MOLECULAR VIEWS OF RECOMBINATION PROTEINS AND THEIR CONTROL

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The efficient repair of double-strand breaks in DNA is critical for the maintenance of genome stability and cell survival. Homologous recombination provides an efficient and faithful pathway of repair, especially in replicating cells, in which it plays a major role in tumour avoidance. Many of the enzymes that are involved in recombination have been isolated, and the details of this pathway are now being unravelled at the molecular level.

HOMOLOGOUS PAIRING
Interactions between DNA molecules of similar sequence.

STRAND EXCHANGE
The transfer of a strand of DNA from one molecule to another.

RECOMBINASES
Proteins that promote DNA–DNA interactions.

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The process of genetic recombination is in danger of becoming popular. Once thought of as an esoteric and rather incomprehensible problem that should be left to intellectuals with a specialist training in fungal genetics, the mechanisms by which DNA molecules interact and exchange information are becoming interesting to anyone who needs to know about DNA and how it replicates. Despite a history that is based on the outcome of genetic crosses, the mechanisms of recombination now fascinate biochemists and structural biologists.

Breakage of the sugar–phosphate backbone of DNA occurs in cells that are irradiated or suffer the effects of genotoxic agents. These single-strand or double-strand DNA breaks (SSBs or DSBs) must be efficiently repaired to restore the integrity and functionality of the genome. In mammalian cells, repair can be promoted either by non-homologous end joining (NHEJ), or by homologous recombination (BOX 1). Homologous recombination also provides a pathway by which stalled or broken replication forks can be re-established, thereby allowing cell survival. Indeed, it has been argued that the *raison d'être* for recombination in a somatic cell is to maintain functional replication forks[1]. The processes of replication, recombination and repair are so entwined that they are becoming indistinguishable — the three Rs of DNA metabolism are becoming one.

Over the past two or three years, our understanding of the mechanisms of recombinational repair, and their importance for genome integrity, has been advanced by significant developments in several areas. First, biochemical and structural studies have shown how key recombination proteins manipulate DNA and catalyse the molecular gymnastics that were once thought to be so very complex. Second, we are beginning to understand that recombination proteins are tightly controlled and relocalize to sites of DNA damage as and when they are required. Third, the discovery that cancer-susceptibility genes such as *BRCA1* and *BRCA2* are required for normal levels of recombinational repair shows the connection between repair efficiency, the ability to maintain genome stability and the potential for tumorigenesis. Finally, the interplay of recombination and replication is now obvious — cells need recombination to be able to fully replicate the genome. The importance of faithful and efficient recombination can no longer be underestimated.

This review summarizes recent developments in the mechanisms and control of DSB repair in eukaryotic cells, with particular emphasis on protein–protein interactions and structural studies of key factors such as RAD52 and BRCA2.

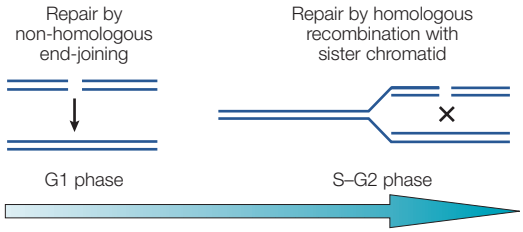
RAD51 — the universal recombinase

In all organisms, the early steps of recombination involving HOMOLOGOUS PAIRING and STRAND EXCHANGE are promoted by proteins belonging to the RecA/RAD51 family of RECOMBINASES. Human RAD51 is a relatively

NUCLEOLYTIC RESECTION
The trimming back of one strand of DNA.

SWI2/SNF2
SWI/SNF proteins are DNA-dependent ATPases that modulate protein–DNA interactions.

Box 1 | Alternative mechanisms for the repair of double-strand breaks



In mammalian cells, double-strand breaks that are caused by radiation or chemical damage can be repaired by two distinct pathways: non-homologous end joining (NHEJ) or homologous recombination (see figure). These pathways differ in their requirement for a homologous DNA template and the fidelity of the repair reaction. Homologous recombination uses an intact sister duplex as the template for repair, so the reaction occurs with high fidelity. By contrast, NHEJ occurs without a template, and involves the simple religation of the broken termini. Any sequences that are lost at the break site will be fixed as a mutation or deletion in the rejoined product. Although they are not mutually exclusive, the relative contribution of each pathway depends on the stage of the cell cycle, with NHEJ dominating in G1, and homologous recombination making a greater contribution in the S and G2 phases[105–107].

NHEJ requires Ku70/Ku80, the catalytic subunit of the DNA-dependent protein kinase (DNA-PK$_{cs}$)[108] and the XRCC4/DNA ligase IV heterodimer[109–111]. The role of DNA-PK$_{cs}$ in this reaction is unclear, especially as there is no identified DNA-PK$_{cs}$ homologue is yeast, although all of the other proteins are conserved. The Ku70/80 protein binds with avidity to DNA termini in a structure-specific manner[112], as contacts between the two subunits lead to the formation of a highly charged channel through which the DNA passes[113]. Ku can promote end alignment, probably by confining DNA movement to a single path. Recently, it was shown that human — but not yeast — Ku70/80 binds inositol hexakisphosphate (InsP$_6$), and that InsP$_6$ is required for NHEJ *in vitro*[114–116]. Binding of InsP$_6$ altered the proteolytic cleavage pattern of Ku70/80, which is indicative of a conformational change. Inositol phosphates also seem to regulate various processes including messenger RNA export[117,118] and ATP-dependent chromatin remodelling by SWI2/SNF2 factors[119,120].

small protein (~38 kDa). It is functional as a long helical polymer, made of hundreds of monomers, that wraps around the DNA to form a nucleoprotein filament[2,3] (FIG. 1). Its closely related homologues in *Escherichia coli* (RecA), archaea (RadA) and *Saccharomyces cerevisiae* (Rad51) also form nucleoprotein filaments; indeed, this unusual filament structure is more highly conserved than the protein sequences.

Although there is no detailed structure of the functional form of RAD51, a good view of this protein has been provided by three-dimensional reconstruction of images provided by electron microscopy[4]. A partial structure has also been gained by crystallographic analysis of a segment of RAD51 (REF. 5). The active form of the RAD51 filament is similar to that formed by RecA, which is a highly ordered right-handed helical structure (pitch 95Å), with the DNA lying along the longitudinal axis of the filament. In this complex, the DNA is extended with a rise per base that is equal to 5.1Å, and with 18 nucleotides of single-stranded (ss)DNA per helical repeat[4].

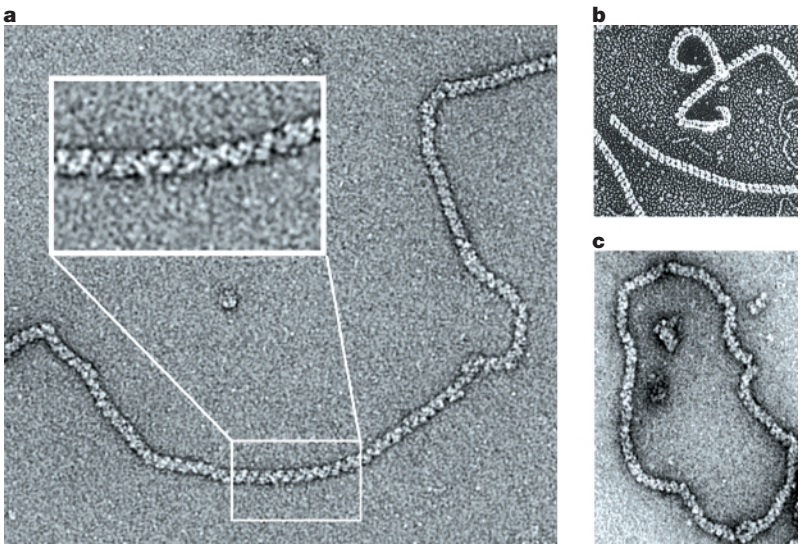


Figure 1 | **Electron-microscopic visualization of nucleoprotein filaments.** Nucleoprotein filaments are shown for **a** | human RAD51, **b** | *Escherichia coli* RecA and **c** | *Archeoglobus fulgidus* RadA protein. Images were provided by A. Stasiak (University of Lausanne, Switzerland).

To initiate the repair of a DSB in DNA, RAD51 needs to bind to the ssDNA that is produced by NUCLEOLYTIC RESECTION at the break site (FIG. 2). The protein shows a weak specificity for binding ssDNA compared with double-stranded (ds)DNA[6,7], but this specificity is enhanced by interactions with another key recombination protein, which is known as RAD52 (REFS 8–11). The importance of RAD52 was first shown in yeast, when it was found that *rad52* mutants show extreme radiation sensitivity and defects in recombination[12]. This phenotype is due to the fact that RAD52 has a dual role in recombination: first, it stimulates RAD51-mediated strand invasion through direct interaction with RAD51 (REFS 13,14) and the ssDNA-binding protein replication protein A (RPA); and second, it promotes single-strand annealing (SSA) reactions independently of RAD51 (REF. 15).

