Regulation of Homologous Recombination in Eukaryotes

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Abstract

Homologous recombination (HR) is required for accurate chromosome segregation during the first meiotic division and constitutes a key repair and tolerance pathway for complex DNA damage, including DNA double-strand breaks, interstrand crosslinks, and DNA gaps. In addition, recombination and replication are inextricably linked, as recombination recovers stalled and broken replication forks, enabling the evolution of larger genomes/replicons. Defects in recombination lead to genomic instability and elevated cancer predisposition, demonstrating a clear cellular need for recombination. However, recombination can also lead to genome rearrangements. Unrestrained recombination causes undesired endpoints (translocation, deletion, inversion) and the accumulation of toxic recombination intermediates. Evidently, HR must be carefully regulated to match specific cellular needs. Here, we review the factors and mechanistic stages of recombination that are subject to regulation and suggest that recombination achieves flexibility and robustness by proceeding through metastable, reversible intermediates.

INTRODUCTION

Homologous recombination (HR): recombination between identical or nearly identical sequences

Homologous recombination (HR) is a key pathway to maintain genomic integrity between generations (meiosis) and during ontogenic development in a single organism (DNA repair). A typical diploid human cell needs to maintain about 6×10^9 base pairs in the correct sequence and chromosomal organization, a formidable task that is usually performed nearly perfectly from one somatic cell generation to the next (44). Recombination is required for the repair or tolerance of DNA damage and the recovery of stalled or broken replication forks (95). However, recombination is also potentially dangerous as it can lead to gross chromosomal rearrangements and potentially lethal intermediates (89). Not surprisingly, defects in HR and associated processes define a number of human cancer predisposition syndromes associated with genome instability (see sidebar, DNA Repair Proteins and Human Genetic Disease). How does the cell achieve the balance between too little and too much recombination? There must be regulation and the answer will depend on the organism, cell type, cell-cycle stage, chromosomal region, as well as the type and level of genotoxic stress.

HR in meiosis is subject to specific regulation that targets recombination events to homologs, establishing crossover outcomes to assist in meiotic chromosome segregation (46, 76). In addition, this volume contains a dedicated review on the RecQ helicases, which provides a much more comprehensive discussion of this important class of proteins than can be achieved here (15). Due to space limitations we refer the reader to recent reviews on how modulation of the DNA substrate affects HR (118, 157), including at specific nuclear territories such as telomeres (34) and the nucleolus (99).

Here, we review how recombinational DNA repair is regulated in mammalian somatic and yeast vegetative cells. We only include examples of meiotic regulation of HR factors that may also be applicable to somatic cells. The focus is on the mechanistic phases of recombination (**Figure 1**) and the factors that execute them (**Table 1**), identifying key regulatory transitions and mechanisms. We elaborate on how HR is modulated by multiple levels of positive and, primarily, negative regulation. Mechanisms of antirecombination appear to be integral to the HR pathway. We suggest that HR gains flexibility and robustness by proceeding through reversible, metastable intermediates.

DNA REPAIR PROTEINS AND HUMAN GENETIC DISEASE

Defects in DNA repair proteins lead to various inherited human diseases sharing common features such as genome instability and cancer predisposition (71). Mutations of several RecQ helicases, BLM, WRN, and RecQ4, cause Bloom, Werner, and Rothmund-Thomson syndromes, respectively. The other two RecQ helicases, RECQ1 and RECQ5, have not yet been implicated in human disease, but cellular studies demonstrate that they function in genome maintenance. Fanconi anemia (FA) is an autosomal recessive disorder characterized by progressive bone marrow failure. Mutations in *FANC* genes affect DNA interstrand crosslink repair; among them, *FANCJ* (= *BRIP*, *BACH1*), *FANCM*, *FANCD1* (= *BRCA2*), *FANCN* (= *PALB2*), and *RAD51C* are involved in HR. *BRCA1* and *BRCA2* are major breast and ovarian tumor suppressor genes and both function in HR. Defective ATM, a key sensor kinase in the DDR pathway, leads to ataxia telangiectasia (A-T), a neurodegenerative disease with severe physical disabilities. Mutations of *MRE11* and *NBS1* in the MRN complex cause similar diseases, A-T–like disorder and Nijmegen breakage syndrome, respectively. Mutations impairing DNA mismatch repair result in hereditary nonpolyposis colorectal cancer (HNPCC), an autosomal dominant disease with highly elevated risk for colon cancer.

MECHANISM OF HOMOLOGOUS RECOMBINATION

Significant strides have been made in identifying the proteins that catalyze HR in eukaryotes and defining their mechanisms of action (69, 91, 129, 161). HR can be conceptually divided into three stages—presynapsis, synapsis, postsynapsis—and we briefly discuss the principal proteins and structures involved (**Figure 1**).

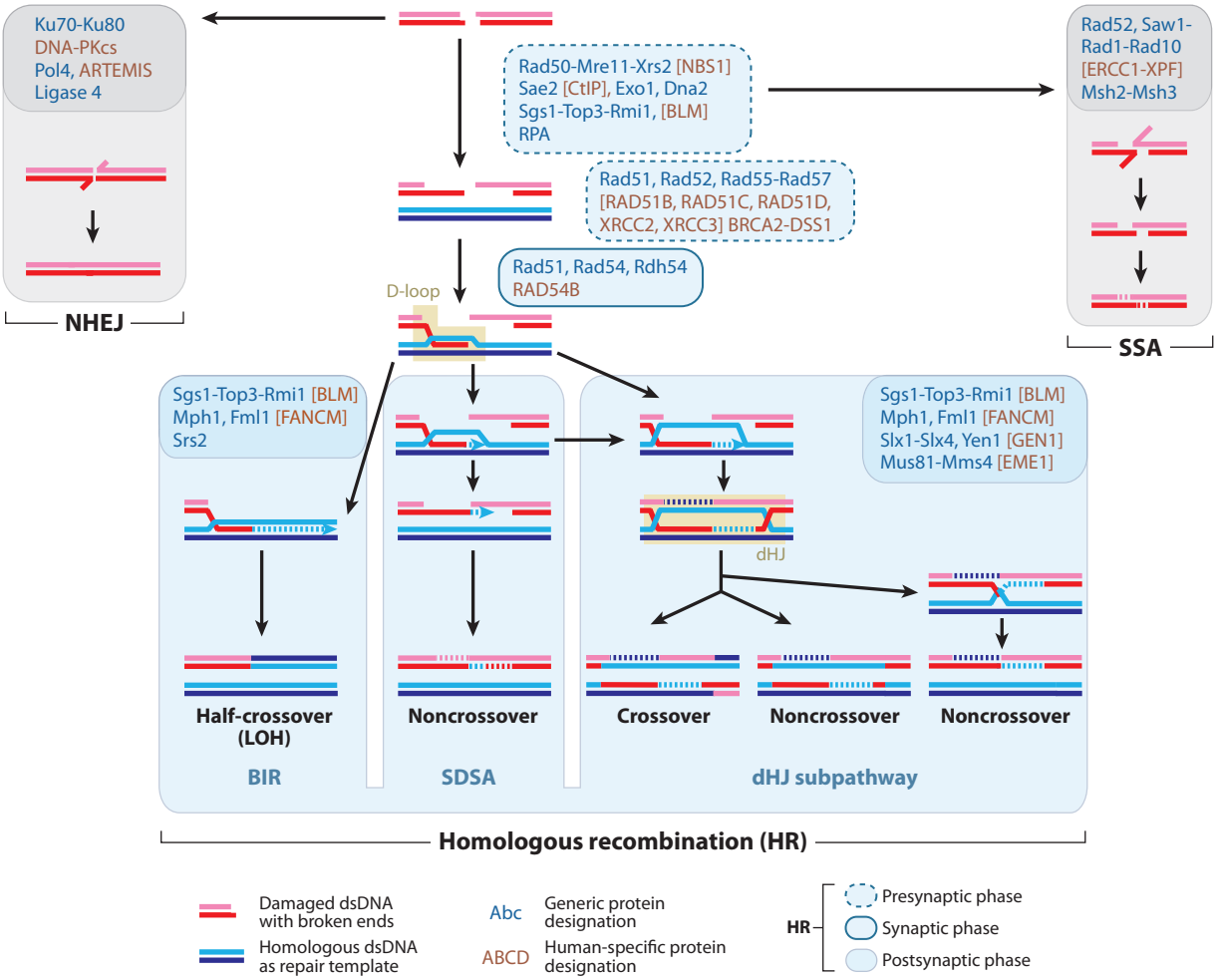

Figure 1

Pathways of double-strand break repair. Protein names refer to the budding yeast *Saccharomyces cerevisiae* (*blue*). Where different in human, names (*brown*) are given in brackets. For proteins without a yeast homolog, brackets for human proteins are omitted. Broken lines indicate new DNA synthesis and stretches of heteroduplex DNA that upon mismatch repair (MMR) can lead to gene conversion. Abbreviations: BIR, break-induced replication; dHJ, double Holliday junction; NHEJ, non-homologous end joining; LOH, loss of heterozygosity; SDSA, synthesis-dependent strand annealing; SSA, single-strand annealing.

