Mechanism of Eukaryotic Homologous Recombination

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Abstract

Homologous recombination (HR) serves to eliminate deleterious lesions, such as double-stranded breaks and interstrand crosslinks, from chromosomes. HR is also critical for the preservation of replication forks, for telomere maintenance, and chromosome segregation in meiosis I. As such, HR is indispensable for the maintenance of genome integrity and the avoidance of cancers in humans. The HR reaction is mediated by a conserved class of enzymes termed recombinases. Two recombinases, Rad51 and Dmc1, catalyze the pairing and shuffling of homologous DNA sequences in eukaryotic cells via a filamentous intermediate on ssDNA called the presynaptic filament. The assembly of the presynaptic filament is a rate-limiting process that is enhanced by recombination mediators, such as the breast tumor suppressor BRCA2. HR accessory factors that facilitate other stages of the Rad51- and Dmc1-catalyzed homologous DNA pairing and strand exchange reaction have also been identified. Recent progress on elucidating the mechanisms of action of Rad51 and Dmc1 and their cohorts of ancillary factors is reviewed here.

Contents

BIOLOGICAL FUNCTIONS OF HOMOLOGOUS RECOMBINATION

Homologous recombination (HR), the exchange of genetic information between allelic sequences, has essential roles in meiosis and mitosis. In meiosis, HR mediates the exchange of information between the maternal and paternal alleles within the gamete

precursor cells and thus generates diversity among the progeny derived from common parents. HR has a second critical function in meiosis; it ensures proper segregation of homologous chromosome pairs at the first meiotic division through the formation of crossovers, resulting in gametes with the correct number of chromosomes. These functions of HR ensure stability of the organism karyotype. Meiotic HR is initiated by Spo11-mediated DNA double-strand breaks (DSBs) (1). HR maintains somatic genomic stability by promoting accurate repair of DSBs induced by ionizing radiation and other agents, repair of incomplete telomeres that arise when the enzyme telomerase is nonfunctional, repair of DNA interstrand crosslinks, and the repair of damaged replication forks. Although cells have alternate DNA repair pathways such as nonhomologous DNA end-joining, these may not be operative at all phases of the cell cycle, they do not always act on injured replication forks, nor are they as precise as HR in the repair of broken chromosomes.

Mutations in genes encoding the enzymatic steps of HR result in extreme sensitivity to DNA-damaging agents such as ionizing radiation in model organisms such as *Saccharomyces cerevisiae* (2). Additionally, these mutant strains are defective in processes that involve the repair of naturally occurring DSBs such as those breaks made during mating-type switching and during meiosis (3, 4). In vertebrate organisms, the equivalent mutations are very often lethal, most likely reflecting the higher occurrence of spontaneous DSBs during somatic growth and the essential role that HR plays in the repair of damaged DNA replication forks (5, 6). HR in higher eukaryotes involves additional factors not found in all model organisms. Heritable mutations in the cancer-prone disease Fanconi anemia and familial breast cancer have turned out to be in some of these factors (7). These are generally hypomorphic mutations as the genes are frequently essential.

The focus of this review is on the factors that promote HR through their action on the

recombinases Rad51 and Dmc1. The Rad51 recombinase mediates the formation of DNA joints that link homologous DNA molecules. It is active in somatic and meiotic cells. A second recombinase, Dmc1, promotes similar associations of homologous DNA molecules, but is active only in meiosis and acts in concert with Rad51. Several DNA helicases have been found to either negatively regulate HR initiation or specifically suppress crossover formation. The biological roles of these DNA helicases and their mechanism of action are the subject of recent reviews (8, 9) and are not covered here.

HOMOLOGOUS RECOMBINATION PATHWAYS AND BIOLOGICAL RELEVANCE

Many HR genes were first identified by mutants that are hypersensitive to DNA-damaging agents that cause DSBs, and by a failure to give viable meiotic products (see below for a more detailed description). From studies of these mutants using recombination reporters, models of HR and classification of HR pathways have emerged. These models are based on the repair of a DSB using a homologous DNA sequence. The first HR model for repair of a DSB was based on observations of transformation in yeast using linear plasmids that carried sequences homologous to yeast chromosomal DNA (10, 11). This model, called the double-strand break-repair (DSBR) model, can explain much of the meiotic segregation in the fungi and linked crossing over and gene conversion as different outcomes of DSB repair (12). Although this model has been modified from its original conception, subsequent models retain several key features. The most important are (*a*) initiation of HR by a DSB, (*b*) processing of the DSB by nucleolytic resection to give single-strand tails with 3'-OH ends, (*c*) formation of a recombinase filament on the ssDNA ends, (*d*) strand invasion into a homologous sequence to form a D-loop intermediate, (*e*) DNA polymerase extension

Crossovers: recombination products that entail an exchange of the arms of the participant chromosomes

Recombinase: an enzyme that mediates the pairing and exchange of DNA strands during homologous recombination

Gene conversion: nonreciprocal homologous recombination between an intact donor duplex and a gapped or broken recipient molecule

from the 3′ end of the invading strand, (*f*) capture of the second DSB end by annealing to the extended D loop, (*g*) formation of two crossed strand or Holliday junctions (HJ)s, and lastly (*h*) resolution of the HJs to give crossover or noncrossover products.

Although the DSBR model explains many observations related to meiotic recombination products in the fungi, one of its main tenets, the linking of gene conversion and crossovers through the resolution of a common intermediate, is not supported by mitotic recombination data where most DSB repair is most frequently unassociated with crossovers. To keep the original DSBR model for mitotic HR would necessitate the imposition of strict rules on HJ resolution in a noncrossover mode only. A second model that avoids this restriction and is based on mitotic DSB repair data in model organisms has been developed (13–15). The essence of this model is a migrating D loop that never leads to capture of the second DSB end. Instead, after the initial steps of DSB resection, DNA strand invasion, and repair DNA synthesis, the invading strand is displaced and anneals with the second resected DSB end. Because no HJ is formed, only noncrossover products are made. Since the model involves DNA synthesis followed by strand annealing, it is called synthesis-dependent strand annealing (SDSA). Although the SDSA model was initially developed to explain mitotic DSB repair, there is now substantial evidence to suggest that SDSA is also important during meiotic HR. Not all meiotic DSBs result in crossover products: only a small fraction of these breaks do. The existing data suggest that there are two waves of meiotic DSB-promoted HR. The first wave proceeds by SDSA and is only noncrossover, whereas the second wave proceeds by DSBR, forms double HJs, and is mainly, if not solely, crossover.

Sometimes a DSB is closely flanked by direct repeats. This DNA organization provides the opportunity to repair the DSB by a deletion process using the repeated DNA sequences, called single-strand annealing (SSA) (16–18). In the SSA process, the DSB ends are resected, but then instead of engaging a homologous DNA sequence for strand invasion, the resected ends anneal to each other. The process is finished by nucleolytic removal of the protruding single-strand tails, and results in deletion of the sequences between the direct repeats and also one of the repeats. Since strand invasion is not involved, SSA is independent of strand invasion and HJ resolution factors (19).

Some DSBs, such as those that can occur at telomeres or at broken replication forks, are single-ended (20–22). These too can participate in HR, through a single-ended invasion process called break-induced replication (BIR) (23–29). In BIR, the DSB end is nucleolytically processed similar to the resection that occurs in other DSB HR repair events. The single-strand tail then invades a homologous DNA sequence, often the sister chromatid or homolog chromosome but sometimes a repeated sequence on a different chromosome. The invading end is used to copy information from the invaded donor chromosome by DNA synthesis. When the sister chromatid or homolog chromosome is used, the repair is accurate. When a repeated sequence on a nonhomologous chromosome is engaged to initiate repair, the result is a nonreciprocal translocation. Most BIR events are dependent on the HR factors used in DSBR and SDSA, but a small fraction can occur independently of these factors that include Rad51. BIR is often used to repair broken or shortened telomeres (26, 29).

The requirement for HR in DNA replication is highlighted by the finding that many replication mutants and mutants in factors required for checkpoint activation when replication is stalled are dependent on HR genes for viability (30–32). This finding suggests that replication checkpoints prevent HR at stalled or damaged forks by stabilizing the replication complex at the fork, thus avoiding the occurrence of HR-promoting or HR-like intermediates. The finding also suggests that defective replication can result

in HR-provoking intermediates, e.g., gaps at or behind the replication fork. Because HR at stalled replication forks can lead to genomic rearrangements, it might be expected that it would be tightly controlled. In the case of collapsed replication forks, HR is used for one-ended strand invasion events using the sister chromatid to reconstruct the fork. This process may be promoted by sister chromatid cohesion complexes.

HR GENES AND PROTEINS

A large proportion of the genes needed for HR were initially identified in the budding yeast *S. cerevisiae* by the classical procedure of mutant isolation (typically based on sensitivity of mutant cells to DNA-damaging agents such as ionizing radiation), in-depth epistasis analyses of the available mutants, and cloning of the corresponding genes by complementation of the mutant phenotype. These genes are collectively known as the *RAD52* epistasis group. The structure and function of the proteins encoded by genes of the *RAD52* group are highly conserved among eukaryotes, from yeast to humans. **Table 1** lists the members of the *RAD52* gene group as first defined in *S. cerevisiae* and their human equivalent.

Generally speaking, in addition to mediating mitotic HR events, members of the *RAD52* group are needed for meiotic recombination as well. Aside from the *RAD52* core group, a whole host of genes that uniquely affect meiotic recombination have been uncovered in screens designed to search for them (1). For instance, the *DMC1* gene, which encodes one of the two recombinase enzymes, was identified as a cDNA species that is strongly upregulated when *S. cerevisiae* cells enter into meiosis. Overall, more is known about the properties of the mitotic HR factors than of meiosis-specific factors. We discuss recent progress in understanding the mechanism of the HR machinery without providing an exhaustive account on the properties of all the known HR factors (reviewed in 4, 8, 19, 33).

