Molecular Cell, Vol. 16, 1–9, October 8, 2004, Copyright ©2004 by Cell Press

Recombination Mechanisms: Fortieth Anniversary Meeting of the Holliday Model

Meeting Review

Wolf-Dietrich Heyer[1,]* and Roland Kanaar[2,]*
[1]Sections of Microbiology and of Molecular and Cellular Biology
Center for Genetics and Development
University of California, Davis
Davis, California 95616
[2]Departments of Cell Biology and Genetics and Radiation Oncology
Erasmus MC, PO Box 1738
3000 DR Rotterdam
The Netherlands

The year 2004 marks the fortieth anniversary of the Holliday junction. This extraordinary DNA structure, originally proposed by Robin Holliday to explain genetic recombination in fungi, now appears to be a pivotal intermediate in many aspects of DNA metabolism. In those forty years the Holliday junction has gone from a hypothetical structure to models for its atomic structure and visualization of its dynamics at the single molecule level.

Introduction

The picturesque setting of the Loire valley was the scene of an EMBO-sponsored workshop on mechanisms of DNA recombination. The approximately 80 attendees were assembled in the secluded environment of "Le Domaine de Seillac" for four and a half days. The meeting celebrated the fortieth anniversary of the "Holliday Model." Back in 1964 Robin Holliday proposed a mechanism for gene conversion based on genetic data obtained in fungi (Holliday, 1964). The salient feature of this imaginative mechanism was a covalent intermediate between the two recombining DNA molecules, later to become known as the "Holliday junction." The Holliday junction has stood the test of time; it has gone from a hypothetical structure to a fact of life and has spawned very active fields of genetic and biochemical analyses of the enzymes required to form and resolve it.

The French countryside was conducive to a congenial atmosphere at the workshop, yet the program was intense since virtually every participant presented his work in a short talk. The workshop covered many types of DNA sequence rearrangements, including homologous recombination, replication-associated recombination, mating type switching, site-specific recombination, transposition, and V(D)J recombination. Aspects of DNA recombination from bacteria to mammals were discussed, and although each species has its own peculiarities, certain features of recombination are remarkably conserved, underscoring the biological importance of recombination. Even though the workshop was focused on DNA recombination, the variety of techniques that are applied to unravel its mechanisms is breathtaking. They range from single molecule biochemistry through live-cell imaging of recombination proteins to the analysis of its biological relevance in mouse models and human disease. Below we give an overview of a few of the many highlights of this exciting and superbly organized workshop.

*Correspondence: wdheyer@ucdavis.edu (W.-D.H.); r.kanaar@erasmusmc.nl (R.K.)

Recombination Mechanisms: From Initiation to Resolution

DNA recombination is an essential cellular process that is involved in many DNA transactions, including preservation of genome integrity, DNA damage checkpoint activation, repair of DNA damage, DNA replication, mating type switching, transposition, immune system development, and meiosis. The most extensively discussed type of recombination was homologous recombination. Discontinuities in double-stranded DNA, such as a DNA double-strand breaks (DSBs), are particularly efficient initiators of homologous recombination. Figure 1 schematically contrasts two mechanistically distinct pathways for DSB repair, homologous recombination, and non-homologous end-joining.

Homologous recombination can be divided into three basic stages (Figure 2). First, during the pre-synapsis stage a recombination proficient DNA (tailed DSB or gap) is generated. Second, during synapsis a joint molecule (D loop) between the recombination proficient DNA and a double-stranded homologous template DNA is formed, which results in heteroduplex DNA and possibly leading to a Holliday junction(s). Third, during the post-synapsis stage contiguous DNA strands are restored, mismatch repair establishes perfect Watson-Crick base pairing, and the separation of the recombined DNA molecules follows from Holliday junction resolution. In contrast to homologous recombination, the non-homologous end-joining pathway does not require a repair template. DNA ends, processed if necessary, are simply ligated together and therefore repair is not always accurate. The immune system has exploited this inaccuracy because repair of DSBs generated by the RAG proteins to initiate V(D)J recombination and TCR gene rearrangements by the non-homologous end-joining proteins results in additional diversity in immunoglobulines and T cell receptors, respectively.

Homologous recombination comprises a number of interrelated pathways that share basic mechanistic aspects. Physical and genetic analyses have led to an elaboration of the original double-strand break repair (DSBR) model proposed by Szostak and colleagues (Szostak et al., 1983) in suggesting that the two ends of the DSB are not equivalent. Only one end of the DSB invades the unbroken target DNA to form a D loop (or single end invasion) intermediate, which represents a bifurcation point to classical DSBR or to synthesis-dependent annealing (SDSA). In DSBR, the D loop is processed to capture the second end of the DSB leading to a double Holliday junction, which can be resolved to crossover and non-crossover outcomes (Figure 2, left).

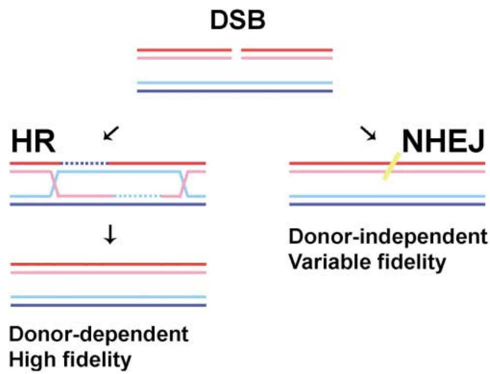

Figure 1. Homologous Recombination versus Nonhomologous End-Joining

Homologous recombination (HR) relies on the presence of a homologous donor sequence to repair a double-strand DNA break (DSB) with high fidelity. Non-homologous end-joining (NHEJ) directly ligates the two ends of the DSB, affording repair in the absence of a donor and incurring associated sequence changes depending on the chemical structure of the DSB.

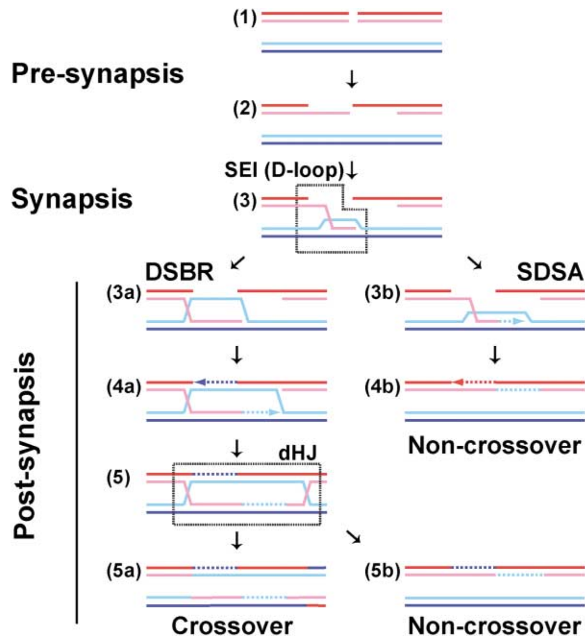

Figure 2. Double-Strand Break Repair and Synthesis-Dependent Strand Annealing Pathway for Homologous Recombination

