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Workshop on Recombination Mechanisms and the Maintenance of Genomic Stability

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Introduction

The accurate repair of DNA double-strand breaks (DSBs) is crucial for the maintenance of genome stability and the prevention of tumours. Homologous recombination (HR) is a complex process used by cells to repair DSBs that arise spontaneously during DNA replication or after exposure to DNA-damaging agents. HR is also fundamental for DSB-initiated DNA transactions, which occur during meiosis, V(D)J recombination and mating-type switching. During the past decade, many HR components have been identified using genetic and biochemical approaches (Jasin, 2002; Keeney, 2001; Lisby & Rothstein, 2004, 2005; Liu & West, 2004; West, 2003); the main challenge now is to define how known HR components function at the molecular level. The first workshop on recombination was held in 1968 and was a highly stimulating meeting that focused on how DNA could be manipulated to achieve recombination. Since then, the field has expanded considerably. This was reflected in the most recent meeting, which brought together scientists studying many areas of DNA metabolism that either use or have a direct effect on HR.

Early recombination steps

The main difference between HR-mediated DSB repair in mitotic and meiotic cells is that repair in mitotic cells occurs through an intact sister chromatid, whereas meiotic cells preferentially use a paired homologue as a template to generate a crossover that is essential for homologue disjunction at the first meiotic division. Meiotic DSBs are generated in a highly controlled manner through the nucleolytic action of the meiosis-specific sporulation protein 11 (Spo11). This process also depends on a complex of interacting proteins including the yeast orthologue (Xrs2) of mammalian Nijmegen breakage syndrome 1 (NBS1), meiotic recombination 2 (Mer2) and recombination 114 (Rec114). S. Keeney (New York, NY, USA) reported on Mer2, which associates with chromosomes prior to synaptonemal complex formation, and is phosphorylated by cell-division cycle 28 (Cdc28)/B cyclin 5 (Clb5) or Clb6 at Ser 30 and Ser 271. Mutation of the phosphorylation sites in Mer2 creates a defect in meiotic DSB formation owing to the failure of Mer2 to form a complex with Rec114 and Xrs2. Therefore, phosphorylation has a crucial role in establishing the protein–protein interactions necessary for the generation of meiotic DSBs. A. Nicolas (Paris, France) reported on the control of the initiation of meiotic recombination by Spo11. A Gal4-binding domain–Spo11 fusion protein can rescue the *spo11* deletion mutant

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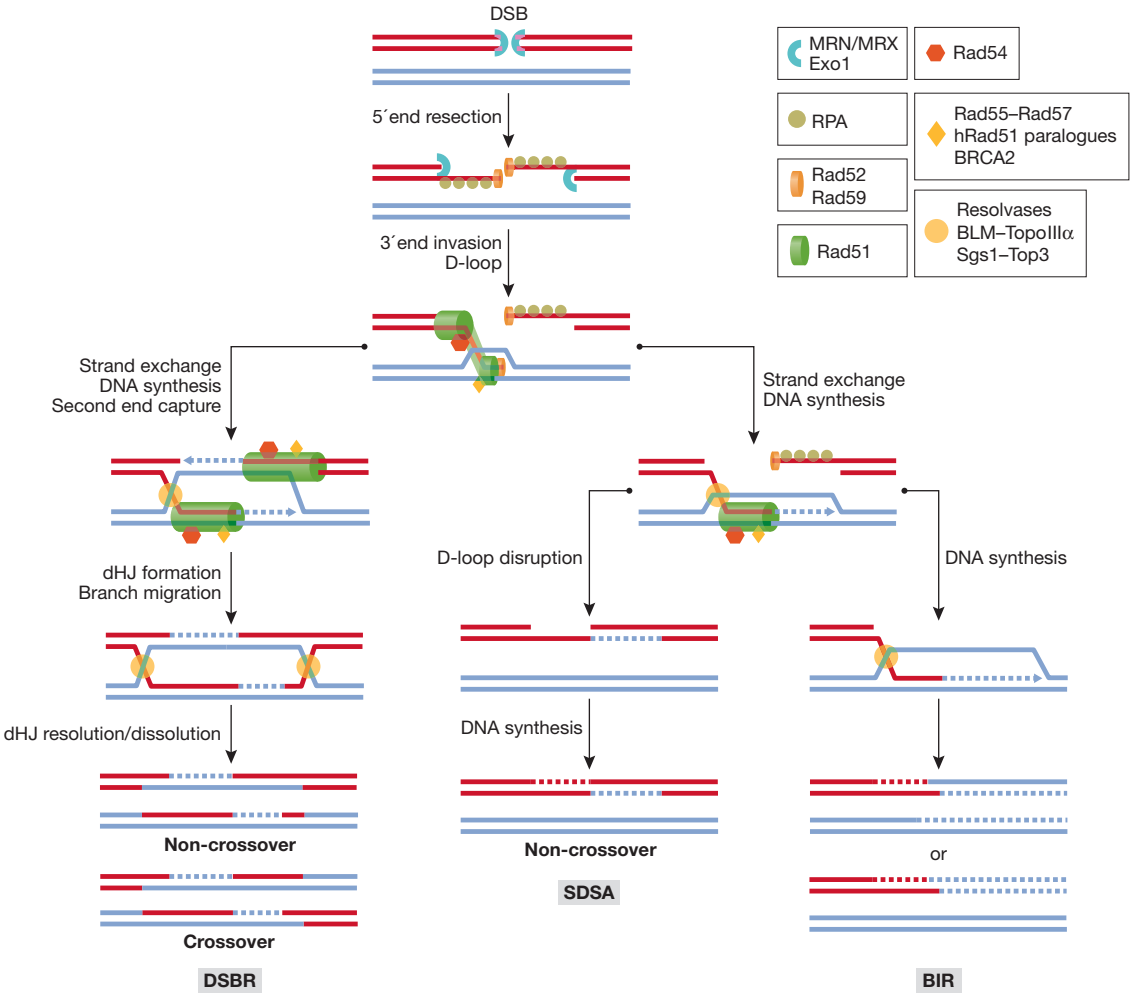