THE RAD51 RECOMBINASE AND PRESYNAPTIC FILAMENT FORMATION

The enzymes that mediate the pairing and shuffling of DNA sequences during HR are called recombinases, and the reaction mediated by these enzymes is termed homologous DNA pairing and strand exchange. Two recombinases, Rad51 and Dmc1, exist in eukaryotes. Rad51 is needed for mitotic HR events such as DSB repair and also for meiotic HR, whereas Dmc1 is only expressed in meiosis so its function is restricted therein. The salient attributes of the *DMC1* gene and encoded protein are discussed in a separate section.

Much of our knowledge on the *RAD51* gene and its encoded protein has been derived from genetic and biochemical studies done in *S. cerevisiae*. The *S. cerevisiae rad51* mutants are highly sensitive to DNA-damaging agents and show defects in mitotic and meiotic recombination. Analysis of the *S. cerevisiae RAD51* gene, which was cloned independently by three different groups, revealed significant homology of its encoded protein to the bacterial recombinase RecA, with particular conservation of those RecA residues that are critical for its recombinase function, including DNA binding and ATP hydrolysis (34–36). The structure of the Rad51 protein has been conserved among eukaryotes. Whereas *S. cerevisiae rad51* mutants are viable mitotically, ablation of the *RAD51* gene in vertebrates engenders mitotic lethality (19), which likely reflects an essential role of Rad51-mediated HR in the repair of damaged DNA replication forks and hence the successful navigation through S phase.

Rad51 and its prokaryotic counterpart RecA exists as a homo-oligomer in solution, being heptameric and hexameric, respectively (33, 37). Just as in the case of RecA, with ATP (or an analogue of ATP) available, *S. cerevisiae* Rad51 protein assembles onto ssDNA or dsDNA to form a right-handed helical polymer that can span thousands of bases or base

Epistasis group: a group of genes that function in the same biological pathway. Epistasis is established by double mutant analyses

Presynaptic filament: the right-handed helical recombinase filament that is assembled on ssDNA

Table 1 Homologous recombination factors

Human	*S. cerevisiae*	Biochemical function	Additional features
Proteins that function with Rad51			
MRN complex: Mre11-Rad50-Nbs1	MRX complex: Mre11-Rad50-Xrs2	DNA binding Nuclease activities	Involved in DNA-damage checkpoints Associated with DSB end resection
BRCA2	(none)	ssDNA binding Recombination mediator	Interacts with RPA, Rad51, Dmc1, PALB2, DSS1 Member of the Fanconi anemia group
Rad52[a]	Rad52	ssDNA binding and annealing Recombination mediator	Interacts with Rad51 and RPA
?[b]	Rad59	ssDNA binding and annealing	Interacts with Rad52 Homology to Rad52
Rad54 Rad54B	Rad54 Rdh54	ATP-dependent dsDNA translocase Induces superhelical stress in dsDNA Stimulates the D-loop reaction	Member of the Swi2/Snf2 protein family Chromatin remodeler Interacts with Rad51 The yeast proteins remove Rad51 from dsDNA
Rad51B-Rad51C Rad51D-XRCC2 Rad51C-XRCC3	Rad55-Rad57	ssDNA binding Recombination mediator activity (Rad55-Rad57 & Rad51B-Rad51C)	Rad51B-Rad51C and Rad51D-XRCC2 form a tetrameric complex Rad51C associates with a Holliday-junction resolvase activity
Hop2-Mnd1	Hop2-Mnd1	Stimulates the D-loop reaction Stabilizes the presynaptic filament Promotes duplex capture	Interacts with Rad51 and Dmc1
Proteins that function with Dmc1			
Hop2-Mnd1	Hop2-Mnd1	Stimulates the D-loop reaction Stabilizes the presynaptic filament Promotes duplex capture	Interacts with Dmc1 and Rad51
?[b]	Mei5-Sae3	Predicted recombination mediator activity	Interacts with Dmc1 Likely functional equivalent of *S. pombe* Sfr1-Swi5
Rad54B	Rdh54	Stimulates the D-loop reaction	Interacts with Dmc1 and Rad51

[a]Recombination mediator activity has been found in the yeast protein only.
[b]No human equivalent has been identified.

pairs. The Rad51-DNA nucleoprotein filament harbors ~18–19 bases or base pairs of DNA and ~6 protein monomers per helical turn. It has a pitch of close to 100 Å, corresponding to an axial rise of 5.2 to 5.5 Å per base or base pair (38, 39). The DNA in this filamentous structure is therefore held in a highly extended conformation, i.e., stretched about 50% when compared to a naked B form duplex molecule. Human Rad51 forms helical filaments on both ssDNA and dsDNA that

exhibit biophysical attributes similar to those described for *S. cerevisiae* Rad51 (40). Biochemical studies have provided clear evidence that only the Rad51-ssDNA nucleoprotein filament species is able to catalyze DNA joint formation (39), which supports the notion that HR in cells is initiated via recruitment of Rad51 to the ssDNA generated via nucleolytic processing of DSBs (**Figure 1**) or ssDNA that is associated with stalled or damaged DNA replication forks. The Rad51-ssDNA

nucleoprotein filament is often referred to as the presynaptic filament, and the biochemical steps that lead to the assembly of the Rad51 filament are collectively known as the presynaptic stage. The formation of Rad51-dsDNA filaments with bulk chromatin could diminish the pool of Rad51 available for HR reactions. As discussed below, the *S. cerevisiae* Rad54 and Rdh54 proteins dissociate Rad51-dsDNA filaments, an activity that is likely critical for the intracellular recycling of Rad51.

Once assembled, the presynaptic filament captures a duplex DNA molecule and searches for homology in the latter. From studies done with RecA (33), it is expected that the homology search process occurs by way of random collisions between the presynaptic filament and the duplex molecule. Thus, segments of the duplex are bound and tested in a reiterative fashion until homology is found. Upon the location of homology in the duplex molecule, the presynaptic filament is able to form DNA joints that are either "paranemic" or "plectonemic" in nature. In the paranemic joint, an internal region of the ssDNA is paired with the duplex molecule via canonical Watson-Crick hydrogen bonds, but the paired DNA strands are not topologically linked. Studies done in the Radding laboratory have shown that the paranemic linkage mostly involves the formation of AT base pairs between the recombining DNA molecules (41). The three-stranded, paranemically paired nucleoprotein intermediate is referred to as the synaptic complex. Recently published work has provided evidence for a role of the Hop2-Mnd1 protein complex in functionally synergizing with the presynaptic filament in the capture of duplex DNA and the assembly of the synaptic complex (42, 43) (see below). Although relatively short-lived, the paranemic joint facilitates the location of a free DNA end to initiate the formation of a plectonemic joint, in which the participant DNA strands are bound by Watson-Crick hydrogen bonds and topologically intertwined (44, 45). The nascent
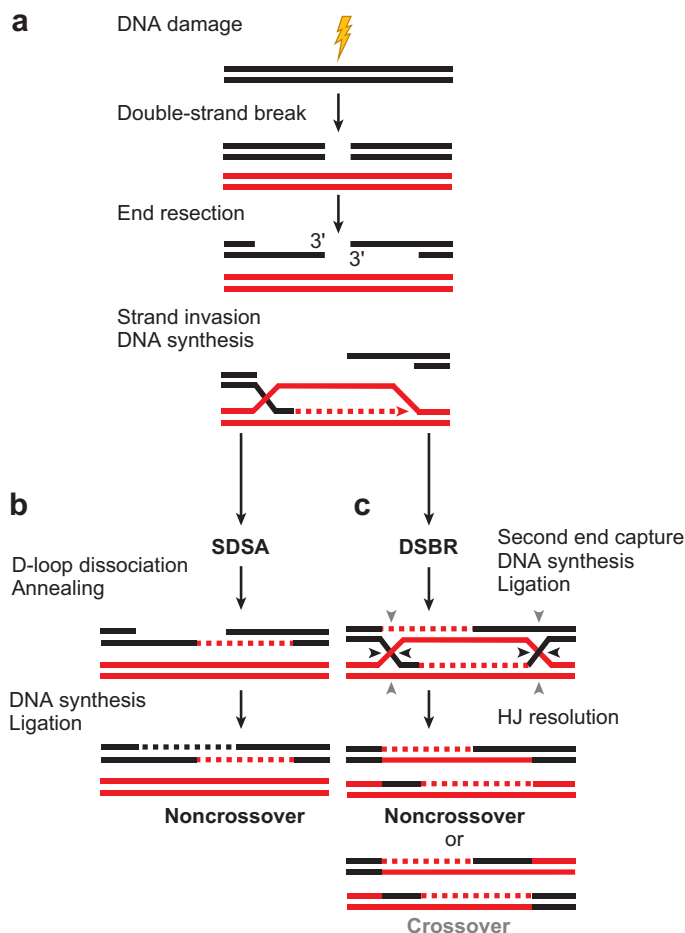
Figure 1

Pathways of DNA double-strand break repair by homologous recombination. Double-strand breaks (DSBs) can be repaired by distinctive homologous recombination (HR) pathways, such as synthesis-dependent strand annealing (SDSA) and double-strand break repair (DSBR). (*a*) After DSB formation, the DNA ends are resected to yield 3′ single-strand DNA (ssDNA) overhangs, which become the substrate for the HR protein machinery to execute strand invasion of a partner chromosome. After a successful homology search, strand invasion occurs to form a nascent D-loop structure. DNA synthesis then ensues. (*b*) In the SDSA pathway, the D loop is unwound and the freed ssDNA strand anneals with the complementary ssDNA strand that is associated with the other DSB end. The reaction is completed by gap-filling DNA synthesis and ligation. Only noncrossover products are formed. (*c*) Alternatively, the second DSB end can be captured to form an intermediate that harbors two Holliday junctions (HJ)s, accompanied by gap-filling DNA synthesis and ligation. The resolution of HJs by a specialized endonuclease can result in either noncrossover (*black triangles*) or crossover products (*gray triangles*).

Synaptic complex:
the ternary complex
of the recombinase
filament, ssDNA,
and dsDNA in which
the DNA molecules
are held in
homologous registry

**Recombination
mediator:** a protein
that facilitates the
assembly of the
recombinase
presynaptic filament
via RPA
displacement from
ssDNA

plectonemic joint can be extended by DNA
strand exchange being catalyzed by the presy-
naptic filament. The DNA strand exchange
reaction is facilitated by the Rad54 protein
(46). Moreover, Rad54 also promotes a spe-
cialized form of DNA strand exchange that
involves the formation of a HJ and migration
of the branch point in the HJ (47).