Meiotic recombination is initiated by Spo11-mediated double-stranded DNA breaks (DSB) (step 1). After DSB processing by the Mre11-Rad50-Xrs2 complex and other enzymes (step 2), RPA, Rad52, and the Rad51 paralogs orchestrate the formation of the Rad51 nucleoprotein filament that is capable of homology search and DNA strand invasion (step 3). Rad54 protein augments Rad51-mediated recombination in D loop formation and may allow access of DNA polymerases to the invading 3′-OH by displacing Rad51 from the product heteroduplex DNA (steps 3b and 4a). The resulting D loop (SEI, single-end invasion) may enter the DSBR pathway (step 3a) and form a double Holliday junction (dHJ; step 5), which can be resolved to crossover (step 5a) and non-crossover (step 5b) products. Resolution to crossover requires a symmetric cleavage of both Holliday junctions in opposite orientations by Holliday junction resolvase. Resolution to non-crossover can also be achieved by the resolvase (cleavage of both junctions in the same orientation) and by a DNA helicase collapsing the dHJ to a hemi-catenane followed by resolution involving a type I topoisomerase activity (e.g., BLM-TOPOIIIα (Wu and Hickson, 2003). Alternatively, the D loop enters the SDSA pathway (step 3b). After extension by DNA polymerase, the invading strand retreats to reanneal with the single-stranded DNA tail that did not form a D loop (step 4b) leading to a non-crossover outcome.

In SDSA, the invading strand of the D loop retreats after priming DNA synthesis and anneals with the second end, leading exclusively to non-crossover outcomes (Figure 2, right). While mechanistically similar, DSBR and SDSA may vary in their substrate choice (sister chromatid versus homolog), may differ in outcome (crossover versus non-crossover), and likely exhibit tissue preference (somatic versus germline), suggesting that regulation is occurring.

Initiation: Double-Strand Breaks and More…

During meiosis, recombination between homologous chromosomes results in genetic diversity. To initiate DNA strand exchange, the DNA strands of one partner chromosome need to be broken. This programmed DSB is catalyzed by Spo11, an evolutionarily conserved endonuclease in eukaryotes. Even though Spo11 contains the catalytically active amino acid residues for DNA strand breakage, at least nine other proteins are required for DSB formation in *S. cerevisiae*. Scott Keeney (Sloan-Kettering Cancer Center, New York) presented a protein-protein interaction map and provided evidence for the role of one of the proteins, the WD40 repeat protein Ski8, in meiotic DSB formation. While Ski8 has a role in cytoplasmic RNA metabolism, Keeney showed that the partners of Ski8 in RNA metabolism are dispensable for meiotic recombination and that Ski8 relocalizes to the nucleus during meiosis and associates with chromosomes. Interaction of Ski8 with Spo11 is required for its relocalization and for DSB formation (Arora et al., 2004; Tesse et al., 2003). During progression of meiosis the chromosomal localization of the Spo11 protein is altered. Kunihiro Ohta (RIKEN Institute, Saitama, Japan) discussed the results of whole *S. cerevisiae* chromosome tiling chromatin immunoprecipitation (ChIP) experiments that showed that Spo11 initially appeared at centromeric regions, while by the time of DNA break formation it can be found on chromosome arms together with Mre11. The biological relevance of the differential localization is not yet clear, since Norio Uematsu from Alain Nicolas' laboratory (Institute Curie, Paris, France) showed that Spo11 can be turned into a site-specific DSB inducer during meiosis by fusing it to the Gal4 DNA binding domain (Pecina et al., 2002). Furthermore, Spo11 can be bypassed altogether. Gerald Smith (Fred Hutchinson Cancer Research Center, Seattle) showed that a palindromic sequence is a hotspot for meiotic recombination in *S. pombe* lacking Spo11. This hotspot requires the nuclease activity of the Mre11 complex, and DSBs dependent on the complex are observed during replication. Presumably, the palindrome can form a hairpin in the single-stranded DNA near the replication fork and be cut by the Mre11 complex. Loss of the Rad2 FEN-1-type flap endonuclease also allows recombination in the absence of Spo11: *rad2*Δ suppresses Spo11 deletion mutants for gene conversion, crossing over,

proper chromosome segregation, and spore viability. Nicks, gaps, and flaps left from incomplete Okazaki fragment sealing in *rad2Δ* mutants apparently provide lesions that promote recombination. These lesions may be the single-strand lesions themselves or DSBs formed by premeiotic replication of single-strand lesions left from mitotic replication.

An important regulatory role in DSB formation during meiosis is provided at the level of chromatin organization. Anne Villeneuve (Stanford University Medical School, Stanford) presented experiments analyzing meiosis in *C. elegans him-17* mutants. HIM-17 is a chromatin-associated protein that shares structural features with proteins that interact genetically with LIN-35/Rb, a component of chromatin-modifying complexes. Like Spo11, HIM-17 is required for meiotic DSB induction, but unlike Spo11, HIM-17 is also required for methylation at lysine 9 in histone H3. Villeneuve suggested that HIM-17 promotes progressive compaction of chromatin needed to acquire competence for DSB formation (Reddy and Villeneuve, 2004). Similarly, in *S. cerevisiae*, Martin Kupiec (University of Tel Aviv, Ramat Aviv, Israel) demonstrated that the nuclease insensitive chromatin structure around repetitive Ty elements correlates with a lack of DSB induction during meiosis (Ben-Aroya et al., 2004). Finally, Denise Zickler (Université Paris-Sud, Orsay, France) pointed out that the importance of meiosis specific DSBs is not limited to initiation of recombination at the DNA level. Experiments in *Sordaria macrospora* uncovered a relationship between DSB formation and the homologous pairing process. In addition to initiating recombination between homologs, DSBs also promote juxtaposition of homologs from long distance interactions to an intimate pre-synaptic co-alignment (Storlazzi et al., 2003).

The function of Spo11 is limited to meiosis. However, Sae2, one of the proteins required together with Spo11 to initiate meiotic DSBs, also functions in mitotic cells. Maria Pia Longhese (University of Milan-Bicocca, Milan, Italy) demonstrated that the checkpoint kinase Mec1 is required for Sae2 phosphorylation during normal cell cycle progression and in response to DNA damage. In addition, the Tel1 kinase and the Mre11 complex are also required for DNA damage-dependent Sae2 phosphorylation. The phosphorylation is likely important for Sae2 function because phosphorylation defective mutants are as defective in DNA repair and recombination as *sae2Δ* mutants (Baroni et al., 2004).

While DSBs are major initiators of homologous recombination, other discontinuities in double-stranded DNA, such as single-stranded gaps, nicks, and stalled replication forks, can also trigger recombination. For example, recombination can be a consequence of RNA-DNA hybrids formed during transcription (Anders Aguilera, University of Seville, Seville, Spain; Huertas and Aguilera, 2003), replication through palindromic sequences (David Leach, University of Edinburgh, Edinburgh, United Kingdom; Cromie et al., 2001) and reduced levels of DNA polymerases (Tom Petes, University of North Carolina, Chapel Hill). During processing many of these discontinuities are probably converted into a DSBs. Benoît Arcangioli (Institut Pasteur, Paris, France) reported how *S. pombe* triggers recombination required for mating type switching; DNA replication through the mating type locus converts a single-strand break into a DSB (Kaykov et al., 2004). Furthermore, in mammalian cells, nicks generated by the RAG proteins, normally involved in initiating non-homologous end-joining during immune system development, can also stimulate homologous recombination (David Roth, New York University School of Medicine, New York; Lee et al., 2004). The importance of active and inactive chromatin states, as measured by different histone modifications, in providing cell specificity for RAG-induced V(D)J recombination was pointed out by Marjorie Oettinger (Massachusetts General Hospital, Boston).

