-promoted gene targeting *(23)*. This type of approach may provide a controlled means of expression of introduced genes, as described for the lox/Cre recombination system *(43)*.

4. RECOMBINATIONAL REPAIR: INTRACHROMOSOMAL RECOMBINATION

The ability of a chromosomal DSB to stimulate gene targeting suggested that a DSB would also induce recombination between two homologous chromosomal sequences. This was first addressed by examining recombination between closely linked homologous sequences in the mammalian genome, i.e., intrachromosomal recombination. Substrates to measure intrachromosomal recombination typically have two homologous sequences oriented as direct repeats (Fig. 2). The direct repeats, consisting of differentially mutated selectable marker genes and located within a few kb of each other, can recombine either by intrachromatid or sister chromatid recombination. Spontaneous recombination between such repeats occurs at a low but detectable frequency, generally 10^{-4} to 10^{-6} *(84,132)*. Although described as "spontaneous," it is likely that a lesion in the chromosome triggers these recombination events. DSBs introduced by I-*Sce*I increased recombination between the direct repeats *(32,79,81,123,140)*. As with gene targeting, the induction of recombination was substantial, usually two to three orders of magnitude, resulting in a recombination frequency of $\geq 10^{-2}$ to 10^{-3}.

Some direct-repeat recombination systems can differentiate between two different products, a deletion or a simple gene conversion. In one system, a deletion is the only repair product that can be selected owing to the marker-gene configuration *(32)*. In this case the I-*Sce*I cleavage site is between the 5′ and 3′ portions of the *hprt* gene and a deletion at the repeats is required to produce *hprt*$^+$ colonies. In three other systems, either recombination product can be selected *(79,123,140),* as shown in Fig. 2. The relative proportion of the two products differs in the three systems. In Taghian and Nickoloff *(140),* simple gene conversions predominate (97%), whereas in Liang et al. *(79),* deletion products predominate, with gene conversions only 25–30% of total recombinants. Both of these systems are composed of *neo* gene repeats separated by a spacer and the experiments were performed in hamster cells. The flip-flop in the relative recovery of the two recombination products may reflect differences in the lengths of the repeats or in the composition or lengths of the spacer sequences. The third system gives results intermediate between these two *(79,123,140)*.

The relative frequency of the deletion and gene-conversion products is important both mechanistically as well as for the consequence to the cell. Conversion events without crossovers are less mutagenic because deletions, by definition, result in loss of sequence information. In considering mechanisms for these events, single-strand annealing, as described earlier for plasmid recombination, would give rise to deletion events. Alternatively, DSB repair by a classical conservative recombination model proposed for yeast predicts an equal frequency of crossover (deletion) and noncrossover

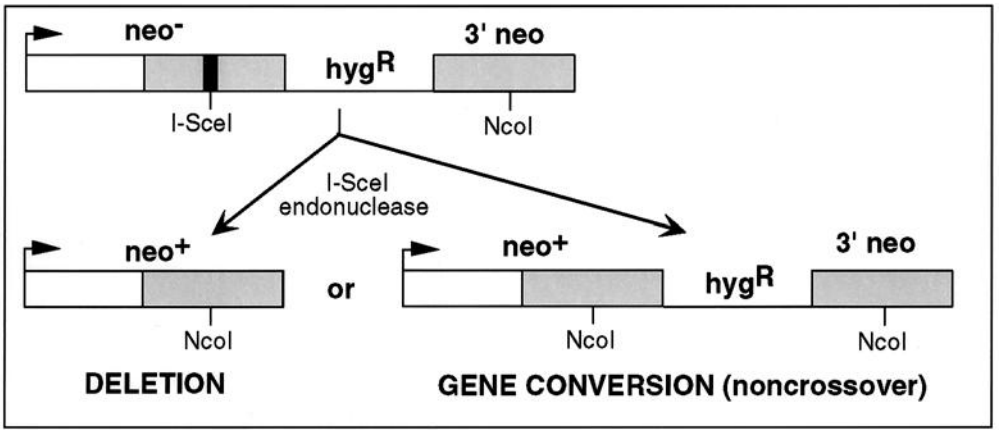

Fig. 2. Direct repeat recombination substrate for DSB repair. Two defective *neo* genes are in direct orientation. DSB-induced recombination results in two different types of products, a deletion or a noncrossover gene conversion. In both cases, the DSB site is converted to an *Nco*I restriction site. The deletion product can occur by single-strand annealing or by a gene conversion with an associated crossover (*see* text).

events *(137).* Another conservative recombination model has also been proposed in yeast and other organisms in which DSB-promoted recombination is tightly coupled to replication, resulting in a predominance of noncrossover gene conversion events (*see* Subheading 8.4. and *59*). This latter model, at least in broad outline, would be consistent with the results of Taghian and Nickoloff *(140),* whereas the results of Liang et al. *(79)* are best explained by the use of two different recombination pathways. Single-strand annealing could give rise to the predominance of deletion products and noncrossover conservative recombination could give rise to simple gene-conversion events.

Most DSB-promoted recombination experiments have been performed in rodent cells. In particular, hamster CHO-K1 cells *(79,81,123,140)* and mouse 3T3 *(122),* EC *(22),* and ES cells *(32,35,99,118,131)* have all been utilized with broadly similar results. Direct repeat recombination has also been examined in human cells, where it has been found to be stimulated by a DSB (A.J. Pierce and M. Jasin, unpublished results; *13*). An alternative to I-*Sce*I, PI-*Sce*I endonuclease (PI for protein intron; *see 48*), was used to introduce DSBs in human cells by Brenneman et al. *(13).* In this case, the endonuclease was directly electroporated into cells rather than expressed from an expression vector. The induction of recombination was only 10-fold, possibly owing to inefficiencies of enzyme electroporation. Because PI-*Sce*I endonuclease cleaves inefficiently and also binds avidly to one end after introducing a DSB in vitro, it is unlikely that this endonuclease will have general utility.

5. RELATIVE CONTRIBUTION OF HOMOLOGOUS RECOMBINATION AND NONHOMOLOGOUS END-JOINING IN MAMMALIAN DSB REPAIR

Three lines of evidence suggested that nonhomologous repair is the dominant DSB repair pathway in mammalian cells (Vol. 2, Chapter 16). When comparing homologous targeting and random integration of transfected DNA, random integrations were found

to dominate, in some cases by several orders of magnitude *(15).* Secondly, linearized plasmids transfected into cells were found to be readily rejoined by nonhomologous end-joining (NHEJ) *(120).* One study that directly compared NHEJ and homologous recombination of plasmid substrates nevertheless found that both types of repair occurred at a similar frequency *(67).* However, because plasmid recombination may not directly reflect chromosomal recombination, these studies were not considered to be conclusive. A third line of evidence came from cell-mutant studies. The first IR-sensitive cell mutants that were examined were found to be defective in NHEJ as demonstrated by their deficiency in V(D)J recombination *(139).* (For more information on repair proteins in V(D)J recombination, *see* Chapter 11.) Because these cells were found to be proficient at homologous recombination between plasmids *(81),* a role for homologous recombination in DSB repair was not obvious.

To determine if homologous recombination is a major pathway for repairing chromosomal DSBs in mammalian cells, DSBs were introduced by the I-*Sce*I endonuclease in a direct repeat recombination substrate (Fig. 2). Individual products of repair were analyzed in clones following nonselective growth and populations of repair products were analyzed by using polymerase chain reaction (PCR) *(79; see also 68a).* Both approaches indicated that 30–50% of DSB repair occurred by homologous recombination, demonstrating that homologous recombination is a major DSB repair pathway in mammalian cells. A portion of nonselected cell clones and the PCR products maintained the I-*Sce*I site in these experiments. This could indicate incomplete cleavage in the transfected cell population or retention of the I-*Sce*I site after repair. Restoration of an intact I-*Sce*I site by DSB repair could occur by either precise ligation of the I-*Sce*I overhangs *(83a)* or by equal sister chromatid recombination, as long as the I-*Sce*I site on the sister chromatid is not also cleaved. As yet, these possibilities cannot be distinguished. Nevertheless, they should be considered when evaluating the contributions of various pathways to DSB repair. In yeast, recent indirect evidence suggests that precise rejoining of HO endonuclease-induced DSBs may comprise as much as 50% of chromosomal DSB repair *(22a).*

In several studies using the I-*Sce*I system, NHEJ products have been sequenced *(79,83a,88,122,123).* Many of the products showed rejoining of the ends with loss of 1 bp or a few bp from the I-*Sce*I overhang and adjacent sequences. The deletions were often at short-sequence overlaps, as if the broken ends aligned prior to repair. Insertions of 1 bp were also recovered *(122).* Larger deletions and insertions were obtained less frequently. Larger deletions were found to have similar types of breakpoint junctions as the smaller deletions *(123).* Insertions came from a variety of sources, including single-copy DNA, repetitive elements in the genome, and the transfected I-*Sce*I expression vector *(79,108,109,123).* Microsatellite $(GT)_n$ repeats in a few instances have been found directly inserted at the chromosome break site *(79,108),* raising the possibility that DSB repair mechanisms contribute to their spread in the genome *(79).* As with the deletions, some insertions occurred at short-sequence overlaps, indicating pairing of a few nucleotides between the broken end and the sequence to be inserted.

6. REPAIR OF DSBs AND SISTER CHROMATID RECOMBINATION

The robust stimulation of homologous recombination between two chromosomal sequences suggests a physiological role for recombination in DSB repair. Recombination

between closely linked direct repeats in model systems can occur between two repeats on the same chromatid or, after DNA replication, between two repeats on sister chromatids. This suggests that sister chromatids and/or natural sequence repeats could be used as repair templates. The large number of repetitive elements in mammalian genomes suggests a role for sequence repeats, although repetitive elements can often be quite divergent. (The effect of sequence divergence on DSB repair is discussed in Subheading 9.) Alternatively, sister chromatids are ideal homologous repair templates because they are identical to each other and they are held in close proximity until anaphase. Sister chromatids have been found to be preferred recombination partners for spontaneous recombination in yeast *(70)* and in mammalian cells *(11).* This section discusses evidence for sister chromatid recombination during DSB repair in mammalian cells.