Nucleation of Rad51 onto ssDNA is a slow
process, which renders presynaptic filament
assembly prone to interference by the ssDNA
binding protein RPA. Certain recombinase
accessory factors, which have been termed
recombination mediators and include the tu-
mor suppressor BRCA2, can overcome the in-
hibitory effect of RPA on the assembly of the
Rad51 presynaptic filament. As such, these re-
combination mediators are critical for the effi-
ciency of HR in vivo. We expand on the mech-
anism of action of the known recombination
mediators below.

THE MEIOSIS-SPECIFIC
RECOMBINASE DMC1

The *DMC1* gene was isolated by Bishop et al.
(48) in a screen for cDNA species specific
for *S. cerevisiae* meiosis. The *DMC1*-encoded
protein is present in almost all eukaryotes in-
cluding humans and is structurally related to
RecA and Rad51 (48, 49). Ablation of *DMC1*
in *S. cerevisiae*, *Arabidopsis thaliana*, and mice
produces a constellation of meiotic abnor-
malities that reflect an indispensable role of
the Dmc1 protein in meiotic recombination
and chromosome segregation (1, 48, 50, 51).
Dmc1 exists as an octamer in solution (52),
and recent biochemical studies have provided
compelling evidence that it too forms right-
handed, helical filaments on ssDNA in an
ATP-dependent manner and catalyzes the ho-
mologous DNA pairing and strand exchange
reaction within the context of these nucle-
oprotein filaments (53–55). Thus, in its ac-
tion as a recombinase, Dmc1 possesses the
same functional attributes as have been docu-
mented for RecA and Rad51.

ROLE OF ATP BINDING AND
HYDROLYSIS IN PRESYNAPTIC
FILAMENT DYNAMICS

Even though Rad51 and Dmc1 hydrolyze
ATP, especially when DNA bound (56–59),
ATP hydrolysis is not needed for the assem-
bly of the presynaptic filament. In fact, bio-
chemical studies have provided evidence that
ATP hydrolysis within the microenvironment
of the presynaptic filament leads to the disso-
ciation of recombinase molecules from DNA
(53, 60–62). As a consequence, the use of a
nonhydrolyzable nucleotide analogue (such as
AMP-PNP) (61, 62), calcium ion (53, 60, 62),
or a Rad51 variant that binds ATP but lacks
ATPase activity (61) leads to the stabilization
of the presynaptic filament. That ATP hydrol-
ysis promotes the turnover of the presynaptic
filament is also a well-known property of RecA
(33, 63). The ATP hydrolysis-linked turnover
of the presynaptic filament could promote
the intracellular recycling of the recombinases
(i.e., preventing the nonproductive associa-
tion of the recombinase protein with DNA)
and to make available the primer end in the
newly made D loop to initiate the repair DNA
synthesis reaction.

OPPOSING EFFECTS OF
RPA IN PRESYNAPTIC
FILAMENT ASSEMBLY

The heterotrimeric RPA (replication protein
A) is an abundant nuclear protein that binds
ssDNA with high affinity and can remove sec-
ondary structure in ssDNA. Depending on
the circumstances, RPA can exert a stimu-
latory or an inhibitory effect on the assem-
bly of the presynaptic filament (64, 65). The
stimulatory action of RPA was noted in 1994
when the recombinase activity of *S. cerevisiae*
Rad51 was first reported (59). Subsequent
studies have provided evidence that RPA facil-
itates the assembly of the presynaptic filament
via the removal of secondary structure in the
ssDNA (66) and also by sequestering ssDNA
generated during the homologous DNA

pairing and strand exchange reaction (67, 68). However, an amount of RPA that is sufficient to saturate the available ssDNA (the ssDNA binding site size of RPA is ∼25 nucleotides per heterotrimeric molecule) strongly suppresses the ssDNA-dependent ATPase and recombinase activities of Rad51 and Dmc1 (64, 65, 69–71). That RPA can exclude the recombinases from the HR substrate has been validated in studies that employed chromatin immunoprecipitation (ChIP) and cytological methods. Several recombination mediators have been shown to counteract the inhibitory action of RPA (see below).

CONSERVED FUNCTIONAL ATTRIBUTES OF THE RECOMBINATION MEDIATORS

As mentioned above, the assembly of the presynaptic filament can be severely impeded by RPA. Studies in several laboratories have led to the identification of recombination mediators capable of overcoming this inhibitory action of RPA. Specifically, the addition of these recombination mediators with the recombinase protein to an RPA-coated ssDNA template permits the efficient assembly of the presynaptic filament (19, 64, 69–72). The recombination mediator activity of a variety of HR factors has also been demonstrated using the restoration of the recombinases' ssDNA-dependent ATPase as the readout (70, 71) or by electron microscopy to directly visualize their effect on presynaptic filament formation (72). Mutations in the recombination mediators invariably impair the delivery of their cognate recombinase to the HR substrate in cells, as revealed in cytological and ChIP experiments (19, 73–77). Since the *K45E* mutation in the largest RPA subunit is associated with a synapsis defect, RPA likely also plays a role in DNA strand invasion during HR (78).

The recombination mediators share common features in that they are all capable of physically interacting with their cognate recombinase and bind ssDNA preferentially over dsDNA (19, 71, 72, 79). In some in-

stances, an interaction of the recombination mediators with RPA has also been noted (80–83). Only a catalytic quantity of the recombination mediators is needed to see reversal of RPA inhibition, which is very likely due to the fact that the addition of recombinase molecules to a nascent presynaptic filament (i.e., filament growth) is sufficient to displace RPA from ssDNA (84). The genetic characteristics and salient features of the various known recombination mediators are reviewed below.

THE *S. CEREVISIAE* RAD52 PROTEIN AND ITS RECOMBINATION MEDIATOR ACTIVITY

The *S. cerevisiae* Rad52 protein has been the most intensely studied recombination mediator to date. Genetically speaking, *S. cerevisiae rad52* mutants are extremely sensitive to a variety of DNA-damaging agents and engender a general defect in all the known pathways of HR, including Rad51-independent reactions such as SSA (see below). Rad52 is a ring-shaped oligomer (81, 85), and oligomerization of monomers to form the protein ring is mediated by the N-terminal portion of the protein (86). Higher-order multimeric structures of Rad52 have also been documented (86). The inclusion of a catalytic quantity of Rad52 leads to highly efficient reversal of RPA-imposed inhibition of the ssDNA-dependent ATPase and recombinase activities of Rad51 (65, 69, 70). In both mitotic and meiotic cells, the recruitment of Rad51 to DSBs is strongly dependent on Rad52, but the DSB recruitment of Rad52 shows no dependence on Rad51 (73, 75–77). Taken together, the genetic and biochemical studies on *S. cerevisiae* Rad52 provide compelling evidence that it helps deliver Rad51 to the ssDNA substrate during HR.

That Rad52 physically associates with the Rad51 protein was first noted in a yeast two-hybrid protein-protein interaction analysis conducted by Milne et al. (87), and this was subsequently confirmed by

Chromatin immunoprecipitation (ChIP): a powerful technique for determining whether a protein associates with a specific region of the genome

coimmunoprecipitation of the two proteins from cell extract (65) and also using purified proteins in affinity pulldown assays (36). The Rad51 interaction domain resides within the carboxyl-terminal portion of the Rad52 protein (87), and truncation mutations (*rad52 Δ327* and *rad52 Δ409–412*) that abolish Rad51 binding attenuate the recombination mediator activity of the latter (70, 88) and compromise HR deficiency in both mitotic and meiotic cells (88, 89). These observations support the premise that complex formation with Rad51 is indispensable for the recombination mediator activity of Rad52. The HR deficiency of the *rad52 Δ409–412* mutant can be largely overcome by the overexpression of Rad51 (88), indicating that the recombination mediator function of ScRad52 can be bypassed when the intracellular concentration of Rad51 is elevated.

Mortensen et al. (90) first reported that ScRad52 has a DNA binding activity that is specific for ssDNA. These authors found that the N-terminal one third of Rad52 harbors a DNA binding function, and extensive subsequent studies have focused on how the N-terminal portion of human Rad52 engages ssDNA (91–94). The structure of the conserved N-terminal protein oligomerization/DNA binding domain of the human Rad52 protein has been analyzed by X-ray crystallography (91, 94). The crystallographic data reveal an undecameric (11-subunit) ring structure with a deep groove on the outer surface, with an abundance of basic and aromatic residues lining this groove (91, 94). Detailed mutational analyses have provided evidence for the involvement of these residues in DNA engagement (91, 92). Mutations of the equivalent residues in the ScRad52 protein compromise DNA repair and HR efficiency, attesting to the importance of the N-terminal DNA binding domain in Rad52 protein function (95). It remains to be determined how these N-terminal mutations affect the recombination mediator activity of ScRad52. Recent studies in the laboratory of the authors have led to the identification of a

second DNA binding domain in the carboxyl terminal portion of ScRad52 (L. Krejci, C. Seong & P. Sung, unpublished observation). How this second DNA binding domain contributes to the known functions of ScRad52 is the subject of ongoing investigations.

Rad52 protein also associates with RPA in *S. cerevisiae* cells (80), and it appears that both the largest and middle subunits of RPA are able to directly bind Rad52 (81, 96). The ability to interact with RPA is conserved in the human Rad52 protein (96). Because Rad52 is unable to overcome inhibition posed by the *Escherichia coli* SSB protein on Rad51-mediated homologous DNA pairing and strand exchange (69), specific association of Rad52 with RPA is very likely necessary for the recombination mediator activity of Rad52.

A model for the recombination mediator function of ScRad52 is presented in **Figure 2**. Among the most pertinent questions regarding the recombination mediator function of Rad52 are the relative contributions of the N-terminal and C-terminal DNA binding domains of this protein and the relevance of protein oligomerization. In this regard, it should be noted that the N-terminal domain of ScRad52 is important for DNA annealing reactions during yeast meiosis (97).