Invasion of a resected end of the DSB into duplex DNA takes place in the RAD51 filament and requires the binding, but not necessarily the hydrolysis, of a high-energy nucleotide cofactor such as ATP[16–20]. Again, studies with yeast proteins have been informative in showing us that the efficiency of this reaction, as measured by the stability of the resultant joint molecule, is stimulated by a SWI2/SNF2 protein that is known as RAD54 (REFS 21,22). But exactly how RAD54 stimulates joint-molecule formation is not clear, and, in fact, there are conflicting views on whether RAD54 plays an early (RAD51-assembly) or late (catalytic) role in the reaction[22–25]. The

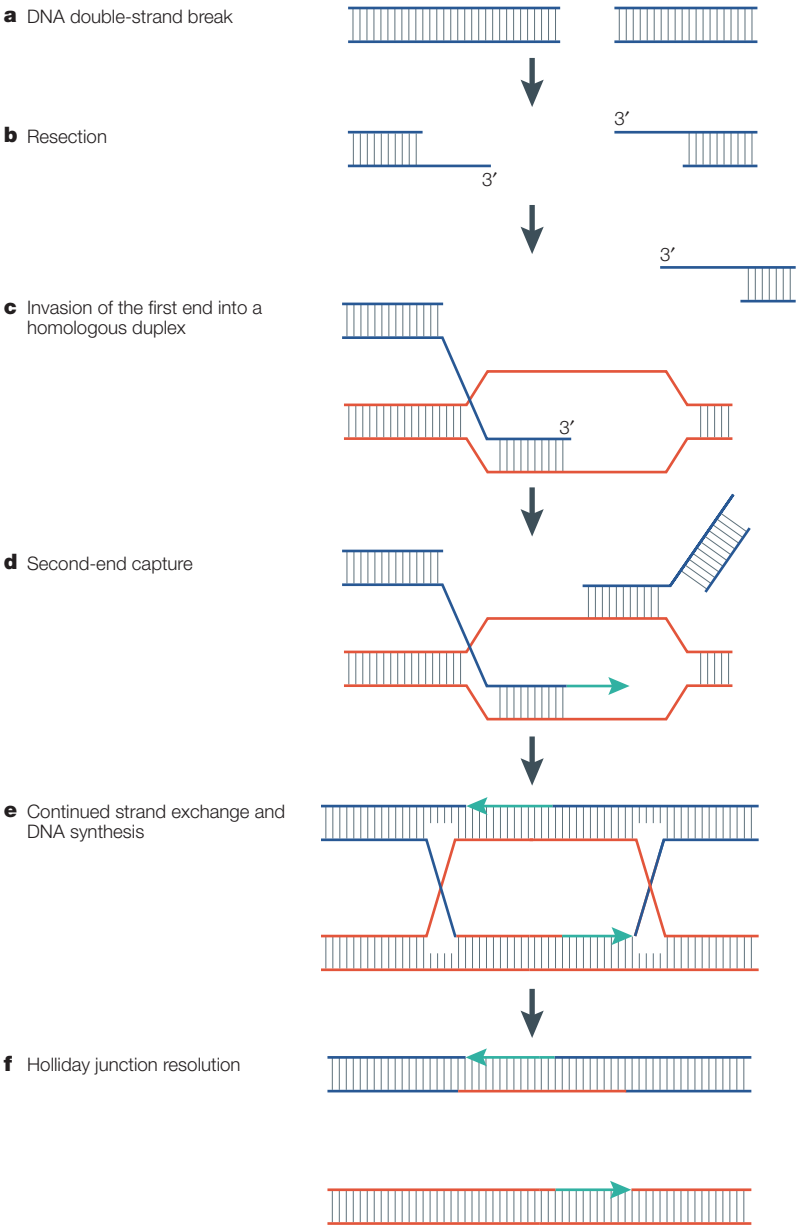


Figure 2 | **Scheme for the repair of a double-strand break by homologous recombination.** **a** | DNA at the break site is **b** | resected to expose single-stranded (ss)DNA. The resulting ssDNA becomes coated by the single-strand-binding protein, replication protein A (RPA), which in turn functions as a target for the binding of RAD52. As RAD51 interacts with RAD52, RAD51 can gain access to the ssDNA–RPA complex. **c** | After assembly of an active nucleoprotein filament on the resected ssDNA tail of the first end, the complex pairs with homologous double-stranded (ds)DNA and strand exchange takes place. **d** | In the next stage, RAD51 and/or RAD52 promote the capture of the second-end ssDNA tail. **d** and **e** | The two invading ends function as primers for DNA resynthesis. **f** | Finally, the DNA crossovers are resolved to allow the repaired duplexes to separate.

most simple explanation, which is based on observations showing that RAD54 induces the introduction of NEGATIVE SUPERCOILS into duplex DNA[26–28], is that RAD54-mediated topological changes to the recipient duplex favour invasion of the incoming single strand and thereby stabilize joint-molecule formation. RAD54 might also help to recycle RAD51 that is bound to duplex DNA[29].

NEGATIVE SUPERCOIL
The twisting or underwinding of DNA resulting in its compaction.

HOLLIDAY JUNCTION
A crossover structure that links recombining DNAs.

The ways in which the next stages of the DSB-repair process occur are less clear, as alternative theories abound[30]. The prototypic break-repair model, which was proposed in 1983 by Jack Szostak, Terry Orr-Weaver, Rodney Rothstein and Frank Stahl[31], favours invasion of the ssDNA tail of the second end, as shown in FIG. 2. This could occur by RAD51/RAD52-mediated strand invasion, as for the first end. Equally, or perhaps more likely, the second ssDNA tail could simply anneal with the displaced strand at the joint, in a reaction that is promoted by the ssDNA-annealing activity of RAD52, without the need for RAD51.

We now have a detailed structure of the ssDNA-annealing domain of human RAD52 protein, and the way in which RAD52 might function in this process is discussed later in this review. Alternatively, the first invading end could function as a primer for DNA replication, followed by strand displacement of the newly synthesized DNA, which would then be available for annealing with the complementary strand of the second-end tail. The latter mechanism is known as synthesis-dependent strand annealing (SDSA), and models such as these are rapidly gaining popularity (for further details, see REF. 30).

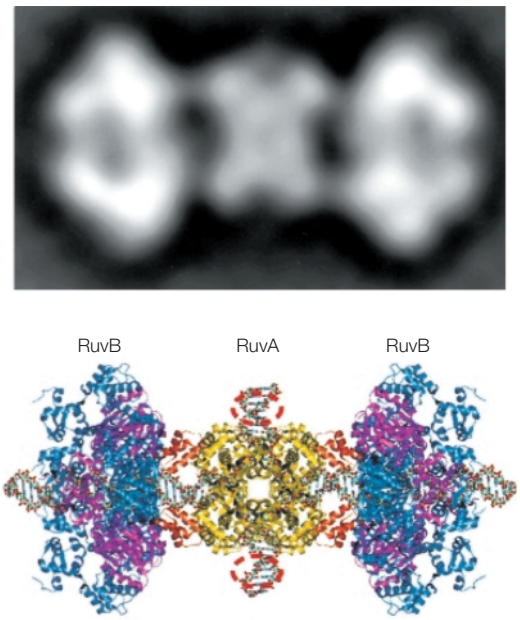
Most models for DSB repair, as indicated in the one that is shown in FIG. 2, involve the formation of a double HOLLIDAY JUNCTION, which needs to be resolved to allow the repaired chromosomes to separate. At present, we have little knowledge of how this process takes place in eukaryotic cells, and our understanding of the late stages of recombinational repair is restricted to the events that occur in bacterial cells.

In prokaryotes, the products of the *ruvA*, *ruvB* and *ruvC* genes act on Holliday junctions[32]. RuvA protein binds Holliday junctions with high affinity and targets RuvB to the site of the junction (BOX 2). Together, RuvAB promote branch migration, and make heteroduplex DNA more rapidly than RecA. The third component of the complex, RuvC, resolves junctions by endonucleolytic cleavage. The mechanism of cutting involves the introduction of symmetrically related nicks in strands of like polarity at the crossover, to produce nicked duplexes that are repaired by DNA LIGASE.

At present, the identification of proteins that carry out RuvABC functions in eukaryotes is something of a Holy Grail. In yeast, RuvC-like Holliday junction resolvases have been identified (*S. cerevisiae* Cce1 and *Schizosaccharomyces pombe* Ydc2), but both function in mitochondria[33–36]. The nuclear resolvase and branch-migration proteins, if there are any, are still to be found. In mammalian cells, branch migration and resolution activities have been observed in extensively fractionated cell-free extracts[37,38]. Biochemical studies show that these activities fit the RuvABC model well, but their identity remains elusive.

Recently, much excitement was generated when it was shown that complexes from yeast or human extracts that contain a protein known as Mus81 could resolve Holliday junctions[39,40]. However, although *mus81* mutants are sensitive to agents that block replication-fork progression, they do not show defects in DSB

Box 2 | **Actions of RuvABC on Holliday junctions**



The RuvABC complex promotes the processing of Holliday junctions in *Escherichia coli*[32]. RuvA and RuvB promote branch migration, whereas RuvC resolves (that is, cuts) the junction to form duplex products. RuvA is tetrameric and RuvB is a hexameric ring protein that translocates along DNA.

The first insight into the mechanism of branch migration was provided by the electron-microscopic visualization of the tripartite RuvA–RuvB–Holliday-junction complex[122]. A more recent high-resolution averaged image of a complex made with *Thermus thermophilus* RuvA and RuvB is shown here, together with a hypothetical structure that was derived from the structure of RuvA and a model of the RuvB hexamer[121,123–127]. RuvA binds specifically to the junction and facilitates the loading of RuvB hexamers onto two arms of the DNA. In a remarkably simple way, the rings lie oppositely orientated across the junction from each other, and function as twin DNA pumps that provide the motive force for branch migration. In the complex, the Holliday junction lies in a square-planar configuration, sandwiched between two tetramers of RuvA. Strand separation actually occurs on the surface of RuvA (rather than within the RuvB rings), as four 'acidic pins' that are located close to the centre of each tetramer function as guides for strands as they pass from one helical axis to another[128].

The resolution of Holliday junctions requires interactions between the RuvAB–Holliday-junction complex and RuvC. It is thought that RuvC might displace one tetramer of RuvA to gain access to one face of the junction[129,130]. The RuvABC–Holliday-junction assembly would then be able to promote branch migration and resolution, as the cleavage sequences (5′-$^{A}/_{T}TT^{G}/_{C}$-3′) preferred by RuvC will pass through the complex. Resolution occurs by a precise mechanism that involves the introduction of symmetrical nicks into two DNA strands of like polarity. The figure is modified with permission from REF. 121 **© Cell Press (2002).**

repair. Moreover, purified recombinant Mus81 complexes show considerably greater activities with various substrates that mimic flap structures and replication forks than they do with Holliday junctions[38,41,42]. Whether or not Mus81 functions as a true resolvase is, at present, a topic of some debate (BOX 3).