Recombination as a Tool in Genome Engineering

Insights derived from studies on the molecular mechanism of recombination are now actively being exploited to engineer whole genomes. Applications range from promoting marker gene excision in rape seed (Rocío Sánchez-Fernández, SunGene GmbH, Gatersleben, Germany) to using DSBs to stimulating recombination in mice (Frédéric Paques, Cellectis S.A., Romainville, France). Particularly useful in this respect are the rare cutting endonucleases, such as I-Sce I. The use of I-Sce I to analyze DSB repair in *E. coli* was discussed by Robert Lloyd (University of Nottingham, Nottingham, United Kingdom). However, for gene targeting this approach requires the presence of an I-Sce I restriction site at the genomic locus that needs to be altered. This problem is circumvented by the ingenious approach presented by Dana Carroll (University of Utah, Salt Lake City). Hybrid proteins were generated between a non-site-specific DNA cleavage domain and zinc finger based DNA recognition domains. By manipulating the zing finger domains the nuclease can be targeted to pre-existing sites in genes of interest (Bibikova et al., 2003). Carroll presented an in vivo proof of principle of this approach by demonstrating increased homologous recombination at the *yellow* locus in *Drosophila*.

Homology Search and DNA Strand Exchange

The genome-wide search for the homologous donor poses a formidable mechanistic challenge for recombinational repair. This is solved by single-stranded DNA-protein filaments of bacterial RecA protein or its eukaryotic counterparts Rad51 and Dmc1, which are able to probe for homology and exchange DNA strands, when sufficient homology has been established (Figure 2). A critical issue is how these filaments are formed and how their assembly, stability, and disassembly is regulated. Formation of the Rad51 filament depends on the Rad52 protein and on Rad51 paralogs (Rad55-Rad57 in *S. cerevisiae*; Xrcc2, Xrcc3, Rad51B, Rad51C, and Rad51D in vertebrates) as shown in elegant large-scale applications involving ChIP and live cell imaging with fluorescent fusion proteins by James Haber (Brandeis University, Waltham) and Rodney Rothstein (Columbia University, New York), respectively (Lisby et al., 2003; Sugawara et al., 2003). These studies provide glimpses into the considerable in vivo dynamics of recombination and Rothstein suggested that Rad52-dependent DSB repair occurs in repair centers, where multiple breaks are being actively colocalized. While aggregation of DSBs is also observed in G1 phase mammalian cells

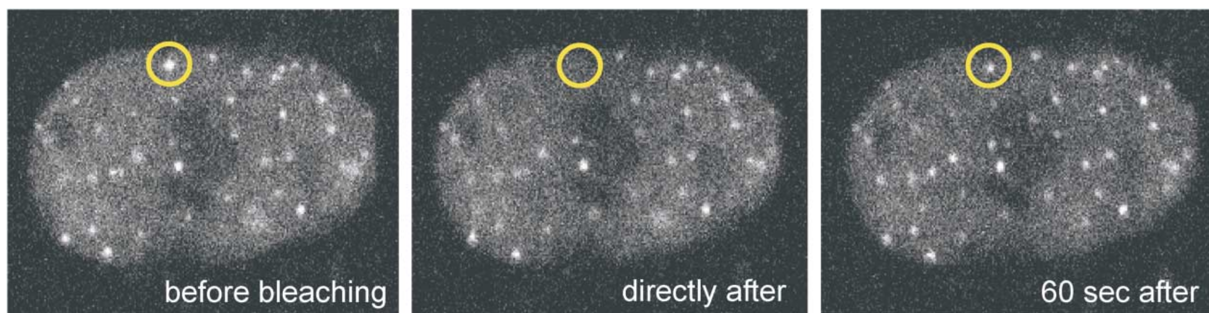

Figure 3. DNA Damage Causes Accumulation of Recombination Proteins in Dynamic Structures

A mammalian cell containing ionizing radiation-induced Rad52-GFP foci is shown. Fluorescence redistribution after photobleaching (FRAP) experiments illustrate the dynamic nature of these foci (Essers et al., 2002). Directly after photobleaching of a single focus (in the circle), fluorescence in the structure is abolished. Redistribution of fluorescence into the structure is observed indicating association and dissociation of the Rad52 protein.

(Aten et al., 2004), no direct evidence for repair centers in mammalian cells is currently available. However, at sites of DNA damage homologous recombination proteins accumulate into highly dynamic structures undergoing continuous association and disassociation of the proteins (Figure 3). Such in vivo analyses provide guidance for detailed mechanistic in vitro work with these proteins. Novel cofactors that may affect the formation, function, or stability of the Rad51 filament are emerging. Dan Camerini-Otero (NIH, Bethesda) reported that the meiosis-specific Hop2 protein is involved in synapsis during meiotic recombination (Petukhova et al., 2003). Matthew Whitby (University of Oxford, Oxford, United Kingdom) and Hidea Shinagawa (Osaka Unviersity Osaka, Japan) illustrated the importance of the fission yeast model system to identify new recombination factors that appear to be lacking in budding yeast. Michael Cox (University of Wisconsin, Madison) proposed that DinI, a protein induced early in the SOS response of *E. coli*, stabilizes RecA filaments by suppressing filament disassembly through blocking protomer exchange (Lusetti et al., 2004). The mechanistic understanding of the meiosis-specific RecA homolog Dmc1 had been hampered by its relative inactivity in standard in vitro recombination assays, but efforts in the laboratories of Douglas Bishop (University of Chicago, Chicago), Akira Shinohara (Osaka University, Osaka, Japan), and Partrick Sung (Yale University School of Medicine, New Haven) are quickly correcting this deficit. Sung reported the discovery of conditions that turn Dmc1 into a respectable DNA strand exchange protein, which is further stimulated by the Swi2/Snf2-like ATPases Rad54 and Rad54B (Sehorn et al., 2004). Beautiful cytological work by Bishop and Shinohara demonstrated a requirement of the yeast Swi2/Snf2-like ATPase Tid1 for the colocalization of the Rad51 and Dmc1 DNA strand exchange proteins during meiosis (Shinohara et al., 2000). Little is known about whether specific factors are required for the assembly and function of Dmc1 filament in meiotic recombination, but these recent breakthroughs make us hopeful that progress is underway. Talks by Albert Pastink (LUMC, Leiden, The Netherlands) and Nancy Hollingsworth (SUNY, Stony Brook) underlined the importance of chromosome structure in homology search, in particular during meiotic recombination. In meiosis, chromosomes undergo extensive remodeling of their structure, which impacts recombination. Moreover, in meiosis, recombination is targeted to the homolog instead of to the sister as in DSB repair in somatic cells, and the factors and mechanisms involved in this regulation are still largely unknown.