6.1. S-Phase Radiation Resistance

Classical radiation studies have suggested a role for sister chromatids in DSB repair because cells irradiated at different points in the cell cycle show different sensitivities to IR *(142).* Using drug synchronization or elutriation, it has been found that cells in S phase can be fivefold more resistant to killing than cells in G1, depending on dose. The S-phase radiation resistance suggests that the presence of sister chromatids protects cells from killing by acting as a homologous repair template for DSB repair. However, it is also possible that expression of repair proteins is induced during DNA replication. Cells in S phase then would more efficiently repair lesions in DNA but by the same nonhomologous mechanisms that are used at other times in the cell cycle.

6.2. Direct Repeat Recombination as a Model for Sister Chromatid Recombination

Sister chromatid recombination can restore a damaged chromatid to its original sequence. This type of event is considered to be "equal" because it occurs between equivalently positioned sequences on sister chromatids. As a result of the precise nature of the repair, such events go undetected. However, unequal sister chromatid recombination can be detected by arranging repeats opposite to that shown in Fig. 2, so that 3′ *neo* is upstream of the *neo* gene. In this case, unequal sister chromatid recombination can create a *neo*$^+$ product that is part of a *neo* gene triplication. DSB-promoted recombination events leading to triplications have been readily observed with this substrate *(68a,68b),* conclusively demonstrating a role for sister chromatids in DSB repair in mammalian cells, and consistent with a role for sister chromatid recombinational repair in S-phase radiation resistance. Although it is still possible that increased expression of proteins involved NHEJ contributes to S-phase radiation resistance, NHEJ mutants show normal levels of survival in S phase *(47),* arguing against a strong contribution of NHEJ repair to S-phase radiation resistance.

7. RECOMBINATION BETWEEN HOMOLOGOUS CHROMOSOMES

7.1. Homologs as Potential Templates for Recombinational Repair

Homologs, like sister chromatids, are potential homologous repair templates for chromosomal sequences with the exception of non-pseudoautosomal regions of the X-Y pair. Although homologs may be somewhat diverged, unlike sister chromatids, they are present throughout the cell cycle and, therefore, provide homology in mitotic cells

at any stage of the cell cycle as well as in nondividing cells. One key difference between homologs and sister chromatids is proximity. Sister chromatids are attached to each other by cohesion proteins until mitosis *(93)*. It is believed that cohesion proteins are assembled during DNA replication and disassemble at the metaphase/anaphase transition. With the exception of the specialized homolog pairing that occurs during meiosis *(106)*, homologs, despite their sequence homology, are generally not any closer to each other than to heterologs *(40)*. There may be exceptions to this, for example, the region of chromosome 15 in humans which is subject to parental imprinting *(74)*. Therefore, chromosome organization in the nucleus does not appear to be random, and during the G1 to G2 transition there is extensive chromosome movement *(40)*. In addition, homologous recombination enzymes may promote a genome-wide homology search, as occurs during yeast meiosis, bringing homologs into close proximity to allow the repair of damaged DNA.

7.2. Evidence for Recombination Between Homologs

Evidence for mitotic recombination between homologs has come from analysis of loss of heterozygosity (LOH) in tumor cells and in model systems *(64)*. LOH results in the genetic information of a particular locus or chromosomal region being derived from only one parent. This can unmask deleterious mutations, as seen with tumor-suppressor genes. LOH can occur by many mechanisms but somatic recombination of homologs has a particularly important contribution *(19)*. LOH arising from recombination between homologs has also been observed in normal tissues where it can encompass large portions of chromosome arms *(64)*. It is unknown whether the frequency of LOH is elevated in tumor tissues, or whether normal cells that undergo LOH of tumor-suppressor genes gain a selective advantage.

LOH can be detected by loss of function of a marker that starts heterozygous but becomes homozygous. Recombination can lead to LOH if a crossover occurs between the centromere and the marker locus at G1 and the two mutant loci cosegregate in the next mitosis. The frequency of these events in normal tissues was found to be as high as 10^{-4} to 10^{-5} *(64)*. Recombination between homologs was also detected in a cell line that is a compound heterozygote for mutations at the *TK* locus. Restoration of a TK^+ gene by recombination in this case required that recombination occurred between the two closeby *TK* mutations. TK^+ revertants were selected and those arising from homolog recombination were identified by LOH of linked markers at a frequency of approx 10^{-9} *(9)*. In a follow-up study, it was demonstrated that IR can induce recombination between the *TK* genes in a dose-dependent manner *(8)*.

The induction of homolog recombination by IR suggests that a DSB may be the lesion that initiates recombination. Because IR causes multiple types of DNA damage, it is also possible that IR activates the recombination machinery. To determine directly if a DSB induces recombination between homologs, the I-*Sce*I system has been utilized in mouse ES cells *(99)*. An I-*Sce*I site was targeted to one allele of chromosome 14, and a DSB was introduced by transient expression of the I-*Sce*I endonuclease. In this system, recombination between homologs at the locus on chromosome 14 was increased two to three orders of magnitude by a DSB, to approx 10^{-5} to 10^{-6}. Homolog recombination in these experiments was verified by LOH of a marker a few kb downstream of the DSB. Polymorphisms were not available further downstream to determine a further

extent of gene conversion. No evidence was obtained for crossing-over between homologs in these experiments, although the lack of polymorphisms does not allow an unequivocal assignment of crossovers and noncrossovers for all recombinants. Interestingly, although the fold-increase is similar to that found with direct repeats, the absolute frequency of homolog recombination was approx three orders of magnitude lower *(99)*. Thus, homologs do not appear to be preferred or frequent repair templates.

8. RECOMBINATION BETWEEN HETEROLOGOUS CHROMOSOMES

8.1. Heterolog Recombination and the Risk of Translocations and Other Chromosomal Abnormalities

Mammalian cells have large numbers of sequence repeats dispersed throughout their genome. Recombination events between repeats on the same chromosome would lead to genome scrambling, including deletions and inversions, if they were resolved as crossovers. If crossovers involved repeats on two different chromosomes (i.e., heterologs), chromosomal translocations or acentric/dicentric chromosome pairs would result. Translocations would occur if the two repeats were oriented in the same direction relative to their respective centromeres. Acentric/dicentric chromosome pairs would result if the repeats were oriented in opposite orientations relative to their respective centromeres, leading to loss of the acentric chromosome and breakage/fusion/bridge cycles involving the dicentric chromosome in dividing cells, unless one of the centromeres becomes inactivated.

8.2. DSB-Induced Heterolog Recombination

To determine if sequence repeats on heterologs can be used as repair templates, two differentially mutated *neo* genes were targeted to chromosomes 17 and 14 in mouse ES cells, with the *neo* gene on chromosome 17 containing an I-*Sce*I site *(118)*. Spontaneous recombination between the *neo* loci on the two different chromosomes was extremely low ($<10^{-9}$) but DSB-induced recombination was readily detectable, occurring at least three orders of magnitude more frequently (10^{-6}). Interestingly, the frequency of DSB-induced recombination between heterologs was only slightly below that for homologs *(118)*. This is consistent with the observation that homologs are not generally any nearer to each other than heterologs in the mammalian nucleus *(40)*.

8.3. Translocation Suppression During DSB-Induced Recombination

The repair products of DSB-induced heterolog recombination were examined to determine if crossovers occurred. The 200 recombinants that were analyzed arose exclusively from gene-conversion events, with no evidence of translocations or other chromosomal abnormalities *(118)*. The majority of events (98%) were simple gene-conversion events confined to the repeated region, with no overall change to the architecture of the locus. In these events, a small amount of sequence information was transferred from the unbroken chromosome to the broken chromosome. The remaining events appeared to have transferred a larger amount of sequence information, extending downstream of the homology. In these events, NHEJ was predicted to complete the recombination event.

The crossover suppression that is seen in heterolog recombination events contrasts with the observation of translocations by nonhomologous mechanisms in other systems *(108,151)*. For example, reciprocal translocations with junctions reflecting NHEJ have

been induced at the *aprt* locus in growth-arrested hamster cells by treatment with bleomycin *(151).* Presumably, two contemporaneous chromosome breaks were improperly rejoined to give rise to the reciprocal recombination products *(151),* contrasting with the single DSB induced by I-*Sec*I in the heterolog recombination experiments. It is possible that translocations are more likely when a cell confronts multiple lesions. Alternatively, homologous repair may in general be less mutagenic than NHEJ and play a greater role in protecting the cell from genome rearrangements. Evidence to support both of these hypotheses has recently been obtained, in which repair of two chromosomal DSBs has been found to lead to frequent translocations using NHEJ and SSA repair mechanisms *(117a).*

8.4. Replication-Based Recombination Mechanisms

The lack of translocations thus far observed in DSB-induced heterolog recombination is not consistent with a recombination model in which both crossover and noncrossover events are equally probable *(137).* Instead the results support a model in which noncrossover gene conversions are the predominant outcome of recombination (*see 49* and refs. therein). In this model, recombination is coupled to repair DNA synthesis. In one version (Fig. 3), the 3′ end from one side of the DSB invades the homologous sequence on the other chromosome and primes DNA synthesis, using the homologous sequence as a template. This leads to a restoration of sequence information originally present at the DSB site. The extended strand can dissociate and then reanneal to the homologous sequences on the broken chromosome. If the synthesis extends past the homology, NHEJ may complete the repair event. Recent evidence lends strong support for this model *(117b).* Although crossover events can be accommodated in this model (e.g., *39*), the majority of events are expected to be noncrossover gene-conversion events. The template remains unchanged during this type of repair.

Similar models have been proposed for yeast *(59), Drosophila (49),* and *Ustilago (39)* recombination, and in bacteriophage T4 replication *(41).* Other evidence for this type of mechanism in mammalian cells comes from experiments in which a gene-targeting vector has been demonstrated to prime synthesis from the target locus, leading to a correction of the mutation in the targeting vector *(124)* and from gap repair experiments of LINE-1 elements *(7).* The predominance of noncrossover gene conversion events in somatic cells contrasts with recombination in meiosis. Crossing-over during meiosis is essential for proper chromosome segregation at the reductional division. However, no such requirement holds in mitotic cells, which only undergo equational divisions. Importantly, noncrossover recombination mechanisms in mitotic cells safeguard the cell against genome scrambling. Other controls may exist in meiosis to prevent deleterious genome rearrangements.