Molecular structure of RAD52

Human RAD52 (46 kDa, 418 amino acids) has been visualized by electron microscopy, and a low-resolution three-dimensional structure has been determined[43,44]. It shows seven subunits arranged in a ring with a large central channel. The yeast Rad52 protein also forms similar ring structures[45]. However, although Rad52 was cloned and sequenced almost 20 years ago, only now are we beginning to understand how it promotes SSA. Surprisingly, the breakthrough came from structural studies of the human homologue.

When ssDNA–RAD52 complexes were probed with HYDROXYL RADICALS, a unique repeating four-nucleotide hypersensitivity pattern was obtained[46]. This pattern was formed independently of the DNA sequence, and was phased from the terminus of the ssDNA. This led to the proposal that the ssDNA–RAD52 complex is precisely organized, with the DNA lying in a groove on the solvent-exposed surface of the protein ring. To determine if this was the case, attempts were made to crystallize RAD52, but initial efforts were unsuccessful. However, sequence comparisons, site-directed mutagenesis and biochemical studies indicated that the SSA activities of RAD52 resided in the highly conserved amino-terminal region[15,47,48] and, fortunately, this region of the protein formed crystals, the structure of which could be solved.

When $RAD52_{1–212}$ and $RAD52_{1–209}$ were crystallized in independent studies, near-identical structures were obtained[49,50]. But surprisingly, the proteins crystallized as undecameric (11-subunit) rather than heptameric (7-subunit) rings (BOX 4). The monomer subunit has two domains, as shown in FIG. 3a. Residues 24–177 (the first 23 amino acids in each structure were disordered) make up domain I, which represents the core of the monomer, whereas the smaller carboxy-terminal domain II (residues 178–209) consists of a flexible extended linker that ends in an α-helix. In the undecameric ring, the smaller domain swaps across the domain boundary to interact with the adjacent subunit (FIG. 3b). Such a mechanism of 'helix swapping' is a common feature that is known to stabilize subunit interactions in other ring-shaped proteins (for example, DNA helicases)[51]. The 11-fold symmetric closed ring has a large channel running through the centre, and the overall dimensions of the ring (120Å diameter, 65Å high) are, rather surprisingly, similar to those reported for the full-length 7-subunit protein.

So how might this protein, which resembles the cap of a mushroom, promote SSA? As yet, a crystal of RAD52 with DNA has not been obtained, so the precise

DNA LIGASE
An enzyme that seals nicks in duplex DNA.

HYDROXYL RADICAL
A small chemical probe that attacks deoxyribose residues in the DNA backbone.

Box 3 | **The story of Mus81**

Mus81 was first identified in yeast two-hybrid assays by its interaction with RAD54 (REF. 131) and with the checkpoint kinase Cds1 (REF. 132). *mus81* mutants are sensitive to the DNA-damaging agents methyl methane sulphonate and ultraviolet radiation, both of which block DNA replication. They are also sensitive to hydroxyurea, which depletes dNTP pools and stalls progression of the replication fork. By contrast, *mus81* mutants are resistant to DNA damage that is caused by ionizing radiation. The *mus81* mutation confers synthetic lethality to cells carrying mutations in *sgs1* (*Saccharomyces cerevisiae*)[133] or its homologue *rqh1* (*Schizosaccharomyces pombe*)[42,132], which encode proteins that are involved in the processing of stalled replication forks. These observations pointed to a role for Mus81 in DNA replication.

Great interest therefore met a report from Paul Russell's laboratory[39] showing that *S. pombe mus81* mutants were defective in a late stage of meiotic recombination, and that this defect could be partially overcome by expression of a bacterial Holliday junction resolvase[39]. Moreover, complexes containing Mus81 were shown to resolve Holliday junctions and fork structures *in vitro*[39]. Although the mechanism of cleavage was rather different from that seen with other resolvases, such as RuvC[134], it was clear that this was an important observation because the human protein carried out very similar reactions[40]. Is Mus81 the elusive Holliday junction resolvase?

Detailed biochemical studies with purified recombinant Mus81 and its partner protein (Mms4 in *S. cerevisiae* or Eme1 in *S. pombe*) showed that the endonucleases actually show a much greater specific activity with 3′-flap or replication-fork structures than with Holliday junctions[41,42]. Similar results were obtained with purified fractions containing Mus81 from human cells[38]. This preference for flaps and forks was perhaps not too surprising, because Mus81 shares sequence homology with the Rad1/XPF subunits of structure-specific endonucleases that function in nucleotide excision repair[131,132]. The pendulum swung further when it was shown that *S. cerevisiae mus81* mutants, in contrast to those from *S. pombe*, did not have such a profound meiotic-recombination deficiency[135].

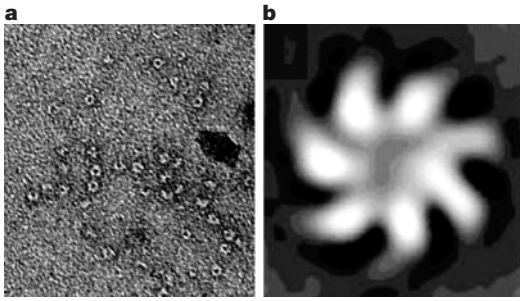
Do these results tell us that the flap/fork endonuclease activity of Mus81/Eme1 is necessary only for the meiotic-recombination pathways that occur in *S. pombe*? Or do they tell us that there is a second (redundant) activity in *S. cerevisiae* that can take over in the absence of Mus81? There are many differences in the mechanism of meiotic recombination between *S. cerevisiae* and *S. pombe*, so differences in mechanism or redundancy are both viable options. But is Mus81 a true Holliday junction resolvase? Well, it might be, but if it is then it resolves junctions in a way that is different from the prototypic resolvase RuvC. Until we know more, the jury is still out.

location of the DNA is unknown. However, the surface electrostatic potential map shows that there is a deep groove running around the outside of the ring. The residues at the base of the cleft are positively charged, whereas those lining the groove are hydrophobic. This groove — the residues of which are highly conserved in RAD52 homologues from other species — would therefore be ideally suited to bind the phosphodiester backbone of ssDNA, with the bases pointing upwards and away from the surface of the protein (FIG. 3c). Because the groove is too narrow to accommodate duplex DNA, annealing by base-pairing with a complementary single strand would be expected to release the bound single strand as a newly formed double helix. Consistent with such a mechanism of SSA, the unique hydroxyl-radical sensitivity of bound ssDNA is lost as annealing takes place[46].

The DNA-damage response

Bacterial cells readily adapt for DNA repair and survival by initiating an 'SOS response', which involves the transcriptional de-repression of more than 20 genes. Many of these genes encode proteins such as RecA, RuvA and RuvB, which play central roles in the repair of DNA damage by homologous recombination. In particular, the induction of RecA is pronounced, with the levels of protein being increased approximately 15-fold within 30 minutes of the introduction of DNA damage. In

Box 4 | **How many subunits are in a RAD52 ring?**



Although it is clear that RAD52 self-associates to form ring structures (see figure, part **a**), there are reports of rings differing both in size[43,136] and the number of subunits[44,136]. Gel-filtration studies and scanning transmission electron microscopy (STEM) indicate that wild-type human RAD52 has a particle size ranging from 175 to 625 kDa. This corresponds to 4–13 subunits. The average molecular mass (330 kDa) indicates that there are seven subunits per ring[44,49], and it is this heptameric form (see figure, part **b**) that shows clear rotational symmetry[44]. Other forms of RAD52, such as $RAD52_{1–192}$ (REFS 136,137) and $RAD52_{1–212}$ (REF. 49) form rings with an average of 10 (decameric) and 11 (undecameric) subunits, respectively. $RAD52_{1–212}$ and $RAD52_{1–209}$ both crystallized in the undecameric ring form[49,50]. Part **a** is reproduced with permission from REF 43 © Elsevier Science (1998) and part **b** is reproduced with permission from REF 44 © Cell Press (2000).

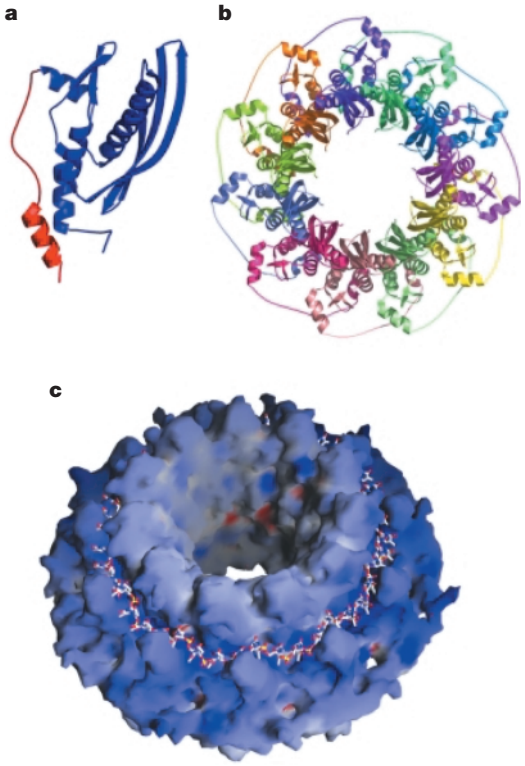


Figure 3 | **Structure of the 11-subunit RAD52$_{1–209}$ ring.** **a** | The RAD52 protein monomer, with domains I (residues 24–177) and II (residues 178–209) indicated in blue and red, respectively. **b** | The undecamer viewed along the axis of symmetry. Each subunit is shown in a different colour. **c** | The molecular surface view of the RAD52$_{1–209}$ ring with single-stranded DNA modelled into the deep groove running across the protein surface (shown in red and grey). Modified with permission from REF. 50 © The National Academy of Sciences (2002).

contrast, eukaryotic cells do not elicit such a repair response, and at most there is only a twofold transcriptional upregulation of proteins such as RAD51. Instead, in response to DNA damage, recombination proteins that are normally found diffused throughout the nucleus are rapidly relocalized and concentrated into subnuclear complexes that are detected microscopically as foci (FIG. 4a). So, the overall effect is the same, in that the local protein concentration of key repair enzymes is increased as the cell prepares for repair.