In yeast, mutations in the *RAD52* gene confer an almost absolute recombination defect that is stronger than a defect in the DNA strand exchange protein Rad51. This is explained by the dual role of Rad52 in Rad51-filament formation and in Rad51-independent annealing of complementary single-stranded DNA (Uffe Mortensen, Danmarks Tekniske Universitet, Lyngby, Denmark). In contrast, in vertebrates mutations in the *RAD52* gene confer minimal recombination defects, and it has been suggested that the breast cancer tumor suppressor protein Brca2 possibly replaces some functions of Rad52 protein in vertebrates (Pellegrini and Venkitaraman, 2004). The importance of Brca2 in predisposition to breast cancer has spawned a wave of studies that firmly established a role of Brca2 in Rad51-mediated homologous recombination (Pellegrini and Venkitaraman, 2004). Four talks by Simon Boulton (Cancer Research UK, South Mimms, United Kingdom), William Holloman (Cornell University, New York), Edward Egelman (University of Virginia, Charlottesville), and Shunichi Takeda (Kyoto University Medical School, Kyoto, Japan) highlighted the immense progress in this area. The mechanistic work had been hampered by the size of human Brca2 with 3,418 amino acids. Although initially thought to be a mammalian-specific protein, Brca2 homologs have been identified in model organisms easily amenable to genetic studies including chicken (Takeda), the nematode *C. elegans* (Boulton), and the smut fungus *U. maydis* (Holloman), which, incidentally, was the model system Robin Holliday preferred for his studies. Even better, some of these Brca2 homologs are considerably smaller than the human protein, for example the *C. elegans* protein is comparably a dwarf with 394 amino acids (Boulton et al., 2004). Undoubtedly, this will help the biochemical analysis of these proteins. Important structural work with a fragment of Brca2 protein revealed an intimate association of Brca2 with the Dss1 protein, a tiny 70 amino acids protein that has been associated with the developmental disorder split hand/split foot malforma-

tion (Yang et al., 2002). Elegant work in *Ustilago* by Holloman and coworkers clearly established a role of Dss1 in recombinational repair, showing that a mutation in the *DSS1* gene essentially was a phenocopy of a mutant in the *BRCA2* homolog (Kojic et al., 2003).

DNA strand exchange by the RecA/Rad51/Dmc1 filaments generates heteroduplex DNA, one of the central intermediates in recombination. When the target and donor DNA differ by polymorphisms or mutations, mismatches (non-Watson-Crick base pairs, deletion/insertion loops) will form that attract the attention of mismatch repair systems. The importance of mismatch repair in diverse DNA metabolic processes ranging from meiotic recombination, mutation avoidance, and repeat stability was highlighted in talks by Francis Fabre (CEA, Fontenay-aux-Roses, France), Eric Alani (Cornell University, Ithaca), and Cynthia McMurray (Mayo Clinic, Rochester).

Holliday Junction Resolution and Crossover Formation

Holliday junctions and their resolution to crossover recombinants was naturally a major topic of discussion in this celebration of the fortieth anniversary of postulating the existence of the "chiasma-like structure," as Holliday described the structure in his original publication (Holliday, 1964). The RuvC and RusA endonucleases of *E. coli* have established a paradigm for Holliday junction-specific endonucleases and elegant biochemical studies have elucidated their mechanism of action and illuminated the intimate relationship between RuvC, the motor protein RuvB, and the Holliday junction-specific binding protein RuvA. However, RusA and RuvC are not found in all bacteria and no apparent counterpart in eukaryotes has been described. Juan Alonso (Campus Universidad Autonoma de Madrid, Madrid, Spain) reported on the *Bacillus subtilis* RecU protein that appears to have taken over the function of RuvC in gram-positive bacteria, although it shares no sequence homology with RuvC protein (Ayora et al., 2004). The identity of the eukaryotic Holliday-junction endonuclease and the mechanisms involved in meiotic crossover formation has been a long-standing interest in the field. Recent progress in two areas was reported on this workshop. Yilun Liu (Cancer Research UK, South Mimms, United Kingdom) from Stephen West's laboratory discussed the purification of a Holliday junction resolvase activity from mammalian cells. Using biochemical and genetic experiments using somatic cell mutant lines, it was shown that Holliday junction resolution depended on the Rad51 paralogs Rad51C and Xrcc3 (Liu et al., 2004). This constitutes a novel, late function for Rad51 paralogs, which were previously demonstrated to function in Rad51 filament formation. Another eukaryotic DNA structure selective DNA endonuclease is Mus81-Mms4/Eme1, which functions in meiotic crossover formation in yeasts and was shown to be able to resolve Holliday junctions. Paul Russell (The Scripps Research Institute, LaJolla) reported on the substrate specificity of bacterially produced fission yeast Mus81-Eme1, which prefers nicked Holliday junctions over alternate substrates like 3′-flaps or intact Holliday junctions (Gaillard et al., 2003). Curiously, the partially purified native enzyme cleaves Holliday junctions much better, suggesting that other cofactors and/or post-translational modifications might be important. However, this peculiar substrate specificity of recombinant Mus81-Eme1 may also hint at the possibility that one pathway for crossover formation may proceed via nicked Holliday junctions rather than the classical closed junctions (Gaillard et al., 2003; Osman et al., 2003). Razqallah Hakem (University of Toronto, Toronto, Canada) underscored the importance of Mus81 in mammalian cells and reported that Mus81 can function as a tumor suppressor protein in mice. Mus81-deficient mice are viable but hyper-sensitive to DNA damage, displaying increased chromosome aberrations and significant cancer predisposition (McPherson et al., 2004). Meiosis in male and female *mus81*$^{-/-}$ appeared normal, suggesting that other pathways for crossover formation are operative in mouse meiosis. Two talks by Neil Hunter (University of California, Davis) and Nancy Kleckner (Harvard University, Cambridge) highlighted the physical analysis of recombination intermediates during yeast meiosis. In a combination of genetic, molecular, and cytological analyses, it was established that the decision to form a meiotic crossover is made well before Holliday junctions are formed, possible even before the initiating DSB is delivered by Spo11 protein (Borner et al., 2004). Meiotic crossover hotspots in mammals and their consequences on human evolution and population genetics were discussed in three talks by Alec Jeffreys (University of Leicester, Leicester, United Kingdom), Frédéric Baudat (CNRS, Montpellier, France), and K.T. Nishant (Indian Institute of Science, Bangalore, India). Laborious analysis of mouse sperm DNA allowed Baudat to identify a mouse meiotic recombination hotspot that is surprisingly small with a maximal crossover density in only a 210 base pair interval (Guillon and de Massy, 2002). Like in yeast recombination hotspots, not only crossovers but also conversion were found at high frequency. Jeffreys discussed the implication of recombination hotspots on linkage disequilibrium analysis and human population genetics, and suggested that linkage disequilibrium analysis cannot reliably detect recombination hotspots, because hotspots emerge quickly during human evolution and have hence not left a mark on a population (Kauppi et al., 2004).