Fig 1 | Pathway of double-stranded break repair by homologous recombination and the factors involved. Recombination is initiated by nucleolytic processing of the DSB to generate 3′ single-stranded DNA (ssDNA) overhangs that rapidly complex with RPA. Rad51 recruitment displaces RPA leading to the formation of a helical nucleoprotein filament with ssDNA, which can search for an intact homologous template and then catalyse invasion of the ssDNA into a donor sister chromatid or homologous chromosome to form a joint molecule. The resulting joint molecule acts as a primer for DNA synthesis to extend the heteroduplex DNA, which, following further processing and resolution of the joint DNA molecules, leads to repair of the DSB and restoration of DNA integrity. BIR, break-induced replication; BRCA2, breast cancer 2; BLM–TopoIIIα, Bloom syndrome protein–topoisomerase IIIα; dHJ, double Holliday junction; DSB, double-stranded break; DSBR, DSB-repair model; Exo1, exonuclease 1; hRad51, human Rad 51; MRN, MRE11–RAD50–NBS1; MRX, Mre11–Rad50–Xrs2; MRE11, meiotic recombination 11; NBS1, Nijmegen breakage syndrome 1; Rad50, radiation sensitive 50; RPA, replication protein A; SDSA, synthesis-dependent strand annealing; Top3, Topoisomerase 3.

and cleaves DNA at similar sites to Spo11. This fusion protein also targets Gal4-binding sites and preferentially generates DSBs approximately 20 nucleotides away, although cleavage can occur within a region of 16–32 nucleotides. Mutation of the strong cleavage site near the Gal4-binding site at the *GAL2* locus was shown to alter the position of the DSB.

In both mitotic and meiotic eukaryotic cells, it is widely believed that the meiotic recombination 11 homologue (MRE11)–radiation sensitive 50 (RAD50)–NBS1 (MRN) complex in mammals and Mre11–Rad50–Xrs2 (MRX) in yeast have important roles in DSB detection (Fig 1) and processing, and in tethering DNA ends to constrain them in an appropriate conformation for DNA repair. J. Tainer (San Diego, CA, USA) reported that Mre11 dimerization is mediated by Rad50–ATP, which has a ring-like structure that can change conformation. R. Kanaar (Rotterdam, The Netherlands) showed that the MRN complex forms intramolecular interactions between Rad50 molecules in the absence of DNA. However, DNA binding by the globular head domain leads to the formation of parallel coiled-coils that preclude intramolecular interactions while favouring intercomplex interactions that are required to tether different DNA ends. Kanaar proposed that the MRN complex functions as 'molecular Velcro' that can tether broken DNA ends through intermolecular interactions between Rad50 coiled-coils mediated by the zinc-hook motifs (Fig 2B).