OTHER FUNCTIONS OF THE *S. CEREVISIAE* RAD52 PROTEIN

Even though the *rad52 Δ327* and *Δ409–412* alleles encode proteins that are defective in Rad51 interaction and consequently lack recombination mediator activity (70, 88), they are not as deficient in mitotic and meiotic HR as is the *rad52* null mutant (88, 89). Moreover, the rad52 Δ327 protein retains residual ability to mediate the recruitment of Rad51 to DSBs (C. Seong & P. Sung, unpublished results). Clearly then, in HR events that are Rad51 dependent, Rad52 protein serves an undefined role that is distinct from its well-characterized recombination mediator activity. Aside from participating in Rad51-dependent HR events, Rad52 is also required for Rad51-independent

SSA and BIR reactions (19, 26). Consistent with its involvement in SSA, Rad52 protein anneals DNA strands that are naked or coated with RPA (81, 82). It has been inferred that Rad52-mediated annealing of RPA-coated DNA strands is relevant for the capture of the second DNA end in the DSBR pathway of HR (98). Rad52 is absent in flies and worms but present in other eukaryotes, including humans.

THE HUMAN RAD52 PROTEIN AND ITS HR FUNCTION

Even though the human Rad52 protein resembles its *S. cerevisiae* counterpart in structure and biochemical attributes (i.e., it is oligomeric and able to bind ssDNA and promote ssDNA annealing), and can under some specialized circumstances enhance Rad51-mediated homologous DNA pairing (99), a recombination mediator activity has not yet been demonstrated for it. In fact, extensive efforts in the laboratory of the authors (P. Sung, unpublished observations) have failed to reveal such an activity. The apparent lack of a recombination mediator activity in hRad52 may explain why it plays only a subsidiary role in HR in vertebrates (19, 100). Alternatively, hRad52 may augment the recombination mediator activity of other factors such as BRCA2 or complexes of the Rad51 paralogues (see below). In this regard, synthetic lethality has been noted for a double mutant of Rad52 and XRCC3 (a Rad51 paralogue; see below) (100). It of course also remains possible that a recombination mediator activity will only be revealed upon the posttranslational modification (such as phosphorylation) of hRad52 or the inclusion of a partner protein.

THE HR ROLE OF THE TUMOR SUPPRESSOR BRCA2

Mutations in the *BRCA2* gene predispose the affected individuals to breast, ovarian, and other cancers (101). Biallelic inactivation of *BRCA2* can cause the cancer-prone dis-
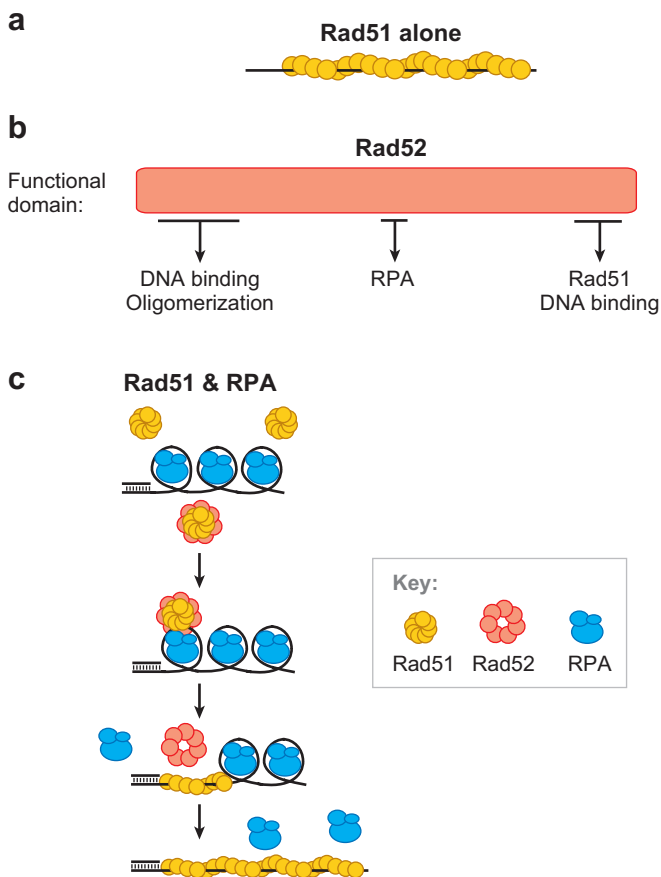
Figure 2

Rad52 and its role as a recombination mediator. (*a*) In the presence of ATP, Rad51 forms the presynaptic filament on ssDNA, but is unable to do so in the presence of replication protein A (RPA). (*b*) The functional domains in *S. cerevisiae* Rad52 are indicated. (*c*) In its recombination mediator role, Rad52 forms a complex with Rad51 and delivers it to RPA-coated ssDNA to seed the assembly of the presynaptic filament. The polymerization of additional Rad51 molecules results in the further displacement of RPA from the DNA.

ease Fanconi anemia (102) (see sidebar: Connection between Fanconi Anemia and Homologous Recombination). Cells from vertebrate, plant, nematode, and fungal species deficient in BRCA2 or its orthologue are sensitive to DNA-damaging agents and impaired for homology-directed elimination of chromosome damage, including interstrand crosslinks and DSBs (51, 101, 103–109). The transport of RAD51 to the nucleus is impaired in cells that harbor a cancer-associated

Paralogue: a protein that is related to another protein in primary structure but not necessarily in function and that arises through gene duplication

CONNECTION BETWEEN FANCONI ANEMIA AND HOMOLOGOUS RECOMBINATION

Fanconi anemia (FA) is an autosomal recessive chromosome fragility syndrome characterized by progressive bone marrow failure, short stature, cancer predisposition, and a severe deficiency in the removal of lesions, interstrand crosslinks in particular, from DNA (209, 210). FA is a complex multigenic disorder comprised of 13 complementation groups (FA- A, B, C, D1, D2, E, F, G, I, J, L, M, and N), and each of the corresponding genes has been identified. FA-D1 and FA-N patients harbor biallelic mutations in BRCA2 and its associated protein PALB2, respectively, thus revealing a functional linkage of BRCA2-/PALB2-dependent homologous recombination to the FA pathway of DNA-damage repair and response (102, 209, 210). Upon DNA damage, a complex of several FANC proteins helps mediate the monoubiquitination of FANCD2 and FANCI, resulting in the chromatin localization of the latter two proteins. It is believed that the chromatin-bound ubiquitinated FANCD2-FANCI complex recruits BRCA2/PALB2 and associated proteins, such as Rad51, to initiate DNA repair by a homology-directed mechanism (102, 209–212). Because FA cells are frequently impaired for HR (209, 210), some of the FA proteins may possibly influence the homologous recombination process via BRCA2/PALB2.

BRCA2 truncation (110). Furthermore, hamster Brca2 mutant cells exhibit an abnormal S phase DNA-damage checkpoint response (104). Brca2-deficient mice suffer embryonic lethality, an observation that highlights the importance of BRCA2-dependent DNA-damage repair and response (107). There is also good evidence that BRCA2 is important for meiotic HR (51, 103, 105, 111). A large body of results point to an important role of BRCA2 in the assembly of presynaptic filaments of Rad51. Specifically, and as expounded below, BRCA2 and its orthologous proteins bind DNA (71, 72, 79, 105), physically interact with Rad51 (101, 107, 112, 113), and are needed for the formation of DNA damage–induced nuclear Rad51 foci (101, 114). Importantly, studies on the *Ustilago maydis* Brh2 protein (the BRCA2 ortho-

Orthologue: the structural and functional equivalent of a protein in a different species

logue in that organism) and a polypeptide that harbors critical functional domains of the human BRCA2 protein have provided direct biochemical evidence for a recombination mediator activity (71, 72) (see below). An ability of human BRCA2 and its *Arabidopsis thaliana* orthologue to interact with Dmc1 has also been demonstrated (115, 116), although whether BRCA2 serves a recombination mediator role in Dmc1-mediated HR reactions remains to be explored. Notably, *S. cerevisiae* and *Schizosaccharomyces pombe* do not possess a BRCA2-like molecule.

SALIENT FEATURES OF BRCA2 ORTHOLOGUES

The BRCA2 orthologues vary greatly in size, ranging from 3418 amino acid residues of the human protein to only 383 residues of the *Caenorhabditis elegans* counterpart (called BRC-2). Human BRCA2 utilizes two separate means to interact with Rad51 protein, through a reiterated motif called the BRC repeat (101, 112, 113, 117) and also via a structurally distinct motif located at its extreme carboxyl terminus (107, 118); the carboxyl terminal Rad51 binding domain will be referred to as the CTRB domain henceforth. The BRC repeat, which comprises about 30 amino acid residues, is highly conserved among the BRCA2 orthologues (117, 119), although there is extreme variance in the number of copies of this motif among the orthologues. For example, human BRCA2 harbors eight BRC repeats, whereas *Ustilago maydis* Brh2 protein and *A. thaliana* Brca2 protein possess a single BRC repeat and four such repeats, respectively. While most of the BRC repeats from different BRCA2 orthologues bind Rad51 with varying affinity, the BRC5 and BRC6 repeats of human BRCA2 do not seem to be functional in this regard (112, 113). The second BRC repeat of the *A. thaliana* Brca2 protein appears to interact with Dmc1 but not Rad51 (115). Biochemical and crystallographic approaches and also electron microscopy have been employed to define the

nature of the BRC-Rad51 complex. The results from these studies indicate that the BRC repeat interacts with the monomeric form of Rad51 [Rad51 by itself is heptameric in solution (37)] in the absence of DNA (120). The atomic structure of a complex between BRC4 of human BRCA2 and the RecA-homology domain of Rad51 has been solved by X-ray crystallography (121). The structure reveals that BRC4 mimics the motif in Rad51 that mediates protein homo-oligomerization, thus providing a structural basis as to why BRC binds the monomeric form of Rad51 (121). Yang et al. (71) have suggested that binding of the BRC repeat to Rad51 leaves one of the Rad51 oligomerization interfaces available for the recruitment of another Rad51 molecule, which could provide a means for the nucleation of Rad51 presynaptic filament assembly in the recombination mediator role of BRCA2. Electron microscopy with accompanying three-dimensional reconstruction analyses have provided evidence that the BRC repeat can interact with a preformed Rad51 presynaptic filament without disruption of the filament, and that BRC3 and BRC4 of human BRCA2 interact with different surfaces of Rad51 in the context of the presynaptic filament (122). The sole BRC repeat of the *C. elegans* BRC-2 protein is bipartite in structure; one region is specific for monomeric Rad51 and the other interacts specifically with and stabilizes the Rad51 presynaptic filament (123).