Recombination and DNA Replication

In addition to generation of genetic diversity and DNA damage repair, DNA recombination is also essential for genomic DNA replication. A constant damage load on the genome from both exogenous and endogenous sources causes problems for DNA replication fork progression. Furthermore, replication can falter at repetitive DNA sequences (Sue Lovett, Brandeis Unviversity, Waltham; Lovett, 2004). When forks are inactivated at DNA damage they need to be reset and this is done with the use of DNA recombination proteins. Mechanistic insights in this process come from genetic and biochemical experiments in *E. coli*. Bénédicte Michel (INRA, Jouy-en-Josas, France) discussed a number of different pathways that can lead to replication fork reset depending on the cause of inactivation. Interestingly, the Holliday junction is also featuring in this aspect of DNA metabolism. Reset pathways make use of a large collec-

tion of DNA recombination proteins, including the strand exchange protein RecA, the DNA end processing helicase and nuclease RecBCD, the gap repair proteins RecFOR, the branch migration motor RuvAB, Holliday junction resolvase RuvC and helicases, including RecG (Michel et al., 2004). The major challenge for the cell is to restore a replication fork in an origin independent manner. A key step in re-building replication forks is loading of the replicative DNA helicase DnaB (Marians, 2004). This accomplished with the help of PriA and PriC proteins (Kenneth Marians, Sloan-Kettering Cancer Center, New York). These proteins are specifically involved in replication restart and are not required for origin-dependent DNA replication or for general recombination functions. While DNA damage can cause problems at DNA replication forks, replication is actually required for repair of certain types of DNA damage. Roland Kanaar (Erasmus MC, Rotterdam, The Netherlands) showed that passages of mammalian cells through S phase is required to convert interstand DNA crosslinks into DSBs, which can then be repaired by homologous recombination. Surprisingly, the structure-specific endonuclease Ercc1-Xpf was not required to create the DSBs, but rather to process them once generated (Niedernhofer et al., 2004).

Holliday junctions, although originally proposed as an intermediate in a specialized form of recombination in meiotic cells, seem to be much more widespread. In addition to being intermediates during specialized replication restart processes, they appear also as intermediates during normal DNA replication in mitotic cells. Chrystele Maric-Antoinat (Institute André Lwoff, Villejuif, France) provided evidence for the existence of normal DNA replication-dependent X-shaped DNA molecules between sister chromatids of *Physarum polycephalum*. These intermediates have branch migrating properties just like Holliday junctions (Benard et al., 2001).

In addition to using replication fork restart to repair or bypass DNA damage, a group of specialized lower fidelity polymerases play important roles in translesion DNA synthesis. These polymerases transiently replace the replicative polymerase at the site of damage. Because there are a number of different translesion DNA polymerases it is of interest to determine how they organize access to the damaged site. Ivan Matic (INSERM, Paris, France) used genetic analysis in *E. coli* to argue that there is a hierarchy among the polymerases, with which they bind to the β clamp DNA polymerase processivity factor that governs the order of their access to the damaged site. To analyze DNA lesion bypass in vertebrates chicken DT40 cells provide a powerful genetic system as demonstrated by Julian Sale (MRC Laboratory of Molecular Biology, Cambridge, United Kingdom). While many enzymes of DNA repair are highly conserved during evolution, there can be significant differences in function between yeast and vertebrate proteins. For example, the Rad18 E3 ubiquitin ligase in yeast controls translesion DNA synthesis by targeting ubiquitin to the processivity clamp PCNA in response to DNA damage, which subsequently promotes lesion bypass. In vertebrates, however, Sale showed that Rad18 does not play such a broad role in translesion synthesis since it appears not to be in the same pathway as the translesion DNA polymerase Rev1, but does function in recombinational repair (Sale, 2004).

Regulation of Recombination

It has been long known that DNA damage induces homologous recombination and that in particular DSBs, nicks, and gaps are highly recombinogenic. The induction was commonly believed to result from overcoming the rate-limiting initiation step by the recombinogenic lesion. Now we know that DNA damage checkpoint are activated after DNA damage and that an increasing number of proteins functioning in recombination are being identified as phosphorylation targets of the DNA damage checkpoint kinases. Hence it is not surprising that novel mechanisms of regulating recombination are being suspected to involve the DNA damage checkpoints. Wolf-Dietrich Heyer (University of California, Davis) reported that the budding yeast Rad51 paralogs Rad55 and Rad57 proteins that function in the assembly of the Rad51 nucleoprotein filament are phosphorylated in response to DNA damage in a DNA damage checkpoint-dependent fashion (Bashkirov et al., 2000). Ablation of Rad55 phosphorylation sites resulted in cellular sensitivity to high levels of genotoxic stress but repair of a single DSB was found to be intact, suggesting that phosphorylation enhances the cellular repair capacity through Rad55 phosphorylation. One of the earliest cellular responses to DSBs is the phosphorylation of histone H2AX on a serine residue at its extreme carboxy-terminus. Michael Lichten (NIH, Bethesda) reported results from detailed ChIP studies of the initial response to DSBs induced by the HO endonuclease in *S. cerevisiae*. H2AX phosphorylation was found to extend for ~25 kb flanking the break site in either direction, marking a chromosomal region that might correspond to a single chromatin loop. Mre11 protein (presumably in a complex with Rad50 and Xrs2) preceded Rad51 protein at the break site (Shroff et al., 2004). The importance of checkpoint signaling in DNA metabolism is not limited to responding to extrinsic DNA damage, as Jean Gautier (Columbia University, New York City) showed that the ATM and ATR DNA checkpoint kinases actively regulate origin firing during normal DNA replication (Shechter et al., 2004). Modification of recombination proteins is not limited to phosphorylation, as shown by Martin Gellert (NIH, Bethesda), who described the auto-ubiquitinilation of the V(D)J recombination-specific enzyme RAG1. The RING-finger domain of RAG1 acts as an ubiquitin ligass and disruption of the RING finger are associated with immunodeficiency in human patients, suggesting that RAG1's ubiquitin ligase is required for its biological role in lymphocyte development (Jones and Gellert, 2003).