In presynapsis, the DNA damage is processed to form an extended region of single-stranded DNA (ssDNA), which is bound by the ssDNA-binding protein RPA (replication protein A). For DNA double-strand breaks (DSBs) in the budding yeast *Saccharomyces cerevisiae*, this step involves a surprising complexity of four nuclease [Mre11-Rad50-Xrs2 (MRX) (human MRE11-RAD50-NBS1[MRN]), Exo1, Dna2, Sae2 (human CtIP)] and a helicase activity Sgs1 (human BLM; see sidebar) (104). Binding of RPA eliminates secondary structures in ssDNA, which is needed for competent Rad51 filaments to assemble. However, RPA bound to ssDNA also forms a kinetic barrier against Rad51 filament assembly, necessitating so-called mediator proteins to allow timely Rad51 filament formation on RPA-covered ssDNA. Three different classes of mediators have been described, but their mechanisms

DSB: double-strand break

MRX, MRN: *Saccharomyces cerevisiae* Mre11-Rad50-Xrs2 complex or human MRE11-RAD50-NBS1 complex

RPA: replication protein A

Table 1 Posttranslational modifications and their effects on proteins involved in homologous recombination

Protein	Organism	PTM	Function and PTM effect	Reference
BLM	*Homo sapiens*		Multiple roles in DNA damage signaling and HR	
		SUMO	BLM-K317/331-SUMO required for full activity and Rad51 focus formation after HU treatment	(47, 120)
BRCA1	*H. sapiens*		E3 ligase involved in HR and NHEJ	
		SUMO	PIAS1/4-dependent SUMO of BRCA1-K119 enhances BRCA1 UBI ligase activity	(107)
BRCA2	*H. sapiens*		RAD51 filament formation	
		PO_4	CDK-mediated PO_4 of S3291 inhibits RAD51 interaction of C-terminal RAD51 interaction site	(49)
CtIP	*H. sapiens*		DSB resection	
		PO_4	CDK-consensus site T847 required for CtIP activity (see also Sae2)	(75)
		PO_4	CDK-consensus site S237 required for BRCA1 binding and HR	(173)
Exo1	*Saccharomyces cerevisiae*		5′-3′ DNA exonuclease	
		PO_4	Rad53-mediated PO_4 of S372, 567, 587, 692 inhibits Exo1 activity	(106)
hEXO1	*H. sapiens*		5′-3′ DNA exonuclease	
		PO_4	ATR-dependent PO_4 leads to degradation	(48)
Nej1	*S. cerevisiae*		DNA ligase 4 cofactor	
		PO_4	Dun1-dependent PO_4 of Nej1-S297/8 enhances binding to Srs2 antirecombinase favoring NHEJ/SSA over HR	(27)
PCNA	*S. cerevisiae*		Processivity clamp	
		SUMO	PCNA-K164 (K127)-SUMO recruits Srs2 antirecombinase	(122, 124)
		UBI	PCNA-K164-UBI prevents SUMO, antirecombination effect by favoring TLS or fork regression	(122, 124)
RAD51	*H. sapiens*		Homology search and DNA-strand invasion	
		PO_4	CHK1-dep. PO_4 on T309 may be required for RAD51 focus formation	(141)
Rad52	*S. cerevisiae*		Rad51 filament formation, SSA	
		SUMO	Sumoylation of K10,11, 220[1] affects protein stability and intranuclear localization	(128, 152)
Rad54	*S. cerevisiae*		Cofactor for Rad51	
		PO_4	Mek1-mediated PO_4 at T132 inhibits Rad51 interaction during meiosis	(115)
Rhp54	*Schizosaccharomyces pombe*		Co-factor for Rad51	
		UBI	APC/C mediated ubiquitylation of Rhp54-K26 leads to proteolysis in G1 cells	(153)
Rad55	*S. cerevisiae*		Rad51 filament assembly/stability	
		PO_4	Rad55-S2,8,14 PO_4 required for full Rad55 activity	(68)
Sae2	*S. cerevisiae*		DSB resection	
		PO_4	CDK-mediated PO_4 at S267 required for Sae2 activity (see also CtIP)	(74)

PTM, post-translational modification; PO_4, phosphorylation; UBI, ubiquitylation; SUMO, sumoylation.

[1]These residues refer to the revised start codon of *RAD52* at methionine 33 (42) as denoted in reference (128), which corresponds to residues 43, 44, 210 in reference (152).

of action and the interplay between them is poorly understood. The Rad51 paralogs constitute a first group and comprise four proteins in two separate complexes in *S. cerevisiae* (Rad55-Rad57, Shu1-Psy3) and five in mammals (RAD51B, RAD51C, RAD51D, XRCC2, XRCC3). These proteins share the RecA core with Rad51, but fail to form extensive filaments on DNA and are unable to perform the range of DNA-pairing reactions catalyzed by Rad51. A second class is typified by the *S. cerevisiae* Rad52 protein, which performs two independent roles: its mediator function, and a second, later function in strand annealing of RPA-bound ssDNA. A third class of mediator proteins, apparently absent in *S. cerevisiae*, is exemplified by BRCA2, the human breast and ovarian cancer tumor suppressor protein. Human BRCA2 contains ssDNA binding motifs (OB-folds), a double-stranded DNA (dsDNA) binding motif (tower domain), and a number of Rad51 binding sites, suggesting that it targets RAD51 filament nucleation to the dsDNA junction at the resected end (168).

During synapsis, the Rad51 filament performs homology search and DNA-strand invasion, generating a displacement loop (D-loop) within which the invading strand primes DNA synthesis (**Figure 1**). The Rad54 motor protein is required for stabilizing the Rad51 filament and enhancing D-loop formation by Rad51, and for promoting the transition from DNA-strand invasion to DNA synthesis by dissociating Rad51 from heteroduplex DNA (70).

Finally, in postsynapsis, the three subpathways of HR are distinguished (**Figure 1**), each with specific enzymatic requirements that have been only partially defined (69, 91, 129, 161). As illustrated in **Figure 1**, the D-loop represents the branching point for the multiple subpathways of HR (BIR, SDSA, dHJ). In the absence of a second end, the D-loop may become a full-fledged replication fork in a process termed break-induced replication (BIR). Although this process restores the integrity of the chromosome, it can lead to loss-of-heterozygosity of all genetic information distal to the DSB. In the presence of a second end, the predominant pathway for DSB repair in somatic cells appears to be synthesis-dependent strand annealing (SDSA), in which the extended D-loop is reversed, leading to annealing of the newly synthesized strand with the resected strand of the second end (**Figure 1**) (123). This pathway inherently avoids crossovers, which reduces the potential for genomic rearrangements. While generation of crossovers by double Holliday junction (dHJ) formation is the purpose of meiotic recombination, recombinational DNA repair in somatic cells is rarely associated with crossovers. Only recently have dHJs been identified as an intermediate in recombinational DNA repair in vegetative (somatic) cells (25). dHJ formation involves capture of the second end, a process that is actively blocked by the Rad51 protein in vitro, suggesting an inherent mechanistic bias toward SDSA (167). The dHJ intermediate could be resolved by endonucleases in a manner described for the bacterial RuvC protein into crossover or noncrossover products (161), but the exact mechanisms and identity of proteins involved remain under debate (see **Figure 1**). Alternatively, dHJs can be dissolved by a complex mechanism involving a RecQ-family DNA motor protein (*S. cerevisiae* Sgs1 or human BLM), topoisomerase 3, and cofactors. The two junctions are migrated toward each other, leading to a hemicatenane that is eliminated by Topo3. Genetically, the end point of dissolution is always a noncrossover, avoiding the potential for rearrangements associated with crossovers (165). Crossovers are defined as recombination events that lead to the exchange of flanking markers (**Figure 1**) generating deletions, inversions, or translocations when non-allelic, repeated DNA sequences are involved.

Displacement loop (D-loop): primary DNA-strand invasion product of the Rad51-ssDNA filament leading to the different HR subpathways (BIR, SDSA, dHJ)

Break-induced replication (BIR): a subpathway of HR where a single-ended DSB invades and establishes a full-fledged replication fork

Holliday junction (HJ): Cross-stranded joint molecule HR intermediate

Synthesis-dependent strand annealing (SDSA): a subpathway of HR where the second end of the DSB anneals with the extended strand of the first end

Double Holliday junction (dHJ): HR intermediate leading to crossovers. Also used here to label the HR subpathway that involves this intermediate

REGULATORY CONTROL POINTS AND IRREVERSIBLE COMMITMENTS

A number of reversible posttranslational modifications on HR proteins, such as phosphorylation, ubiquitylation, and sumoylation, have recently been identified (14, 17)

CDK: cyclin-dependent kinase

Single-strand annealing (SSA): a mode of homology-directed DNA repair that does not involve Rad51-mediated DNA-strand invasion but does involve DNA reannealing

NHEJ: nonhomologous end joining

(**Table 1**). Some of these posttranslational modifications may lead to novel protein interactions, as indicated by the presence of phospho-, ubiquitin-, and SUMO-specific protein interaction motifs in factors that function in the DDR, DNA replication, DNA repair or HR (134). Irreversible modifications include proteolytic control of HR proteins (*S. cerevisiae* Rad52, *Schizosaccharomyces pombe* Rhp54) (**Table 1**) (128, 153) and exonucleolytic degradation of DNA and endonucleolytic processing of DNA junction intermediates (**Figure 1**). Several key HR intermediates, such as the Rad51-ssDNA filament, the D-loop, and the dHJ, constitute reversible intermediates and hence likely regulatory control points. Below, we provide a detailed discussion of regulatory targets and processes, as well as their mechanistic consequences for HR. While transcriptional regulation is at the heart of the bacterial DNA damage response (called SOS response) (36), there is little evidence of biologically significant transcriptional induction of HR genes by DNA damage in eukaryotes (30, 33, 86).