8.5. Relative Contributions of Sister Chromatid, Homolog, and Heterolog Interactions During DSB-Induced Recombination

DSBs induce sister chromatid, homolog, and heterolog recombination each by approx two to three orders of magnitude. Clearly different, however, is the absolute level of recombination of these three substrates, with sister chromatid recombination occurring two to three orders of magnitude more frequently than either homolog or heterolog recombination *(68a).* Thus, in mammalian cells as in yeast, sister chromatids are

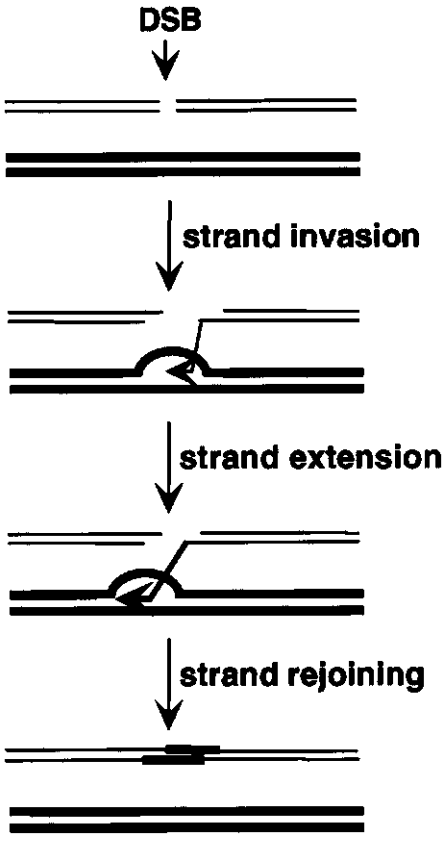

Fig. 3. Replication-based recombination. After a DSB occurs in one duplex, one of the 3′ ends that is produced invades a region of homology and primes DNA synthesis. A "D-loop" is formed by the strand invasion. The invaded homology region serves as a template for repair DNA synthesis, resulting in migration of the D-loop. The extended strand then reanneals to the other broken strand and the duplexes are sealed by ligation. This is the simplest version of several models that have been proposed. *See* ref. *(118)* and refs. therein.

preferred substrates for recombinational repair of DSBs, probably owing to their close proximity.

Interestingly, sister chromatid recombination induced by a DSB also has a predominately noncrossover outcome, like interchromosomal recombination *(68a,118).* These results parallel those obtained in spontaneous recombination experiments. Spontaneous recombination was measured at an immunoglobulin locus in a mouse hybridoma cell line. Recombination between direct repeats occurred at a rate of 10^{-4}/cell generation *(4),* somewhat higher than that found in other systems. However, recombination between homologs occurred orders of magnitude less frequently, about 10^{-8}/cell generation *(130)* and recombination between unlinked sequences likely to be on different chromosomes was also significantly less frequent, about 10^{-7}/cell generation *(3).* Thus, although spontaneous recombination is much lower than DSB-promoted recombination, the relative contribution of sequences located in different chromosomal positions to recombination rates is broadly similar.

9. SEQUENCE DIVERGENCE AND DSB REPAIR

9.1. Repetitive Elements in Mammalian Genomes

The large component of repetitive elements in mammalian genomes together with the observation of recombination between direct repeats raise the possibility that the endogenous sequence repeats are used for homologous repair of chromosome DSBs. In humans repetitive elements constitute fully one-third of the genome, with *Alu* elements, the most abundant class of dispersed repeat sequences, estimated at 10^6 copies *(125).* Complicating an analysis of recombination between repetitive elements is that elements within each class display heterogeneity in terms of length and degree of identity. *Alu* elements, for example, have 70–98% sequence identity with the consensus *Alu* sequence. A few examples of *Alu-Alu* recombination have been reported that are associated with the etiology of various diseases *(28).* Although these recombination events clearly involve sequence homology, it is not clear if they are *bona fide* homologous recombination events involving Rad51 and other recombination proteins (*see* Subheading 12.), or nonhomologous events in which the limited sequence identity is used in an alignment step prior to rejoining. One noncrossover gene conversion between *Alu* elements has been reported in what appears to be a *bona fide* recombination event *(71).* Because such conversions within *Alu* elements would not be expected to lead to gene mutations, they are likely to be underrepresented in tallies of *Alu-Alu* recombination events. At least a portion of events involving *Alu* elements occur by nonhomologous mechanisms, i.e., when single *Alu* elements become joined to unrelated sequences.

In addition to sequence divergence, relative location of *Alu* elements needs to be considered in evaluating the potential for *Alu-Alu* recombination in DSB repair. *Alu* elements can be located nearby each other, within a few kb or less, similar to the direct repeat recombination substrates described above. This suggests that nearby *Alu* elements (or other repetitive elements) may be used for recombination if the barrier arising from sequence divergence can be overcome. Alternatively, the large number of *Alu* elements found on other chromosomes or at greater distance on the same chromosome suggests recombination may be possible between more distantly located elements, as observed for interchromosomal recombination in ES cells (Subheading 8.2). In the mouse, recombination between diverged LINE-1 elements has been observed in experiments in which one element is on a transfected plasmid *(7,116).* More recently, a DSB in a chromosomal LINE element has been shown to be repaired by gene conversion with various endogenous LINE elements *(142a).*

9.2. Effect of Sequence Divergence on Recombination

In model systems examining the effect of sequence heterology, spontaneous recombination rates have been shown to be very dependent on the degree of identity of the recombining sequences, for both direct repeat chromosomal recombination *(150)* and gene targeting *(141).* The effect of sequence heterology on DSB-promoted recombination has been examined using the I-*Sce*I system *(35,140).* In DSB-promoted gene targeting in ES cells, increasing numbers of single bp polymorphisms were found to lead to progressively lower frequencies of recombination *(35).* The range in the amount of heterology was narrow, between 0.8 and 1.2%, with the decrease in recombination estimated to be between 2.5- and 6-fold, respectively. Because a 20-fold decrease in spon-

taneous gene targeting is observed for substrates with an estimated 0.6% divergence *(141),* spontaneous gene targeting may be more sensitive to sequence divergence. However, in these experiments the 0.6% heterology included small sequence insertions and deletions, which may have a greater effect on recombination than single bp polymorphisms. Interestingly, the barrier to recombination between the diverged sequences is relaxed when the mismatch repair gene *msh2* is mutated *(30),* consistent with results obtained in other organisms (*see* e.g., *113*).

In addition to the overall amount of heterology, the length of perfect homology is also important in spontaneous recombination. A decrease from 232 to 134 bp of uninterrupted homology decreased recombination 20-fold *(149).* There are indications that this is also true for DSB-promoted events, although less so *(35).* The position of polymorphisms relative to the DSB may also affect recombination, because a single polymorphism interrupting the homology adjacent to a DSB was shown to consistently lower the frequency of recombination *(35).*

The precise position of the DSB introduced by I-*Sce*I allows a determination of the extent of gene conversion from a DSB. Similar results have been obtained with direct repeats *(140)* and gene targeting *(35).* Gene-conversion tract lengths were found to be short, typically less than 100 bp, even when homology increased from 0.7 kb *(35)* to 1.4 kb *(140).* The short tract lengths imply that chromosome ends are protected from extensive degradation. In homolog recombination, in which homology extends the length of the chromosome, longer gene-conversion tracts were observed in some recombinants *(99).*

10. OTHER FACTORS THAT INFLUENCE RECOMBINATION

10.1. Transcription and DSB-Induced Recombination

Transcription has been shown both in yeast and mammalian cells to stimulate direct-repeat recombination *(100).* It has been postulated that transcription increases the number of initiating events, either directly by increasing the frequency of lesions or indirectly by increasing accessibility to recombination enzymes. To address this question, the effect of transcription on DSB-induced recombination was examined *(140).* No further stimulation of DSB-induced recombination was found when the recombination substrates were highly transcribed. In addition, the spectrum of gene-conversion events was found to be similar under conditions of low and high transcription *(140).* These results are consistent with a role for transcription in increasing the number of initiating events, rather than affecting later steps in the recombination pathway.

10.2. Hairpin Structures

Hairpin structures in chromosomes are susceptible to strand breakage and, therefore, are a potential source of genetic instability. These structures can form at the center of symmetry of perfect inverted repeats (palindromes), inverted repeats separated by spacers, or quasipalindromic sequences such as triplet repeats. Palindromes manifest a higher degree of instability than imperfect inverted repeats, presumably owing to more efficient hairpin extrusion and subsequent strand breakage. Single-strand breaks or DSBs can be introduced at hairpin tips by nicking enzymes which appear to be ubiquitous in mammalian cells *(76).* The Rad50 complex (*see* Subheading 12.3.), which has

been shown to introduce nicks into hairpins in vitro *(104),* has been proposed to be involved in this process in vivo *(1).* Strand breaks have also been presumed to arise at the base of hairpins. Because hairpins impede the progression of replication forks, breaks are likely to arise owing to stalled replication, as described in *E. coli* and yeast *(50,75).* In humans, quasipalindromic triplet repeats are very unstable presumably as a result of replication slippage at stalled replication forks *(2).*

In the mouse germline, instability of palindromes leading to both nonhomologous and homologous rearrangements is observed *(1,26).* Nonhomologous rearrangements are frequently deletions at the center of symmetry, consistent with hairpin-induced strand breaks that are nonhomologously rejoined. Because the deletions are not symmetrical, the palindrome is resolved into an imperfect inverted repeat that is genetically more stable than a perfect palindrome. Homologous recombination is also increased at palindromes, including gene conversions within the palindromic repeats and other types of recombination *(1).*

10.3. Transposable Elements

Transposable element excision in some organisms can be the source of DSBs that cause genetic instability. For example, P-element excision in *Drosophila (36)* and Tc 1 excision in *C. elegans (111)* result in DSBs that induce a high frequency of recombination between homologs. Although active endogenous transposons have not yet been identified in mammalian cells, transposable elements of the mariner family have been suggested to be responsible for creating a recombination hotspot that is responsible for some inherited neuropathies *(114).* Transposable elements in mammalian genomes are much less abundant than retrotransposable elements such as *Alu* repeats, and those that have been identified are transpositionally inactive owing to the accumulation of mutations *(72).* However, it has been possible to reconstruct an active vertebrate mariner transposon by eliminating inactivating mutations, raising the possibility that active elements exist *(61).*