As mentioned above, BRCA2 also binds Rad51 through the CTRB domain (107, 118). An allele of mouse Brca2 lacking the CTRB domain is associated with reduced viability, hypersensitivity to the DNA interstrand crosslinking agent mitomycin C, chromosomal instability (124), and a modest impairment of the HR-directed repair of a site-specific DSB (106). Thus, the CTRB domain appears to make a significant contribution toward the HR function of BRCA2 but is not essential for this function. Unlike the BRC repeat, the CTRB domain interacts with the oligomeric form of Rad51 in the absence of DNA (125, 126), and appears to bind the interface of two adjacent Rad51 monomers in the context of the presynaptic filament (126). The CTRB domain harbors a serine residue (serine 3291) that becomes phosphorylated by cyclin-dependent kinases (CDKs) in a cell cycle-dependent fashion, a modification that blocks the interaction with Rad51. It has been suggested that the CDK-mediated phosphorylation of the CTRB domain serves as a molecular switch in regulating Rad51-mediated recombination (127). Note that the *U. maydis* Brh2 protein also harbors a Rad51 binding domain in its carboxyl terminus. This C-terminal Rad51 binding domain, which is crucial for the HR and DNA repair functions of Brh2 in vivo, is unrelated to the BRC repeat (128). Whether this Brh2 domain is structurally related to the CTRB domain of BRCA2 is unclear. Dmc1 also interacts with the CTRB domain in human BRCA2, albeit more weakly than Rad51 (116).

Although human BRCA2 and its *A. thaliana* equivalent both interact with Dmc1, the primary Dmc1 binding domain appears to be different in the two proteins. None of the BRC repeats in the human BRCA2 protein has any affinity for Dmc1, and the main Dmc1 interaction site in human BRCA2 has been mapped to a 26-amino-acid region (residues 2386–2411) termed the PhePP motif. This motif is highly conserved in mammalian BRCA2 species but absent in the BRCA2 orthologue in *A. thaliana* and other eukaryotes (116). In contrast, *A. thaliana* Brca2 associates with Dmc1 through BRC2, the second of four BRC repeats in this protein (115).

Aside from Rad51 interaction, BRCA2 has also been found to associate with RPA (83) by several biochemical criteria. RPA binding is mediated by the extreme N-terminal region of BRCA2, and a cancer-associated mutation, Y42C, in BRCA2 attenuates or abolishes the ability to bind RPA (83).

The DNA binding activity of BRCA2 was first revealed in a combined biochemical and structural examination of the mouse Brca2 (mBrca2) protein by Yang et al. (79).

The BRCA2 DNA binding domain (BRCA2 DBD) is thought to harbor five subdomains: three oligonucleotide/oligosaccharide-binding (OB) folds (OB1, OB2, and OB3), a helix-turn-helix motif that is appended to OB2 and called the Tower domain, and a proximal alpha-helical region termed the helical domain (79). The OB folds of BRCA2 are structurally similar to the OB folds present in RPA. Embedded within the helical domain and OB1 is a surface that mediates complex formation with a small, highly acidic protein DSS1 (see below for a more detailed description of DSS1). The mBrca2 DBD-Dss1 complex binds ssDNA but not dsDNA and appears to recognize the duplex-ssDNA transition in various DNA substrates (79). In this regard, the *U. maydis* Brh2-Dss1 complex has a high affinity for a duplex-ssDNA junction that bears the 3′ overhang polarity of a resected DSB (71). Crystallographic and biochemical evidence has implicated all three OB folds of BRCA2 in DNA engagement (79).

RECOMBINATION MEDIATOR ACTIVITY OF *U. MAYDIS* BRH2 AND HUMAN BRCA2 PROTEINS

The *U. maydis* Brh2 protein in complex with Dss1 was found to possess a recombination mediator activity (71). Using several biochemical approaches, Yang et al. demonstrated that an amount of Brh2-Dss1 substoichiometric to that of the *U. maydis* Rad51 protein is sufficient to seed the assembly of the presynaptic filament on DNA precoated with RPA. The Brh2-Dss1 pair is particularly adept at mediating Rad51 filament assembly at a duplex-ssDNA junction that has a 3′ overhang polarity, which is in congruence with the ability of this protein complex to specifically recognize such a DNA junction. These results suggest that Brh2-Dss1 preferentially seeds Rad51 presynaptic filament assembly at the duplex-ssDNA junction of a resected DSB or another DNA lesion in vivo.

The sole BRC repeat of Brh2 can interact with the human Rad51 protein and, accordingly, the Brh2-Dss1 complex is functional as a recombination mediator with human Rad51 (71).

Owing to its uncommonly large size, full-length human BRCA2 protein has not yet been purified. To circumvent this problem, San Filippo et al. (72) fused the BRC3 and BRC4 repeats of BRCA2 to the DNA binding domain of this protein and devised a method to purify the BRCA2-derived polypeptide (termed BRC3/4-DBD) to near homogeneity. BRC3/4-DBD binds both ssDNA and dsDNA but with a distinct preference for the former. As expected, BRC3/4-DBD interacts with Rad51 but not RecA. A quantity of BRC3/4-DBD substoichiometric to Rad51 promotes the assembly of the Rad51 presynaptic filament on RPA-coated ssDNA efficiently, but this BRCA2-derived polypeptide is completely inactive toward RecA (72). Aside from its ability to restore presynaptic filament assembly, BRC3/4-DBD also specifically targets Rad51 to ssDNA when an excess of dsDNA is present. Polypeptides that harbor just the BRC3 and BRC4 repeats or the DBD, when used alone or in combination, do not exhibit recombination mediator or ssDNA targeting activity, indicating that both the BRC repeats and the DBD are needed for biological efficacy and they act in *cis* (i.e., both entities have to be present on the same polypeptide). Thus, BRC3/4-DBD, and by inference BRCA2 protein, possesses two distinct functional properties, i.e., an ability to target Rad51 to ssDNA and a recombination mediator activity, that are germane for the promotion of Rad51-mediated HR reactions. That BRC3/4-DBD possesses recombination mediator activity is congruent with a recent finding that fusion proteins comprising RPA and either a single BRC repeat or multiple such repeats can improve the HR proficiency of Brca2-deficient hamster cells and enable these cells to form Rad51 foci upon DNA damaging treatment (129).
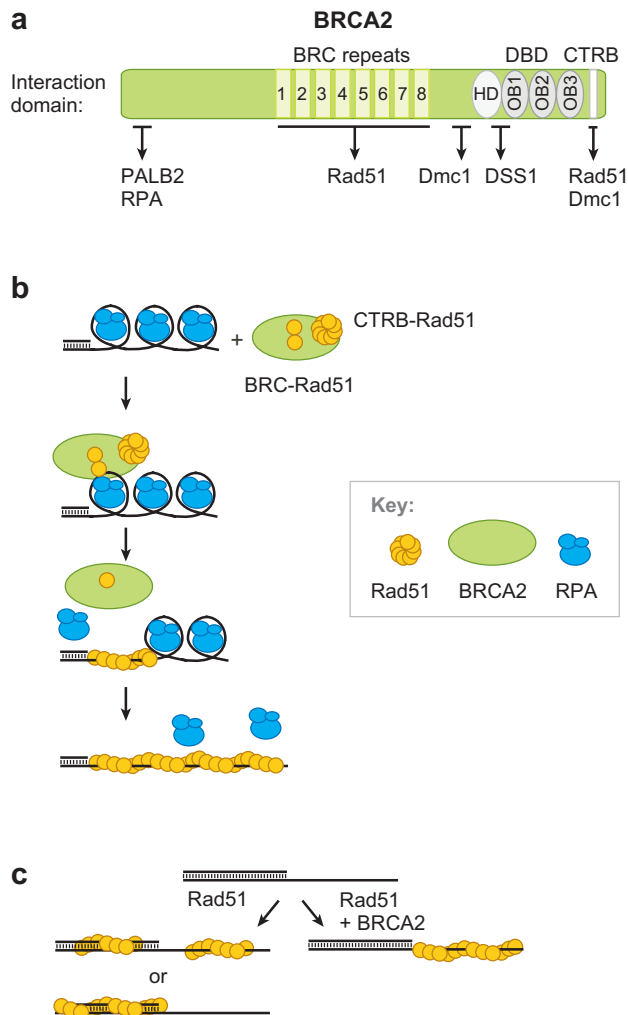
A MODEL FOR THE BRCA2 RECOMBINATION MEDIATOR ACTIVITY AND SOME QUESTIONS

We present in **Figure 3***a* model that incorporates all the biochemical attributes believed to be germane for the recombination mediator activity of BRCA2. Of special note is the cooperative action of the BRC repeats and CTRB domain in the assembly of the nascent Rad51 filament, with the former acting as the initial depositor of monomeric Rad51 onto the HR substrate and the latter being a recruiter of an intact Rad51 oligomer to seed the assembly of the nascent presynaptic filament.

As reviewed above, recent studies have provided evidence for functional differences among the BRC repeats of BRCA2 and possibly a presynaptic filament stabilizing activity in some (or all) of these repeats (122, 123). Future studies will address the contributions of the individual BRC repeats toward presynaptic filament assembly and preservation and the mechanistic basis for their action. BRCA2 is also able to interact with Dmc1 (115, 116), and studies in *A. thaliana* have provided direct evidence for functional interactions between Brca2 and Dmc1 in meiotic HR (51). It seems reasonable to suggest that BRCA2 also serves to nucleate Dmc1 presynaptic filament assembly on resected DSBs during meiotic HR. Even though the CTRB domain of BRCA2 interacts with Rad51 more avidly than Dmc1, the phosphorylation of S3291 in this domain attenuates Rad51 binding without significantly affecting the association with Dmc1 (116). It will be interesting to test whether CDK-mediated phosphorylation of S3291 in BRCA2 provides a means for the differential regulation of BRCA2-Rad51 and BRCA2-Dmc1 interactions.