DOUBLE-STRAND BREAK REPAIR: COMPETITION BETWEEN HOMOLOGOUS RECOMBINATION, SINGLE-STRAND ANNEALING, AND NONHOMOLOGOUS END JOINING

HR, single-strand annealing (SSA), and nonhomologous end joining (NHEJ) are the principal pathways for DSB repair, and the balance between them depends on the species, cell type, cell-cycle stage, and type of DNA damage. NHEJ is a specialized ligation reaction with varying accuracy that depends on the end structure (**Figure 1**) (138). SSA is a homology-directed DNA repair pathway that promotes recombination between tandemly repeated DNA sequences, and involves reannealing of RPA-covered ssDNA by the Rad52 protein (**Figure 1**) (91). SSA does not involve DNA-strand invasion and is therefore independent of Rad51. This process leads to deletion of the interstitial DNA and one of the repeated homologous sequences.

How is the balance between NHEJ, SSA, and HR regulated? SSA and NHEJ can occur within the context of a single DNA molecule (**Figure 1**), whereas HR is a template-dependent process, typically involving two independent DNA molecules (sister chromatids, chromosomes). Studies in *S. cerevisiae* have demonstrated that the sister chromatid is the preferred template over a homolog, when given the choice (84). Sister chromatid cohesion likely provides the mechanistic underpinning for this preference (111). This could suggest that HR is entirely restricted to the S- and G2 phases of the cell cycle when a sister chromatid is present, but HR has also been demonstrated to occur in the G1 phase of budding yeast, using the homolog as a template (50). HR in G1 can only occur in diploid cells, and most organisms, including *S. cerevisiae*, are naturally diploid. The fission yeast *S. pombe*, on the other hand, is a naturally haploid organism, precluding recombinational DNA repair in the G1 phase. This provides a possible rationale as to why HR in fission yeast is downregulated in the G1 phase by targeting Rhp54 (fission yeast Rad54) for ubiquitin-mediated degradation (153).

In the budding yeast *S. cerevisiae*, the mating-type locus provides an example of complex regulation of HR in response to ploidy (156). The diploid-specific Mata1-Matα2 corepressor turns off haploid-specific genes and induces diploid-specific genes. One gene it regulates is Nej1, a cofactor for the principal NHEJ ligase, DNA ligase 4, which also recruits the Srs2 antirecombinase (see below) to resected ends. Downregulation of Nej1 thus shifts the balance from NHEJ or SSA to HR in diploid cells (5, 27, 53, 93). This also explains results of early radiobiological studies establishing that a/α diploid cells, which contain the Mata1-Matα2 corepressor, are more radiation-resistant than haploid cells or a/a or α/α diploid cells, which lack this co-repressor (109).

DSB resection is a key commitment step for homology-directed repair as both SSA and HR

depend on ssDNA. As discussed in detail below, DSB resection is highly regulated and low in the G1 phase, favoring NHEJ over HR and SSA (54). In budding and fission yeast, the NHEJ DNA end-binding factors Ku70-Ku80 inhibit DSB resection (92, 151). In vertebrate cells, the MRN complex, BRCA1, DNA PKcs, and ATM function in both NHEJ and HR (138). The MRN complex and BRCA1 are connected to resection, providing a possible regulatory target. Using elegant substrate design, it was shown that SSA and HR compete for the repair of DSBs in budding yeast and mammals (80, 143). Since SSA requires sufficient resection to expose direct repeats as ssDNA (**Figure 1**), the balance is expected to be highly locus and assay dependent.

How Is the Balance Between the Subpathways of Homologous Recombination (Break-Induced Replication, Synthesis-Dependent Strand Annealing, Double Holliday Junction) Regulated During Double-Strand Break Repair?

BIR, SDSA, and the dHJ subpathways of HR (**Figure 1**) lead to repair of a DSB but are associated with different genetic consequences. BIR can lead to loss-of-heterozygosity distal to the break site, which can have detrimental consequences if it creates two identical alleles bearing a deleterious mutation. dHJ formation has the potential to create genomic rearrangements if HR occurs in nonallelic sites. SDSA, which does not have these deleterious consequences, is the favored subpathway (123).

Experiments in *S. cerevisiae* demonstrated that the SDSA pathway outcompetes BIR in mitotic DSB repair, because BIR is a much slower process (100). Using an ingenious experimental setup, Haber and colleagues (81, 145) demonstrated that BIR is suppressed at the DNA synthesis step for over five hours after DSB formation. This suppression requires Sgs1, but surprisingly not its helicase activity (81). Mec1 kinase, the master regulator of the DNA damage response (DDR) in budding yeast (**Figure 3**), is not required to suppress BIR. Close proximity of the second end suppresses BIR (81), but it is unclear how this is communicated to the D-loop to prevent replication fork assembly. This may involve the end-tethering function of the MRX complex (41). Unlike meiotic HR, dHJs are formed only at low levels during mitotic DSB repair (25), consistent with the low association of mitotic DSB repair with a crossover outcome (78).

DNA GAP REPAIR: COMPETITION BETWEEN HOMOLOGOUS RECOMBINATION, TRANSLESION SYNTHESIS, AND FORK REGRESSION

Replication fork stalling leads to gaps resulting from downstream reinitiation by DNA polymerases on the leading and lagging strands (17, 67, 98). Stalled forks and gaps can be recovered by different pathways, including translesion synthesis (TLS), template switching by fork regression, or HR (17) (**Figure 2**). Although the accuracy of TLS is lesion and polymerase dependent (126), template switching by fork regression and HR is inherently highly accurate. TLS is favored by mono-ubiquitination of proliferating cell nuclear antigen (PCNA) on K164 by the Rad6-Rad18 E2-E3 complex (**Figure 2**), which enhances the intrinsic affinity of Y-family TLS polymerases (Pol eta) for PCNA through their ubiquitin binding motifs (126). In *S. cerevisiae*, subsequent polyubiquitylation of PCNA by Ubc13-Mms2 (E2) and Rad5 (E3) controls fork regression by a mechanism that is not understood (126). Alternatively, K164 (and K127) can be sumoylated by Ubc9, which leads to recruitment of the Srs2 antirecombinase through its SUMO binding motif (122, 124). As discussed in more detail below, Srs2 dissociates Rad51 from ssDNA, antagonizing Rad51-ssDNA filament formation (90, 158). It is unclear whether PCNA ubiquitylation and sumoylation can coexist in a hetero-trimeric PCNA ring, and the relationship between HR and these ubiquitylation and

DDR: DNA damage response

Translesion synthesis (TLS): DNA synthesis by specialized DNA polymerases that bypasses a template lesion without repairing it