11. OTHER OUTCOMES OF REPAIR OF INDUCED DSBs

11.1. Telomere Addition

The addition of new telomeres to the ends of broken chromosomes, termed chromosome healing, has been extensively studied in organisms such as *Tetrahymena.* This process has been shown to be dependent on telomerase *(156).* Telomerase, a reverse transcriptase-like enzyme, has an RNA cofactor that templates the addition of simple sequence repeats onto the ends of chromosomes *(157).* In addition to being required for proper DNA replication of chromosome ends, telomeres protect chromosomes from undergoing end-to-end fusions that lead to breakage/fusion/bridge cycles *(91)* by forming specialized structures, called t loops *(53).* A telomere binding protein, TRF2, is apparently critical for this structure because disruption of its binding can lead to end-to-end fusions of chromosomes in human cells *(145).*

To detect chromosome healing after DSB repair in mammalian cells, an I-*Sce*I site and a *TK* gene were integrated adjacent to a telomere in ES cells *(133).* Upon expression of I-*Sce*I, terminal deletions were identified by selecting *TK*$^{-}$ cell clones, analysis of which showed that telomeric repeats were added to the break site, in some cases

directly to the I-*Sce*I overhang. The number of repeats initially was less than in the parental cell line, but they increased after continued cell culture. Two different mechanisms of telomere healing are possible: the *de novo* synthesis of new telomeric repeats by telomerase, or the addition (or copying) of pre-existing telomeres. The contribution of these two mechanisms has not conclusively been determined, although results are consistent with *de novo* telomere synthesis *(133).*

11.2. Gene Amplification

An infrequent outcome of DSB repair is gene amplification. Breaks induced at fragile sites are known to trigger breakage/fusion/bridge cycles, presumably owing to the fusion of broken sister chromatids *(29).* After mitosis, the fused sister chromatids break asymmetrically, doubling the copy number of sequences near the fragile site in one of the daughter cells (*see* also *89*). Continued rounds of amplification lead to larger copy numbers. A hallmark of this type of amplification is loss of sequences distal to the break site.

Taking advantage of the insertion of an I-*Sce*I site distal to the dihydrofolate reductase gene *(DHFR)* in hamster cells, it has been possible to select amplification events as a result of I-*Sce*I-induced DSB repair *(109).* The structure of the amplification events after I-*Sce*I cleavage were compared with those that were induced at a fragile site located distal to the *DHFR* gene *(29).* As with the fragile site, amplifications induced with I-*Sce*I led to loss of sequences distal to the I-*Sce*I site. Because the I-*Sce*I site is closer to the *DHFR* gene than the fragile site, a larger portion of the distal region of the chromosome was lost with I-*Sce*I-induced amplification, confirming that they were initiated from the I-*Sce*I site. Thus, it appears that a DSB induced by I-*Sce*I is able to initiate the same sequence of events leading to *DHFR* amplification as a DSB occurring at a fragile site. This suggests that amplifications initiated at fragile sites reflect the high probability of breakage, rather than some other contribution to the amplification process *(109).*

12. ANALYSIS OF DSB REPAIR MUTANTS AND OVEREXPRESSION OF RECOMBINATION PROTEINS

IR-sensitive mammalian cell mutants have been identified that are defective in nonhomologous repair processes (Vol. 2. Chapter 16). The demonstration that homologous recombination is a major repair pathway implies that some IR-sensitive mutants may have defects in recombination proteins. This is indeed the case both for mutants obtained from targeted mutagenesis and from more standard mutant screens for IR-sensitive cell lines.

12.1. Rad51

Rad51 knockout mice display the most severe phenotype to date for disruption of a purported DSB repair gene. Rad51 is a RecA homolog *(98,129),* and has strand-transferase activity *(5)* which is expected to result in the formation of recombination intermediates (*see* Chapter 6). Disruption of the mouse *Rad51* gene leads to very early embryonic lethality *(82,144).* This contrasts with mouse mutants that are defective in nonhomologous repair process, which exhibit a late embryonic lethality *(42,46)* or are viable *(45,54,78,101,138).* Although cell lines cannot be established from the embryos,

short-term cultures of cells recovered from the dying *Rad51*$^{-/-}$ embryos are IR-sensitive and have sharply reduced chromosome numbers *(82).* These results point to a key role for Rad51 in the repair of endogenous and exogenous DNA damage.

Overexpression studies as well as mouse knockouts are consistent with a role for Rad51 in recombination. Human cell lines immortalized by SV40 T antigen have increased expression of Rad51 over control lines and a parallel increase in spontaneous intrachromosomal recombination, up to seven fold *(153).* Similarly, overexpression of hamster Rad51 in CHO cells increases intrachromosomal recombination by 20-fold *(147).*

12.2 Rad51-Related Proteins

Mammalian Rad51 shares approx 70% sequence identity with the yeast Rad51 protein. A number of other Rad51-related proteins have been identified in mammalian cells that share much less sequence identity (*see* Chapter 15). These proteins have been shown to interact with each other and with Rad51 in a number of pairwise combinations *(33,86)* possibly acting together in a complex *(128a).* Three of the Rad51-related proteins, Rad51B *(17,115),* Rad51C *(33),* and Rad51D *(110),* have been identified by database searches and although cell lines mutated for the genes have yet to be reported, mutation of at least two of the genes results in embryonic lethality *(110a,129a).* Two other Rad51-related proteins, XRCC2 and XRCC3, were identified as being defective in hamster cell mutants *(18,86).* The cell mutants, irs1 and irs1SF exhibit chromosomal instability, a weak IR sensitivity, and an extreme sensitivity to DNA crosslinking agents *(44,69).* These cell lines also have chromosome segregation defects *(52a).* The *XRCC2* mutation in irs1 cells is apparently a null allele *(86)* indicating that, in contrast to Rad51, the XRCC2 protein is not necessary for cell viability. DSB-promoted recombination has been examined recently in both the irs1 *(68b)* and irs1SF *(13a,108a)* cells lines and has been found to be significantly reduced in both, although not totally abolished. It is possible that these proteins act as accessory proteins to Rad51 function, similar to the Rad51 related proteins in yeast *(58,68,135),* rather than having Rad51-like strand transferase activity.

12.3. Other Homologs of Yeast Rad52 Epistasis Group Proteins

In yeast, Rad51 is member of the Rad52 epistasis group of proteins that is involved in recombinational repair of DSBs (Vol. 1, Chapter 16). Other members of this group, Rad54 and Rad52, have mammalian homologs that have been disrupted in mice. Disruption of *Rad54* results in IR sensitivity and decreased gene targeting in ES cells, although *Rad54*$^{-/-}$ mice are viable and fertile *(37).* A defect in sister chromatid recombination has also been reported recently *(33a).* Disruption of *Rad52* has an even milder effect than *Rad54,* with only slightly reduced gene targeting (30% decrease) and no sensitivity to IR *(119).* Overexpression of human Rad52 in monkey CV1 cells results in a two-fold increase in spontaneous direct repeat recombination, smaller than with Rad51 overexpression *(102).*

Two other mammalian homologs to Rad52 epistasis group members have been cloned, Rad50 *(31)* and Mre11 *(107).* These two proteins are complexed with a third protein called p95 or NBS1 *(16),* which is deficient in patients with Nijmegen breakage syndrome *(16,146),* one of the chromosome instability syndromes (*see* Subheading 12.5.). This complex in yeast has multiple functions, being involved in both meiotic and

mitotic homologous recombination and nonhomologous end-joining *(55).* It is expected that its role in mammalian cells will be similarly complex, although little information is currently available. (For more information about these proteins, *see* Chapter 7.)

12.4. Chromosome-Instability Syndromes

Cell lines derived from patients with chromosome-instability syndromes (Vol. 2 Chapter 19) provide an additional source of mutants in which to study recombination. Common to these syndromes is a high frequency of chromosome breakage and IR sensitivity. For one of these syndromes, ataxia telangiectasia (A-T), a high level of spontaneous intrachromosomal recombination has been observed *(92).* The hyper-recombination phenotype may be the result of a higher frequency of chromosome breaks or their longer persistence, with recombination mechanisms *per se* not being affected. Further analysis is necessary to establish this point. Homologous recombination has yet to be examined in cell lines from patients with the other syndromes. NHEJ is not affected in these syndromes, as extrapolated from proficiency in V(D)J recombination *(60).*

13. RECOMBINATION GENES AS CARETAKERS AGAINST TUMORIGENESIS

13.1. Mutator Phenotypes and Cancer

Tumor-suppressor genes can be broadly divided into two groups termed "gatekeepers" and "caretakers" *(73).* Gatekeepers are genes that regulate cell proliferation and cell death, and, thus, have a direct role in guarding a cell from becoming tumorigenic. Caretakers play an indirect role by maintaining genetic integrity. Several genetic "hits" apparently need to occur for a cell to become tumorigenic *(96),* yet low cellular mutation rates suggest that this should be nearly impossible. However, cells that have acquired a mutator phenotype, by definition, have higher mutation rates and thus can be expected to accumulate the necessary number of mutations *(87).* This is supported by mutations in caretaker genes in several familial cancer syndromes *(148),* including in genes involved in mismatch repair (colon cancer; *10*) and nucleotide excision repair (skin cancer; *12*).

