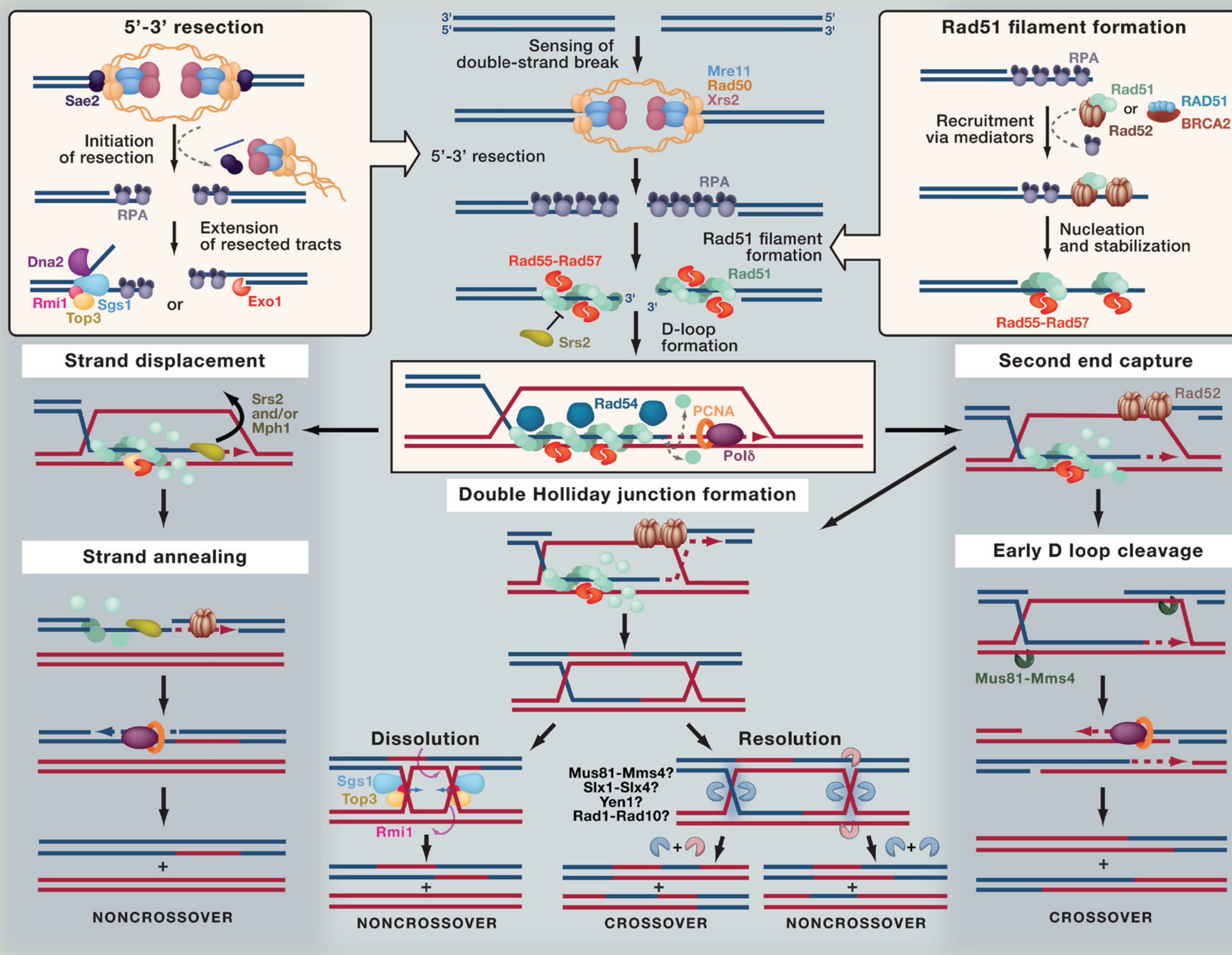
Cell

SnapShot: Homologous Recombination in DNA Double-Strand Break Repair

Gerard Mazón, Eleni P. Mimitou, and Lorraine S. Symington
Department of Microbiology and Immunology, Columbia University Medical Center, New York, NY 10032, USA



Saccharomyces cerevisiae

RESECTION	Mre11-Rad50-Xrs2 (MRX) Sae2 Replication protein A (RPA) Exo1 Sgs1-Top3-Rmi1 Dna2
SYNAPSIS	Rad51 Rad52 Rad55-Rad57 Rad54
DNA SYNTHESIS	DNA Polymerase δ PCNA
STRAND DISPLACEMENT	Srs2 Mph1
DISSOLUTION/ RESOLUTION	Sgs1-Top3-Rmi1 Mus81-Mms4 Yen1 Slx1-Slx4 Rad1-Rad10

Mammals

RESECTION	MRE11-RAD50-NBS1 (MRN CtIP RPA EXO1 BLM-TopoIIIα-RMI1 DNA2
SYNAPSIS	RAD51 BRCA2-PALB2 RAD51B, RAD51C, RAD51D, XRCC2, XRCC3 RAD54, RAD54B RAD51AP1
DNA SYNTHESIS	DNA Polymerase δ DNA Polymerase η PCNA
STRAND DISPLACEMENT	RTEL1 FANCM
DISSOLUTION/ RESOLUTION	BLM-TopoIIIα-RMI1-RMI2 MUS81-EME1 GEN1 SLX1-SLX4 XPF-ERCC1

See online version for legend and references.