PCNA: proliferating cell nuclear antigen

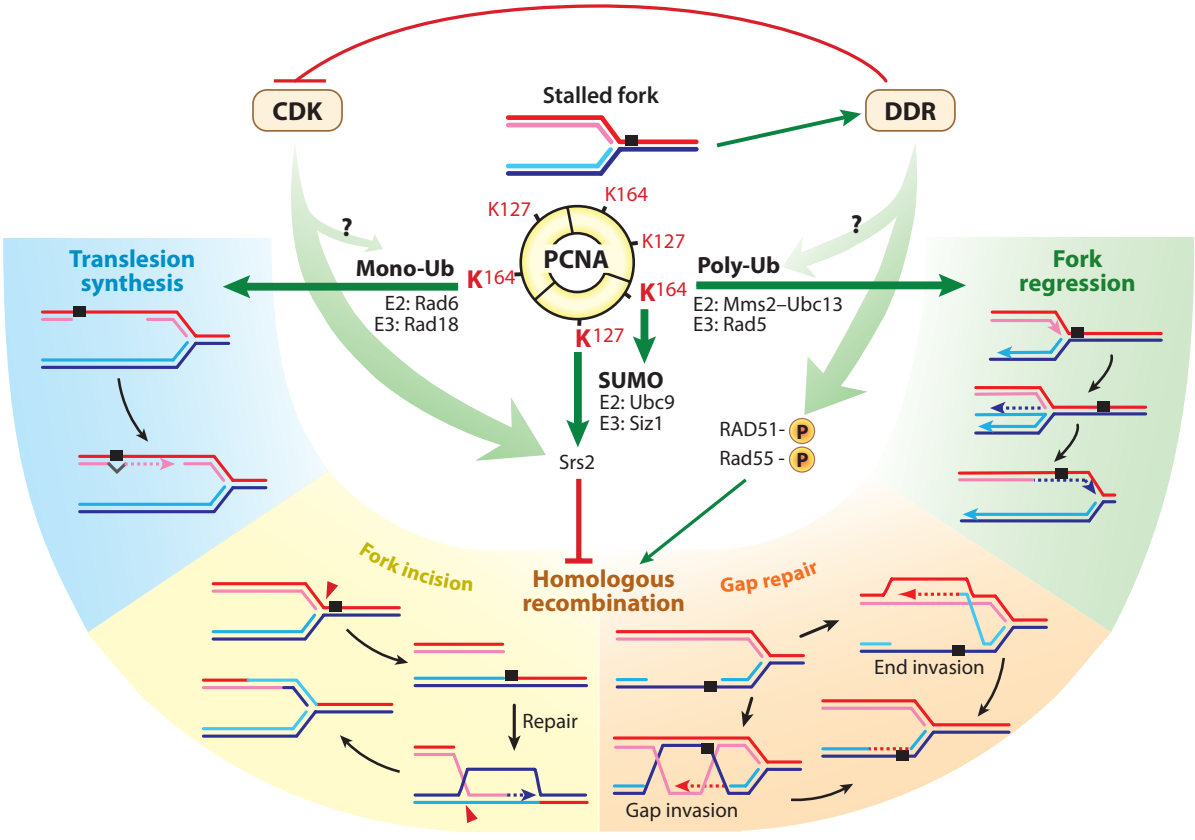

Figure 2

Pathways and regulation at stalled replication forks. Proliferating cell nuclear antigen (PCNA) modification regulates the choice of competing pathways for stalled replication fork recovery. A stalled fork triggers the DNA damage response (DDR), which directly activates homologous recombination (HR). The relationship between the DDR and cell-cycle control to PCNA sumoylation/ubiquitylation has not yet been determined.

sumoylation pathways (**Figure 2**) is still poorly understood (18, 19).

How is the balance between TLS, fork regression, and HR regulated? Genetic evidence in budding yeast favors the model that TLS and fork regression are primary pathways. At least initially, HR is actively repressed, but the sensitivity of HR mutants to fork stalling agents suggests that this inhibition is temporary. Mutations in *RAD6* or *RAD18* disable TLS and fork regression, leading to severe DNA damage sensitivity. An additional mutation in *SRS2* (suppressor of rad six) suppresses the sensitivity to a significant degree by relieving the inhibition of HR (2, 132). These data suggest that Rad6-Rad18 binding to RPA-covered ssDNA (39) is kinetically favored over Rad51 filament formation. Possibly, PCNA sumoylation marks a later phase where Srs2 actively removes Rad51 filaments. What regulates PCNA ubiquitylation or sumoylation and whether DDR signaling is involved remain to be determined.

SIGNALING BY THE CELL-CYCLE MACHINERY AND THE DNA DAMAGE RESPONSE

Two signaling systems intersect in the control of HR: the cell-cycle control machinery and the DDR (**Figure 3**) (17). In *S. cerevisiae*, the Cdc28 CDK drives directional progress through the cell cycle, dependent on the expression of

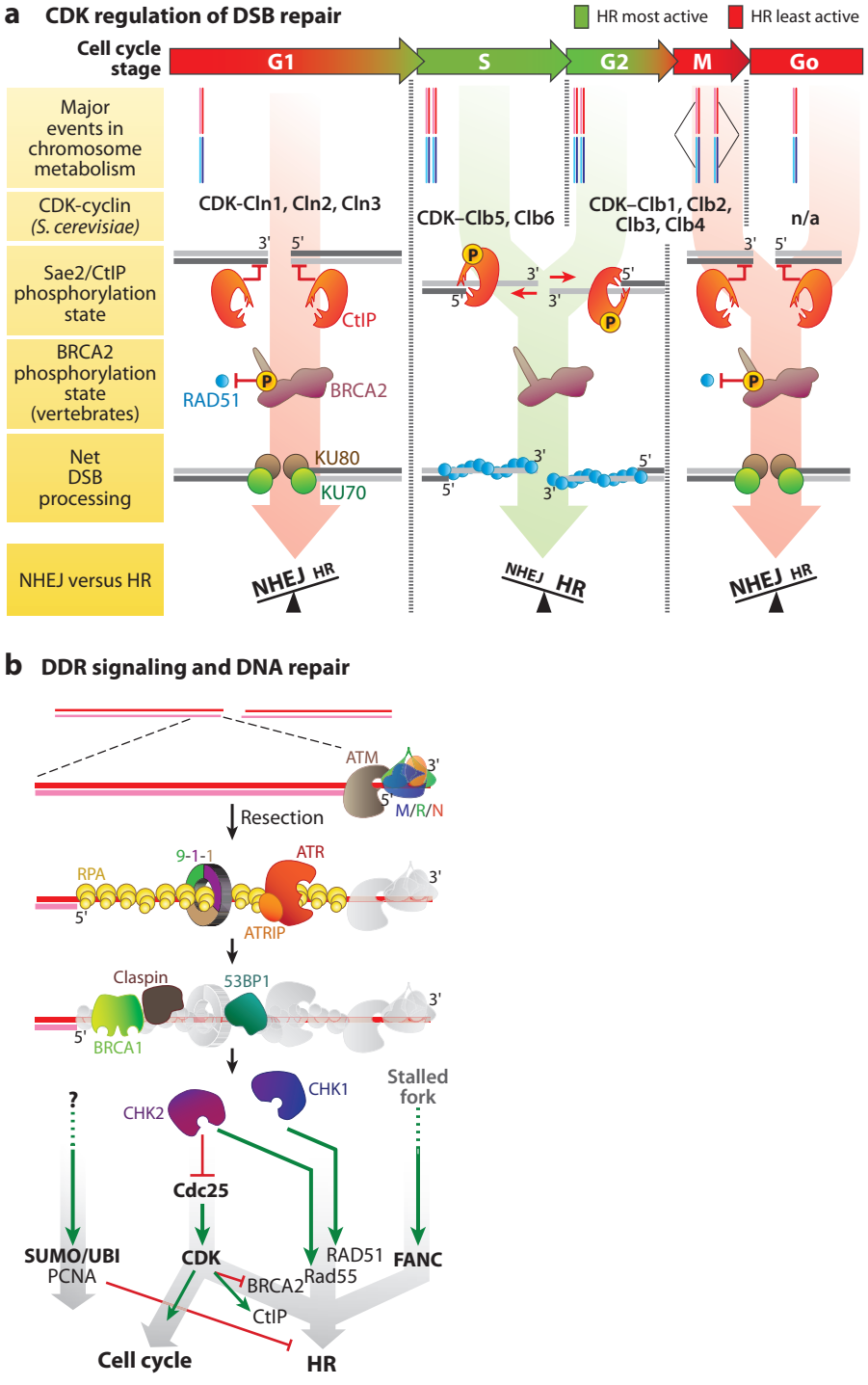

Figure 3

Homologous recombination (HR) is regulated by cell-cycle control and DNA damage signaling. (*a*) The cell cycle controls the competition between nonhomologous end joining (NHEJ) and HR in double-strand break (DSB) repair. Cdc28 is the sole cyclin-dependent kinase (CDK) responsible for cell-cycle progression in *Saccharomyces cerevisiae*, and partners with the indicated cyclins. In mammals, six CDKs drive cell-cycle progression, and their relative importance varies in different tissue types. (*b*) The DNA damage response (DDR) results in HR activation and inhibition of cell-cycle progression. The relationship between the DDR and the Fanconi anemia (FANC) pathway as well as proliferating cell nuclear antigen (PCNA) sumoylation/ubiquitylation is poorly understood. Abbreviations: NHEJ, non-homologous end joining; HR, homologous recombination.

IR: ionizing radiation

stage-specific cyclins that modulate CDK activity and impart substrate specificity (101, 163). As discussed below, CDK phosphorylates HR proteins to positively and negatively regulate HR. The availability of sister chromatids largely determines whether HR is a primary pathway, explaining why HR is favored in the S and G2 phases but not in the G0, G1, or M phases (**Figure 3*a***). Stalled replication forks and DNA damage trigger the DDR signaling cascade that activates DNA repair and pauses cell-cycle momentum (**Figure 3*b***) (17). The key intermediate is RPA-bound ssDNA, which serves as a platform for DDR signaling, recruitment of ubiquitylation and, likely, sumoylation factors, as well as for Rad51 filament formation. DDR signaling is required for efficient DNA damage–induced HR (13, 108, 141). In addition, the DDR affords time for HR to be completed by leading to a transient cell-cycle arrest, which in most organisms, but not *S. cerevisiae*, is achieved by downregulating CDK activity (116).