THE BRCA2-ASSOCIATED PROTEINS DSS1 AND PALB2 (FANCN)

BRCA2 forms a complex with the small acidic protein DSS1 (130), which is needed for HR

Figure 3

BRCA2 and its role in homologous recombination. (*a*) The functional domains in human BRCA2 are indicated. (*b*) In this model for the BRCA2 recombination mediator activity, the BRC repeats each bind a Rad51 monomer (for simplicity, only two of the six functional BRC repeats are depicted as being associated with Rad51), while the CTRB is charged with an intact Rad51 heptamer. One of the BRC repeats and the CTRB then cooperate to assemble a nascent Rad51 filament, followed by the further displacement of RPA by the growing Rad51 filament. (*c*) Recent results (72) suggest a function of BRCA2 in targeting Rad51 specifically to the ssDNA region of a resected DSB.

efficiency in vivo (131, 132). In *U. maydis*, the Dss1 orthologue is thought to maintain Brh2 in an active state by preventing the formation of Brh2 homo-oligomers (128). Whether mammalian DSS1 serves a similar role has not

yet been explored. Since the DSS1 interaction surface resides within the DNA binding domain of BRCA2 (79), DSS1 may well modulate the DNA binding properties of the latter. Even though a homologue of DSS1 (called Sem1) is present in the budding yeast, it appears to affect diverse processes such as exocytosis (133) and DSB repair by HR and NHEJ (134) via an association with the proteasome (134, 135).

A novel BRCA2-associated protein called PALB2 was recently identified by Xia et al. (136). PALB2 promotes the proper localization and maintains the stability of BRCA2 in chromatin and nuclear matrix and appears to be critical for the DNA repair and checkpoint functions of BRCA2 (136). The PALB2 interaction domains lies within the amino terminus of BRCA2, and the cancer-associated Y42C mutation that affects the interaction of BRCA2 with RPA (83) also ablates PALB2 binding (136). PALB2 possesses a series of WD40 repeats at its carboxyl terminus (136, 137) that are indispensable for complex formation with BRCA2 and the biological activity of PALB2 (137). Importantly, mutations in PALB2 are associated with familial breast cancer (138, 139) and biallelic inactivation of PALB2 can cause Fanconi anemia (FA) of complementation group N (140, 141) (see sidebar). Owing to its FA connection, PALB2 is also known as FANCN. How the HR function and specifically how the recombination mediator activity of BRCA2 may be influenced by PALB2 will undoubtedly be a hotly pursued area of research in the near future.

THE *S. CEREVISIAE* RAD55-RAD57 COMPLEX AND ITS RECOMBINATION MEDIATOR ACTIVITY

Mutants of *RAD55* and *RAD57* genes share the uncommon property of being cold sensitive for HR and DNA repair (19, 142), and both genes are required for the delivery of Rad51 to HR substrates in cells (73, 76, 77). Overexpression of Rad51 or Rad52 partially overcomes the DNA repair and HR deficits of *rad55* and *rad57* mutant cells, and simultaneous overexpression of Rad51 and Rad52 leads to further suppression of the mutant phenotype (143, 144). The *RAD55*- and *RAD57*-encoded proteins are regarded as paralogues of Rad51 as they exhibit significant homology to Rad51, including sequence motifs that are thought to confer the ability to bind and hydrolyze ATP (19, 145). Rad55 protein is phosphorylated in a checkpoint-dependent manner when DNA damage occurs, and this modification appears to be important for Rad55 function upon genome-wide genotoxic stress (146, 147). Interactions between Rad55 and Rad57 and of Rad55 with Rad51 have been noted in the yeast two-hybrid system (143, 144). The majority or all of the Rad55 and Rad57 proteins in yeast cell extract can be coimmunoprecipitated, indicating that they are associated as a stable complex in cells (148). When coexpressed in yeast cells, the Rad55 and Rad57 proteins assemble into a heterodimeric complex that has ssDNA binding activity and the ability to interact with Rad51 (148; P. Sung, unpublished results). Even though results from a genetic study suggest that biological activity of the Rad55 protein is influenced by ATP (144), the Rad55-Rad57 complex does not seem to have a significant ATPase activity and its DNA binding function appears to be refractory to ATP (P. Sung, unpublished results). Despite the similarity of both Rad55 and Rad57 to Rad51, the Rad55-Rad57 complex has no recombinase activity (148). Addition of an amount of the purified Rad55-Rad57 complex substoichiometric to Rad51 overcomes the inhibitory effect of RPA on Rad51-mediated homologous DNA pairing and strand exchange (148), indicative of a recombination mediator activity. As deduced from the analysis of a *RAD51* allele (*RAD51 I345T*) that affords partial suppression of the *rad55* and *rad57* mutant phenotype, the Rad55-Rad57 complex possibly also stabilizes the already assembled Rad51 presynaptic filament (149).

Much remains to be learned about the HR function of the Rad55-Rad57 complex. Most notably, whether the recombination mediator activity of Rad55-Rad57 involves a specific interaction with RPA, as has been demonstrated for Rad52, is not yet known. The role, if any, of Rad55 phosphorylation and of ATP in the modulation of the recombination mediator function of Rad55-Rad57 are also important questions that need to be answered.

THE HUMAN RAD51B-RAD51C COMPLEX AND ITS RECOMBINATION MEDIATOR ACTIVITY

The *RAD51B* and *RAD51C* genes code for two of the five Rad51 paralogues (the remaining three Rad51 paralogue-encoding genes are *RAD51D*, *XRCC2*, and *XRCC3*) in vertebrate cells (19). There is good evidence that all the Rad51 paralogues participate in HR by influencing the assembly and/or maintenance of the Rad51 presynaptic filament (19, 150), and the overexpression of human Rad51 protein suppresses the defects of chicken DT40 cells that lack any one of the five Rad51 paralogues (151). The Rad51B and Rad51C proteins interact in the yeast two-hybrid system (152) and form a stable complex as evidenced by coimmunoprecipitation and other means (153, 154). The Rad51B-Rad51C complex has been purified to near homogeneity and found to possess ssDNA binding activity and a modest ATPase activity that is stimulated by DNA, ssDNA in particular, but the DNA binding activity of this complex seems to be refractory to ATP (154). The DNA binding and ATPase activities of the Rad51B-Rad51C complex are derived from both Rad51B and Rad51C proteins (155). Biochemical experiments (154) revealed a recombination mediator activity in the Rad51B-Rad51C complex. Like other recombination mediators that have been characterized to date, Rad51B-Rad51C acts in a catalytic fashion in that optimal recombination mediator activity requires a relatively small amount of the complex (154).

Rad51B-Rad51C enhances the homologous DNA pairing activity of Rad51 (154), an effect that could stem from the ability of Rad51C to promote the melting of duplex DNA (155). Whether Rad51B-Rad51C interacts with RPA in nucleating Rad51 presynaptic filament assembly and if ATP binding and hydrolysis by the protein complex influence its recombination mediator function are still unanswered.

Yeast two-hybrid and biochemical studies have found a higher-order complex consisting of the Rad51B, Rad51C, Rad51D, and XRCC2 proteins, termed the BCDX2 complex (153). The BCDX2 complex has the highest affinity for branched DNA (156) and can recognize nicks in DNA (153). Rad51D and XRCC2 on their own form a heterodimeric complex that has DNA binding activity (157) but apparently no recombination mediator activity (W. Bussen & P. Sung, unpublished results). It will be particularly interesting to test whether the BCDX2 complex has enhanced recombination mediator activity compared to the Rad51B-Rad51C complex. Rad51C also combines with the XRCC3 protein to form a distinct protein complex that seems to be associated with a nuclease activity capable of resolving the HJ (158). This latter observation is consistent with an apparent late role of the XRCC3 protein in HR (159) and the fact that, in chicken DT40 cells, simultaneously ablating the *Xrcc3* and *Rad51D* genes engenders a mutant phenotype more severe than that of the respective single mutants (150).

THE *S. POMBE* SWI5-SFR1 COMPLEX AND ITS RECOMBINATION MEDIATOR ACTIVITY

As determined by a combination of yeast two-hybrid, coimmunoprecipitation, and genetic analyses, *S. pombe* Swi5 protein combines with either Swi6 or Sfr1 to form two separate complexes that have distinct functions. Specifically, the Swi5-Swi6 complex plays a role

in mating-type switching (160), whereas the Sfr1-Swi5 complex is needed for mitotic and meiotic HR (64, 160). The *sfr1* and *swi5* null mutants are partially impaired for the ability to assemble DNA damage–induced foci of Rph51 (which is the *S. pombe* Rad51 orthologue), and the DNA repair defects of these mutant cells can be partially suppressed by the overexpression of Rhp51. The Sfr1-Swi5 complex appears to provide a function in HR similar to that of the Rhp55-Rhp57 complex (which is orthologous to the *S. cerevisiae* recombination mediator Rad55-Rad57 complex), as *swi5*, *rph57* double mutant cells are more severely HR impaired and deficient in DNA damage–induced Rph51 focus formation than the single mutants (161). Taken together, the genetic and cytological observations suggest that Swi5-Sfr1 regulates Rph51 presynaptic filament assembly and/or maintenance, and that it acts independently of the Rph55-Rph57 complex in this regard (161).

The Sfr1-Swi5 complex (which harbors one Sfr1 molecule and two Swi5 molecules) has been expressed in *E. coli*, purified, and characterized by Haruta et al. (64). The Sfr1-Swi5 complex physically interacts with both Rph51 and Dmc1 through Sfr1 (64, 160). Sfr1-Swi5 enhances the homologous DNA pairing and strand exchange activity of Rph51 and Dmc1 and can function in conjunction with both recombinases in the displacement of RPA from ssDNA. Thus, the biochemical analyses of Haruta et al. (64) reveal a recombination mediator activity in the Sfr1-Swi5 complex and also an ability of this complex to stimulate the homologous DNA pairing and strand exchange potential of the two recombinases.