Novel Systems, Models, and Techniques

Besides the traditional model systems for recombination studies including *E. coli,* yeasts, fungi, and *Drosophila*, new model organisms add breadth and exciting new biology to the mix. The chicken DT40 cell line, an avian leukosis virus-induced, p53-negative B cell line, has been developed primarily by the efforts in the Buerstedde and Takeda laboratories into a powerhouse of vertebrate somatic cell genetics. The development of a tightly regulated promoter system in DT40 cells by

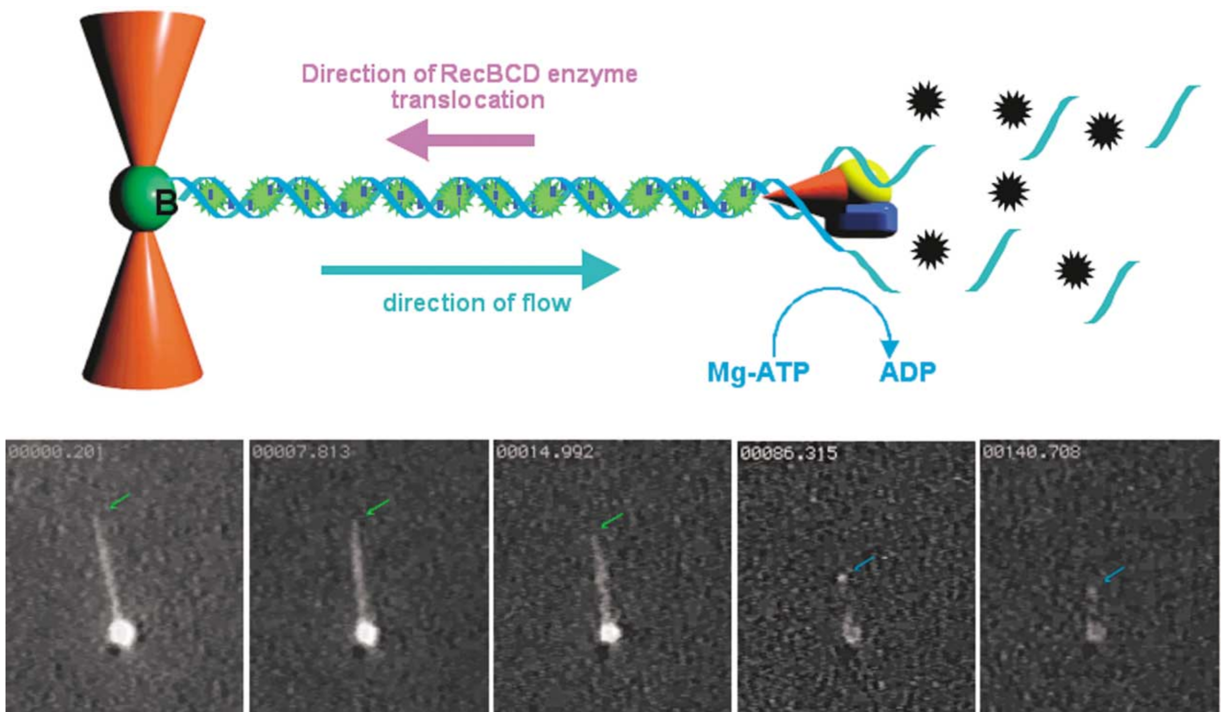

Figure 4. Single-Molecule Analysis of the RecBCD DNA Helicase/Nuclease

Schematic representation of single molecule analysis is shown on top. A streptavidin-coated bead is loaded with biotinylated double-stranded DNA, which is visualized by fluorescence with the double-strand-specific dye YOYO. RecBCD loads at the free end, starts unwinding after the addition of ATP, and produces short single-stranded digestion products. At the bottom, a time sequence (in seconds) illustrates the translocation of RecBCD, as visualized by the shortening of the duplex DNA (green arrow indicates tip of double-stranded DNA). The last two pictures show RecBCD after it encountered its regulatory site Chi (χ). After a short pause at chi, RecBCD translocates at a reduced speed and accumulates DNA at the translocating tip. For more details see Spies et al. (2003).

Shunichi Takeda (Kyoto University Medical School, Kyoto, Japan) even allows the analysis of essential genes (Sonoda et al., 1998). DT40 cells are particularly well suited for homologous recombination studies, as the biology of chicken immunoglobulin diversification affords a natural recombination assay. Unlike mice and human B and T cells that engage site-specific V(D)J recombination to assemble their final B- and T cell receptors, chicken cells utilize homologous recombination between an expressed gene locus and a pool of pseudogenes to make new sequence variants. Hiroshi Arakawa (GSF, Neuherberg, Germany) reported data showing that the activation-induced cytosine deaminase (AID) initiates immunoglobulin recombination and somatic hypermutation through a common intermediate (Arakawa et al., 2004). His model suggests that AID induces a DNA structure/lesion that can lead to Rad51-mediated gene conversion with pseudo-V gene donors or to error-prone DNA synthesis leading to targeted hypermutations in expressed V gene. The elegant beauty of meiotic cytology in *C. elegans* (*nomen est omen*) was demonstrated in talks by Anne Villeneuve (Stanford University Medical School, Stanford) and Simon Boulton (Cancer Research UK, South Mimms, United Kingdom), allowing new details of meiotic chromosome behavior to be analyzed. *C. elegans* engages p53-dependent apoptosis in response to disruptions in meiotic chromosome metabolism, a pathway not found in the yeast model systems. The growing impact of genomics on the recombination field was clearly visible in two talks on plant recombination genes by Charles White (CNRS, Aubière, France) and Giora Simchen (The Hebrew University of Jerusalem, Jerusalem, Israel).

The recombination field has always been at the front of technological developments and spawned an entire industry in the application of recombination enzymes and mechanisms in genome manipulations. While site-specific recombination systems have led the way in mechanistic and structural studies with detailed atomic resolution structures of many site-specific recombinases, this meeting showed in talks by John Tainer (The Scripps Research Institute, LaJolla) and Dale Wigley (Cancer research UK, South Mimms, United Kingdom) that X-ray crystallography is becoming increasingly useful in the analysis of homlgous recombination enzymes. In a beautiful one-two punch Tainer and John Petrini (Sloan-Ketering Cancer Center, New York City) reported on the structure and function of the Zink hook in the Rad50 protein that connects two Rad50 monomers to its functional form (Hopfner et al., 2002). The novel atomic resolution structures of archaeal Rad51 by Tainer (Shin et al., 2003) and of yeast Rad51 by Phoebe Rice (Conway et al., 2004) provide a rich basis for future mechanistic studies with these central homologous pairing and DNA strand exchange proteins.

Another breakthrough is the development of tech-

niques that allow analysis of single DNA and/or protein molecules and their application to recombination proteins and structures, which was showcased in talks by David Sherratt (Unversity of Oxford, Oxford, United Kingdom), Stephen Kowalczykowski (University of California, Davis), and David Lilley (University of Dundee, Dundee, United Kingdom). Kowalczykowski developed a technique that allows watching a single RecBCD DNA helicase molecule in action on a single DNA molecule (Figure 4). RecBCD is a "two-wheel drive" DNA helicase with two DNA helicase subunits (RecB, RecD) of opposing polarity that move coordinately in the same absolute direction of the Watson and Crick strands of duplex DNA (Dillingham et al., 2003; Taylor and Smith, 2003). While confirming previous bulk measurements on the processivity and rate of DNA unwinding by RecBCD, the single molecule analysis allowed entirely novel insights through the analysis of individual DNA helicase molecules unwinding DNA (Spies et al., 2003). RecBCD pauses at its regulatory site χ (Chi) and then continues at about one-half of its original speed, likely owing to the disengagement (but not ejection) of the RecD motor subunit. It was only befitting that Lilley's talk on single molecule analysis of the dynamics of Holliday junctions concluded the meeting on the fortieth anniversary of the Holliday model. Using fluorescence resonance energy transfer (FRET) techniques the rates of interconversion between different conformational forms of the Holliday junctions were determined, suggesting that electrostatic stabilization of the stacked structures dictates the interconversion rates between different structural forms (Joo et al., 2004).