DOUBLE-STRAND BREAK RESECTION AND ITS REGULATION

Resection of DSB ends seems deceptively straightforward in principle, but in *S. cerevisiae*, resection involves four nucleases (Mre11, Sae2, Exo1, Dna2), dependent on the specific chemical structure encountered at the DSB (hairpins, modified bases, covalent protein–DNA adducts) (104). A current view proposes that the Mre11 subunit of the MRX complex, recruited or supported by the endonuclease Sae2, catalyzes initial end processing that results in the removal of about 50–100 nucleotides (104). Sae2 is thought to clip DNA ends in preparation for the more processive nucleases that catalyze the extended resection responsible for 3′-ssDNA tail generation (94). The 3′ → 5′ polarity of the Mre11 nuclease appears unsuited to conduct the 5′ → 3′ resection (154), but it could act as an endonuclease in this context. Extended resection is achieved by the 5′ → 3′ exonuclease activity of Exo1 or the helicase activity of Sgs1 in cooperation with the endonuclease activity of Dna2 (103, 174). How these options for extended DNA resection (Exo1 alone or Sgs1 with Dna2 or Exo1) or the extent of resection are regulated is unknown. Compounding the complexity associated with the collaboration of multiple nucleases to achieve end resection is the question of their regulation by posttranslation modification.

Activation of DSB Resection by CDK-Mediated Sae2/CtIP Phosphorylation

In haploid *S. cerevisiae* cells, limited end resection can restrict repair of a DSB by HR in the G1 phase of the cell cycle (7, 79). In yeast, end resection is primarily regulated by CDK-dependent phosphorylation of the Sae2 nuclease (74, 79) (**Figure 3**) (**Table 1**), which determines whether a DSB is channeled into NHEJ or HR. The pivotal phosphorylation occurs at serine 267, located in one of three Sae2 CDK consensus sites (74). An endonuclease-mediated DSB at the *MAT* locus is poorly resected in an *S. cerevisiae sae2-Δ* mutant; a *sae2* mutant in which serine 267 has been substituted with alanine (*sae2-S267A*) phenocopies the *sae2-Δ* strain for unresected DSB ends. In contrast, a Sae2 phosphomimic mutant in which serine 267 has been replaced with aspartic acid (*sae2-S267E*) is hypermorphic for DSB resection, sidestepping a requirement for CDK activity to sanction DSB resection.

These observations are mirrored by results from human cells, where CtIP, the human homolog of Sae2, is also required for DSB resection (130). Phosphorylation on threonine 847 is required for ssDNA generation and RPA phosphorylation in response to the topoisomerase I inhibitor camptothecin, laser-induced DNA damage, or ionizing radiation (IR) (75). A transfected phosphomimic CtIP-T847E resects DSBs even after CDK inhibition, whereas the nonphosphorylatable CtIP-T847A mutant impairs resection (75). CDK phosphorylation of Sae2/CtIP therefore appears to be conserved in eukaryotes as a key switch in determining

whether DSB ends are sanctioned for resection and HR. In addition to the conserved mechanism described for *S. cerevisiae* Sae2, human CtIP function also appears to be regulated by an additional CDK phosphorylation at serine 327, a modification that enhances CtIP interaction with the BRCT domain of BRCA1 and is critical for HR (171, 173). The function of BRCA1 in HR remains enigmatic. It is interesting to observe that BRCA1 is sumoylated by PIAS1/4 to enhance its ubiquitin ligase activity (**Table 1**) (57, 107) and that CtIP appears to be one of its native ubiquitylation targets (172), implying a potential regulatory role of BRCA1 in resection.

Sae2-S267 in *S. cerevisiae* is unlikely to be the exclusive target of CDK relevant to end resection, because the *sae2-S267E* phosphorylation-mimic mutation does not completely restore resection to wild-type levels (74). Although Mre11 and Xrs2 have CDK phosphorylation consensus sites, no resection phenotype has been observed when these sites are mutated (79). Thus, additional targets remain to be discovered.

A second signaling pathway, which depends on cell-cycle controls, also regulates Sae2, as Mec1/Tel1 consensus sites, which are essential for meiotic recombination (26, 150), are also required for full Sae2 function during DNA repair in mitotic cells (12). In summary, two signaling pathways, the cell-cycle control machinery and DDR signaling, converge on Sae2/CtIP to regulate end resection.

Inhibition of Exo1 Activity by the DDR Kinase Rad53

Exo1 is one of the nucleases that generates the ssDNA that is a defining intermediate of DSB processing in HR. ssDNA also occurs at telomeres that are uncapped during end-replication in S phase and in mutants (e.g., *cdc13–1*) that lose the protective T-loop and associated factors (34). Four serines in the *S. cerevisiae* Exo1 C-terminus are targets for regulatory phosphorylation, presumably by Rad53, because Exo1 phosphorylation is absent in a *rad53-K227A* kinase-defective mutant (106). Overexpression of Exo1 results in hyperactivation of the DDR, consistent with the generation of excess ssDNA. The same phenotype is observed in mutants in which all four serines are substituted by alanine, suggesting that Rad53-dependent phosphorylation reduces Exo1 activity. Unlike Sae2/CtIP activation by CDK, Exo1 phosphorylation limits resection of ssDNA at uncapped telomeres and consequently minimizes further activation of the DDR. The inhibition of Exo1 activity is not limited to pathological situations such as telomere uncapping. Exo1 is also phosphorylated in *yku70-Δ* mutant cells following bleomycin treatment (106). Repression of Exo1 activity by DDR signaling is likely involved in the avoidance of fork regression at stalled replication forks (**Figure 2**) (35). Another mechanism of negative regulation of EXO1 is observed in human cells challenged with the replication inhibitor hydroxyurea (HU), where phosphorylation by ATR targets EXO1 for destruction (48). This may reflect a prohibition of resection at ssDNA gaps associated with stalled replication forks. These results suggest that Exo1 is not required for the generation of ssDNA to allow DDR signaling and that fork regression and potentially HR may require more extensive stretches of ssDNA generated by Exo1.

HU: hydroxyurea

Regulation of Human BLM Helicase by Sumoylation

Biochemical and genetic evidence demonstrate an involvement of the RecQ helicases Sgs1 (yeast) and BLM (human) in DSB resection (62, 103, 114, 174) besides functions in joint reversal, dHJ dissolution, and DDR signaling (15). Sumoylation of BLM may exert positive regulation, as BLM is normally sumoylated on several lysine residues, and BLM lacking sumoylation on lysine K317 and K331 (**Table 1**) only partly complemented the genetic defects in BLM-deficient cells (47). The observation that cells with SUMO-deficient BLM exhibit a defect in RAD51 focus formation after HU treatment (120) may suggest

that sumoylation is required for a prorecombination activity of BLM, possibly resection (48, 106).

In summary, posttranslational modification of factors involved in DSB resection is paramount to the regulation of eukaryotic HR. Two regulatory pathways, cell cycle control and the DDR, exert positive and negative control, respectively, directly phosphorylating two nucleases (Sae2/CtIP and Exo1). CDK-dependent modification of yeast Sae2/human CtIP demonstrates that pathway choice for DSB repair depends to a large extent on commitment to resection.

THE RAD51 FILAMENT: A BALANCE BETWEEN FORMATION AND DISRUPTION

The Rad51-ssDNA filament performs the central functions: homology search, and DNA-strand exchange (**Figure 1**). Not surprisingly, this crucial role is reflected in an elaborate regulation of the balance between Rad51 filament formation and disruption.

CDK- and DDR-Mediated Phosphorylation of RPA

RPA functions at the nexus of all DNA metabolic processes because of its high affinity for ssDNA. RPA-covered ssDNA is the physiological target for assembly of the Rad51-ssDNA filament. Rad51 filament formation competes with other processes that occur on RPA-covered ssDNA such as recruitment of the Rad6-Rad18 ubiquitylation complex, TLS, fork regression (**Figure 2**), and ATR signaling (**Figure 3*b***). RPA2, the middle subunit of RPA, undergoes cell cycle–dependent and DNA damage–induced phosphorylation in yeast and human cells (52). RPA phosphorylation does not appear to affect its DNA binding properties, but likely modulates protein interactions that may affect its intranuclear localization (52).

Positive Control by DDR-Mediated Rad51 Phosphorylation

In response to HU, human RAD51 is phosphorylated by CHK1 kinase within a consensus site at threonine 309 (**Figure 2**) (**Table 1**) (141). Cells depleted for CHK1 activity by UCN-01-mediated inhibition or siRNA display a defect in RAD51 focus formation in response to HU, which is consistent with positive regulation of HR by CHK1. Targets other than RAD51 may be involved as well. Expression of the RAD51-T309A phosphorylation-defective mutant, but not the wild-type protein, causes dominant hypersensitivity to HU, supporting an activating role of threonine 309 phosphorylation (141).

Negative Control by CDK Phosphorylation of BRCA2

The tumor suppressor protein BRCA2 (see sidebar) plays a key role in the formation of the RAD51 filament (129). CDK-cyclin A can phosphorylate BRCA2 on serine 3291 in vitro, and this residue is also phosphorylated in vivo, peaking during M phase (**Figure 2**) (**Table 1**) (49). S3291 of BRCA2 is near the C-terminal RAD51 interaction site (residues 3196–3226) (135), and phosphorylation of this residue or mutation of serine to alanine ablates the interaction of the BRCA2 C-terminus with RAD51 (49). These data led to the model that CDK-mediated BRCA2 phosphorylation precludes HR during M phase, where it could interfere with chromosome segregation (49). Furthermore, BRCA2 and the RAD51 paralog, RAD51C, are also involved in nuclear transport of RAD51 after DNA damage (40, 61).