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Homologous recombination (HR) provides an important mechanism to repair both accidental and programmed DNA double-strand breaks (DSBs) during mitosis and meiosis. Defects in HR are associated with mutagenesis and predispose to cancer, highlighting the importance of this pathway for preserving genome integrity (Moynahan and Jasin, 2010). HR is active in the S and G2 phases of the cell cycle where it promotes repair of a broken chromatid from an intact sister chromatid, ensuring error-free repair. The DNA transactions associated with HR are accompanied by modifications to histones, most notably phosphorylation of H2A/H2AX and chromatin remodeling. This SnapShot shows the yeast proteins directly involved in mitotic DSB repair; their mammalian counterparts are shown on the right.

The central reaction in homologous recombination is the pairing and exchange of strands between two homologous DNA molecules. This step is catalyzed by the conserved Rad51/RecA family of proteins (Chen et al., 2008; San Filippo et al., 2008). To generate the substrate for binding of Rad51, DNA ends are processed to yield 3′ single-stranded DNA tails (5′–3′ resection). End resection occurs through a biphasic mechanism: first, the Mre11-Rad50-Xrs2 (MRX) complex and Sae2 clip 50–100 nucleotides from the 5′ ends of DNA, and then Exo1 or Sgs1-Top3-Rmi1 and Dna2 process the early intermediate to form extensive regions of single-stranded DNA (Mimitou and Symington, 2009). Replication protein A (RPA) binds to the single-stranded DNA tails but is displaced by Rad51 aided by the mediator proteins Rad52 or BRCA2 (Rad51 filament formation) (San Filippo et al., 2008). The Rad51 paralogs in the budding yeast *Saccharomyces cerevisiae*, Rad55 and Rad57, are thought to extend or stabilize the Rad51 nucleoprotein filament (San Filippo et al., 2008). Once formed, the complex of Rad51 and single-stranded DNA searches for a homologous sequence in double-stranded DNA and then promotes invasion of the single-stranded DNA into donor double-stranded DNA to form a joint molecule with a displaced strand (D loop). Homologous pairing by Rad51 is stimulated by Rad54, a member of the Swi2/Snf2 family of chromatin remodeling proteins/ATPases (San Filippo et al., 2008). Rad54 displaces Rad51 from double-stranded DNA in vitro, and this could be important for turnover of Rad51, or for uncovering the 3′ end of paired intermediates to allow initiation of DNA synthesis (San Filippo et al., 2008). DNA polymerase δ extends the 3′ end from the broken chromosome using the donor strand as a template and replacing nucleotides lost by end resection (Li et al., 2009). To resolve the intermediate by synthesis-dependent strand annealing, the invading strand that has been extended by DNA synthesis is displaced (strand displacement) and anneals to complementary sequences exposed by 5′–3′ resection of the other side of the break forming noncrossover products exclusively (strand annealing). In addition to preventing initiation of inappropriate recombination events by disrupting Rad51 nucleoprotein filaments, the Srs2 DNA helicase can disrupt D loop intermediates to promote noncrossover recombination (Dupaigne et al., 2008; San Filippo et al., 2008). The Mph1 and RTEL1 helicases also dissociate D loop intermediates to facilitate synthesis-dependent strand annealing (Barber et al., 2008; Prakash et al., 2009).

In the canonical DNA double-strand break repair model, the other end of the break interacts with the displaced strand of the strand invasion intermediate (second end capture) and the 3′ end primes DNA synthesis, forming a double Holliday junction (dHJ) intermediate (double Holliday junction formation). The dHJ intermediates can be dissolved or resolved to yield separate intact duplex molecules. Alternatively, the extended D loop structure could be cleaved by the Mus81-Mms4 nuclease prior to formation of a mature dHJ intermediate (early D loop cleavage). Mus81-Mms4 exhibits higher activity toward D loop and nicked Holliday junction intermediates than intact Holliday junctions and could promote crossovers by cleaving the strand invasion intermediate directly. Dissolution of dHJ intermediates requires the combined activity of the BLM/Sgs1 helicase, which drives migration of the constrained Holliday junctions, and the Top3-Rmi1 complex, which decatenates the interlinked strands between the two Holliday junctions eventually leading to noncrossover products (Wu and Hickson, 2003). On the other hand, resolution through nucleolytic cleavage of the Holliday junctions can yield crossover (cutting inner strands of one Holliday junction and outer strands of the other) or noncrossover (cutting both junctions in the same plane) products. To date, four resolvases have been determined to cleave Holliday junction intermediates in vitro: Mus81-Mms4, Slx1-Slx4, Yen1, and Rad1-Rad10 (Svendsen and Harper, 2010). Factors governing the choice of a resolvase are not yet clear, and a given species may preferentially use one of these resolvases in meiosis. For example, Mus81-Mms4 plays an essential role in the resolution of meiotic recombination intermediates in the fission yeast *Schizosaccharomyces pombe*, whereas SLX4/MUS312 is the central player in the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* (Svendsen and Harper, 2010). Following cleavage of Holliday junctions, the ends are ligated to complete the reaction.

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