Perspectives

The word recombination in a seminar title used to ensure a low turnout, except in the few places where recombination research was cherished. This workshop showed that these times are over and that recombination has become en vogue. The impressive span of techniques and levels of analysis, from single molecules to population genetics, captivated the attendants of the workshop until the very last talk of the last session. New important themes are emerging that demonstrate that the biological importance of recombination far exceeds meiotic recombination and the generation of genetic diversity, posing new and unanswered questions. The role of recombination in the recovery of stalled replication forks and DNA repair is at the heart of maintaining genomic stability. Not surprisingly, defects in recombination proteins are increasingly implicated in cancer predisposition. Yet we know little about the events leading to fork stalling, the structure of stalled forks, and the pathways leading to recovery. Regulation by DNA damage checkpoint and cell cycle kinases adds a new level of complexity in studying recombination pathways and mechanisms. What are the mechanistic consequences of phosphorylating recombination proteins? The effect of chromatin structure and histone modifications on recombination is entering a stage where the effects of individual histone modifications can now be studied.

Also some very old questions still await satisfactory answers. Forty years after the publication of the Holliday model, we still ask what are the pathways leading to crossovers and what are the proteins and mechanisms involved in eukaryotes, despite the impressive progress in this area. Particularly apparent at this workshop was the high degree of integration between the three Rs, recombination, replication, and repair. What was previously thought to be organized in neat pathways and epistasis groups is looking upon closer inspection more like a highly interconnected web of competing activities. How is this regulated? Do different organisms have different strategies? Do the same enzymes play different roles in different organisms or even tissues? Although certain features of recombination are remarkably well conserved, this deviation from reductionist thinking needs to be considered.

Acknowledgments

The EMBO Workshop "Recombination Mechanisms: 40th Anniversary Meeting of the Holliday Model" took place at Domaine de Seillac, Seillac, France, between May 24 and 29, 2004. The workshop was organized by Alain Nicolas and Stephen C. West. We thank J. Essers and S. Kowalczykowski for kindly providing Figures 3 and 4, respectively.

References

Arakawa, H., Saribasak, H., and Buerstedde, J.M. (2004). Activation-induced cytidine deaminase initiates immunoglobulin gene conversion and hypermutation by a common intermediate. PLoS Biology *2*, E179.

Arora, C., Kee, K., Maleki, S., and Keeney, S. (2004). Antiviral protein Ski8 is a direct partner of Spo11 in meiotic DNA break formation, independent of its cytoplasmic role in RNA metabolism. Mol. Cell *13*, 549–559.

Aten, J.A., Stap, J., Krawczyk, P.M., van Oven, C.H., Hoebe, R.A., Essers, J., and Kanaar, R. (2004). Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. Science *303*, 92–95.

Ayora, S., Carrasco, B., Doncel, E., Lurz, R., and Alonso, J.C. (2004). Bacillus subtilis RecU protein cleaves Holliday junctions and anneals single-stranded DNA. Proc. Natl. Acad. Sci. USA *101*, 452–457.

Baroni, E., Viscardi, V., Cartagena-Lirola, H., Lucchini, G., and Longhese, M.P. (2004). The functions of budding yeast Sae2 in the DNA damage response require Mec1- and Tel1-dependent phosphorylation. Mol. Cell. Biol. *24*, 4151–4165.

Bashkirov, V.I., King, J.S., Bashkirova, E.V., Schmuckli-Maurer, J., and Heyer, W.D. (2000). DNA repair protein Rad55 is a terminal substrate of the DNA damage checkpoints. Mol. Cell. Biol. *20*, 4393–4404.

Ben-Aroya, S., Mieczkowski, P.A., Petes, T.D., and Kupiec, M. (2004). The compact chromatin structure of a Ty repeated sequence suppresses recombination hotspot activity in Saccharomyces cerevisiae. Mol. Cell *15*, 221–231.

Benard, M., Maric, C., and Pierron, G. (2001). DNA replication-dependent formation of joint DNA molecules in Physarum polycephalum. Mol. Cell *7*, 971–980.

Bibikova, M., Beumer, K., Trautman, J.K., and Carroll, D. (2003). Enhanced gene targeting with designed zinc finger nucleases. Science *300*, 764.

Borner, G.V., Kleckner, N., and Hunter, N. (2004). Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell *117*, 29–45.

Boulton, S.J., Martin, J.S., Polanowska, J., Hill, D.E., Gartner, A., and Vidal, M. (2004). BRCA1/BARD1 orthologs required for DNA repair in Caenorhabditis elegans. Curr. Biol. *14*, 33–39.

Conway, A.B., Lynch, T.W., Zhang, Y., Fortin, G.S., Fung, C.W.,

Symington, L.S., and Rice, P.A. (2004). Crystal structure of a Rad51 filament. Nat Struct Mol Biol *11*, 791–796.

Cromie, G.A., Connelly, J.C., and Leach, D.R. (2001). Recombination at double-strand breaks and DNA ends: conserved mechanisms from phage to humans. Mol. Cell *8*, 1163–1174.

Dillingham, M.S., Spies, M., and Kowalczykowski, S.C. (2003). RecBCD enzyme is a bipolar DNA helicase. Nature *423*, 893–897.

Essers, J., Houtsmuller, A.B., van Veelen, L., Paulusma, C., Nigg, A.L., Pastink, A., Vermeulen, W., Hoeijmakers, J.H., and Kanaar, R. (2002). Nuclear dynamics of RAD52 group homologous recombination proteins in response to DNA damage. EMBO J. *21*, 2030–2037.

Gaillard, P.-H., Noguchi, E., Shanahan, P., and Russell, P. (2003). The endogenous Mus81-Eme1 complex resolves Holliday junctions by a nick and counternick mechanism. Mol. Cell *12*, 747–759.

Guillon, H., and de Massy, B. (2002). An initiation site for meiotic crossing-over and gene conversion in the mouse. Nat. Genet. *32*, 296–299.

Holliday, R. (1964). A mechanism for gene conversion in fungi. Genet. Res. *5*, 282–304.

Hopfner, K.P., Craig, L., Moncalian, G., Zinkel, R.A., Usui, T., Owen, B.A.L., Karcher, A., Henderson, B., Bodmer, J.L., McMurray, C.T., et al. (2002). The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. Nature *418*, 562–566.

Huertas, P., and Aguilera, A. (2003). Cotranscriptionally formed DNA:RNA hybrids mediate transcription elongation impairment and transcription-associated recombination. Mol. Cell *12*, 711–721.

Jones, J.M., and Gellert, M. (2003). Autoubiquitylation of the V(D)J recombinase protein RAG1. Proc. Natl. Acad. Sci. USA *100*, 15446–15451.

Joo, C., McKinney, S.A., Lilley, D.M.J., and Ha, T. (2004). Exploring rare conformational species and ionic effects in DNA Holliday junctions using single-molecule spectroscopy. J. Mol. Biol. *341*, 739–751.

Kauppi, L., Jeffreys, A.J., and Keeney, S. (2004). Where the crossovers are: Recombination distributions in mammals. Nat. Rev. Genet. *5*, 413–424.