The identification of homologous recombination as a major DNA repair pathway in mammalian cells implies that disruptions in the recombination machinery might lead to a mutator phenotype *(62).* Consistent with this, patients with chromosome-instability syndromes have increased frequencies of a variety of malignancies (Chapter 19, Vol. 2) although the role that recombination plays in the etiology of these malignancies has yet to be established. Several types of mutations may be predicted to occur when homologous recombination is altered, such as increased frequencies of deletions, insertions, and possibly translocations, owing to a greater reliance on NHEJ, and unequal sister chromatid exchanges or LOH, owing to altered outcomes of recombination events. The latter idea is particularly appealing owing to the observance of LOH in tumor cells *(19).*

13.2. Hereditary Breast-Cancer Syndromes

The most striking connection between recombination and tumorigenesis is in hereditary breast-cancer disease (*see* Chapter 10). Proteins encoded by the genes

associated with these cancers, BRCA1 and BRCA2, have been shown to interact with Rad51. The interaction between BRCA2 and Rad51 is direct, being detected both by two-hybrid assays *(94,127)* and co-immunoprecipitation *(20,21,90).* For BRCA1, the interaction between BRCA1 and Rad51 may be indirect, possibly mediated by BRCA2 *(20,126).*

Like the Rad51 knockouts, mouse knockouts of BRCA1 and BRCA2 show early embryonic lethality *(52,56,85,127,128,136).* When cells are recoverable from these mutants, they are sensitive to DNA damaging agents and exhibit chromosome abnormalities *(27,51,97, 103,128,154,155).* Taken together, these results strongly suggest that Rad51-mediated homologous recombination is disrupted in cells with mutated *BRCA1* or *BRCA2* genes. Direct evidence for a role for BRCA1 homologous recombination has recently been obtained *(99a).* It will be important to determine if and how recombination defects can lead to tumorigenesis. Loss of BRCA1 protein has also been associated with sporadic breast carcinomas *(152),* suggesting that disrupted recombination may be important in the pathogenesis of both sporadic and hereditary breast and ovarian cancers.

14. CONCLUSION

The last few years have witnessed a tremendous growth in our understanding of homologous recombination in mammalian cells. It is now clear that homologous recombination is a major DNA repair pathway in mammalian cells. Of the available templates for repair in the cell, sister chromatids are preferred although recombination can occur between two different chromosomes. The ability of DSBs to stimulate recombination suggests a role for homologous recombination in maintaining genetic integrity after exposure to DNA damaging agents. A number of the genes involved in recombination have been identified, either by homology with yeast genes involved in recombination, or as genes mutated in radiation-sensitive cell lines, and study of these genes will help elucidate mechanisms of recombination. Finally, a caretaker role appears likely for homologous repair proteins in preventing cells becoming tumorigenic. Further research in this area offers much promise in the future for understanding the maintenance of genomic integrity.

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REFERENCES

1. Akgün, E., J. Zahn, S. Baumes, G. Brown, F. Liang, P. J. Romanienko et al. 1997. Palindrome resolution and recombination in the mammalian germ line. *Mol. Cell. Biol.* **17(9):** 5559–5570.
2. Ashley, C. T., and S. T. Warren. 1995. Trinucleotide repeat expansion and human disease. *Ann. Rev. Genet.* **29:** 703–728.
3. Baker, M. D., and L. R. Read. 1992. Ectopic recombination within homologous immunoglobulin mu gene constant regions in a mouse hybridoma cell line. *Mol. Cell. Biol.* **12(10):** 4422–4432.
4. Baker, M. D., and L. R. Read. 1995. High-frequency gene conversion between repeated C mu sequences integrated at the chromosomal immunoglobulin mu locus in mouse hybridoma cells. *Mol. Cell. Biol.* **15(2):** 766–771.

5. Baumann, P., F. E. Benson, and S. C. West. 1996. Human Rad51 protein promotes ATP-dependent homolgous pairing and strand transfer reactions in vitro. *Cell* **87:** 757–766.
6. Belfort, M. 1997. Homing endonucleases: keeping the house in order. *Nucleic Acids Res.* **25:** 3379–3388.
7. Belmaaza, A., E. Milot, J. F. Villemure, and P. Chartrand. 1994. Interference of DNA sequence divergence with precise recombinational DNA repair in mammalian cells. *EMBO J.* **13(22):** 5355–5360.
8. Benjamin, M. B., and J. B. Little. 1992. X rays induce interallelic homologous recombination at the human thymidine kinase gene. *Mol. Cell. Biol.* **12(6):** 2730–2738.
9. Benjamin, M. B., H. Potter, D. W. Yandell, and J. B. Little. 1991. A system for assaying homologous recombination at the endogenous human thymidine kinase gene. *Proc. Natl. Acad. Sci. USA* **88(15):** 6652–6656.
10. Boland, C. R. 1998. Hereditary nonpolyposis colorectal cancer, in *The Genetic Basis of Human Cancer* (Vogelstein, B. and K. W. Kinzler, eds.), McGraw-Hill, New York, NY, pp. 333–346.
11. Bollag, R. J., and R. M. Liskay. 1991. Direct-repeat analysis of chromatid interactions during intrachromosomal recombination in mouse cells. *Mol. Cell. Biol.* **11(10):** 4839–4845.
12. Bootsma, D., K. H. Kraemer, J. E. Cleaver, and J. H. J. Hoeijmakers. 1998. Nucleotide excision repair systems: xeroderma pigmentosa, Cockayne syndrome, and trichothiodystrophy, in *The Genetic Basis of Human Cancer* (Vogelstein, B. and K. W. Kinzler, eds.), McGraw-Hill, New York, NY, pp. 245–274.
13. Brenneman, M., F. S. Gimble, and J. H. Wilson. 1996. Stimulation of intrachromosomal homologous recombination in human cells by electroporation with site-specific endonucleases. *Proc. Natl. Acad. Sci. USA* **93(8):** 3608–3612.

13a. Brenneman, M. A., A. E. Weiss, J. A. Nickoloff, and D. J. Chen. 2000. XRCC3 is required for efficient repair of chromosome breaks by homologous recombination. *Mutat. Res.* **459:** 89–97.

14. Bryant, F. E. 1989. Restriction enonuclease- and radiation-induced DNA double-strand breaks and chromosomal aberrations: similarities and differences, in *Chromosomal Aberrations: Basic and Applied Aspects* (Obe G. and A. T. Natarajan, eds.), Springer, Berlin, pp. 61–69.
15. Capecchi, M. R. 1989. Altering the genome by homologous recombination. *Science* **244:** 1288–1292.
16. Carney, J. P., R. S. Maser, H. Olivares, E. M. Davis, M. Le Beau, J. R. Yates, 3rd, et al. 1998. The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell* **93(3):** 477–486.
17. Cartwright, R., A. M. Dunn, P. J. Simpson, C. E. Tambini, and J. Thacker. 1998. Isolation of novel human and mouse genes of the recA/RAD51 recombination-repair gene family. *Nucleic Acids Res.* **26(7):** 1653–1659.
18. Cartwright, R., C. E. Tambini, P. J. Simpson, and J. Thacker. 1998. The XRCC2 DNA repair gene from human and mouse encodes a novel member of the recA/RAD51 family. *Nucleic Acids Res.* **26(13):** 3084–3089.
19. Cavenee, W. K., T. P. Dryja, R. A. Phillips, W. F. Benedict, R. Godbout, B. L. Gallie, et al. 1983. Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. *Nature* **305:** 779–784.
20. Chen, J., D. P. Silver, D. Walpita, S. B. Cantor, A. F. Gazdar, G. Tomlinson, F. J. Couch, B. L. Weber, T. Ashley, D. M. Livingston, and R. Scully. 1998. Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. *Mol. Cell* **2(3):** 317–328.
21. Chen, P. L., C. F. Chen, Y. Chen, J. Xiao, Z. D. Sharp, and W. H. Lee. 1998. The BRC repeats in BRCA2 are critical for RAD51 binding and resistance to methyl methanesulfonate treatment. *Proc. Natl. Acad. Sci. USA* **95(9):** 5287–5292.

22. Choulika, A., A. Perrin, B. Dujon, and J.-F. Nicolas. 1995. Induction of homologous recombination in mammalian chromosomes by using the I-*Sce*I system of *Saccharomyces cerevisiae. Mol. Cell. Biol.* **15:** 1963–1973.
22a. Clikeman, J. A., G. J. Khalsa, S. L. Barton, and J. A. Nickoloff. 2001. Homologous recombinational repair of double-strand breaks in yeast is enhanced by *MAT* heterozygosity through yKu-dependent and independent mechanisms. *Genetics* (in press).
23. Cohen-Tannoudji, M., S. Robine, A. Choulika, D. Pinto, F. El Marjou, C. Babinet, D. Louvard, and F. Jaisser. 1998. I-*Sce*I-induced gene replacement at a natural locus in embryonic stem cells. *Mol. Cell. Biol.* **18(3):** 1444–1448.
24. Colleaux, L., L. d'Auriol, M. Betermier, G. Cottarel, A. Jacquier, F. Galibert, and B. Dujon. 1986. Universal code equivalent of a yeast mitochondrial intron reading frame is expressed into *E. coli* as a specific double strand endonuclease. *Cell* **44:** 521–533.
25. Colleaux, L., L. d'Auriol, F. Gailbert, and B. Dujon. 1988. Recognition and cleavage site of the intron-encoded *omega* transposase. *Proc. Natl. Acad. Sci. USA* **85:** 6022–6026.
26. Collick, A., J. Drew, J. Penberth, P. Bois, J. Luckett, F. Scaerou, A. Jeffreys, and W. Reik. 1996. Instability of long inverted repeats within mouse transgenes. *EMBO J.* **15:** 1163–1171.
27. Connor, F., D. Bertwistle, P. J. Mee, G. M. Ross, S. Swift, E. Grigorieva, et al. 1997. Tumorigenesis and a DNA repair defect in mice with a truncating Brca2 mutation. *Nature Genet.* **17(4):** 423–430.
28. Cooper, D. N., M. Krawczak, and S. E. Antonarakis. 1998. The nature of human gene mutation, in *The Genetic Basis of Human Cancer* (Vogelstein, B. and K. W. Kinzler, eds.), McGraw-Hill, New York, pp. 64–92.
29. Coquelle, A., E. Pipiras, F. Toledo, G. Buttin, and M. Debatisse. 1997. Expression of fragile sites triggers intrachromosomal mammalian gene amplification and sets boundaries to early amplicons. *Cell* **89(2):** 215–225.
30. de Wind, N., M. Dekker, A. Berns, M. Radman, and H. te Riele. 1995. Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell* **82:** 321–330.
31. Dolganov, G. M., R. S. Maser, A. Novikov, L. Tosto, S. Chong, D. A. Bressan, and J. H. J. Petrini. 1996. Human Rad50 is physically associated with human Mre11: identification of a conserved multiprotein complex implicated in recombinational DNA repair. *Mol. Cell. Biol.* **16:** 4832–4841.
32. Donoho, G., M. Jasin, and P. Berg. 1998. Analysis of gene targeting and intrachromosomal homologous recombination stimulated by genomic double-strand breaks in mouse embryonic stem cells. *Mol. Cell. Biol.* **18:** 4070–4078.
33. Dosanjh, M. K., D. W. Collins, W. Fan, G. G. Lennon, J. S. Albala, Z. Shen, and D. Schild. 1998. Isolation and characterization of RAD51C, a new human member of the RAD51 family of related genes. *Nucleic Acids Res.* **26(5):** 1179–1184.
33a. Dronkert, M. L. G, H. B. Beverloo, R. D. Johnson, J. H. J. Hoeijmakers, M. Jasin, and R. Kanaar. 2000. Mouse *RAD54* affects DNA double-strand break repair and sister chromatid exchange. *Mol. Cell. Biol.* **20:** 3147–3156.
34. Dujon, B. 1989. Group I introns as mobile genetic elements: facts and mechanistic speculations: a review. *Gene* **82:** 91–114.
35. Elliott, B., C. Richardson, J. Winderbaum, J. A. Nickoloff, and M. Jasin. 1998. Gene conversion tracts from double-strand break repair in mammalian cells. *Mol. Cell. Biol.* **18:** 93–101.
36. Engels, W. R., D. M. Johnson-Schlitz, W. B. Eggleston, and J. Sved. 1990. High-frequency P element loss in Drosophila is homolog dependent. *Cell* **62(3):** 515–525.
37. Essers, J., R. W. Hendriks, S. M. A. Swagemakers, C. Troelstra, J. de Wit, D. Bootsma, et al. 1997. Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination. *Cell* **89:** 195–204.
38. Fairhead, C., and B. Dujon. 1993. Consequences of unique double-stranded breaks in yeast chromosomes: death or homozygosis. *Mol. Gen. Genet.* **240:** 170–178.