RELATIONSHIP OF THE *S. CEREVISIAE* MEI5-SAE3 COMPLEX TO THE *S. POMBE* SWI5-SFR1 COMPLEX

The *S. cerevisiae* *MEI5-* and *SAE3*-encoded proteins are structurally related to the *S. pombe* Sfr1 and Swi5 proteins, respectively (74). Un-like their *S. pombe* counterpart, the expression of Mei5 and Sae3 proteins is restricted to meiosis (74, 162). Deletion of *MEI5* or *SAE3* impairs meiotic HR and the ability to mount nuclear Dmc1 foci in response to meiotic DSB formation. Taken together, the mutant and cytological analyses provide evidence that Mei5, Sae3, and Dmc1 proteins operate in the same recombination pathway and suggest a critical role of Mei5 and Sae3 in the delivery of Dmc1 to the HR substrate (74, 162). Mei5 and Sae3 proteins form a complex that physically interacts with Dmc1 (74). Considering what is known about the functional properties of the *S. pombe* Sfr1-Swi5 complex (64), it will be particularly relevant to test whether the Mei5-Sae3 complex enhances the recombinase activity of Dmc1 and Rad51 and provides a recombination mediator activity for the two recombinases.

BIPARTITE ACTION OF THE HOP2-MND1 COMPLEX IN RECOMBINASE ENHANCEMENT

That *HOP2* and *MND1* genes are critical for meiotic recombination was demonstrated in genetic studies in *S. cerevisiae* and mice (163–169). Based on extensive genetic analyses in *S. cerevisiae*, it has been deduced that the Hop2 and Mnd1 proteins function with Rad51 and Dmc1 to ensure the timely formation of DNA intermediates critical for the completion of meiotic recombination (163–165, 167, 168). Although the expression of *HOP2* and *MND1* is restricted to meiosis in *S. cerevisiae*, these genes are also expressed in somatic tissues in plants, mice, and humans (165, 169–171). This latter observation hints at the possibility that in higher eukaryotes, the *HOP2-* and *MND1*-encoded products influence mitotic HR as well.

The Hop2 and Mnd1 proteins can be coimmunoprecipitated from meiotic *S. cerevisiae* cell extract, indicating that they exist in a complex (167). Consistent with this finding, when coexpressed in *E. coli*, Hop2 and Mnd1

proteins assemble into a stable, heterodimeric complex (163, 172, 173). The Hop2-Mnd1 complex binds dsDNA preferentially over ss-DNA (42, 163, 173) and appears to have an even higher affinity for branched DNA (172). Studies using purified components revealed that the mouse Hop2-Mnd1 complex directly interacts with Rad51 and Dmc1 (174) but not with *E. coli* RecA (42). Although Hop2 and Mnd1 proteins can individually bind DNA and interact with Rad51 and Dmc1 (42, 173), Hop2 has much higher affinity for DNA and Mnd1 possesses greater avidity for Rad51 (42). The Hop2-Mnd1 complex from mammalian and yeast species strongly stimulates the recombinase activity of Dmc1 (163, 172, 174), and the mouse and human Hop2-Mnd1 complexes are just as active toward Rad51 in this regard (172, 174). Because Hop2-Mnd1 does not enhance the recombinase activity of the *E. coli* RecA protein (42), physical association of Hop2-Mnd1 with Rad51 and Dmc1 is likely important for functional interaction to occur.

Recent studies by Chi et al. (42) and Pezza et al. (43) have revealed that recombinase enhancement afforded by the Hop2-Mnd1 complex occurs at two critical stages of the homologous DNA pairing reaction. First, Hop2-Mnd1 stabilizes the presynaptic filament of Rad51 and Dmc1, as shown using a variety of approaches (42, 43) when the presynaptic filament is rendered stable by the use of a nonhydrolyzable ATP analogue, calcium ion, or the Rad51 K133R protein (which binds but does not hydrolyze ATP), Hop2-Mnd1 still exerts a strong stimulatory effect on DNA joint formation, leading to the deduction that it must also act at a stage subsequent to presynaptic filament assembly. Importantly, Chi et al. and Pezza et al. have shown that the Hop2-Mnd1 complex works in conjunction with the presynaptic filament to capture duplex DNA molecule to facilitate the assembly of the synaptic complex (42, 43; P. Chi & P. Sung, unpublished data). Duplex capture by Hop2-Mnd1 and Rad51 or Dmc1 is not de-pendent on homology in the incoming duplex molecule (42, 43) but requires a functional presynaptic filament (42). Thus, Hop2-Mnd1 acts in a bipartite fashion in Rad51/Dmc1-mediated homologous DNA pairing: stabilization of the presynaptic filament and duplex capture to enhance synaptic complex formation.

Figure 4 presents a model that depicts the bipartite action of the Hop2-Mnd1complex in its enhancement of Rad51 and Dmc1 activity. Future studies will determine the relative importance of the presynaptic filament stabilization and duplex capture roles of Hop2-Mnd1 in HR reactions. Since the Hop2-Mnd1 complex appears to have a high affinity for branched DNA structures (172; J. San Filippo & P. Sung, unpublished results), it remains possible that Hop2-Mnd1 recognizes and stabilizes the nascent DNA loop formed by the two recombinases.

THE MULTIFUNCTIONAL ROLE OF THE DNA MOTOR PROTEIN RAD54 IN HR

Rad54 is a member of the Swi2/Snf2 super-family of proteins and, similar to other members of that family, has dsDNA-dependent ATPase, DNA translocase, DNA supercoiling and chromatin remodeling activities. Recent reviews (175, 176) have summarized some of the roles of this multifunctional factor in HR. Notably, Rad54 interacts with Rad51 and is required at multiple stages in HR, in the early stages to promote a search for DNA homology, chromatin remodeling, and D-loop formation, and in the postsynaptic stage to catalyze the removal of Rad51 protein from dsDNA. The ability of Rad54 to remove Rad51 from dsDNA is believed to prevent the nonspecific association of Rad51 with bulk chromatin and to provide DNA polymerases access to the 3′-OH primer terminus in the nascent D loop to initiate the repair DNA synthesis reaction (175). Rad54 also mediates the ATP hydrolysis-driven

Figure 4

The bipartite action of the Hop2-Mnd1 complex in recombinase enhancement. Hop2-Mnd1 acts in two critical steps to enhance the recombinase activity of Rad51. Hop2-Mnd1 first stabilizes the presynaptic filament and then cooperates with the presynaptic filament to capture a dsDNA molecule (42). Hop2-Mnd1 also functionally interacts with Dmc1 in the same fashion (43).
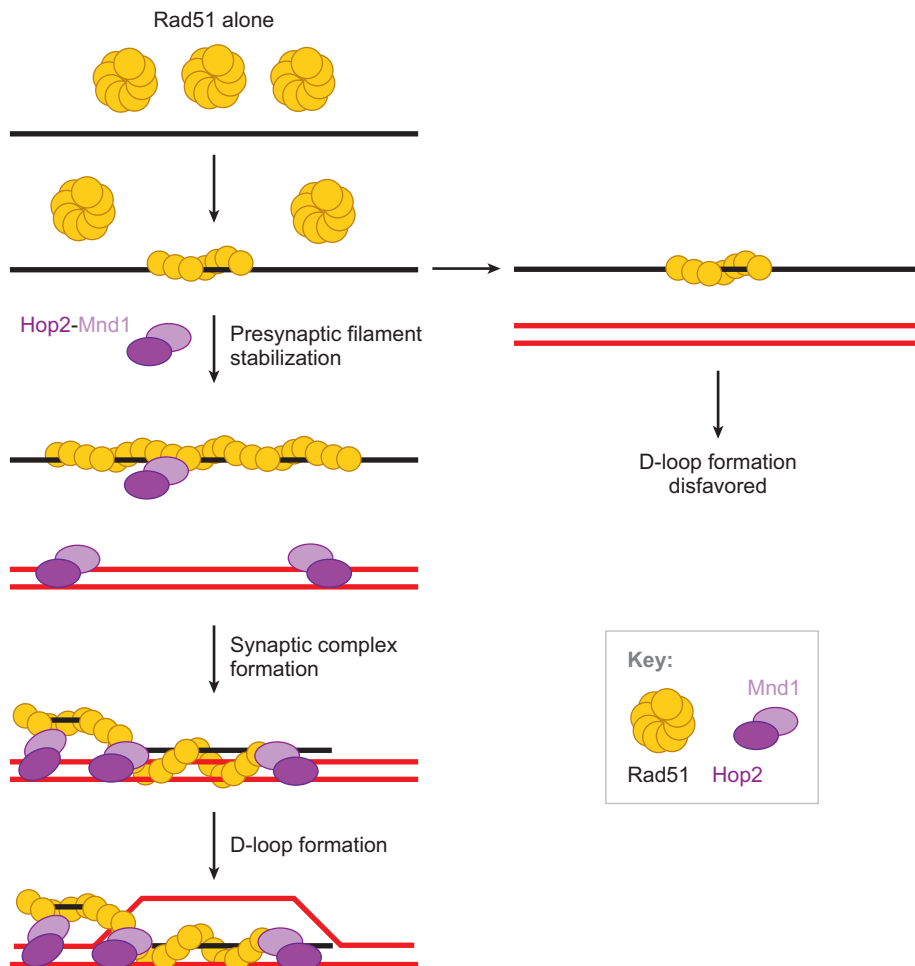
migration of branched DNAs including the HJ and acts in conjunction with Rad51 to promote a DNA strand exchange reaction that involves two duplex molecules (47). Under certain in vitro conditions, Rad54 can dissociate the D-loop intermediate, an activity thought to be relevant for the promotion of SDSA (177). By ChIP, the synapsis of the *MAT* initiator and *HML* donor sequences can be detected in the absence of Rad54 (76). Whether this reflects a role of Rad54 in plectonemic DNA joint formation or the initiation of repair DNA synthesis remains to be established (76).