HO and ISceI endonucleases induce site-specific DSBs *in vivo*, and have been used to analyse the interplay between DNA-damage sensing and repair proteins. The ability to induce DNA breaks by other means at specific genomic locations represents an important

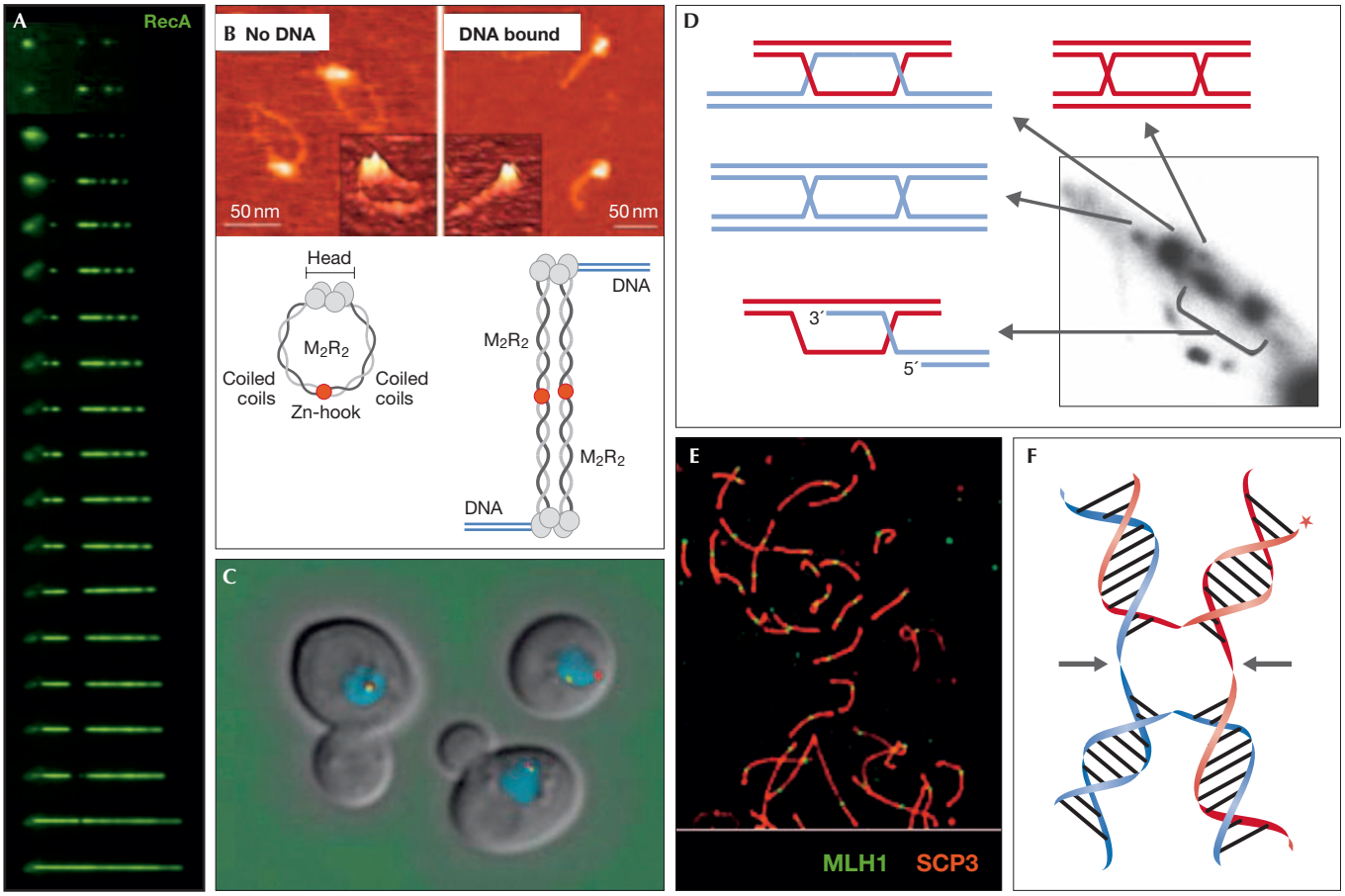

Fig 2 | Current methods for visualizing the different stages of homologous recombination. **(A)** Direct visualization of the assembly of fluorescently labelled recombination A (RecA) on single-stranded DNA (image courtesy of S. Kowalczykowski; Galletto *et al*, 2006). **(B)** Visualization of Mre11/Rad50 complexes with or without DNA by atomic-force microscopy (image courtesy of R. Kanaar; Moreno-Herrero *et al*, 2005). **(C)** Co-localization of two fluorescently marked double-stranded breaks (shown in yellow and red) with a single Rad52-repair centre in living yeast cells (image courtesy of R. Rothstein and M. Lisby; Lisby & Rothstein, 2005). **(D)** Detection of meiotic recombination intermediates by two-dimensional gel electrophoresis (image courtesy of N. Hunter; Hunter & Kleckner, 2001). **(E)** MLH1 (green) marks the sites of crossover-recombination events in mouse spermatocytes. The synaptonemal complex component 3 (SCP) is also shown (red; image courtesy of S. West). **(F)** Illustration depicting a Holliday junction (image courtesy of S. West); the asterisk indicates the 5′ end. MLH1, MutL homologue 1; Mre11, meiotic recombination 11; Rad50, radiation sensitive 50.