Nuclear foci that contain RAD51 form in response to various DNA-damaging treatments[52,53]. Other proteins that colocalize with RAD51 include the recombination-repair proteins RAD52 (REFS 54–56) and RAD54 (REFS 27,56), the ssDNA-binding protein RPA[57], and the tumour suppressors BRCA1 (REF. 58) and BRCA2 (REF. 59). The kinetics of localization differ from one protein to another, which indicates that there is a sequential assembly of one protein dependent on another. Consistent with this interpretation, cell lines that are defective in BRCA2 fail to accumulate RAD51 foci after DNA-damaging treatments[60,61]. The assembly of a RAD51 focus and the capacity of the cell to repair is therefore compromised.

RAD51 foci are also found in undamaged S-phase cells, in which they are thought to identify sites at which stalled or broken replication forks undergo repair[57,62]. The S-phase and damage-induced foci seem to be distinct from each other, because BRCA2 is not required for the formation of RAD51 foci in unirradiated S-phase cells[63].

What are the functions of RAD51 foci? The way that nuclear foci accumulate in response to DNA damage, and the demonstration that they contain ssDNA[57], makes it tempting to speculate that these foci represent sites where DNA repair is taking place. It is, however, difficult to relate the number of foci to the number of damage-induced breaks[55], so it is unlikely that a focus represents a single repair event. It is more likely that nuclear foci represent assemblies of proteins that can elicit repair as required, but the way in which they form and gather their target DNA molecules remains unknown.

The dynamic nature of recombination proteins in radiation-induced foci has been studied using the technique of laser photobleaching (fluorescence recovery after photobleaching; FRAP). Proteins tagged with green fluorescent protein (GFP) and contained within a single focus were photobleached, and the kinetics of fluorescence recovery were followed[56]. It was found that GFP–RAD52 and GFP–RAD54 returned to a bleached focus and restored the fluorescence signal quite quickly (FIG. 4b). These results showed that the bleached proteins in the focus were replaced or supplemented with unbleached molecules from the nucleus. However, the rate of recovery was quite different, with half the original fluorescence returning within 0.5 s for RAD54 and 26 s for RAD52. In contrast to RAD52 and RAD54, GFP–RAD51 fluorescence failed to recover over time, implying that RAD51 is relatively immobile in the focus, in which it is present as a nucleoprotein filament.

Involvement of BRCA1 and BRCA2

The tumour suppressors BRCA1 and BRCA2 colocalize with RAD51 in damage-induced nuclear foci[53,58,59]. In ground-breaking studies, BRCA1 was found to interact with RAD51 in pull-down assays that were carried out using cell-free extracts[58]. However, it subsequently became clear that it is BRCA2 that interacts with RAD51 (REFS 64,65) and, because BRCA1 and BRCA2 interact, it is now thought that the observed interactions between BRCA1 and RAD51 might have been mediated through BRCA2.

Both BRCA1 and BRCA2 are required for normal levels of homologous recombination and DSB repair[66–68]. Cell lines that are defective in BRCA1 or BRCA2 show gross chromosomal rearrangements, and there are indications of chromosome breakage[69,70]. Similarly, cells that are derived from tumours taken from people who are predisposed to cancer through *BRCA1* and *BRCA2* germline mutations show evidence of genome instability.

People who carry mutations in *BRCA1* or *BRCA2* are predisposed to breast and/or ovarian cancers[71,72].

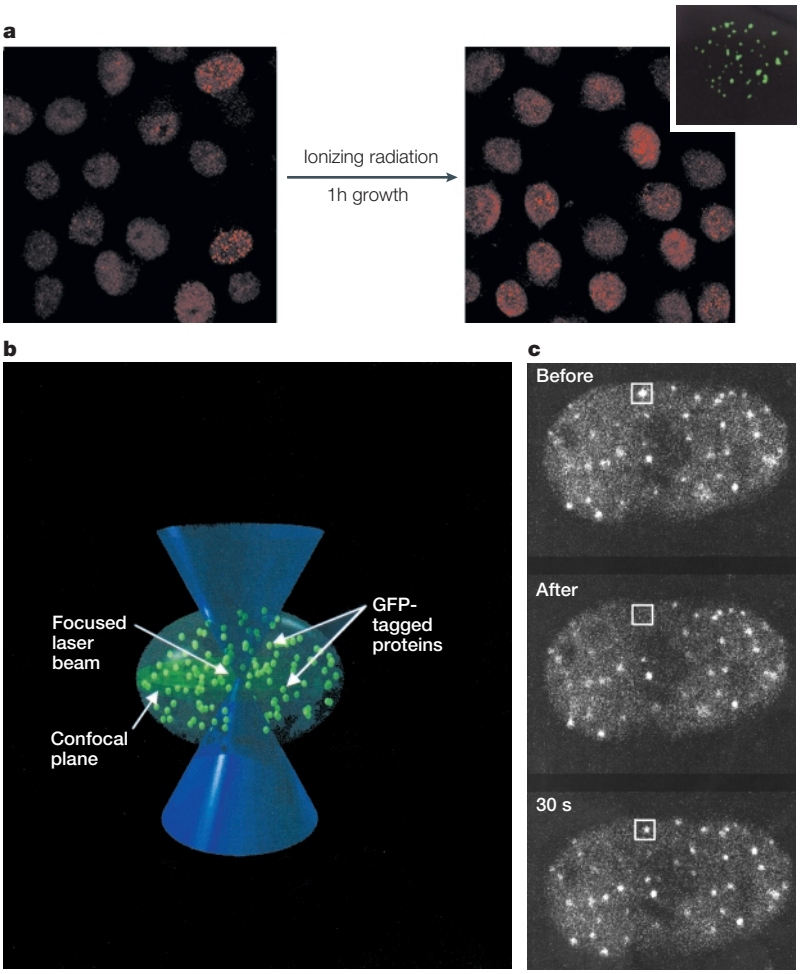


Figure 4 | **Localization of RAD51 and RAD52 to nuclear foci. a** | Human RAD51 protein as visualized by immunofluorescence in unirradiated (left) and irradiated (right) HeLa cells. The inset (top right) shows RAD51 foci in a single irradiated nucleus. Images provided by M. Tarsounas (Cancer Research UK). **b** | The dynamic nature of nuclear foci has been analysed by fluorescence recovery after photobleaching (FRAP). **c** | A single RAD52 repair focus, visualized by immunofluorescence, can be photobleached by laser treatment. Images taken before and after laser treatment show the dynamic nature by which the RAD52 signal is quickly restored by protein from elsewhere in the nucleus (30 s). Part **b** is modified with permission from REF. 138 © The American Association for the Advancement of Science (1999). Part **c** is modified with permission from REF. 56 © The European Molecular Biology Organization (2002).

Approximately 20% of breast cancers are familial, and of these about one-third have been linked to mutations in *BRCA1* or *BRCA2*. In the United Kingdom alone, there are approximately 80,000 predisposed people, of whom more than half will develop cancer. One defective copy of *BRCA1* or *BRCA2* in the genome is enough to confer cancer predisposition, and the loss of the second allele is often observed in tumour cells that are isolated from predisposed people. Clearly both *BRCA1* and *BRCA2* are required for maintaining genome integrity, and could therefore be defined as tumour suppressors. The interaction of BRCA2 with RAD51, together with the recombination/repair-defective phenotype of *BRCA1*- or *BRCA2*-defective cell lines, indicates that the main defect leading to genome instability in these cells lies in RAD51-mediated DNA-repair systems.

But *BRCA1* or *BRCA2* mutations result in a remarkably pleiotropic phenotype, which indicates that the two proteins are involved in, or affect, many basic cellular processes in addition to DNA repair. These processes include cell-cycle control, chromatin remodelling and transcription[73]. Both proteins are highly interactive and are thought to be components of large nuclear complexes[74–80]. A few of the more important protein–protein interactions that have been found are illustrated in FIG. 5.

Both BRCA1 and BRCA2 are very large proteins (208 kDa and 384 kDa, respectively). BRCA1 has a RING-FINGER DOMAIN at its amino terminus and, in association with BARD1 (BRCA1-associated RING domain protein 1)[81], this region is important for the E3 UBIQUITIN LIGASE activity of BRCA1 (REFS 82–84). In response to ionizing radiation, BRCA1 is required for the monoubiquitylation of the FANCD2 protein. Defects in *FANCD2* result in an autosomal-recessive disorder that is known as Fanconi anaemia (FA), which is characterized by cancer predisposition, congenital abnormalities and bone-marrow failure[85]. Cell lines that are derived from individuals with FA are uniquely hypersensitive to crosslinking agents such as mitomycin C or *cis*-platinum, which indicates that they have a DNA-repair defect[86,87]. The modification of FANCD2, involving BRCA1, is necessary for the targeting of FANCD2 to RAD51 foci[88]. However, it has yet to be shown that the E3 ubiquitin ligase activity of BRCA1 is directly responsible for ubiquitylation of FANCD2.

Another important region in BRCA1 — the BRCA1 carboxy-terminal (BRCT) domain[89,90] — is thought to mediate interactions with other proteins such as BACH1, which is a putative DNA helicase[91]. Interestingly, it was shown that cellular overexpression of a dominant-negative form of BACH1 resulted in sensitivity to ionizing radiation, indicating that wild-type BACH1 is also required for DSB repair. BRCA1 also interacts with the MRE11/RAD50/NBS1 (M/R/N) complex[74,77], which functions at an early stage in recombinational repair as the DSB is resected.

The interactions of BRCA2 with RAD51

BRCA2 contains a series of eight degenerate motifs[92], six of which have been shown to bind the RAD51 recombinase[65,93]. These motifs, which are known as the BRC repeats, are approximately 30 amino acids long, and are interspersed along 1,200 amino acids of the central region of the protein that is encoded by exon 11. In addition, there is an unrelated RAD51-binding site at the carboxyl terminus of BRCA2. The BRC repeats are well conserved in sequence and spacing from mammals to birds[94], and, remarkably, a BRC motif has also been found in the *Ustilago maydis* (smut fungus) homologue of BRCA2, which is known as Brh2 (REF. 95). Overexpression of a single BRC repeat in rodent cells is enough to cause decreased homologous recombination, radiation hypersensitivity and a loss of G2/M checkpoint control, presumably because it functions as a dominant negative by sequestering RAD51 (REFS 20,96).