Rad51 and Rad54 enhance each other's activities (47, 175). Given the multifaceted role

of Rad54 in HR, one might expect *RAD54* to be essential to HR and DSB repair. While yeast *rad54* mutants are extremely sensitive to ionizing radiation and other types of DNA damage that induce DSBs and are severely reduced in spontaneous and induced mitotic recombination (2, 3), *rad54* mutants are among the least debilitated in meiosis of the HR mutants. In contrast to *rad51* and *rad52* mutants, *rad54* mutants are able to form viable meiotic products, although with reduced efficiency compared to wild type (178, 179). The lack of a strong meiotic phenotype of *S. cerevisiae rad54* mutant cells can be attributed to the Rad54-related protein Rdh54 (179; see below). Nonetheless, overexpression of Rad54

can suppress the meiotic defects caused by the *dmc1*Δ mutation (180). In mouse ES cells, loss of Rad54 results in a slightly reduced HR frequency, sensitivity to ionizing radiation and mitomycin C, and aberrant repair of DNA damage, whereas *rad54*$^{-/-}$ mice appear to be normal (183, 184). This stands in contrast to other mammalian HR genes such as *RAD51*, as loss of Rad51 in mouse ES cells is lethal and the mouse *rad51*$^{-/-}$ genotype is an embryonic lethal (6, 185).

The stronger mitotic phenotype of *rad54* mutants in yeast and mammals may reflect a preferential action of Rad54 in promoting DSB repair between sister chromatids. Indeed, genetic studies in *S. cerevisiae* have suggested that Rad54 acts in sister chromatid recombination (186). The modest knockout phenotype in vertebrates may reflect the predominant use of nonhomologous end joining (NHEJ) to repair DSBs. This suggestion is supported by the finding that chromosome loss and rearrangements are increased in *rad54* mutants in yeast and mammalian cells, and that loss of the NHEJ pathway in combination with a *rad54* mutation has a synergistic effect on chromosome instability, DNA-damage sensitivity, and cell growth (187). This shows that mammalian Rad54 is critical for DSB repair and for maintenance of genomic stability. Several *hRAD54* mutations that reduce or eliminate Rad54 activity in vitro have been found in human tumors, suggesting an important role of Rad54 in cancer avoidance (188–191).

RAD54-RELATED DNA MOTOR PROTEINS: *S. CEREVISIAE* RDH54 AND HUMAN RAD54B

RAD54 paralogues exist in both yeast and mammals. The mammalian paralogue is called *RAD54B*. The Rad54B protein has biochemical activities that are similar to those of Rad54 (191–193). Rad54B interacts with Rad51, has a dsDNA-dependent ATPase activity, and can translocate on duplex DNA to result in topological changes in the DNA and the transient opening of the DNA strands. Similar to Rad54, Rad51 enhances the activities of Rad54B, and Rad54B promotes D-loop formation by Rad51 (193). The recombinase activity of Dmc1 is also stimulated by Rad54B (55, 194).

Mouse ES cells deficient in Rad54B have no overt HR defect as measured by gene-targeting frequencies, but *Rad54*$^{-/-}$ *Rad54B*$^{-/-}$ cells are decreased in gene-targeting efficiency below that of the *Rad54*$^{-/-}$ single mutant, showing that Rad54B functions in HR and this is revealed only when Rad54 is absent (193). Studies of ionizing radiation and mitomycin C sensitivities showed that mouse Rad54B has a role in repairing DNA damage caused by these agents. Mice that are deficient in both Rad54 and Rad54B are very sensitive to mitomycin C treatment, with particular damage to the bone marrow. While *Rad54*$^{-/-}$ mice show some abnormalities in meiotic chromosome structure, *Rad54*$^{-/-}$, *Rad54B*$^{-/-}$, and *Rad54*$^{-/-}$ *Rad54B*$^{-/-}$ mice are fertile (193).

S. cerevisiae also harbors a *RAD54* paralogue, called *RDH54* or *TID1* (179, 195). However, based on mutant phenotypes, particularly the meiotic mutant phenotype, *RDH54* is not equivalent to *RAD54B*. Rdh54 protein has functional attributes similar to those of Rad54, including dsDNA-dependent ATPase, DNA translocase, DNA branch migration, and DNA supercoiling activities, and also the ability to enhance the Rad51-mediated D-loop reaction (196–199). It also can remove Rad51 from duplex DNA in an ATP hydrolysis-dependent fashion (196), an activity that may become important in the later stages of HR (196), and in the adaptation from a DSB-induced checkpoint arrest (200). Rdh54 seems to be able to remove Dmc1 from nonrecombinogenic chromatin sites as well (201; P. Chi & P. Sung, unpublished results).

Rdh54 interacts with Dmc1 (202). Although the meiotic role of Rdh54 is not fully understood, *rdh54* mutants are severely

reduced in meiotic viability and the double mutant *rad54 rdh54* fails to form viable meiotic progeny or repair meiotic DSBs (179, 203). The mitotic phenotype of the double mutant is most visible in diploid cells, seen as a failure to grow uniformly owing to spontaneous DNA damage and checkpoint arrest. *rdh54* mutants are also defective in resuming growth after a DSB-induced checkpoint arrest (200). These findings indicate that aside from some functional overlap, *RAD54* and *RDH54* have independent functions in HR, DSB repair, and other processes.

Genetic studies by several groups have suggested that in meiosis Rad54 may have a prominent role in promoting DSB repair through HR between sister chromatids, whereas Rdh54 is required for interhomologue HR (180–182, 186). How this distinction is made at the molecular level is not known.

While Rad54 and Rdh54 can move Rad51 bound to duplex DNA and can remodel chromatin in vitro, in vivo other DNA or chromatin binding proteins may well be targets of these proteins. A recent study (204) has found that the meiotic-specific sister chromatid cohesion factor Rec8 and the mitotic sister chromatid cohesion factor Mcd1/Scc1 shows aberrant distribution on chromosomes in mutant *rdh54* meioses. Chromosomes are mis-segregated in both of the meiotic divisions, which is attributed to a failure of sister chromatid separation. Whether Rdh54 acts directly to remodel cohesin through Mcd1/Scc1 and Rec8 is not known. However, loading of cohesin at DSBs is critical for repair of mitotic DSBs (205–208), so it is conceivable that cohesin loading and remodeling at meiotic DSBs by Rdh54 is critical for proper meiotic interhomologue HR. Mcd1/Scc1association on chromatin during meiosis in the absence of Rdh54 appears to be the cause of chromosome mis-segregation. One intriguing possibility is that Mcd1/Scc1-mediated cohesion is important in distinguishing sister from nonsister chromatids in

HR. If true, then the deficiency of *rdh54* mutant cells in interhomologue HR could arise from an inability of nonsister chromatids to interact due to persistent cohesion of the sister chromatids.

CONCLUSIONS

Defects in HR cause genomic instability. When the instability leads to aberrant expression or regulation of tumor suppressors or oncogenes, cell transformation and cancer may ensue. Because HR can give rise to alterations in the genomic configuration, it must be finely controlled to avoid deleterious chromosome rearrangements and the generation of pathological intermediates (8, 9).

As detailed here and elsewhere (8, 9, 33), the basic HR machinery and its mechanism and regulation are remarkably conserved among eukaryotes. The HR reaction mediated by either Rad51 or Dmc1 has at least two rate-limiting steps, assembly of the presynaptic filament and capture of duplex DNA by the presynaptic filament. As has been reviewed in this article, recent biochemical studies have unveiled recombinase accessory factors that function to overcome these rate-limiting steps.

One of the most exciting developments in understanding HR mechanism and its health relevance is the identification of the tumor suppressor BRCA2 as a recombination mediator. Aside from interactions with Rad51, Dmc1, RPA, and DNA, additional domains within BRCA2 mediate its association with other factors, such as PALB2, that are important for its biological functions. Whether these factors influence the recombination mediator activity of BRCA2 is not yet known. Moreover, how the BRCA2-PALB2 complex functionally links the HR machinery to the FA pathway of DNA-damage repair and response remains to be delineated.

Studies on the Swi2/Snf2-like DNA motor proteins Rad54, Rdh54 and Rad54B have begun to elucidate their multifaceted role in HR. Rad54 and Rdh54 help determine the

selection of the sister chromatid or nonsister chromatid as HR substrate. The genetic consequences of recombination between the sister or nonsister chromatids have a profound effect on sequence variation and meiotic chromosome segregation. How this distinction is made or regulated will be a particularly interesting problem to tackle.

SUMMARY POINTS

1. Homologous recombination is required for DNA double-strand break repair, repair of damaged replication forks, repair of incomplete telomeres, and correct segregation of homologous chromosomes in meiosis.

2. Homologous recombination is critical for suppression of genome instability and tumor formation.

3. Homologous recombination is mediated by recombinases, a conserved group of proteins from bacteria to humans. The eukaryotic recombinases, orthologues of *E. coli* RecA, are Rad51 and Dmc1.

4. Rad51 and Dmc1 mediate the homologous DNA pairing and strand exchange reaction through the presynaptic filament, single-stranded DNA coated with Rad51 or Dmc1.

5. Presynaptic filament assembly is slow and prone to interference by the single-stranded DNA binding protein RPA and so requires the involvement of recombination mediator proteins, such as BRCA2, for enhancement.

6. Synaptic complex formation by Rad51 and Dmc1 are enhanced by the Hop2-Mnd1 complex, revealing that capture of homologous duplex DNA is a rate-limiting step in homologous recombination.

7. Several DNA motor proteins, such as Rad54 and Rdh54, function at several steps in homologous recombination. They promote homologous pairing and strand exchange on naked DNA and chromatinized DNA and help in recycling the recombinases by removing them from duplex DNA before and after the DNA strand invasion step.

FUTURE ISSUES

1. Which DNA polymerase(s) is used in vivo in the repair DNA synthesis step of homologous recombination and is this difference dependent on which recombination pathway is used?

2. Which nuclease(s) generates the ssDNA during mitotic homologous recombination?

3. How does resolution of homologous recombination intermediates occur and what is the biochemical role of the Rad51 paralogues in this reaction?

4. How do chromosome architecture and chromatin modifications influence the biochemical steps in homologous recombination?

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Errata

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