Rad52 Sumoylation Affects Protein Stability and Intranuclear Localization

Rad52 is the lynchpin of HR in *S. cerevisiae* and is essential both for HR and SSA (69, 91, 129, 161). Sumoylation of a significant fraction of yeast Rad52 protein is induced in meiosis or after DNA damage, dependent on the MRX complex (128). A triple mutant at lysine residues

10, 11, and 220 ablated Rad52 sumoylation, leading to faster proteasome-dependent protein turnover (128) (**Table 1**). While SUMO-deficient Rad52 protein is largely proficient for HR, the mutant displayed a 2.5-fold reduction in direct repeat recombination (128). Live cell imaging revealed that sumoylation controls nucleolar localization of the Rad52 protein. In wild-type cells, Rad52 protein is excluded from the nucleolus, the nuclear compartment containing the rDNA repeats. The Rad52 SUMO-deficient mutant overcomes the nucleolar exclusion and forms foci within the nucleolus, resulting in slightly elevated rDNA recombination (152), opposite to the effect on nuclear repeat recombination (128).

Phosphorylation of Rad55 Serines 2, 8, and 14 Is Required for Optimal HR

In yeast, the Rad51 paralog complex consisting of Rad55 and Rad57 facilitates the formation or stabilization of Rad51 filaments (97, 147). Rad55 is phosphorylated in response to DNA damage on multiple residues by Mec1 (serine 378), Rad53 (serine 14), and an unidentified kinase (serines 2 and 8) (13, 68, 82). The N-terminal phosphorylation mutant (Rad55-S2,8,14A) displays strong defects in growth and survival in response to the alkylating agent methyl methanesulfonate. These conditions lead to replication fork stalling, and Rad55 phospho-deficient mutants exhibit a defect in the recovery of stalled replication forks (68) (**Table 1**).

Disruption of Rad51-ssDNA Filaments by Antirecombinogenic DNA Helicases

The yeast helicase Srs2 is the prototype for antirecombination helicases capable of disrupting Rad51-ssDNA filaments (90, 158) (**Figure 2**), exerting biologically significant antirecombination activity (1, 29, 132). During S-phase, Srs2 is recruited to replication forks by sumoylated PCNA (**Table 1**) (122, 124). Similarly, the yeast NHEJ factor Nej1 recruits Srs2 to DSBs to repress HR and favor NHEJ or SSA, and this interaction is enhanced by DNA damage–induced phosphorylation of Nej1 by Dun1 kinase (**Table 1**) (27). Srs2 has no direct ortholog in mammals, but genetic studies with human FBH1 in budding yeast have led to the proposal that FBH1 is the mammalian counterpart of yeast Srs2 (32). This is consistent with the original identification of Fbh1 as a suppressor of a hypomorphic mutant in the *S. pombe rad22* gene (homolog of *S. cerevisiae RAD52*) (119). Moreover, overexpression of human FBH1 in human cells impaired recruitment of human RAD51 to ssDNA and suppressed HR, whereas FBH1 depletion caused an increase in sister chromatid exchanges (SCEs) (56), which is consistent with an antirecombination role of FBH1.

RECQ5, a RecQ family helicase in mammals (see sidebar), may play an antirecombinogenic role (15). It interacts with RAD51 and displaces RAD51 from ssDNA to inhibit D-loop formation in vitro (73). In addition, a defect in RECQ5 causes increased levels of spontaneous RAD51 foci, as well as elevated frequencies of spontaneous DSBs and HR between direct repeats (73).

BLM, another mammalian RecQ family member (see sidebar), also interacts with RAD51 and is capable of disrupting RAD51-ssDNA filaments in vitro (23, 166). The biological relevance of this observation is uncertain because BLM only disrupts filaments in conditions containing Mg^{2+}, which has been interpreted as targeting the ADP-bound, inactive form of RAD51 and does not dissociate the ATP-bound RAD51 in the presence of Ca^{2+} (22, 23).

FANCJ, a component of the Fanconi Anemia pathway (see sidebar) (105), exhibits a 5′ → 3′ directionality, in contrast to the RecQ family helicases. As with BLM, human FANCJ disrupts RAD51-ssDNA filaments in vitro but dissociates only the inactive, ADP-bound form of RAD51 from ssDNA in vitro (140). No specific interaction between FANCJ and RAD51 has been reported. The biological significance of RAD51 dissociation by FANCJ remains unclear, as a mutant in *dog-1*, the *FANCJ* homolog

SCE: sister chromatid exchange

in *Caenorhabditis elegans*, shows no significant increase in Rad51 foci (169).

In summary, the Rad51-ssDNA filament is controlled by a balance between mediator proteins that promote assembly and antirecombinogenic DNA helicases that promote disassembly. Cell cycle–dependent and DNA damage–inducible posttranslational modifications of these factors impinge on both assembly and disassembly of the Rad51 filament.

REGULATION OF HOMOLOGY SEARCH AND DNA-STRAND INVASION

Homology search and DNA-strand invasion generate D-loops, a key intermediate for all subpathways of HR (**Figure 1**). These reactions are catalyzed by Rad51, which interacts with the dsDNA motor protein Rad54 (70). In meiosis, a critical protein interaction (Rad51-Rad54) is targeted to assert negative regulation of HR by Mek1-mediated phosphorylation of Rad54-T132 (115) (**Table 1**). This mechanism is independent of Hed1 (115), a meiosis-specific repressor of the Rad51-Rad54 interaction that binds to Rad51 protein (24, 155). Both mechanisms are active in mitotic cells when Hed1 or the Rad54-T132D phosphomimic mutant are ectopically expressed (24, 115, 155).

REVERSION OF D-LOOPS AND EXTENDED D-LOOPS: PRO- AND ANTIRECOMBINOGENIC FUNCTIONS

Disruption of a D-loop prior to extension of the primer strand by DNA polymerases is a potentially powerful mechanism of antirecombination. A number of DNA helicases/translocases, including human FANCM, its *S. cerevisiae* homolog Mph1 and *S. pombe* homolog Fml1, metazoan RTEL1, mammalian RECQ1 and BLM, as well as Rad54, are capable of disrupting D-loops in vitro (**Figure 4**) (8, 11, 20, 21, 59, 146). However, reversion of an extended D-loop is also inherent to the SDSA pathway and constitutes in this context a prorecombination activity (**Figure 1**). In some genetic assays such an activity can be scored to suppress crossovers, constituting a mechanism of anticrossover other than dHJ dissolution (**Figure 1**).

Rad54 is essential for HR in budding yeast and is required for in vitro D-loop formation by the yeast Rad51 protein (70). However, Rad54 can also dissociate D-loops in vitro (21), the very product it forms in conjunction with Rad51, making it difficult to test the biological significance of this activity.

In yeast, genetic studies on Sgs1 have provided critical insights on the cellular functions and regulation of the related human RecQ helicases in HR (15). However, the multiple functions of Sgs1 in DDR signaling, DSB resection, dHJ dissolution, and potentially other steps of HR complicate interpretation of the genetic data. Importantly, Sgs1 suppresses crossovers during mitotic and meiotic recombination (78, 117). This role of Sgs1 correlates with the ability of the human BLM-TOPO3alpha-RMI complex to dissolve dHJs into noncrossover products (165). dHJ dissolution by human BLM helicase also explains the elevated levels of SCE in BLM-deficient cells (see sidebar). BLM may also contribute to a noncrossover outcome by promoting the SDSA pathway, as indicated by genetic studies in *Drosophila* (3). Human BLM interacts with human RAD51 protein and can dissociate mobile D-loops (8, 166), but not D-loops during an ongoing RAD51-mediated in vitro reaction (114). This leaves open the question of how BLM may promote SDSA.