39. Ferguson, D. O., and W. K. Holloman. 1996. Recombinational repair of gaps in DNA is asymmetric in *Ustilago maydis* and can be explained by a migrating D-loop model. *Proc. Natl. Acad. Sci. USA* **93:** 5419–5424.
40. Ferguson, M., and D. C. Ward. 1992. Cell cycle dependent chromosomal movement in premitotic human T-lymphocyte nuclei. *Chromosoma* **101(9):** 557–565.
41. Formosa, T., and B. M. Alberts. 1986. DNA synthesis dependent on genetic recombination: characterization of a reaction catalyzed by purified bacteriophage T4 proteins. *Cell* **47(5):** 793–806.
42. Frank, K. M., J. M. Sekiguchi, K. J. Seidl, W. Swat, G. A. Rathbun, H. L. Cheng, et al. 1998. Late embryonic lethality and impaired V(D)J recombination in mice lacking DNA ligase IV. *Nature* **396(6707):** 173–177.
43. Fukushige, S., and B. Sauer. 1992. Genomic targeting with a positive-selection *lox* integration vector allows highly reproducible gene expression in mammalian cells. *Proc. Natl. Acad. Sci. USA* **89:** 7905–7909.
44. Fuller, L. F., and R. B. Painter. 1988. A Chinese hamster ovary cell line hypersensitive to ionizing radiation and deficient in repair replication. *Mutat. Res.* **193:** 109–121.
45. Gao, Y., J. Chaudhuri, C. Zhu, L. Davidson, D. T. Weaver, and F. W. Alt. 1998. A targeted DNA-PKcs-null mutation reveals DNA-PK-independent functions for KU in V(D)J recombination. *Immunity* **9(3):** 367–376.
46. Gao, Y., Y. Sun, K. M. Frank, P. Dikkes, Y. Fujiwara, K. J. Seidl, et al. 1998. A critical role for DNA end-joining proteins in both lymphogenesis and neurogenesis. *Cell* **95(7):** 891–902.
47. Giaccia, A., R. Weinstein, J. Hu, and T. D. Stamato. 1985. Cell cycle-dependent repair of double-strand DNA breaks in a gamma-ray- sensitive Chinese hamster cell. Somat. *Cell Mol. Genet* **11(5):** 485–491.
48. Gimble, F. S., and J. Thorner. 1992. Homing of a DNA endonuclease gene by meiotic gene conversion in *Saccharomyces cerevisiae. Nature* **357:** 301–306.
49. Gloor, G. B., and D. H. Lankenau. 1998. Gene conversion in mitotically dividing cells: a view from Drosophila. *Trends Genet* **14(2):** 43–46.
50. Gordenin, D. A., and M. A. Resnick. 1998. Yeast ARMs (DNA at-risk motifs) can reveal sources of genome instability. *Mutat Res* **400(1–2):** 45–58.
51. Gowen, L. C., A. V. Avrutskaya, A. M. Latour, B. H. Koller, and S. A. Leadon. 1998. BRCA1 required for transcription-coupled repair of oxidative DNA damage. *Science* **281:** 1009–1012.
52. Gowen, L. C., B. L. Johnson, A. M. Latour, K. K. Sulik, and B. H. Koller. 1996. *Brcal* deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. *Nature Genet* **12:** 191–194.
52a. Griffin, C. S., P. J. Simpson, C. R. Wilson, and J. Thacker. 2000. Mammalian recombination-repair genes XRCC2 and XRCC3 promote correct chromosome segregation. *Nat. Cell. Biol.* **2:** 757–761.
53. Griffith, J. D., L. Comeau, S. Rosenfield, R. M. Stansel, A. Bianchi, H. Moss, and T. de Lange. 1999. Mammalian telomeres end in a large duplex loop. *Cell* **97:** 503–514.
54. Gu, Y., K. J. Seidl, G. A. Rathbun, C. Zhu, J. P. Manis, N. van der Stoep, et al. 1997. Growth retardation and leaky SCID phenotype of Ku70-deficient mice. *Immunity* **7:** 653–665.
55. Haber, J. E. 1998. The many interfaces of Mre11. *Cell* **95(5):** 583–586.
56. Hakem, R., J. L. de la Pompa, C. Sirard, R. Mo, M. Woo, A. Hakem, et al. 1996. The tumor suppressor gene *Brca1* is required for embryonic cellular proliferation in the mouse. *Cell* **85:** 1009–1023.
57. Hasty, P., J. Rivera-Perez, and A. Bradley. 1992. The role and fate of DNA ends for homologous recombination in embryonic stem cells. *Mol. Cell. Biol.* **12(6):** 2464–2474.
58. Hays, S. L., A. A. Firmenich, and P. Berg. 1995. Complex formation in yeast double-strand break repair: Participation of Rad51, Rad52, Rad55, and Rad57 proteins. *Proc. Natl. Acad. Sci.* **92:** 6925–6929.
59. Holmes, A. M., and J. E. Haber. 1999. Double-strand break repair in yeast requires both leading and lagging strand DNA polymerases. *Cell* **96(3):** 415–424.

60. Hsieh, C. L., C. F. Arlett, and M. R. Lieber. 1993. V(D)J recombination in ataxia telangiectasia, Bloom's syndrome, and a DNA ligase I-associated immunodeficiency disorder. *J. Biol. Chem.* **268(27):** 20,105–20,109.
61. Ivics, Z., P. B. Hackett, R. H. Plasterk, and Z. Izsvak. 1997. Molecular reconstruction of Sleeping Beauty, a Tc1-like transposon from fish, and its transposition in human cells. *Cell* **91(4):** 501–510.
62. Jasin, M. 2000. Chromosome breaks and genomic instability. *Cancer Invest.* **18:** 78–76.
63. Jasin, M. 1996. Genetic manipulation of genomes with rare-cutting endonucleases. Trends *Genet.* **12:** 224–228.
64. Jasin, M. 1999. LOH and mitotic recombination, In DNA Alterations in Cancer: Genetic and Epigenetic Changes. (Ehrlich, M. eds.), Eaton Publishing, Natick, MA.
65. Jasin, M., and P. Berg. 1988. Homologous integration in mammalian cells without target gene selection. *Genes Dev.* **2:** 1353–1363.
66. Jasin, M., J. deVilliers, F. Weber, and W. Schaffner. 1985. High frequency of homologous recombination in mammalian cells between endogenous and introduced SV40 genomes. *Cell* **43:** 695–703.
67. Jasin, M., and F. Liang. 1991. Mouse embryonic stem cells exhibits high levels of extrachromosomal homologous recombination in a chloramphenicol acetyltransferase assay system. *Nucleic Acids Res.* **19:** 7171–7175.
68. Johnson, R. D., and L. S. Symington. 1995. Functional differences and interactions among the putative RecA homologs Rad51, Rad55, and Rad57. *Mol. Cell. Biol.* **15:** 4843–4850.
68a. Johnson, R. D., and M. Jasin. 2000. Sister-chromatid gene conversion is a prominent DNA repair pathway in mammalian cells, *EMBO J.* **19:** 3398–3407.
68b. Johnson, R. D., N. Liu, and M. Jasin. 1999. Mammalian XRCC2 promotes the repair of DNA double-strand breaks by homologous recombination. *Nature* **401:** 397–399.
69. Jones, N. J., R. Cox, and J. Thacker. 1987. Isolation and cross-sensitivity of X-ray-sensitive mutants of V79-4 hamster cells. *Mut. Res.* **183:** 279–286.
70. Kadyk, L. C., and L. H. Hartwell. 1992. Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae. Genetics* **132:** 387–402.
71. Kass, D. H., M. A. Batzer, and P. L. Deininger. 1995. Gene conversion as a secondary mechanism of short interspersed element (SINE) evolution. *Mol. Cell. Biol.* **15(1):** 19–25.
72. Kazazian, H. H., Jr., and J. V. Moran. 1998. The impact of L1 retrotransposons on the human genome. *Nature Genet* **19(1):** 19–24.
73. Kinzler, K. W., and B. Vogelstein. 1998. Familial cancer syndromes: the role of caretakers and gatekeepers, in (Vogelstein, B. and K. W. Kinzler, eds.), *The Genetic Basis of Human Cancer* McGraw-Hill, New York, NY, pp. 241–242.
74. LaSalle, J. M., and M. Lalande. 1996. Homologous association of oppositely imprinted chromosomal domains. *Science* **272(5262):** 725–728.
75. Leach, D. R. F. 1994. Long DNA palindromes, cruciform structures, genetic instability and secondary structure repair. *BioEssays* **16:** 893–900.
76. Lewis, S. 1994. P nucleotide insertions and the resolution of hairpin DNA structures in mammalian cells. *Proc. Natl. Acad. Sci. USA* **91:** 1332–1336.
77. Lewis, S. M. 1994. The mechanism of V(D)J joining: lessons from molecular, immunological and comparative analyses. *Adv. Immunol.* **56:** 27–149.
78. Li, G. C., H. Ouyang, X. Li, H. Nagasawa, J. B. Litle, D. J. Chen, et al. 1998. *Ku70:* a candidate tumor suppressor gene for murine T cell lymphoma. *Mol. Cell* **2:** 1–8.
79. Liang, F., M. Han, P. J. Romanienko, and M. Jasin. 1998. Homology-directed repair is a major double-strand break repair pathway in mammalian cells. *Proc. Natl. Acad. Sci. USA* **95:** 5172–5177.
80. Liang, F., and M. Jasin. 1996. Ku80 deficient cells exhibit excess degradation of extrachromosomal DNA. *J. Biol. Chem.* **271:** 14,405–14,411.
81. Liang, F., P. J. Romanienko, D. T. Weaver, P. A. Jeggo, and M. Jasin. 1996. Chromosomal double-strand break repair in Ku80 deficient cells. *Proc. Natl. Acad. Sci. USA* **93:** 8929–8933.