Kaykov, A., Holmes, A.M., and Arcangioli, B. (2004). Formation, maintenance and consequences of the imprint at the mating-type locus in fission yeast. EMBO J. *23*, 930–938.

Kojic, M., Yang, H., Kostrub, C.F., Pavletich, N.P., and Holloman, W.K. (2003). The BRCA2-interacting protein DSS1 is vital for DNA repair, recombination, and genome stability in Ustilago maydis. Mol. Cell *12*, 1043–1049.

Lee, G.S., Neiditch, M.B., Salus, S.S., and Roth, D.B. (2004). RAG proteins shepherd double-strand breaks to a specific pathway, suppressing error-prone repair, but RAG nicking initiates homologous recombination. Cell *117*, 171–184.

Lisby, M., Mortensen, U.H., and Rothstein, R. (2003). Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. Nat. Cell Biol. *5*, 572–577.

Liu, Y.L., Masson, J.Y., Shah, R., O'Regan, P., and West, S.C. (2004). RAD51C is required for Holliday junction processing in mammalian cells. Science *303*, 243–246.

Lovett, S.T. (2004). Encoded errors: mutations and rearrangements mediated by misalignment at repetitive DNA sequences. Mol. Microbiol. *52*, 1243–1253.

Lusetti, S.L., Voloshin, O.N., Inman, R.B., Camerini-Otero, R.D., and Cox, M.M. (2004). The DinI protein stabilizes RecA protein filaments. J. Biol. Chem. *279*, 30037–30046.

Marians, K.J. (2004). Mechanisms of replication fork restart in Escherichia coli. Philos. Trans. R. Soc. Lond. B Biol. Sci. *359*, 71–77.

McPherson, J.P., Lemmers, B., Chahwan, R., Pamidi, A., Migon, E., Matysiak-Zablocki, E., Moynahan, M.E., Essers, J., Hanada, K., Poonepalli, A., et al. (2004). Involvement of mammalian Mus81 in genome integrity and tumor suppression. Science *304*, 1822–1826.

Michel, B., Grompone, G., Flores, M.J., and Bidnenko, V. (2004). Multiple pathways process stalled replication forks. Proc. Natl. Acad. Sci. USA.

Niedernhofer, L.J., Odijk, H., Budzowska, M., van Drunen, E., Maas, A., Theil, A.F., de Wit, J., Jaspers, N.G., Beverloo, H.B., Hoeijmakers, J.H., and Kanaar, R. (2004). The structure-specific endonuclease Ercc1-Xpf is required to resolve DNA interstrand cross-link-induced double-strand breaks. Mol. Cell. Biol. *24*, 5776–5787.

Osman, F., Dixon, J., Doe, C.L., and Whitby, M.C. (2003). Generating crossovers by resolution of nicked Holliday junctions: A role of Mus81-Eme1 in meiosis. Mol. Cell *12*, 761–774.

Pecina, A., Smith, K.N., Mezard, C., Murakami, H., Ohta, K., and Nicolas, A. (2002). Targeted stimulation of meiotic recombination. Cell *111*, 173–184.

Pellegrini, L., and Venkitaraman, A. (2004). Emerging functions of BRCA2 in DNA recombination. Trends Biochem. Sci. *29*, 310–316.

Petukhova, G.V., Romanienko, P.J., and Camerini-Otero, R.D. (2003). The Hop2 protein has a direct role in promoting interhomolog interactions during mouse meiosis. Dev. Cell *5*, 927–936.

Reddy, K.C., and Villeneuve, A.M. (2004). C. elegans HIM-17 Links Chromatin Modification and Competence for Initiation of Meiotic Recombination. Cell *118*, 439–452.

Sale, J.E. (2004). Immunoglobulin diversification in DT40: a model for vertebrate DNA damage tolerance. DNA Repair (Amst.) *3*, 693–702.

Sehorn, M.G., Sigurdsson, S., Bussen, W., Unger, V.M., and Sung, P. (2004). Human meiotic recombinase Dmc1 promotes ATP-dependent homologous DNA strand exchange. Nature *429*, 433–437.

Shechter, D., Costanzo, V., and Gautier, J. (2004). ATR and ATM regulate the timing of DNA replication origin firing. Nat. Cell Biol. *6*, 648–655.

Shin, D.S., Pellegrini, L., Daniels, D.S., Yelent, B., Craig, L., Bates, D., Yu, D.S., Shivji, M.K., Hitomi, C., Arvai, A.S., et al. (2003). Full-length archaeal Rad51 structure and mutants: mechanisms for RAD51 assembly and control by BRCA2. EMBO J. *22*, 4566–4576.

Shinohara, M., Gasior, S.L., Bishop, D.K., and Shinohara, A. (2000). Tid1/Rdh54 promotes colocalization of rad51 and dmc1 during meiotic recombination. Proc. Natl. Acad. Sci. USA *97*, 10814–10819.

Shroff, R., Arbel-Eden, A., Pilch, D., Ira, G., Bonner, W.M., Petrini, J.H., Haber, J.E., and Lichten, M. (2004). Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. Curr. Biol., in press.

Sonoda, E., Sasaki, M.S., Buerstedde, J.M., Bezzubova, O., Shinohara, A., Ogawa, H., Takata, M., Yamaguchilwai, Y., and Takeda, S. (1998). Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. EMBO J. *17*, 598–608.

Spies, M., Bianco, P.R., Dillingham, M.S., Handa, N., Baskin, R.J., and Kowalczykowski, S.C. (2003). A molecular throttle: the recombination hotspot chi controls DNA translocation by the RecBCD helicase. Cell *114*, 647–654.

Storlazzi, A., Tesse, S., Gargano, S., James, F., Kleckner, N., and Zickler, D. (2003). Meiotic double-strand breaks at the interface of chromosome movement, chromosome remodeling, and reductional division. Genes Dev. *17*, 2675–2687.

Sugawara, N., Wang, X., and Haber, J.E. (2003). In vivo roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. Mol. Cell *12*, 209–219.

Szostak, J.W., Orr-Weaver, T.L., Rothstein, R.J., and Stahl, F.W. (1983). The double-strand-break repair model for recombination. Cell *33*, 25–35.

Taylor, A.F., and Smith, G.R. (2003). RecBCD enzyme is a DNA helicase with fast and slow motors of opposite polarity. Nature *423*, 889–893.

Tesse, S., Storlazzi, A., Kleckner, N., Gargano, S., and Zickler, D. (2003). Localization and roles of Ski8p protein in Sordaria meiosis and delineation of three mechanistically distinct steps of meiotic homolog juxtaposition. Proc. Natl. Acad. Sci. USA *100*, 12865–12870.

Wu, L.J., and Hickson, I.D. (2003). The Bloom's syndrome helicase suppresses crossing-over during homologous recombination. Nature *426*, 870–874.

Yang, H., Jeffrey, P.D., Miller, J., Kinnucan, E., Sun, Y., Thoma, N.H., Zheng, N., Chen, P.L., Lee, W.H., and Pavletich, N.P. (2002). BRCA2 function in DNA binding and recombination from a BRCA2–DSS1-ssDNA structure. Science *297*, 1837–1848.