RING-FINGER DOMAIN
A loop structure that is found in more than 200 proteins.

E3 UBIQUITIN LIGASE
Protein ubiquitylation is important for protein activation or degradation. The E3 ligase attaches ubiquitin to the protein that has been targeted for degradation.

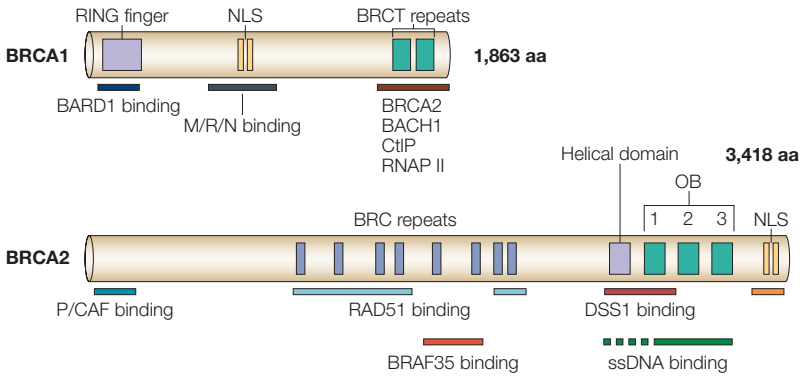


Figure 5 | **Functional domains of BRCA1 and BRCA2.** Both proteins are large polypeptides (1,863 and 3,418 amino acids, respectively) that interact with each other and with several other proteins. For example, BRCA1 interacts with: the BARD1 (BRCA1-associated RING finger domain) protein, which is required for ubiquitin-ligase activity; the MRE11/RAD50/NBS1 (M/R/N) recombination/repair complex; a putative DNA helicase that is known as BACH1; the CtBp-interacting protein CtIP; and RNA polymerase II (RNAP II). The sites of the RING finger domain, the nuclear-localization signals (NLS) and the BRCA1 carboxy-terminal (BRCT) domains are indicated. Similarly, BRCA2 interacts with the histone acetylase P/CAF, the 35-kDa BRCA2-associated factor BRAF35, and DSS1 (a protein that is deleted in split-hand/split-foot syndrome). The sites of the eight BRC repeats (six of which interact with RAD51), the oligonucleotide-binding (OB) domains that interact with single-stranded DNA and the carboxy-terminal NLS are indicated.

To investigate the effects that are imposed by BRC interaction on RAD51 activity, the DNA-binding properties of RAD51 were analysed in the presence of synthetic peptides corresponding to the BRC repeats[97]. The results showed that interactions between RAD51 and BRC3 or BRC4 blocked RAD51 from forming nucleoprotein filaments on DNA. The protein was therefore inactivated. Using a BRC4 peptide, it was shown that inactivation of RAD51 was the consequence of direct interactions between a monomer of RAD51 and a single BRC4 peptide, such that a stable RAD51–BRC4 heterodimer was formed.

Thanks to a recent landmark paper from Pellegrini *et al.*[5] describing the crystallographic structure of a RAD51–BRC4 complex, we might now know how these events occur (FIG. 6). To overcome the tendency of RAD51 to self-aggregate, a RAD51–BRC4 fusion protein was constructed. Although the RAD51 that was used in these experiments was not the full-sized protein (amino acids 1–96 were deleted), it was reassuring to see that the structure of the ATPase domain was topologically identical to that of RecA. The authors found that the BRC4 region remained in continuous contact with RAD51 over a stretch of 28 amino acids. This interaction impaired the ability of one RAD51 monomer to interact with another, so filament formation was blocked. The BRC4 polypeptide seemed to mimic the structure of the interaction domain of the adjacent RAD51 monomer. This concept, which is based on studies with short isolated BRC repeats, is likely to be overly simplistic. However, it is reasonable to think that these peptide studies give us some insight into the interactions that occur with full-size BRCA2, and that a new negative control mechanism of BRCA2 over RAD51 has been uncovered.

The role of BRCA2 in recombinational repair, however, cannot simply be one of negative control. It is known that BRCA2 is required for the accumulation of RAD51 at damage-induced foci[60,63], so it is plausible that BRCA2 provides a scaffold that keeps RAD51 inactive until the moment when DNA damage occurs. At that time, BRCA2 and RAD51 relocalize to repair foci, in which the RAD51 needs to be released for the formation of nucleoprotein filaments. BRCA1 (REFS 53,98) and RAD51 (REFS 99–101) are phosphorylated in response to ionizing radiation, so it is possible that modification events are important in the release of RAD51 from BRCA2. The possibility that BRCA2 itself might be modified in response to DNA damage has yet to be determined.

As the BRCA field is progressing rapidly, it comes as no surprise that the structure of a second region of BRCA2 was also described recently. Yang *et al.*[102] presented the crystal structure of an 800-amino-acid carboxy-terminal domain of mouse BRCA2 bound to a 70-amino-acid protein that is known as DSS1 (a protein of unknown function, deletion of which results in split-hand/split-foot syndrome[103]). The structure showed that BRCA2 contains ssDNA-binding motifs, and a structure of a BRCA2–DSS1–oligo(dT)$_9$ complex was obtained. The carboxy-terminal domain of BRCA2 was also found to stimulate the homologous pairing and strand-exchange activities of RAD51 *in vitro*[102].

In addition to its interaction with the BRC repeats of BRCA2, RAD51 can also interact with the carboxy-terminal region of BRCA2, which raises the possibility that the BRC repeats and the carboxy-terminal RAD51 interaction domain have opposite functions. One could imagine a situation in which the BRC repeats provide negative control over RAD51, whereas the domain that

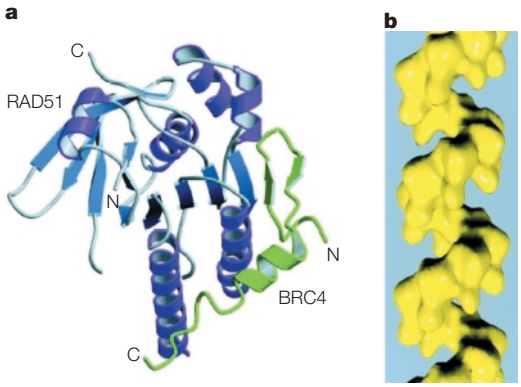


Figure 6 | **The molecular basis by which BRCA2 controls RAD51.** The RAD51 protein interacts with BRCA2 through a series of BRC repeats that are distributed throughout the region that is encoded by exon 11. **a** | Recent crystallographic studies have determined the structure of a complex that is formed between RAD51 and a peptide corresponding to BRC repeat 4 (BRC4). Importantly, the BRC4 peptide seems to mimic a region of RAD51 that is involved in the monomer–monomer interactions that are necessary for the formation of a RAD51 nucleoprotein filament. Modified with permission from REF. 5 *Nature* © Macmillan Magazines Ltd (2002). **b** | Three-dimensional reconstruction of a RAD51 filament. Modified with permission from REF. 4 © The National Academy of Sciences (2001).

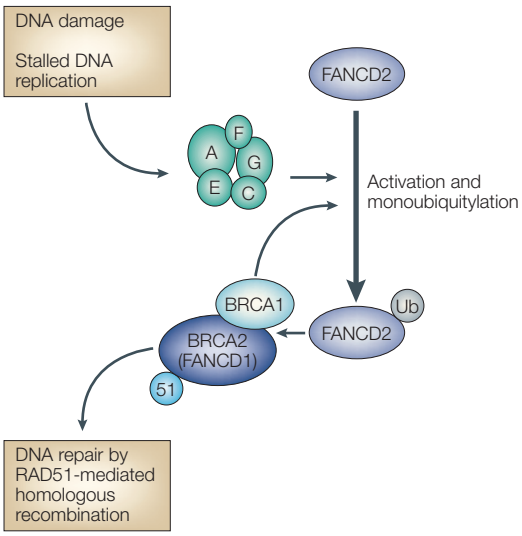


Figure 7 | **Interactions between the FA and BRCA proteins.** The Fanconi anaemia (FA) proteins FANCA, FANCC, FANCE, FANCF and FANCG form a nuclear complex. In response to DNA damage, and during DNA replication, the complex can be activated, which results in the monoubiquitylation (Ub) of FANCD2. BRCA1 is required for ubiquitylation of FANCD2. The activated FANCD2 protein is then seen to colocalize with BRCA1 in nuclear foci, in which it might interact with other repair proteins. BRCA1 is known to interact with BRCA2 (which is also known as FANCD1), which, in turn, interacts with the RAD51 recombinase. RAD51 protein has a direct role in DNA repair, so completing the cycle. FANCB, which might also be related to BRCA2 is not shown. Modified with permission from REF. 139 © Elsevier Science (2003).

is located towards the carboxyl terminus might facilitate RAD51 loading and activation.

A significant discovery was made recently when it was shown that cell lines derived from people with FA complementation group D1 (FANCD1) carry biallelic mutations in *BRCA2*. Most importantly, the *FANCD1*-defective cell lines were complemented by *BRCA2*, indicating that *FANCD1* and *BRCA2* are one and the same[104]. However, this remarkable connection between FA and *BRCA2* was not entirely unexpected because of the way that BRCA1 is required for the monoubiquitylation of FANCD2 (REF. 88; FIG. 7). Moreover, mutations in *BRCA2* have also been found in *FANCB*-defective cell lines[104]. Unfortunately, we know much less about the remaining five FA genes (*FANCA*, *FANCC*, *FANCE*, *FANCF* and *FANCG*), other than that they encode proteins that interact to form a complex that is required for the repair of interstrand crosslinks in DNA and for genome stability[85].

Conclusions and perspectives

Remarkable progress has been made in the past two or three years. Our understanding of how proteins act on DNA to drive the recombination reactions that are critical for cell growth and survival is progressing rapidly. This is due, in part, to the convergence of genetic, biochemical, structural and cell-biological approaches. But we still have some way to go to find out how recombination proteins are controlled and activated. What are the signals for protein relocalization after DNA damage, and how are proteins directed into repair foci? What really happens inside a RAD51 focus? Clearly there are complex protein–protein and protein–DNA interactions that are necessary for repair, but should we consider nuclear foci as 'repairosomes', or simply as sites in which individual proteins accumulate and function sequentially? We do not yet know the answers.