82. Lim, D. -S., and P. Hasty. 1996. A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. *Mol. Cell. Biol.* **16:** 7133–7143.
83. Lin, F. -L., K. Sperle, and N. Sternberg. 1984. Model for homologous recombination during transfer of DNA into mouse L cells: Role for DNA ends in the recombination process. *Mol. Cell. Biol.* **4:** 1020–1034.
83a. Lin, Y., T. Lukacsovich, and A. S. Waldman. 1999. Multiple pathways of repair of DNA double-strand breaks in mammalian chromosomes. *Mol. Cell. Biol.,* **19:** 8353–8360.
84. Liskay, R. M., and J. L. Stachelek. 1983. Evidence for intrachromosomal gene conversion in cultured mouse cells. *Cell* **35(1):** 157–165.
85. Liu, C. Y., A. Fleskin-Nikitin, S. Li, Y. Zeng, and W. H. Lee. 1996. Inactivation of the mouse BRCA1 gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. *Genes Dev.* **10:** 1835–1843.
86. Liu, N., J. E. Lamerdin, R. S. Tebbs, D. Schild, J. D. Tucker, M. R. Shen, et al. 1998. XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol. Cell* **1:** 783–793.
87. Loeb, L. A. 1991. Mutator phenotype may be required for multistage carcinogenesis. *Cancer Res* **51(12):** 3075–3079.
88. Lukacsovich, T., D. Yang, and A. S. Waldman. 1994. Repair of a specific double-strand break generated within a mammalian chromosome by yeast endonuclease I-*Sce*I. *Nucleic Acids Res.* **22:** 5649–5657.
89. Ma, C., S. Martin, B. Trask, and J. L. Hamlin. 1993. Sister chromatid fusion initiates amplification of the dihydrofolate reductase gene in Chinese hamster cells. *Genes. Dev.* **7(4):** 605–620.
90. Marmorstein, L. Y., T. Ouchi, and S. A. Aaronson. 1998. The BRCA2 gene product functionally interacts with p53 and RAD51. *Proc. Natl. Acad. Sci. USA* **95(23):** 13,869–13,874.
91. McClintock, B. 1941. The stability of broken ends of chromosomes in Zea mays. *Genetics* **41:** 234–282.
92. Meyn, M. S. 1993. High spontaneous intrachromosomal recombination rates in ataxia-telangiectasia. *Science* **260(5112):** 1327–1330.
93. Miyazaki, W. Y., and T. L. Orr-Weaver. 1994. Sister-chromatid cohesion in mitosis and meiosis. *Ann. Rev. Genet.* **28:** 167–187.
94. Mizuta, R., J. M. LaSalle, H. L. Cheng, A. Shinohara, H. Ogawa, N. Copeland, et al. 1997. RAB22 and RAB163/mouse BRCA2: proteins that specifically interact with the RAD51 protein. *Proc. Natl. Acad. Sci. USA* **94(13):** 6927–6932.
95. Monteilhet, C., A. Perrin, A. Thierry, L. Colleaux, and B. Dujon. 1990. Purification and characterization of the *in vitro* activity of I-*Sce*I, a novel and highly specific endonuclease encoded by a group I intron. *Nucleic Acids Res.* **18:** 1407–1413.
96. Moolgavkar, S. H., and A. G. Knudson, Jr. 1981. Mutation and cancer: a model for human carcinogenesis. *J. Natl. Cancer Inst.* **66(6):** 1037–1052.
97. Morimatsu, M., G. Donoho, and P. Hasty. 1998. Cells deleted for Brca2 COOH terminus exhibit hypersensitivity to gamma- radiation and premature senescence. *Cancer Res.* **58(15):** 3441–3447.
98. Morita, T., Y. Yoshimura, A. Yamamoto, K. Kurata, M. Mori, H. Yamamoto, and A. Matsushiro. 1993. A mouse homolog of the *Escherichia coli recA* and *Saccharomyces cerevisiae RAD51* genes. *Proc. Natl. Acad. Sci. USA* **90:** 6577–6580.
99. Moynahan, M. E., and M. Jasin. 1997. Loss of heterozygosity induced by a chromosomal double-strand break. *Proc. Natl. Acad. Sci. USA* **94:** 8988–8993.
99a. Moynahan, M. E., J. W. Chiu, B. H. Koller, and M. Jasin. 1999. Brcal controls homology-directed DNA repair. *Molecular Cell* **4:** 511–518.
100. Nickoloff, J. A. 1992. Transcription enhances intrachromosomal homologous recombination in mammalian cells. *Mol. Cell. Biol.* **12(12):** 5311–5318.

101. Nussenzweig, A., C. Chen, V. da Costa Soares, M. Sanchez, K. Sokol. et al. 1996. Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. *Nature* **382:** 551–555.
102. Park, M. S. 1995. Expression of human RAD52 confers resistance to ionizing radiation in mammalian cells. *J. Biol. Chem.* **270(26):** 15,467–15,470.
103. Patel, K. J., V. P. Vu, H. Lee, A. Corcoran, F. C. Thistlethwaite, M. J. Evans, et al. 1998. Involvement of Brca2 in DNA repair. *Mol. Cell.* **1(3):** 347–357.
104. Paull, T. T., and M. Gellert. 1998. The 3′ to 5′ exonuclease activity of Mre 11 facilitates repair of DNA double-strand breaks. *Mol. Cell* **1(7):** 969–979.
105. Perrin, A., M. Buckle, and B. Dujon. 1993. Asymmetrical recognition and activity of the I-*Sce*I endonuclease on its site an on intron-exon junctions. *EMBO J.* **12:** 2939–2947.
106. Petes, T. D., R. E. Malone, and L. S. Symington. 1991. Recombination in yeast, in *The Molecular and Cellular* Biology of the Yeast *Saccharomyces:* genome Dynamics, Protein Synthesis, and Energetics. (Broach, J. R., J. R. Pringle, and E. W. Jones, eds.), Cold Spring Harbor Laboratory Press, *Cold Spring Harbor, NY,* pp. 407–521.
107. Petrini, J. H. J., M. E. Walsh, C. DiMare, J. R. Korenberg, X. -N. Chen, and D. T. Weaver. 1995. Isolation and characterization of the human MRE11 homologue. *Genomics* **29:** 80–86.
108. Phillips, J. W., and W. F. Morgan. 1994. Illegitimate recombination induced by DNA double-strand breaks in a mammalian chromosome. *Mol. Cell. Biol.* **14:** 5794–5803.
108a. Pierce, A. J., R. D. Johnson, L. H. Thompson, and M. Jasin. 1999. The Rad51-related protein XRCC3 promotes homology-directed repair of damage in mammalian cells. *Genes Dev.* **13:** 2633–2638.
109. Pipiras, E., A. Coquelle, A. Bieth, and M. Debatisse. 1998. Interstitial deletions and intrachromosomal amplification initiated from A double-strand break targeted to a mammalian chromosome. *EMBO J.* **17(1):** 325–333.
110. Pittman, D. L., L. R. Weinberg, and J. C. Schimenti. 1998. Identification, characterization, and genetic mapping of Rad51d, a new mouse and human RAD51/RecA-related gene. *Genomics* **49(1):** 103–111.
110a. Pittman, D. L., and J. C. Schimenti. 2000. Midgestation lethality in mice deficient for the RecA-related gene, Rad51d/Rad5113. *Genesis* **26:** 167–173.
111. Plasterk, R. H. 1991. The origin of footprints of the Tc1 transposon of Caenorhabditis elegans. *EMBO J.* **10(7):** 1919–1925.
112. Plessis, A., A. Perrin, J. E. Haber, and B. Dujon. 1992. Site-specific recombination determined by I-*Sce*I, a mitochondrial group I intron-encoded endonuclease expressed in the yeast nucleus. *Genetics* **130:** 451–460.
113. Rayssiguier, C., D. S. Thaler, and M. Radman. 1989. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature* **342:** 396–401.
114. Reiter, L. T., T. Murakami, T. Koeuth, L. Pentao, D. M. Muzny, R. A. Gibbs, and J. R. Lupski. 1996. A recombination hotspot responsible for two inherited peripheral neuropathies is located near a mariner transposon-like element. *Nature. Genet.* **12(3):** 288–297.
115. Rice, M. C., S. T. Smith, F. Bullrich, P. Havre, and E. B. Kmiec. 1997. Isolation of human and mouse genes based on homology to REC2, a recombinational repair gene from the fungus Ustilago maydis. *Proc. Natl. Acad. Sci. USA* **94(14):** 7417–7422.
116. Richard, M., A. Belmaaza, N. Gusew, J. C. Wallenburg, and P. Chartrand. 1994. Integration of a vector containing a repetitive LINE-1 element in the human genome. *Mol. Cell. Biol.* **14(10):** 6689–6695.
117. Richardson, C., B. Elliott, and M. Jasin. 1999. Chromosomal double-strand breaks introduced in mammalian cells by expression of I-*Sce*I endonuclease, in *DNA Repair Protocols: Eukaryotic Systems* (Henderson, D. S. ed.), Humana Press, Totowa, NJ, pp. 453–464.