Human FANCM protein is a core component in the Fanconi Anemia pathway that is critical for the repair of interstrand DNA crosslinks (105). FANCM and its homologs form an evolutionarily deeply rooted family that includes the archaeal Hef nuclease/helicase, budding yeast Mph1, and fission yeast Fml1. The eukaryotic enzymes either lost or degenerated their nuclease domain (162). FANCM-deficient cells display elevated levels of spontaneous SCE, consistent with the ability of FANCM protein to dissociate mobile D-loops (9, 59, 127). The FANC pathway is

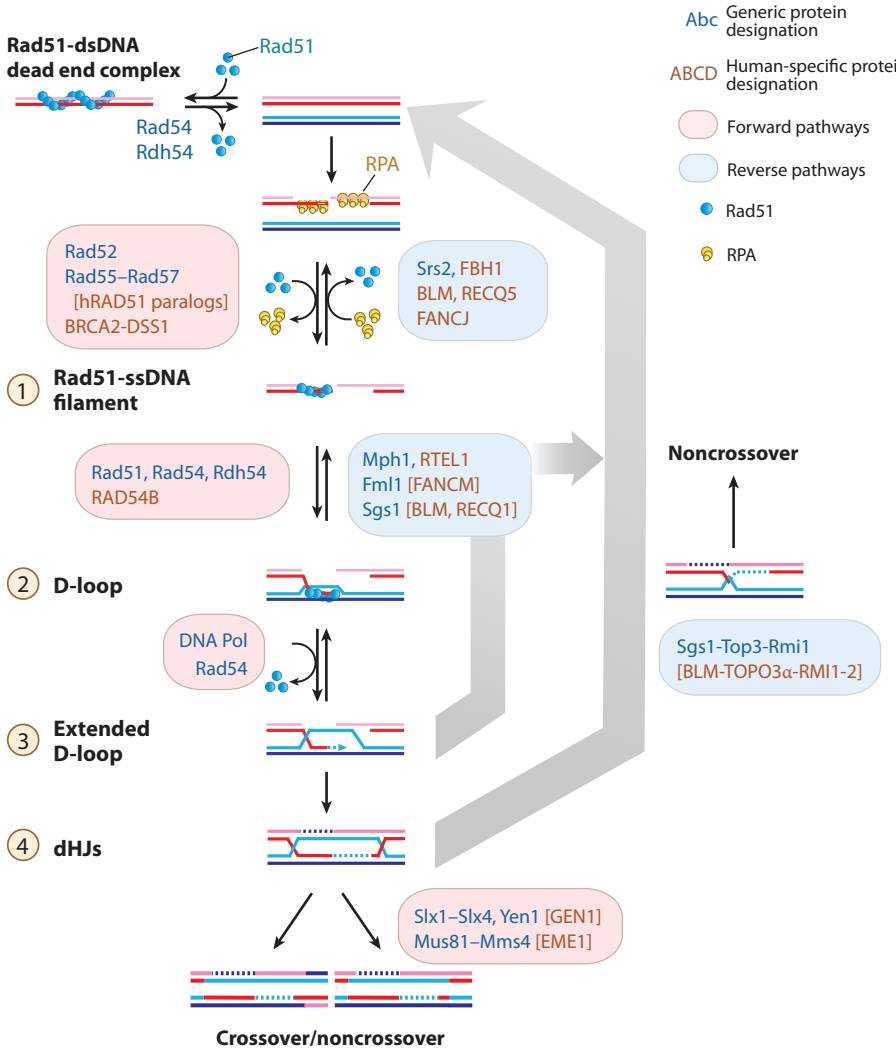

Figure 4

Reversible, metastable intermediates in homologous recombination (HR). HR is proposed to involve key intermediates that are reversible and metastable including (*1*) the Rad51-ssDNA filament, (*2*) the initial D-loop, (*3*) the extended D-loop, and (*4*) the double Holliday junction (dHJ). The dead-end complex of Rad51/Dmc1 with dsDNA, although not an HR intermediate, can be added to this list of reversible HR protein–DNA complexes (72).

negatively regulated in mitosis by polo-like kinase PLK1 phosphorylation of FANCM, leading to its ubiquitin-mediated degradation (88). Similar to human FANCM, Mph1 and Fml1 dissociate D-loops in vitro (125, 146). A defect in the yeast FANCM orthologs, Mph1 or Fml1, also causes a three- to fourfold increase in crossovers. Epistasis analysis in both fission and budding yeast suggests that Mph1 and Fml1 act independently of Srs2 or Sgs1 in suppressing crossovers (10, 125, 146). Both proteins promote Rad51-dependent recombination at stalled replication forks (121, 133, 146). Using an inducible replication fork stalling system

MMR: mismatch repair

Homeologous recombination: recombination between similar but not identical sequences, as found in repeated DNA

in fission yeast, Whitby and colleagues (146) showed a requirement for Fml1 in spontaneous and fork stalling–induced HR. Moreover, in *S. cerevisiae*, mutants in *MPH1* have the same mutator phenotype as HR mutants (*rad51*), and this effect is epistatic with an HR defect, suggesting that Mph1 functions in concert with HR to avoid Rev3-dependent mutagenesis (133). Mph1 appears to function late in HR, as the synthetic lethality with *srs2* is suppressed by mutations in *rad51*, *rad55*, *rad57*, and *rad52* (133). However, Mph1 has also been suggested to promote gross chromosomal rearrangements by inhibiting HR through stabilizing RPA on ssDNA (10).

RECQ1, another mammalian RecQ family helicase, is required for genome stability in mouse and human cells, as RECQ1 deficiency leads to aneuploidy, chromosomal instability, and hypersensitivity to DNA damage (IR, camptothecin) (136, 137). RECQ1-deficient cells exhibit increased levels of spontaneous DNA damage as suggested by an increase in spontaneous gamma-H2AX foci and elevated SCE levels. In vitro, RECQ1 disrupts D-loops, with significant preference for D-loops resulting from invasion of the 5′-end (20). Given that DNA polymerase cannot extend such a D-loop, it constitutes a potential dead-end complex. This activity provides a plausible mechanism for RecQ1 function in reversing a potentially toxic HR intermediate.

The RAD3-like helicase RTEL1 was isolated in a screen for functional analogs of Srs2 in *C. elegans* (11). A defect in *RTEL1* causes synthetic lethality when combined with mutations in *BLM*, *RECQ5*, or *MUS81*, as well as a fourfold increase in meiotic crossovers and DNA damage sensitivity to interstrand crosslinks and the topoisomerase I inhibitor camptothecin (11, 170). Depletion of RTEL1 in human cells causes a fourfold increase in DSB-mediated intrachromosomal repeat recombination that is improbably explained by a defect in crossover suppression, as well as hypersensitivity to the crosslinking agent mitomycin C but not IR (11). In vitro, RTEL1 dissociates D-loops, which could explain the antirecombination and anticrossover phenotype (11, 170). The DNA damage sensitivity profile of *RTEL1* mutants is more consistent with a defect in HR, suggesting that RTEL1 may play a role in SDSA (170). In *C. elegans*, mutations in *RTEL1* show a synthetic phenotype with a defect in the HELQ-1 DNA helicase, leading to accumulation of HR intermediates in the double mutant as deduced from the persistence of meiotic RAD-51 foci (159). Unlike yeast Srs2, RTEL1 cannot dissociate RAD51 from ssDNA (11, 90, 158). Interestingly, Ira et al. (78) postulated that Srs2 exerts its anticrossover effect through a function in SDSA and suggested that Srs2 dissociates D-loops. However, this biochemical activity has not been observed in vitro (90, 158). The stimulation of Srs2 helicase activity by Rad51 bound to dsDNA suggests the possibility that Srs2 targets two HR intermediates, Rad51-ssDNA filaments and (extended?) D-loops (45).

In summary, a number of proteins are capable of dissociating D-loops, which may function in HR to favor SDSA and suppress crossovers or may be a mechanism of antirecombination. The mutant phenotypes suggest potentially complex roles involving pro- and antirecombination functions for RTEL1, Srs2, Sgs1/BLM, and the FANCM helicases in HR.

MISMATCH REPAIR EDITS RECOMBINATION FIDELITY

Mismatch repair (MMR) edits replication errors, and mismatch correction in heteroduplex DNA achieves gene conversion during HR (see **Figure 1**). More critical to the regulation of HR, however, is that MMR proteins help to discriminate homology from homeology (partial homology) (65). This MMR-mediated screening of recombination fidelity favors HR between perfectly homologous sequences and actively opposes homeologous recombination, responding to the degree of homology.

Genetic studies in *S. cerevisiae* systematically surveyed the effects of homeology on HR using an elegant intron-based assay (37, 38). Remarkably, even a single mismatch reduced

spontaneous recombination rates by fourfold relative to substrates with 100% identity. Most importantly, defects in MMR suppressed the effects on spontaneous HR rates when homeology was up to 15% sequence divergence. MMR factors therefore regulate whether HR is sanctioned over given sequences when the interacting sequences are 85–100% similar. Three yeast complexes function in both replication-associated MMR and in negative regulation of HR: MutSα (Msh2-Msh6), MutSβ (Msh2-Msh3), and MutLα (Mlh1-Pms1) (37, 38). In addition, the nucleases Rad1-Rad10 and Exo1, and the helicases Sgs1 and Srs2 function in the MMR-mediated barrier to HR between homeologous sequences (113, 160). MMR not only affects the frequency of HR but also influences the crossover/noncrossover outcome of HR (**Figure 1**) (160). This characteristic of the MMR-mediated editing of HR may be particularly useful to suppress crossovers between slightly divergent repeated DNA sequences, where crossovers would lead to genome rearrangements.