What are the precise roles of BRCA1, BRCA2 and interacting proteins such as BACH1? Is BRCA2 simply a scaffold to which proteins like RAD51 bind, or does its ability to bind DNA indicate that it is an essential mediator that delivers these proteins to repair sites? Does the interaction of BRCA1 with the MRE11/RAD50/NBS1 complex, which is one of the earliest proteins found at DSBs, lead to the rapid targeting of the BRCA2/RAD51 complex to the DSB? And, if so, how are these proteins released once they have been targeted?

The recombination/repair field is a vibrant one, and we are entering an era in which an understanding of the mechanisms of recombinational repair and genomic stability, and its impact on cancer predisposition, is well within our grasp. For any young scientists looking for an arena to make new discoveries in basic research that will have a profound impact on human disease, this area of research should be high on their list.

1. Cox, M. M. Recombinational DNA repair of damaged replication forks in *Escherichia coli*: questions. *Annu. Rev. Genet.* **35**, 53–82 (2001).
2. Ogawa, T., Yu, X., Shinohara, A. & Egelman, E. H. Similarity of the yeast Rad51 filament to the bacterial RecA filament. *Science* **259**, 1896–1899 (1993).
The similarity between *E. coli* RecA and yeast Rad51 was seen by electron microscopy and indicated that the nucleoprotein filament structure that is important for recombination in bacteria has been conserved throughout evolution.
3. Benson, F. E., Stasiak, A. & West, S. C. Purification and characterisation of the human RAD51 protein, an analogue of *E. coli* RecA. *EMBO J.* **13**, 5764–5771 (1994).
4. Yu, X., Jacobs, S. A., West, S. C., Ogawa, T. & Egelman, E. H. Domain structure and dynamics in the helical filaments formed by RecA and Rad51 on DNA. *Proc. Natl Acad. Sci. USA* **98**, 8419–8424 (2001).
5. Pellegrini, L. *et al.* Insights into DNA recombination from the structure of a RAD51–BRCA2 complex. *Nature* **420**, 287–293 (2002).
Describes the crystal structure of a RAD51–BRC fusion protein, and illustrates how the interaction with a BRC repeat might 'cap' RAD51 in the monomeric form and inhibit nucleoprotein-filament formation.
6. Mazin, A. V., Zaitseva, E., Sung, P. & Kowalczykowski, S. C. Tailed duplex DNA is the preferred substrate for Rad51 protein-mediated homologous pairing. *EMBO J.* **19**, 1148–1156 (2000).
7. McIlwraith, M. J. *et al.* Reconstitution of the strand invasion step of double-strand break repair using human RAD51, RAD52 and RPA proteins. *J. Mol. Biol.* **304**, 151–164 (2000).
8. Benson, F. E., Baumann, P. & West, S. C. Synergistic actions of RAD51 and RAD52 in genetic recombination and DNA repair. *Nature* **391**, 401–404 (1998).
9. Sung, P. Function of yeast Rad52 protein as a mediator between replication protein-A and the Rad51 recombinase. *J. Biol. Chem.* **272**, 28194–28197 (1997).
10. New, J. H., Sugiyama, T., Zaitseva, E. & Kowalczykowski, S. C. Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein-A. *Nature* **391**, 407–410 (1998).
11. Shinohara, A. & Ogawa, T. Stimulation by Rad52 of yeast Rad51-mediated recombination. *Nature* **391**, 404–407 (1998).
12. Game, J. C. DNA double-strand breaks and the *RAD50–RAD57* genes in *Saccharomyces*. *Semin. Cancer Biol.* **4**, 73–83 (1993).
13. Milne, G. T. & Weaver, D. T. Dominant-negative alleles of *RAD52* reveal a DNA repair recombination complex including Rad51 and Rad52. *Genes Dev.* **7**, 1755–1765 (1993).
14. Shen, Z. Y., Cloud, K. G., Chen, D. J. & Park, M. S. Specific interactions between the human RAD51 and RAD52 proteins. *J. Biol. Chem.* **271**, 148–152 (1996).
15. Mortensen, U. H., Bendixen, C., Sunjevaric, I. & Rothstein, R. DNA strand annealing is promoted by the yeast Rad52 protein. *Proc. Natl Acad. Sci. USA* **93**, 10729–10734 (1996).
The first evidence that Rad52 protein promotes single-strand-annealing reactions *in vitro*.