new avenue of research. A. Aguilera (Seville, Spain) described a new *in vivo* assay in *Saccharomyces cerevisiae* for the induction of site-specific single-strand breaks, and showed that they are converted into DSBs by replication. These are repaired preferentially by sister-chromatid exchange, which requires MRX, cohesins and the structural maintenance of the chromosome 5–6 (SMC5–6) complex. Aguilera showed that SMC5–6 is recruited to HO-induced DSBs and proposed that SMC proteins have a role in holding chromatids together to facilitate repair. C. Sjögren (Stockholm, Sweden) reported that the SMC5–6 complex also localizes to HO-induced DSBs and collapsed replication forks in an MRX complex-dependent manner. Sjögren proposed that loading of the SMC5–6 ring structure by the MRX complex at DSBs facilitates repair between sister chromatids. M. Lisby (Copenhagen, Denmark) reported that bleomycin-induced DSBs are recognized by Mre11 in the nucleolus, whereas loading of Rad52 onto DSBs occurs exclusively outside the nucleolus. An HO-induced DSB within the ribosomal DNA was exported from the nucleolus before association with Rad52. Lisby proposed that HR is excluded from the nucleolus to prevent recombination between repetitive sequences. Indeed, *smc5–6* mutants exhibit ribosomal DNA instability and contain Rad52 foci on the periphery or inside the nucleolus. P. Jeggo (Brighton, UK) reported on the role of Artemis in facilitating repair of a subset (~5–10%) of infrared-induced DSBs that require extensive end-processing before repair. Artemis is a single-strand overhang endonuclease in the presence of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and ATP. Jeggo showed that DNA-PKcs autophosphorylation, rather than Artemis phosphorylation, is required for Artemis to function as an endonuclease.

The human activation-induced cytidine deaminase (AID) provides an interesting mechanism for the natural induction of recombinogenic DNA lesions in mitotically growing cells. Previous reports have shown that AID induces abasic sites that trigger immunoglobulin gene conversion and hypermutation during V(D)J recombination. S. Takeda (Kyoto, Japan) reported that AID-induced damage is repaired by either HR or error-prone polymerases. Nbs1-deficiency in DT40 cells abolishes gene conversion, which

can be reverted by AID overexpression. This indicates that the MRN complex might directly process single-stranded DNA (ssDNA) breaks rather than DSBs to facilitate recombination.

Intermediate recombination steps

HR is initiated by resection of the DSB to generate a 3′ single-stranded overhang that is bound by the recombination A (RecA)/Rad51 recombinase to form a presynaptic nucleoprotein filament, which is responsible for homology searching and invasion of the processed DSB into intact duplex DNA. Single-molecule analyses represent an exciting avenue of research that is being used to explore the dynamics and behaviour of the RecA/Rad51 nucleoprotein filament (Fig 1). S. Kowalczykowski (Davis, CA, USA) directly visualized fluorescently labelled RecA to monitor its assembly and disassembly on ssDNA (Fig 2A). RecA assembles initially as a cluster at several sites on double-stranded DNA (dsDNA) with a preference for (A+T)-rich DNA. Clusters consist of nuclei comprising four or five monomers of RecA that can grow bidirectionally. RecA nucleation is independent of ATP hydrolysis and dependent on nucleotide triphosphates. E. Egelman (Charlottesville, VA, USA) reported on the structural analysis of the carboxy-terminal RadA/Rad51/Dmc1 (for radiation sensitive A/Rad51/disrupted meiotic cDNA 1) domain compared with the C-terminal-specific RecA domain. Egelman also showed how the interaction between the cI repressor of bacteriophage λ and RecA is different from that between LexA and RecA, even though both repressors are structural homologues.