What is the mechanism of the MMR-mediated barrier to HR between divergent sequences? Suppression of homeologous recombination by MMR could function during DNA-strand exchange, heteroduplex DNA extension, or even later, in joint molecule resolution (**Figure 1**). Paradigmatic biochemical studies with bacterial RecA, MutS, and MutL proteins suggest that heteroduplex DNA extension may be the decisive stage (164). Similar biochemical work with eukaryotic proteins has not been reported, but genetic evidence from *S. cerevisiae* is consistent with this scenario. Mitotic and meiotic gene conversion tracts in *msh2 msh3* mutants are ~50% longer than in wild-type cells, indicating that heteroduplex DNA extension may be blocked by Msh2-Msh3 binding to mismatches in vivo (31). *S. cerevisiae* Sgs1 and Mph1 are candidates for motor proteins active in heteroduplex DNA rejection (110, 149). *sgs1* mutants allowed an increased rate of homeologous HR (substrate with 91% sequence identity), synergistic with MMR mutants (142). This increase in homeologous recombination was also linked to a role for Sgs1 in suppression of gross chromosomal translocations (110). In addition, Sgs1 and Msh2-Msh6 suppress SSA between homeologous sequences (144).

In summary, MMR is a key regulator of HR in the distinction between allelic sites and ectopic sites. This editing function is sufficiently sensitive to discriminate allelic targets on sister chromatids from allelic targets on homologs. The importance of MMR in focusing HR to perfect sequence identity (allelic sites on sister chromatids) suggests that MMR defects in tumors not only increase the rates of point mutations, but also increase rates of inappropriate HR between homeologous sequences leading to genome rearrangements.

NUCLEOLYTIC PROCESSING OF STALLED REPLICATION FORKS AND DOUBLE HOLLIDAY JUNCTIONS

A number of DNA joint molecules are intermediates at which regulatory decisions can be made, providing successive opportunities to decide whether HR is initiated, aborted, or sanctioned for a specific genetic outcome (crossover or noncrossover). The regulation of HR relevant to two specific joint molecules, replication forks and dHJs, is elaborated here.

Stalled replication forks are potentially substrates for HR, but the mechanisms by which HR promotes fork restart and recovery remain unclear. Fork incision to generate a single-sided DSB end or a ssDNA gap could initiate HR (**Figure 2**). The relative significance of fork incision versus gap repair is uncertain, although Fabre et al. (51) suggested that breaks are rare in S phase and that ssDNA gaps are the primary substrates for replication-associated HR in *S. cerevisiae*. Nevertheless, Hanada et al. (63, 64) and Froget et al. (55) implicate MUS81-EME1 in fork incision in human cells and *S. pombe*, respectively. DSBs are observed after 18 h of chronic HU challenge, dependent on human MUS81-EME1 (63). Interestingly, *S. pombe* Mus81 dissociates from chromatin in response to HU treatment, although Mus81 is required

Crossover/noncrossover: describes outcome of HR with respect to the flanking DNA, which is either in parental (noncrossover) or nonparental (crossover) configuration

for resistance to HU (16, 85). It was proposed that fork incision by Mus81-Mms4/EME1 represents a last resort for fork recovery, and it may be negatively regulated under some circumstances of replication stress (85).

dHJs are intermediates during mitotic DSB repair by HR (25). Alternative mechanisms for removing dHJs determine whether the genetic products result in a crossover or noncrossover outcome (**Figure 1**). A number of conserved eukaryotic endonucleases have been proposed to cut Holliday junctions or their precursors in vivo, including *S. cerevisiae* Mus81-Mms4 (human MUS81-EME1), *S. cerevisiae* Yen1 (human GEN1), and Slx1-Slx4 (**Figure 1**) (104). In addition to endonucleolytic resolution, dHJs can be dissolved by the concerted activities of a helicase-topoisomerase complex (**Figure 1**) (165). How endonucleolytic resolution of dHJs is regulated relative to dissolution is unknown. Caspari et al. (28) suggested that CDK phosphorylates Top3 in *S. pombe*, dependent on interaction with the DDR mediator Crb2. Loss of Top3 function results in hyper-recombination and cell death after IR, perhaps associated with an inability to optimally resolve dHJs.

In summary, nucleolytic processing of stalled replication forks and dHJs determines whether HR is initiated after fork stalling and whether the genetic outcome of HR is potentially a crossover. The processes and proteins involved, and their specific function and regulation, still need to be determined.

MODEL: HOMOLOGOUS RECOMBINATION: A PATHWAY WITH METASTABLE, REVERSIBLE INTERMEDIATES TO ACHIEVE FLEXIBILITY AND ROBUSTNESS

DNA repair is a formidable task that requires quality control to balance accuracy of the repair event with the potential for genome rearrangements (87). It has been proposed that reversibility of HR intermediates provides robustness to the pathway (87, 148). In biology, the concept of robustness has been largely discussed in the context of mutational robustness, keeping an organism's phenotype constant in spite of mutations (43). In the present discussion, however, the term robustness applies more in the engineering sense, where a system or algorithm does not break down easily, continues to operate despite single application failures, and recovers quickly from, and holds up under, exceptional circumstances (4, 131).

What can we learn from the analysis of the regulation of HR about the mechanism of HR and how it achieves robustness? One aspect is protein interactions. The myriad of direct protein-protein interactions between HR proteins have been previously projected into a single time point and interpreted as a stable recombinosome (66). Further analysis now suggests that these interactions can be regulated by reversible posttranslational modifications, are transient, and occur sequentially (69, 91, 129, 161), which provides significantly more plasticity. A second aspect is pathway flexibility. While the HR pathway is typically portrayed as a linear sequence, **Figure 1** reveals bifurcations, where identical intermediates (D-loop, dHJ) can enter different subpathways and fates. Finally, the abundance of motor proteins that dissociate recombination intermediates suggests that apparent antirecombination mechanisms are integral parts of the HR pathway. Four key intermediates in HR that are reversible by the action of motor proteins include the Rad51-ssDNA filament, the initial D-loop, the extended D-loop, and the dHJ (**Figure 4**). These intermediates also appear to be metastable, because they can be visualized cytologically (Rad51 foci) (97) or identified physically (25, 76, 77).

There is significant evidence that key HR intermediates are reversible in vivo and that this feature is important for HR. Reversal of extended D-loops is central to the SDSA model (**Figure 1**). There is compelling genetic evidence for multiple, sequential DNA-strand invasion events during HR, implying dissociation of D-loops or extended D-loops (3, 139). Promiscuous joint formation, at least in meiotic HR in *S. cerevisiae*, is not rare and needs active reversal by Sgs1 helicase (83, 117) and

suppression by MMR (see above). Another indication that antirecombination mechanisms are an integral part of the HR pathway is provided by the complex phenotypes of mutations in HR motor proteins. Mutations in *S. cerevisiae SGS1* show increased spontaneous recombination that appears unrelated to crossover suppression, which is consistent with the antirecombination role of Sgs1, but reduced DNA damage–induced recombination, suggesting a prorecombination role (58). Likewise, yeast Srs2 was shown to have anti- and prorecombination phenotypes, as was suggested for human FBH1 (6, 56, 78, 158). A defect in *C. elegans* and human RTEL1 causes hyper-recombination but also a DNA damage–sensitivity profile that suggests a defect in HR (11). The FANCM-related proteins (human FANCM, *S. pombe* Fml1, *S. cerevisiae* Mph1) can reverse D-loops in vitro and, depending on the assay, mutants display anti- or prorecombination phenotypes (10, 60, 102, 121, 125, 133, 162). Furthermore, the phenomenon of recombination-dependent lethality, where the synthetic lethality of certain double-mutant combinations (e.g., *S. cerevisiae srs2 sgs1*, *mus81 sgs1*) can be suppressed by an HR defect, demonstrates the occurrence of potentially toxic HR intermediates that require resolution by nucleases or motor proteins (51, 58).

In summary and as depicted in **Figure 4**, we suggest that the HR pathway proceeds through a series of metastable, reversible intermediates that are under active positive and negative regulation to allow flexibility for the repair outcomes (crossover versus noncrossover), accommodation of the unforeseen (e.g., absence of a second end and switch to BIR) (**Figure 1**), and recovery from unwanted intermediates (e.g., independent invasions of both ends of a DSB into different targets), which are all aspects that define robustness of a well-engineered system that is essential for maintaining a stable genome. Reversibility entails the destruction of potentially normal intermediates (87), and the MMR barrier, for example, affects HR even between perfectly homologous sequences (112). While counterintuitive at first, it appears that reactions that reverse recombination intermediates are required for the optimal functioning of HR, as the stochastic nature of the process will favor accurate pathway progression.

SUMMARY POINTS

1. Recombinational DNA repair is not constitutive but is highly modulated by positive and a preponderance of negative regulatory mechanisms.
2. Two signaling systems, the cell cycle control machinery and the DDR, intersect in the control of HR.
3. In DSB repair, end resection is a major commitment point to HR, regulated by CDK-dependent phosphorylation of Sae2/CtIP.
4. The Rad51 filament is a major regulatory control point of HR governed by mechanisms that favor its assembly (mediators and their posttranslational modifications) or disassembly (antirecombinogenic motor proteins).
5. Several mechanisms, including MMR, extended D-loop reversion, and dHJ dissolution, enforce an anticrossover bias during DSB repair in somatic (mitotic) cells.
6. Antirecombination mechanisms mediated by DNA motor proteins appear to be integral to the HR pathway, providing flexibility and robustness through reversible, metastable intermediates.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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