16. Sung, P. Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast Rad51 protein. *Science* **265**, 1241–1243 (1994).
Shows that Rad51 promotes strand-exchange reactions that are similar to those catalysed by *E. coli* RecA.
17. Baumann, P., Benson, F. E. & West, S. C. Human RAD51 protein promotes ATP-dependent homologous pairing and strand transfer reactions *in vitro*. *Cell* **87**, 757–766 (1996).
18. Sung, P. & Stratton, S. A. Yeast Rad51 recombinase mediates polar DNA strand exchange in the absence of ATP hydrolysis. *J. Biol. Chem.* **271**, 27983–27986 (1996).
19. Morrison, C. *et al.* The essential functions of human RAD51 are independent of ATP hydrolysis. *Mol. Cell. Biol.* **19**, 6891–6897 (1999).
20. Stark, J. M. *et al.* ATP hydrolysis by mammalian RAD51 has a key role during homology-directed DNA repair. *J. Biol. Chem.* **277**, 20185–20194 (2002).
21. Petukhova, G., Stratton, S. & Sung, P. Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. *Nature* **393**, 91–94 (1998).
22. Van Komen, S., Petukhova, G., Sigurdsson, S., Stratton, S. & Sung, P. Superhelicity-driven homologous DNA pairing by yeast recombination factors Rad51 and Rad54. *Mol. Cell* **6**, 563–572 (2000).
23. Mazin, A. V., Bornarth, C. J., Solinger, J. A., Heyer, W.-D. & Kowalczykowski, S. C. Rad54 protein is targeted to pairing loci by the Rad51 nucleoprotein filament. *Mol. Cell* **6**, 583–592 (2000).
24. Solinger, J. A., Lutz, G., Sugiyama, T., Kowalczykowski, S. C. & Heyer, W. D. Rad54 protein stimulates heteroduplex DNA formation in the synaptic phase of DNA strand exchange via specific interactions with the presynaptic Rad51 nucleoprotein filament. *J. Mol. Biol.* **307**, 1207–1221 (2001).
25. Van Komen, S., Petukhova, G., Sigurdsson, S. & Sung, P. Functional cross-talk among Rad51, Rad54, and replication protein A in heteroduplex DNA joint formation. *J. Biol. Chem.* **277**, 43578–43587 (2002).
26. Petukhova, G., Van Komen, S., Vergano, S., Klein, H. & Sung, P. Yeast Rad54 promotes Rad51-dependent homologous DNA pairing via ATP hydrolysis-driven change in DNA double helix conformation. *J. Biol. Chem.* **274**, 29453–29462 (1999).
27. Tan, T. L. R. *et al.* Mouse RAD54 affects DNA conformation and DNA-damage-induced RAD51 foci formation. *Curr. Biol.* **9**, 325–328 (1999).
28. Sigurdsson, S., Van Komen, S., Petukhovan, G. & Sung, P. Homologous DNA pairing by human recombination factors RAD51 and RAD54. *J. Biol. Chem.* **277**, 42790–42794 (2002).
29. Solinger, J. A., Kiianitsa, K. & Heyer, W. D. Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments. *Mol. Cell* **10**, 1175–1188 (2002).
30. Paques, F. & Haber, J. E. Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **63**, 349–404 (1999).
A comprehensive review of recombination pathways and mechanisms in yeast.
31. Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J. & Stahl, F. W. The double-strand break repair model for recombination. *Cell* **33**, 25–35 (1983).
Describes the prototypic model for repair that is initiated by a double-strand break.
32. West, S. C. Processing of recombination intermediates by the RuvABC proteins. *Annu. Rev. Genet.* **31**, 213–244 (1997).
33. Kleff, S., Kemper, B. & Sternglanz, R. Identification and characterization of yeast mutants and the gene for a cruciform cutting endonuclease. *EMBO J.* **11**, 699–704 (1992).
34. White, M. F. & Lilley, D. M. J. The structure-selectivity and sequence-preference of the junction-resolving enzyme Cce1 of *Saccharomyces cerevisiae*. *J. Mol. Biol.* **257**, 330–341 (1996).
35. Whitby, M. C. & Dixon, J. A new Holliday junction resolving enzyme from *Schizosaccharomyces pombe* that is homologous to Cce1 from *Saccharomyces cerevisiae*. *J. Mol. Biol.* **272**, 509–522 (1997).
36. Oram, M., Keeley, A. & Tsaneva, I. Holliday junction resolvase in *Schizosaccharomyces pombe* has identical endonuclease activity to the Cce1 homolog Ydc2. *Nucleic Acids Res.* **26**, 594–601 (1998).
37. Constantinou, A., Davies, A. A. & West, S. C. Branch migration and Holliday junction resolution catalyzed by activities from mammalian cells. *Cell* **104**, 259–268 (2001).
Shows that mammalian cells have activities that promote coupled branch migration and Holliday junction resolution in reactions that are similar to those catalysed by *E. coli* RuvABC.
38. Constantinou, A., Chen, X.-B., McGowan, C. H. & West, S. C. Holliday junction resolution in human cells: two junction endonucleases with distinct substrate specificities. *EMBO J.* **21**, 5577–5585 (2002).
39. Boddy, M. N. *et al.* Mus81–Eme1 are essential components of a Holliday junction resolvase. *Cell* **107**, 537–548 (2001).
40. Chen, X. B. *et al.* Human MUS81-associated endonuclease cleaves Holliday junctions *in vitro*. *Mol. Cell* **8**, 1117–1127 (2001).
41. Kaliraman, V., Mullen, J. R., Fricke, W. M., Bastin-Shanower, S. A. & Brill, S. J. Functional overlap between Sgs1–Top3 and the Mms4–Mus81 endonuclease. *Genes Dev.* **15**, 2730–2740 (2001).
42. Doe, C. L., Ahn, J. S., Dixon, J. & Whitby, M. C. Mus81–Eme1 and Rqh1 involvement in processing stalled and collapsed replication forks. *J. Biol. Chem.* **277**, 32753–32759 (2002).
43. Van Dyck, E., Hajibagheri, N. M. A., Stasiak, A. & West, S. C. Visualisation of human RAD52 protein and its complexes with RAD51 and DNA. *J. Mol. Biol.* **284**, 1027–1038 (1998).
44. Stasiak, A. Z. *et al.* The human RAD52 protein exists as a heptameric ring. *Curr. Biol.* **10**, 337–340 (2000).
45. Shinohara, A., Shinohara, M., Ohta, T., Matsuda, S. & Ogawa, T. Rad52 forms ring structures and cooperates with RPA in single-strand DNA annealing. *Genes Cells* **3**, 145–156 (1998).
46. Parsons, C. A., Baumann, P., Van Dyck, E. & West, S. C. Precise binding to single-stranded DNA termini by RAD52 protein. *EMBO J.* **19**, 4175–4181 (2000).
47. Kagawa, W., Kurumizaka, H., Ikawa, S., Yokoyama, S. & Shibata, T. Homologous pairing promoted by the human RAD52 protein. *J. Biol. Chem.* **276**, 35201–35208 (2001).
48. Park, M. S., Ludwig, D. L., Stigger, E. & Lee, S. H. Physical interaction between human RAD52 and RPA is required for homologous recombination in mammalian cells. *J. Biol. Chem.* **271**, 18996–19000 (1996).
49. Kagawa, W. *et al.* Crystal structure of the homologous-pairing domain from the human RAD52 recombinase in the undecameric form. *Mol. Cell* **10**, 359–371 (2002).
50. Singleton, M. R., Wentzell, L. M., Liu, Y., West, S. C. & Wigley, D. B. Structure of the single-strand annealing domain of human RAD52 protein. *Proc. Natl Acad. Sci. USA* **99**, 13492–13497 (2002).
References 49 and 50 present the X-ray crystal structure of the single-strand-annealing domain of human RAD52.
51. Singleton, M. R., Sawaya, M. R., Ellenberger, T. & Wigley, D. B. Crystal structure of T7 gene 4 ring helicase indicates a mechanism for sequential hydrolysis of nucleotides. *Cell* **101**, 589–600 (2000).
52. Haaf, T., Golub, E. I., Reddy, G., Radding, C. M. & Ward, D. C. 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 (1995).
53. Scully, R. *et al.* Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. *Cell* **90**, 425–435 (1997).
54. Liu, Y. & Maizels, N. Coordinated response of mammalian RAD51 and RAD52 to DNA damage. *EMBO Rep.* **1**, 85–90 (2000).
55. Lisby, M., Rothstein, R. & Mortensen, U. H. Rad52 forms DNA repair and recombination centers during S phase. *Proc. Natl Acad. Sci. USA* **98**, 8276–8282 (2001).
56. Essers, J. *et al.* Nuclear dynamics of RAD52 group homologous recombination proteins in response to DNA damage. *EMBO J.* **21**, 2030–2037 (2002).
57. Raderschall, E., Golub, E. I. & Haaf, T. Nuclear foci of mammalian recombination proteins are located at single-stranded DNA regions formed after DNA damage. *Proc. Natl Acad. Sci. USA* **96**, 1921–1926 (1999).
58. Scully, R. *et al.* Association of BRCA1 with RAD51 in mitotic and meiotic cells. *Cell* **88**, 265–275 (1997).
59. Chen, J. J. *et al.* Stable interaction between the products of the *BRCA1* and *BRCA2* tumor suppressor genes in mitotic and meiotic cells. *Mol. Cell* **2**, 317–328 (1998).
References 58 and 59 show the colocalization of RAD51 recombinase with the BRCA1 and BRCA2 tumour suppressors, leading to the concept that certain inheritable breast cancers might be due to defects in repair by homologous recombination.
60. Yuan, S.-S. F. *et al.* BRCA2 is required for ionizing radiation-induced assembly of RAD51 complex *in vivo*. *Cancer Res.* **59**, 3547–3551 (1999).
61. Godthelp, B. C., Artwert, F., Joenje, H. & Zdzienicka, M. Z. Impaired DNA damage-induced nuclear RAD51 foci formation uniquely characterizes Fanconi anemia group D1. *Oncogene* **21**, 5002–5005 (2002).
62. Tashiro, S. *et al.* S phase specific formation of the human RAD51 protein nuclear foci in lymphocytes. *Oncogene* **12**, 2165–2170 (1996).
63. Tarsounas, M., Davies, D. & West, S. C. BRCA2-dependent and independent formation of RAD51 nuclear foci. *Oncogene* **22**, 1115–1122 (2003).
64. Sharan, S. K. *et al.* Embryonic lethality and radiation hypersensitivity mediated by RAD51 in mice lacking BRCA2. *Nature* **386**, 804–810 (1997).
65. Wong, A. K. C., Pero, R., Ormonde, P. A., Tavtigian, S. V. & Bartel, P. L. RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene *BRCA2*. *J. Biol. Chem.* **272**, 31941–31944 (1997).
66. Moynahan, M. E., Chiu, J. W., Koller, B. H. & Jasin, M. BRCA1 controls homology-directed DNA repair. *Mol. Cell* **4**, 511–518 (1999).
67. Moynahan, M. E., Pierce, A. J. & Jasin, M. BRCA2 is required for homology-directed repair of chromosomal breaks. *Mol. Cell* **7**, 263–272 (2001).
68. Xia, F. *et al.* Deficiency of human BRCA2 leads to impaired homologous recombination but maintains normal nonhomologous end joining. *Proc. Natl Acad. Sci. USA* **98**, 8644–8649 (2001).
References 67 and 68 provide the first direct evidence that *BRCA2*-defective cells are compromised in their ability to promote double-strand-break repair by homologous recombination.
69. Xu, X. *et al.* Centrosome amplification and a defective G2–M cell cycle checkpoint induce genetic instability in *BRCA1* exon 11 isoform-deficient cells. *Mol. Cell* **3**, 389–395 (1999).
70. Yu, V. P. C. C. *et al.* Gross chromosomal rearrangements and genetic exchange between non-homologous chromosomes following BRCA2 inactivation. *Genes. Dev.* **14**, 1400–1406 (2000).
71. Rahman, N. & Stratton, M. R. The genetics of breast cancer susceptibility. *Annu. Rev. Genet.* **32**, 95–121 (1998).
72. Welcsh, P. L. & King, M. C. BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. *Hum. Mol. Genet.* **10**, 705–713 (2001).
73. Kerr, P. & Ashworth, A. New complexities for BRCA1 and BRCA2. *Curr. Biol.* **11**, R668–R676 (2001).
74. Wang, Y. *et al.* BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes Dev.* **14**, 927–939 (2000).
75. Anderson, S. E., Schlegel, B. P., Nakajima, T., Wolpin, E. S. & Parvin, J. D. BRCA1 protein is linked to the RNA polymerase II holoenzyme complex via RNA helicase A. *Nature Genet.* **19**, 254–256 (1998).
76. Yarden, R. I. & Brody, L. C. BRCA1 interacts with components of the histone deacetylase complex. *Proc. Natl Acad. Sci. USA* **96**, 4983–4988 (1999).
77. Zhong, Q. *et al.* Association of BRCA1 with the RAD50–MRE11–p95 complex and the DNA damage response. *Science* **285**, 747–750 (1999).
78. Bochar, D. A. *et al.* BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. *Cell* **102**, 257–265 (2000).
79. Marmorstein, L. Y. *et al.* A human BRCA2 complex containing a structural DNA binding component influences cell cycle progression. *Cell* **104**, 247–257 (2001).
80. Chiba, N. & Parvin, J. D. Redistribution of BRCA1 among four different protein complexes following replication blockage. *J. Biol. Chem.* **276**, 38549–38554 (2001).
81. Wu, L. C. *et al.* Identification of a RING protein that can interact *in vivo* with the *BRCA1* gene product. *Nature Genet.* **14**, 430–440 (1996).
82. Ruffner, H., Joazeiro, C. A. P., Hemmati, D., Hunter, T. & Verma, I. M. Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. *Proc. Natl Acad. Sci. USA* **98**, 5134–5139 (2001).
83. Mallery, D. L., Vandenberg, C. J. & Hiom, K. Activation of the E3 ligase function of the BRCA1/BARD1 complex by polyubiquitin chains. *EMBO J.* **21**, 6755–6762 (2002).
84. Baer, R. & Ludwig, T. The BRCA1/BARD1 heterodimer, a tumor suppressor complex with ubiquitin E3 ligase activity. *Curr. Opin. Genet. Dev.* **12**, 86–91 (2002).
85. D'Andrea, A. D. & Grompe, M. The Fanconi anaemia/BRCA pathway. *Nature Rev. Cancer* **3**, 23–34 (2002).
86. Grompe, M. & D'Andrea, A. D. Fanconi anemia and DNA repair. *Hum. Mol. Genet.* **10**, 2253–2259 (2001).
87. Joenje, H. & Patel, K. J. The emerging genetic and molecular basis of Fanconi anaemia. *Nature Rev. Genet.* **2**, 446–457 (2001).
88. Garcia-Higuera, I. *et al.* Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol. Cell* **7**, 249–262 (2001).
The first indication of the inter-relationship between Fanconi anaemia and BRCA gene products.
89. Ekblad, C. M. S. *et al.* Characterisation of the BRCT domains of the breast cancer susceptibility gene product BRCA1. *J. Mol. Biol.* **320**, 431–442 (2002).