Several HR factors have been shown to facilitate the formation of the Rad51 presynaptic filament in yeast, including Rad52 and the Rad51 paralogues Rad55/57 (Fig 1). It is believed that these proteins facilitate recruitment or loading of Rad51 onto DSBs and in some cases might also modulate the strand-exchange enzymatic activity of Rad51. In addition, the Srs2 and Sgs1 DNA helicases of the RecQ family have also been shown to impact on strand exchange. L. Symington (New York, NY, USA) reported that heterozygosity at the *mating-type* locus (*MAT*) increases spontaneous and DSB-induced HR between homologues, but not between sisters. *MAT* heterozygosity suppressed the irradiation sensitivity and the DSB-repair defect of *rad55* and *rad57* mutants, but not the spontaneous direct-repeat recombination defect of *rad57*. Symington proposed that the Rad55/57 complex functions during the early steps of spontaneous and DSB-induced HR that require strand invasion, rather than during a late step in stalled forks, which involve template switching. R. Rothstein (New York, NY, USA) described a screen for increased/decreased spontaneous Rad52 foci formation in yeast. He found that yeast cells with altered numbers of Rad52 foci were defective in various processes, including DNA replication and recombination, sister-chromatid cohesion, and transcription and silencing. Comparative analysis of direct-repeat versus inter-homologue recombination revealed specific effects of several mutants for each type of recombination event, which is consistent with the idea that they occur by different mechanisms. H. Klein (New York, NY, USA) showed that the deleterious effect of Rad51 overexpression in the *srs2* helicase mutant is dependent on Rad55, and that Rad52 provides some protection against Rad51 overexpression in *srs2* mutants. P. Russell (La Jolla, CA, USA) described *Schizosaccharomyces pombe* Sws1, which interacts with Srs2 in yeast two-hybrid assays. Sws1 is a conserved protein with a SWIM domain [$CXC(X)_nCXH$] consisting of a potential zinc-chelating domain that is essential for Sws1 function. The *sws1* mutation rescues the camptothecin sensitivity of the helicase mutants *srs2* and *rqh1* (RecQ-like helicase 1) and, remarkably, also rescues the synthetic lethality of *rqh1 srs2*. Russell proposed that Sws1 promotes the formation of toxic recombination intermediates in *rqh1* and *srs2* mutants. The fact that the *S. pombe* homologue of ScRad52 (Rad22) foci was reduced in *sws1* mutants indicates that Sws1 has an early role in HR.

In meiosis, Rad51-dependent strand exchange is modulated by specific meiotic factors such as Dmc1, which is a Rad51-related protein, the function of which is not fully understood. H. Tsubouchi, from S. Roeder's group (New Haven, CT, USA), reported that DSBs persist in *dmc1* mutants for 60 h, owing to the inhibition of Rad51 by Hed1 in the absence of Dmc1. In *dmc1 hed1* strains, meiotic DSBs are formed and repaired in a Rad51-dependent manner, but do not associate well with crossovers, in a similar manner to mitotic HR. Hed1 localization in meiosis mirrors that of Rad51, on which it is dependent. Two-hybrid analysis revealed that Hed1 interacts with itself and with Rad51, and that the Hed1–Hed1 interaction is required for Rad51 inhibition.

The tumour suppressor protein breast cancer 2 (BRCA2) is also believed to perform mediator functions in HR through its ability to bind directly to Rad51 and ssDNA. Recent work has focused on how BRCA2 promotes Rad51 recruitment onto DNA and how it might influence Rad51 recombinase activities. M.-P. Doutriaux (Paris, France) reported on the *Arabidopsis thaliana* BRCA2, which interacts with both Rad51 and Dmc1. The knockout of AtBRCA2 is sterile, accumulates chromosome aggregates in meiosis and fails to load Dmc1 onto meiotic DSBs, which is consistent with a mediator function. P. Sung (New Haven, CT, USA) also showed that a polypeptide comprising the DNA-binding domain and Rad51-interacting motifs (BRC3 and BRC4) of human BRCA2 binds ssDNA and Rad51, and is able to promote the strand-exchange activity of Rad51 *in vitro*. The BRC motifs and the DNA-binding domain do not act in *trans* in this regard.

Late recombination steps

Branch migration and resolution of Holliday junctions (HJs; Fig 1) are crucial steps in the final outcome of meiotic recombination, and are under the control of several proteins including a particular subset of DNA helicases, resolvases and proteins of the MSH (MutS homologue) family. Deciphering the role and mode of actions of these factors is providing new clues to improve understanding of crossover control.

Crossover formation during yeast meiosis requires Msh4 and Msh5, Zip1–4, Mei3 (meiosis defective 3) and Spo16, as well as Exo1 (exonuclease 1), Mlh1 (MutL homologue 1) and Mlh3, which also act in mitosis. N. Hunter (Davis, CA, USA) reported that a mutation in the *sgs1* helicase alleviates the meiotic crossover defect of *msh5* mutants. Two-dimensional gel analysis provided direct *in vivo* evidence that Sgs1 can prevent the formation of double HJs (dHJs). Hunter proposed that meiotic cells have evolved factors, such as Msh5, specifically to antagonize the anti-crossover activity of Sgs1. M. Jasin (New York, NY, USA) reported on an analysis of *ataxia telangiectasia-mutated* (*Atm*) mutants in mice in which the *Spo11* gene is heterozygous. This analysis revealed that ATM is required for normal crossover control during meiosis, and seems to link meiotic chromosome axis integrity to crossover control.