117a. Richardson, C., and M. Jasin. 2000. Frequent chromosomal translocations induced by DNA double-strand breaks, *Nature* **405:** 697–700.
117b. Richardson, C., and M. Jasin. 2000. Coupled homologous and nonhomologous repair of a double-strand break preserves genomic integrity in mammalian cells. *Mol. Cell. Biol.* **20:** in press.
118. Richardson, C., M. E. Moynahan, and M. Jasin. 1998. Double-strand break repair by interchromosomal recombination: suppression of chromosomal translocations. *Genes Dev.* **12:** 3831–3842.
119. Rijkers, T., J. Van Den Ouweland, B. Morolli, A. G. Rolink, W. M. Baarends, P. P. Van Sloun. et al. 1998. Targeted inactivation of mouse RAD52 reduces homologous recombination but not resistance to ionizing radiation. *Mol. Cell. Biol.* **18(11):** 6423–6429.
120. Roth, D. B., and J. H. Wilson. 1985. Mechanisms of nonhomologous recombination in mammalian cells. *Mol. Cell. Biol.* **5:** 2599–2607.
121. Rouet, P., F. Smih, and M. Jasin. 1994. Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells. *Proc. Natl. Acad. Sci. USA* **91:** 6064–6068.
122. Rouet, P., F. Smih, and M. Jasin. 1994. Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. *Mol. Cell. Biol.* **14:** 8096–8106.
123. Sargent, R. G., M. A. Brenneman, and J. H. Wilson. 1997. Repair of site-specific double-strand breaks in a mammalian chromosome by homologous and illegitimate recombination. *Mol. Cell. Biol.* **17(1):** 267–277.
124. Scheerer, J. B., and G. M. Adair. 1994. Homology dependence of targeted recombination at the Chinese hamster APRT locus. *Mol. Cell. Biol.* **14(10):** 6663–6673.
125. Schmid, C. W. 1996. Alu: structure, origin, evolution, significance and function of one-tenth of human DNA. *Prog. Nucleic Acid Res. Mol. Biol.* **53:** 283–319.
126. Scully, R., J. Chen, A. Plug, Y. Xiao, D. Weaver, J. Feunteun, T. Ashley, and D. M. Livingston. 1997. Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell* **88(2):** 265–275.
127. Sharan, S. K., M. Morimatsu, U. Albrecht, D. -S. Lim, E. Regel, C. Dinh, A. Sands, G. Eichele, P. Hasty, and A. Bradley. 1997. Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking *Brca2. Nature* **386:** 804–810.
128. Shen, S. X., Z. Weaver, X. Xu, C. Li, M. Weinstein, L. Chen, X. Y. Guan, T. Ried, and C. X. Deng. 1998. A targeted disruption of the murine Brca1 gene causes gamma-irradiation hypersensitivity and genetic instability. *Oncogene* **17(24):** 3115–3124.
128a. Schild, D., Y. Lio, D. W. Collins, T. Tsomondo, T., and D. J. Chen. 2000. Evidence for simultaneous protein interactions between human Rad51 paralogs. *J. Biol. Chem.* **275:** 16443–16449.
129. Shinohara, A., H. Ogawa, and T. Ogawa. 1992. *Rad51* protein involved in repair and recombination in *S. cerevisiae* is a *recA*-like protein. *Cell* **69:** 457–470.
129a. Shu Z., S. Smith, L. Wang, M. C. Rice, and E. B. Kmiec. 1999. Disruption of muREC2/RAD51L1 in mice results in *early embryonic lethality which can Be partially rescued in a p53 (–/–) background. Mol. Cell. Biol.* **19:** 8686–8693.
130. Shulman, M. J., C. Collins, A. Connor, L. R. Read, and M. D. Baker. 1995. Interchromosomal recombination is suppressed in mammalian somatic cells. *EMBO J.* **14(16):** 4102–4107.
131. Smih, F., P. Rouet, P. J. Romanienko, and M. Jasin. 1995. Double-strand breaks at the target locus stimulate gene targeting in embryonic stem cells. *Nucleic Acids Res.* **23:** 5012–5019.
132. Smith, A. J., and P. Berg. 1984. Homologous recombination between defective neo genes in mouse 3T6 cells. *Cold Spring Harb. Symp. Quant. Biol.* **49:** 171–181.
133. Sprung, C. M., G. E. Reynolds, M. Jasin, and J. P. Murnane. 1999. Chromosome healing in mouse embryonic stem cells. *Proc. Natl. Acad. Sci. USA* **96:** 6781–6786.
134. Subramani, S., and B. L. Seaton. 1988. Homologous recombination in mitotically dividing mammalian cells, in *Genetic Recombination,* (Kucherlapati, R. and G. R. Smith, eds.), American Society for Microbiology, Washington, DC, pp. 549–573.

135. Sung, P. 1997. Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. *Genes Dev* **11(9):** 1111–1121.
136. Suzuki, A., J. L. de la Pompa, R. Hakem, A. Elia, R. Yoshida, R. Mo, et al. 1997. Brca2 is required for embryonic cellular proliferation in the mouse. *Genes Dev* **11(10):** 1242–1252.
137. Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein, and F. W. Stahl. 1983. The double-strand-break repair model for recombination. *Cell* **33:** 25–35.
138. Taccioli, G. E., A. G. Amatucci, H. J. Beamish, D. Gell, X. H. Xiang, M. I. Torres Arzayus, et al. 1998. Targeted disruption of the catalytic subunit of the DNA-PK gene in mice confers severe combined immunodeficiency and radiosensitivity. *Immunity* **9(3):** 355–366.
139. Taccioli, G. E., G. Rathbun, E. Oltz, T. Stamato, P. A. Jeggo, and F. W. Alt. 1993. Impairment of V(D)J recombination in double-strand break repair mutants. *Science* **260:** 207–210.
140. Taghian, D. G., and J. A. Nickoloff. 1997. Chromosomal double-strand breaks induce gene conversion at high frequency in mammalian cells. *Mol. Cell. Biol.* **17:** 6386–6393.
141. te Riele, H., E. R. Maandag, and A. Berns. 1992. Highly efficient gene targeting in embryonic stem cells through homologous recombination with isogenic DNA constructs. *Proc. Natl. Acad. Sci. USA* **89:** 5128–5132.
142. Terasima, T., and L. J. Tolmach. 1961. Variations in several responses of HeLa cells to X-irradiation during the division cycle. *J. Biophys.* **3:** 11–33.
142a. Tremblay, A., M. Jasin, and P. Chartrand. 2000. A double-strand break in a chromosomal LINE element can be repaired by gene conversion with various endogenous LINE elements in mouse cells. *Mol. Cell. Biol.* **20:** 54–60.
143. Thierry, A., A. Perrin, J. Boyer, C. Fairhead, B. Dujon, B. Frey, and G. Schmitz. 1991. Cleavage of yeast and bacteriophage T7 genomes at a single site using the rare cutter endonuclease I-*Sce*I. *Nucleic Acids Res.* **19:** 189–190.
144. Tsuzuki, T., Y. Fujii, K. Sakumi, Y. Tominaga, K. Nakao, M. Sekiguchi, A. Matsushiro, Y. Yoshimura, and T. Morita. 1996. Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc. Natl. Acad. Sci. USA* **93:** 6236–6340.
145. van Steensel, B., A. Smogorzewska, and T. de Lange. 1998. TRF2 protects human telomeres from end-to-end fusions. *Cell* **92(3):** 401–413.
146. Varon, R., C. Vissinga, M. Platzer, K. M. Cerosaletti, K. H. Chrzanowska, K. Saar, et al. 1998. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell* **93(3):** 467–476.
147. Vispe, S., C. Cazaux, C. Lesca, and M. Defais. 1998. Overexpression of Rad51 protein stimulates homologous recombination and increases resistance of mammalian cells to ionizing radiation. *Nucleic Acids Res.* **26(12):** 2859–2864.
148. Vogelstein, B., and K. W. Kinzler (eds.). 1998. *The Genetic Basis of Human Cancer.* McGraw-Hill, New York.
149. Waldman, A. S., and R. M. Liskay. 1988. Dependence of intrachromosomal recombination in mammalian cells on uninterrupted homology. *Mol. Cell. Biol.* **8:** 5350–5357.
150. Waldman, A. S., and R. M. Liskay. 1987. Differential effects of base-pair mismatch on intrachromosomal versus extrachromosomal recombination in mouse cells. *Proc. Natl. Acad. Sci. USA* **84(15):** 5340–5344.
151. Wang, P., R. H. Zhou, Y. Zou, C. K. Jackson-Cook, and L. F. Povirk. 1997. Highly conservative reciprocal translocations formed by apparent joining of exchanged DNA double-strand break ends. *Proc. Natl. Acad. Sci. USA* **94(22):** 12,018–12,023.
152. Wilson, C. A., L. Ramos, M. R. Villasenor, K. H. Anders, M. F. Press, K. Clarke, et al. 1999. Localization of human BRCA1 and its loss in high-grade, non-inherited breast carcinomas. *Nature Genet.* **21(2):** 236–240.

153. Xia, S. J., M. A. Shammas, and R. J. Shmookler Reis. 1997. Elevated recombination in immortal human cells is mediated by HsRAD51 recombinase. *Mol. Cell. Biol.* **17(12):** 7151–7158.
154. Xu, X., K. U. Wagner, D. Larson, Z. Weaver, C. Li, T. Ried, et al. 1999. Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. *Nat. Genet.* **22(1):** 37–43.
155. Xu, X., Z. Weaver, S. P. Linke, C. Li, J. Gotay, X. W. Wang, et al. 1999. Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. *Mol. Cell* **3(3):** 389–395.
156. Yu, G. L., and E. H. Blackburn. 1991. Developmentally programmed healing of chromosomes by telomerase in Tetrahymena. *Cell* **67(4):** 823–832.
157. Yu, G. L., J. D. Bradley, L. D. Attardi, and E. H. Blackburn. 1990. In vivo alteration of telomere sequences and senescence caused by mutated Tetrahymena telomerase RNAs. *Nature* **344(6262):** 126–132.