90. Williams, R. S., Green, R. & Glover, J. N. M. Crystal structure of the BRCT repeat region from the breast cancer-associated protein BRCA1. *Nature Struct. Biol.* **8**, 838–842 (2001).
91. Cantor, S. B. *et al.* BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell* **105**, 149–160 (2001).
92. Bork, P., Blomberg, N. & Nilges, M. Internal repeats in the BRCA2 protein sequence. *Nature Genet.* **13**, 22–23 (1996).
93. Chen, P. L. *et al.* The BRC repeats in BRCA2 are critical for RAD51 binding and resistance to methyl methanesulfonate treatment. *Proc. Natl Acad. Sci. USA* **95**, 5287–5292 (1998).
94. Takata, M. *et al.* Conserved domains in the chicken homologue of BRCA2. *Oncogene* **21**, 1130–1134 (2002).
95. Kojic, M., Kostrub, C. F., Buchman, A. R. & Holloman, W. K. BRCA2 homolog required for proficiency in DNA repair, recombination, and genome stability in *Ustilago maydis*. *Mol. Cell* **10**, 683–691 (2002).
A BRCA2-like protein found in a smut fungus provides evidence for the universality of BRCA2 and its potential role in the control of RAD51 recombinase.
96. Chen, C. F., Chen, P. L., Zhong, Q., Sharp, Z. D. & Lee, W. H. Expression of BRC repeats in breast cancer cells disrupts the BRCA2–RAD51 complex and leads to radiation hypersensitivity and loss of G2/M checkpoint control. *J. Biol. Chem.* **274**, 32931–32935 (1999).
97. Davies, A. A. *et al.* Role of BRCA2 in control of the RAD51 recombination and DNA repair protein. *Mol. Cell* **7**, 273–282 (2001).
The first indication that interactions between RAD51 and a BRC repeat could block nucleoprotein-filament formation and thereby inhibit recombinase activity.
98. Cortez, D., Wang, Y., Qin, J. & Elledge, S. J. Requirement of ATM-dependent phosphorylation of BRCA1 in the DNA damage response to double-strand breaks. *Science* **286**, 1162–1166 (1999).
99. Yuan, Z. M. *et al.* Regulation of RAD51 function by c-Abl in response to DNA damage. *J. Biol. Chem.* **273**, 3799–3802 (1998).
100. Chen, G. *et al.* Radiation-induced assembly of RAD51 and RAD52 recombination complex requires ATP and c-Abl. *J. Biol. Chem.* **274**, 12748–12752 (1999).
101. Saintigny, Y., Dumay, A., Lambert, S. & Lopez, B. S. A novel role for the Bcl-2 protein family: specific suppression of the RAD51 recombination pathway. *EMBO J.* **20**, 2596–2607 (2001).
102. Yang, H. J. *et al.* BRCA2 function in DNA binding and recombination from a BRCA2–DSS1–ssDNA structure. *Science* **297**, 1837–1848 (2002).
Provided the first high-resolution structure of a segment of BRCA2 and showed that BRCA2 has the ability to bind DNA.
103. Marston, N. J. *et al.* Interaction between the product of the breast cancer susceptibility gene *BRCA2* and DSS1, a protein functionally conserved from yeast to mammals. *Mol. Cell. Biol.* **19**, 4633–4642 (1999).
104. Howlett, N. G. *et al.* Biallelic inactivation of *BRCA2* in Fanconi anemia. *Science* **297**, 606–609 (2002).
Showed that the Fanconi anaemia D1 gene (*FANCD1*) is the same as *BRCA2*.
105. Liang, F., Han, M. G., Romanienko, P. J. & Jasin, M. Homology-directed repair is a major double-strand break repair pathway in mammalian cells. *Proc. Natl Acad. Sci. USA* **95**, 5172–5177 (1998).
106. Takata, M. *et al.* Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *EMBO J.* **17**, 5497–5508 (1998).
107. Essers, J. *et al.* Homologous and non-homologous recombination differentially affect DNA damage repair in mice. *EMBO J.* **19**, 1703–1710 (2000).
108. Smith, G. C. M. & Jackson, S. P. The DNA-dependent protein kinase. *Genes Dev.* **13**, 916–934 (1999).
109. Chu, G. Double-strand break repair. *J. Biol. Chem.* **272**, 24097–24100 (1997).
110. Critchlow, S. E. & Jackson, S. P. DNA end-joining: from yeast to man. *Trends Biochem. Sci.* **23**, 394–398 (1998).
111. Kanaar, R., Hoeijmakers, J. H. J. & van Gent, D. C. Molecular mechanisms of DNA double-strand break repair. *Trends Cell Biol.* **8**, 483–489 (1998).
112. Dynan, W. S. & Yoo, S. Interaction of Ku protein and DNA-dependent protein kinase catalytic subunit with nucleic acids. *Nucleic Acids Res.* **26**, 1551–1559 (1998).
113. Walker, J. R., Corpina, R. A. & Goldberg, J. Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* **412**, 607–614 (2001).
114. Hanakahi, L. A., Bartlet-Jones, M., Chappell, C., Pappin, D. & West, S. C. Binding of inositol phosphate to DNA-PK and stimulation of double-strand break repair. *Cell* **102**, 721–729 (2000).
115. Hanakahi, L. A. & West, S. C. Specific interaction of IP_6 with human Ku70/80, the DNA-binding subunit of DNA-PK. *EMBO J.* **21**, 2038–2044 (2002).
116. Ma, Y. M. & Lieber, M. R. Binding of inositol hexakisphosphate (IP_6) to Ku but not to DNA-PK_{cs}. *J. Biol. Chem.* **277**, 10756–10759 (2002).
117. Odom, A. R., Stahlberg, A., Wente, S. R. & York, J. D. A role for nuclear inositol 1,4,5-trisphosphate kinase in transcriptional control. *Science* **287**, 2026–2029 (2000).
118. York, J. D., Odom, A. R., Murphy, R., Ives, E. B. & Wente, S. R. A phospholipase C-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export. *Science* **285**, 96–100 (1999).
119. Steger, D. J., Haswell, E. S., Miller, A. L., Wente, S. R. & O'Shea, E. K. Regulation of chromatin remodelling by inositol polyphosphates. *Science* **299**, 114–116 (2003).
120. Shen, X., Xiao, H., Ranallo, R., Wu, W.-H. & Wu, C. Modulation of ATP-dependent chromatin-remodeling complexes by inositol polyphosphates. *Science* **299**, 112–114 (2003).
121. Yamada, K. *et al.* Crystal structure of the RuvA–RuvB complex: a structural basis for the Holliday junction migrating motor machinery. *Mol. Cell* **10**, 671–681 (2002).
122. Parsons, C. A., Stasiak, A., Bennett, R. J. & West, S. C. Structure of a multisubunit complex that promotes DNA branch migration. *Nature* **374**, 375–378 (1995).
The first visualization of the *E. coli* RuvAB–Holliday-junction complex, which led to the proposal of a model for branch migration mediated by two opposing motor proteins.
123. Rafferty, J. B. *et al.* Crystal structure of DNA recombination protein RuvA and a model for its binding to the Holliday junction. *Science* **274**, 415–421 (1996).
124. Hargreaves, D. *et al.* Crystal structure of *E. coli* RuvA with bound DNA Holliday junction at 6Å resolution. *Nature Struct. Biol.* **5**, 441–446 (1998).
125. Roe, S. M. *et al.* Crystal structure of an octameric RuvA–Holliday junction complex. *Mol. Cell* **2**, 361–372 (1998).
126. Ariyoshi, M., Nishino, T., Iwasaki, H., Shinagawa, H. & Morikawa, K. Crystal structure of the Holliday junction DNA in complex with a single RuvA tetramer. *Proc. Natl Acad. Sci. USA* **97**, 8257–8262 (2000).
127. Yamada, K. *et al.* Crystal structure of the Holliday junction migration motor protein RuvB from *Thermus thermophilus* HB8. *Proc. Natl Acad. Sci. USA* **98**, 1442–1447 (2001).
128. Ingleston, S. M., Sharples, G. J. & Lloyd, R. G. The acidic pin of RuvA modulates Holliday junction binding and processing by the RuvABC resolvasome. *EMBO J.* **19**, 6266–6274 (2000).
129. Whitby, M. C., Bolt, E. L., Chan, S. N. & Lloyd, R. G. Interactions between RuvA and RuvC at Holliday junctions: inhibition of junction cleavage and formation of a RuvA–RuvC–DNA complex. *J. Mol. Biol.* **264**, 878–890 (1996).
130. van Gool, A. J., Shah, R., Mézard, C. & West, S. C. Functional interactions between the Holliday junction resolvase and the branch migration motor of *Escherichia coli*. *EMBO J.* **17**, 1838–1845 (1998).
131. Interthal, H. & Heyer, W. D. *MUS81* encodes a novel helix-hairpin-helix protein involved in the response to UV- and methylation-induced DNA damage in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **263**, 812–827 (2000).
132. Boddy, M. N. *et al.* Damage tolerance protein Mus81 associates with the FHA1 domain of checkpoint kinase Cds1. *Mol. Cell. Biol.* **20**, 8758–8766 (2000).
133. Mullen, J. R., Kaliraman, V., Ibrahim, S. S. & Brill, S. J. Requirement for three novel protein complexes in the absence of the Sgs1 DNA helicase in *Saccharomyces cerevisiae*. *Genetics* **157**, 103–118 (2001).
134. West, S. C. The RuvABC proteins and Holliday junction processing in *Escherichia coli*. *J. Bacteriol.* **178**, 1237–1241 (1996).
135. de los Santos, T., Loidl, J., Larkin, B. & Hollingsworth, N. M. A role for *MMS4* in the processing of recombination intermediates during meiosis in *Saccharomyces cerevisiae*. *Genetics* **159**, 1511–1525 (2001).
136. Ranatunga, W. *et al.* Human RAD52 exhibits two modes of self-association. *J. Biol. Chem.* **276**, 15876–15880 (2001).
137. Lloyd, J. A., Fiorget, A. L. & Knight, K. L. Correlation of biochemical properties with the oligomeric state of human RAD52 protein. *J. Biol. Chem.* **277**, 46172–46178 (2002).
138. Houtsmuller, A. B. *et al.* Action of DNA repair endonuclease ERCC1/XPF in living cells. *Science* **284**, 958–961 (1999).
139. West, S. C. Cross-links between Fanconi anaemia and BRCA2. *DNA Repair (Amst.)* **2**, 281–284 (2003).

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