A. Shinohara (Osaka, Japan) reported on Spo16, the mutation of which leads to a defect in crossover formation in meiosis. *Spo16* mutants are epistatic with Zip1, and exhibit defects in synaptonemal

complex elongation and synapsis. Spo16 forms a punctate staining pattern and often co-localizes with Zip3. Spo16 localization requires Zip1 and Zip3, but not Msh4. Zip3 is a RING (Really Interesting New Gene) finger/U-box protein with E3-ubiquitin ligase activity. Conjugated ubiquitylation occurs during meiosis but the targets and relevance of this modification are unknown. A. Villeneuve (Palo Alto, CA, USA) reported on meiosis-specific chromosomal proteins in *Caenorhabditis elegans* that colocalize during much of meiotic prophase, but exhibit notable reciprocal localization at late prophase when the synaptonemal complex disassembles. The relationship of this reciprocal localization to crossover recombination is now being investigated. S. Lovett (Waltham, MA, USA) reported on the *Escherichia coli* RecA paralogue, RadA. Mutants of RadA are weakly sensitive to hydroxyurea, methyl methane sulphonate, mitomycin C and phleomycin, and this sensitivity is increased in a *radA recG* double-mutants. HR is weakly decreased in single *radA* mutants, but synergistically in *radA recG*, indicating a late role for RadA in recombination.

It is apparent that cells have evolved various ways to process recombination intermediates and complete recombination. Previous work from I. Hickson (Oxford, UK) established a role for the Bloom syndrome protein (BLM) and topoisomerase IIIα (TopoIIIα) in the dissolution of dHJs. At the meeting, Hickson reported RMI1/BLAP75 (for RecQ-mediated genome instability 1 homologue) to be a BLM-associated protein. He showed that RMI1 stimulates the dissolution activity of BLM and TopoIIIα, indicating a late role for RMI1 in recombination. S. Sharan (Bethesda, MD, USA) reported that mouse knockouts for Rad51C, which is one of five Rad51 paralogues in mammals, confers inviability. However, a hypomorphic allele with reduced protein expression retains viability but causes infertility with spermatocytes arrested at prophase I. Oocytes are cytologically normal at metaphase I, but sister chromatids undergo premature separation at metaphase II, in a similar manner to cohesin mutants. This phenotype suggests a late role for Rad51C in HR.

From replication to recombination

Increasing evidence indicates that HR in vegetatively growing cells is a co-replicative and post-replicative DNA-repair pathway devoted to the repair of lesions that impede DNA replication. DSBs can therefore arise when DNA replication forks collapse on encountering natural barriers in DNA, such as regions of heterochromatin, tightly bound non-histone proteins or DNA lesions. Different studies on the role of recombination in rescuing stalled replication forks is providing new clues about the *in vivo* relevance of HR as a mechanism to bypass DNA-replication problems. B. Lloyd (Nottingham, UK) reported that ultraviolet treatment of *E. coli* cells delays ongoing DNA replication for approximately 20 min. DnaC is essential for replication to resume, implying that the restart depends on DnaB loading and the assembly of new replisomes rather than the resumption of synthesis by existing replisomes. The replication origin (OriC) continues to fire, masking the extent of the delay. UvrA-dependent excision of ultraviolet lesions occurs during the delay; as a result, replication can proceed once it has been restarted. B. Michel (Gif-sur-Yvette, France) showed that the presence of replication termination ectopic Ter sites can block replication with the formation of linear DNA as a result of re-firing from OriC. The viability of this strain required RecA, the helicase–nuclease complex RecBCD and the RuvABC resolvase, indicating that HR is important for the restart of replication. Michel also showed that the UvrD helicase is essential for removal of the replication barrier generated by binding of the Tus protein to the Ter sites. D. Sherratt (Oxford, UK) proposed the 'split' replisome model to explain chromosome organization in *E. coli* and the observation that newly replicated sister chromosomes remain together for only a short period of time before separating. In addition, Sherratt showed that fluorescent TetR and LacI can block replication by binding to DNA. Rapid replication restart after repressor binding does not require replication proteins and occurs in temperature-sensitive dnaB mutants.

Inducible site-specific replication fork barriers, such as RuraR in *S. pombe*, are excellent substrates for the analysis of fork stabilization, repair and restart (Lambert & Carr, 2005). A. Carr (Brighton, UK) reported that the RuraR barrier does not produce detectable levels of DSBs, but does stimulate Rad22 (ScRad52)-dependent HR between direct repeats located at RuraR. Replication stalling at palindromic sequences leads to chromosome rearrangements. Yeast cells that contain palindromic *URA4* sequences within RuraR have poor viability, and accumulate acentric and dicentric DNA fragments. M. Whitby (Oxford, UK) also described a direct-repeat reporter containing a replication-stall site in fission yeast. He observed a 50-fold increase in HR frequency at this reporter, with the detection of both deletion and gene-conversion events that are Rad51-dependent and Rad22 (ScRad52)-dependent. HR was increased even further in several helicase mutants, including *rqh1* and *srs2*, indicating that the cell uses various strategies to limit HR at stalled forks.

Recombination initiated by one-ended DSBs results in the synthesis of long stretches of DNA after strand invasion in a process termed break-induced replication (BIR; Fig 1). BIR is therefore one possible mechanism for the reconstitution of replication forks, although evidence for this theory is lacking at present. J. Haber (Waltham, MA, USA) reported on the *in vivo* dynamics of BIR in budding yeast chromosomes containing an active HO site. Among the different replication mutants studied, proliferating cell nuclear antigen (PCNA) is required for BIR, but Mcm4 (minichromosome maintenance-deficient 4), Pol α-primase and the Cdc7 kinase are not. Nevertheless, in G2-arrested cells, Polα (*pol1–17*) and primase (*pri2–1*) conditional mutants block BIR. Pol32, which is a non-essential subunit of Pol ∂, is required for BIR but not during S phase. BIR is more efficient in *exo1 rad30 sae2* mutants, indicating that 5′-end resection is important. P. Pasero (Montpellier, France) reported that the checkpoint function of Mrc1 can be separated from its role in the maintenance of stalled replication forks, and that Mrc1-activated Rad53 prevents the formation of Rad52 foci but not HR. J. Petrini (New York, NY, USA) reported that a yeast Rad53TA (Thr to Ala) mutant was sensitive to hydroxyurea, and used this allele to identify BMH1 (Brain modulosignalin homologue 1) and BMH2 as multicopy suppressors of Rad53TA. BMH2 interacts with Rad53, and Petrini identified an amino-acid residue that abolished this interaction and phenocopied Rad53 hypomorphism.

In complex eukaryotes, physical barriers to replication-fork progression trigger the activation of the Fanconi anaemia (FA) pathway, which is believed to orchestrate lesion repair through HR and/or translesion bypass pathways. However, the way in which this is achieved is poorly understood. K. Patel (Cambridge, UK) reported that the FA core in DT40 cells is present constitutively during the cell cycle and depends on the presence of the E3-ubiquitin ligase subunit FANCL for FA core-complex formation. This complex forms in the presence of a ligase-dead form of FANCL and retains its ability to associate with chromatin. However, the mutant fails to rescue the mitomycin C-sensitivity of FANCL-knockout cells and is unable to mono-ubiquitylate FANCD2. FANCL was also shown to auto-ubiquitylate, and is able to

ubiquitylate FANCD2 *in vitro*. S. Boulton (South Mimms, UK) reported on the identification of a novel S-phase checkpoint component that impacts on Chk1 (Checkpoint homologue 1) function downstream of the ATM and Rad3-related (ATR) kinase. Similar to ATR, this factor is also required for the mono-ubiquitylation of FANCD2 and its subsequent recruitment to repair foci, as well as for the activation of HR at the level of recruitment of Rad51 to sites of replication stress.

Genome rearrangements and DSB repair

An important consequence of DSB repair by HR is its influence on genome stability. Depending on the effectiveness of HR, unrepaired DNA substrates can remain, which might be a source of DNA rearrangements induced by HR with an illegitimate partner or by non-homologous types of DNA recombination. Using a genome-wide approach, T. Petes (Durham, NC, USA) described the effect of γ-ray-induced DSBs on chromosome aberrations in yeast. A duplication of approximately 60 Kbp and several translocations were detected, many of which involved ∂ sequences (the long terminal repeats of Ty retrotransposons; 32%) or intact Tys residues (47%). Most translocations were non-reciprocal and some Tys residues were preferred sites for chromosome rearrangements relative to others. More than 90% of the breakpoints involved repetitive DNA. T. de Lange (New York, NY, USA), reported on POT1, which is a ssDNA-binding protein that is required in fission yeast to prevent the degradation of telomeric DNA. The two POT1 orthologues in mice localize to telomeres. Furthermore, POT1a is essential in early embryogenesis, whereas POT1b is not. POT1 double-knockout mice have occasional telomere fusions; however, POT1b-knockout mice have a 10-fold excess of telomeric TTAGGG repeats, indicating that POT1b might be important for the regulation of processing of 3′-ssDNAs present at telomere ends.

Transposition or site-specific recombination can also be a source of chromosome rearrangements. N. Craig (Baltimore, MD, USA) summarized the *in vitro*-transposition mechanism of the transposon hAT purified from *E. coli*. This involves a nick at the 5′ end, hairpin formation and 3′-end joining to the target. mHermes transposition was also studied in a new yeast genetic assay. Transposition could be analysed genetically and rejoining after transposition was found to occur by single-strand annealing, gene conversion and non-homologous end joining (NHEJ). D. Roth (New York, NY, USA) proposed that RAG-transposition activity might be more robust than previously thought. His group analysed a number of hairpin structures and found that several could stimulate efficient transposition *in vivo*; surprisingly, one structure proved to be a potent transposition inhibitor. D. Carroll (Salt Lake City, UT, USA) reported high-efficiency Rad51-dependent targeting of the *Drosophila rosy* gene using custom-engineered zinc-finger nucleases. In *C. elegans*, zinc-finger nuclease-induced mutations were also analysed. At both extrachromosomal and genomic targets, mutations occurred by NHEJ, and included both fill-ins and small deletions. These observations raised the possibility that zinc-finger nucleases could be used to generate heritable site-specific mutations in the worm genome.

Finally, the identification of the mechanism by which particular organisms have an exceptional ability to deal with massive DSBs provided important clues to improve our understanding of DSB repair and HR. On the basis that *Deinococcus radiodurans* is highly resistant to the DNA-damaging effects of X-rays, M. Cox (Madison, WI, USA) asked whether it was possible to make *E. coli* highly resistant through natural selection. After 21 cycles of irradiation and selection, four independent mutants were isolated and Cox discussed two of these. Both were mutated in genes involved in replication and repair; however, the pattern of gene alterations observed was different for the two mutants, implying that no single genetic combination is responsible for high X-ray resistance. This work indicates that large changes in X-ray resistance affect gene regulation and might occur by various paths. M. Radman (Paris, France) explored the possible mechanisms responsible for extreme γ-ray resistance in *D. radiodurans*. The presented data supported a two-stage model for deinococcal DNA repair: an 'extended' synthesis-dependent strand-annealing step involving massive PolA-dependent DNA synthesis, followed by maturation of long linear DNA-repair intermediates into circular chromosomes by RecA-dependent HR.

In summary, the workshop provided an excellent opportunity to discuss new results concerning the structure and function of recombination proteins. At present, the field is heading towards a more complete understanding of HR in the context of the whole cell. Multidisciplinary approaches using genetics, biochemistry, structural, molecular and cellular biology, nanotechnologies and genome-wide screenings should lead to an integrated view of HR mechanisms and their relationship with the cell cycle, checkpoint control, DNA replication and chromosome segregation. Collectively, these approaches will contribute to the ultimate aim of understanding how HR influences cell proliferation and differentiation.

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REFERENCES

Galletto R, Amitani I, Baskin RJ, Kowalczykowski SC (2006) Direct observation of individual RecA filaments assembling on single DNA molecules. *Nature* **19:** 875–878

Hunter N, Kleckner N (2001) The single-end invasion: an asymmetric intermediate at the double-strand break to double-Holliday junction transition of meiotic recombination. *Cell* **106:** 59–70

Jasin M (2002) Homologous repair of DNA damage and tumorigenesis: the BRCA connection. *Oncogene* **21:** 8981–8993

Keeney S (2001) Mechanism and control of meiotic recombination initiation. *Curr Top Dev Biol* **52:** 1–53

Lambert S, Carr AM (2005) Checkpoint responses to replication fork barriers. *Biochimie* **87:** 591–602

Lisby M, Rothstein R (2004) DNA damage checkpoint and repair centers. *Curr Opin Cell Biol* **16:** 328–334

Lisby M, Rothstein R (2005) Localization of checkpoint and repair proteins in eukaryotes. *Biochimie* **87:** 579–589

Liu Y, West SC (2004) Happy Hollidays: 40th anniversary of the Holliday junction. *Nat Rev Mol Cell Biol* **5:** 937–944

Moreno-Herrero F, de Jager M, Dekker NH, Kanaar R, Wyman C, Dekker C (2005) Mesoscale conformational changes in the DNA-repair complex Rad50/Mre11/Nbs1 upon binding DNA. *Nature* **437:** 440–443

West SC (2003) Molecular views of recombination proteins and their control. *Nat Rev Mol Cell Biol* **4:** 435–445

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