Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

DNA helicases in homologous recombination repair

Dana Branzei[1,2] and Barnabas Szakal[1]

Helicases are in the spotlight of DNA metabolism and are critical for DNA repair in all domains of life. At their biochemical core, they bind and hydrolyze ATP, converting this energy to translocate unidirectionally, with different strand polarities and substrate binding specificities, along one strand of a nucleic acid. In doing so, DNA and RNA helicases separate duplex strands or remove nucleoprotein complexes, affecting DNA repair and the architecture of replication forks. In this review, we focus on recent advances on the roles and regulations of DNA helicases in homologous recombination repair, a critical pathway for mending damaged chromosomes and for ensuring genome integrity.

Addresses
[1] IFOM, the FIRC Institute of Molecular Oncology, Via Adamello 16, 20139, Milan, Italy

[2] Istituto di Genetica Molecolare, Consiglio Nazionale delle Ricerche (IGM-CNR), Via Abbiategrasso 207, 27100, Pavia, Italy

Corresponding author: Branzei, Dana (dana.branzei@ifom.eu)

Current Opinion in Genetics & Development 2021, **71**:27–33

This review comes from a themed issue on **Mechanisms of Homologous Recombination**

Edited by **Eric C Greene** and **Rodney Rothstein**

For a complete overview see the Issue and the Editorial

Available online 14th July 2021

https://doi.org/10.1016/j.gde.2021.06.009

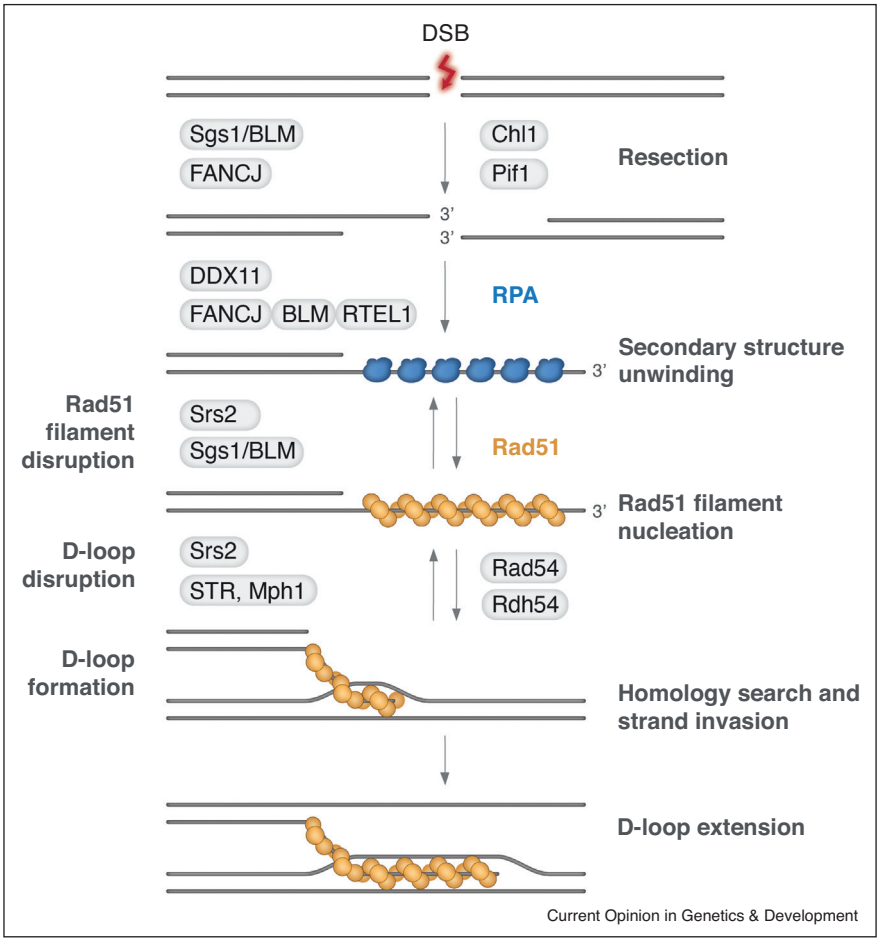
Introduction

Homologous recombination (HR) is critical for normal development and for stable propagation of the newly replicated genome. Defects in this process give rise to debilitating disorders, including predisposition to cancers, premature aging, reduced fertility and other congenital and developmental defects [1,2]. Functionally, HR is required to repair double strand breaks (DSBs), which arise due to replication errors or as a result of exogenous and endogenous DNA damage. In addition, HR factors stabilize stalled replication forks and guide filling of gaps arising during replication to the newly synthesized chromatid to facilitate replication completion [3]. The presence of daughter-strand gaps, rather than stalled or collapsed forks or DSBs, has recently emerged as the underlying feature of HR-defective cancers and of their chemotherapeutic sensitivity [4]. HR is most of the time error-free in outcome as the newly synthesized chromatid or the homologous chromosome is used as template for DNA repair [3]. However, in certain cases, HR can cause genome rearrangement and instability, for instance when induced at DNA repeat elements [5]. Importantly, formation, maturation and processing of HR intermediates, induced by both breaks and stalled replication forks, is intricately mediated or reversed by DNA helicases, which thus play critical roles in genome stability [6,7].

HR repair can be divided in several pathways, and the underlying mechanisms include gene conversion, when both sides of the break are homologous to the donor and participate in repair, and break-induced replication, when only one end of the break can find a homologous template [5]. The latter pathway of break-induced replication, recently discussed in a dedicated review [8], relates to situations arising at eroded telomeres or when replication forks collapse at nicked DNA. Another critical context for HR repair is the one of gap-filling by template switching, in which replication-associated DNA gaps ensured by repriming downstream the lesion [9,10] are being engaged by postreplicative repair coordinated by PCNA polyubiquitylation and HR factors. In HR-mediated gap-filling, the information from the newly synthesized sister chromatid is used to bypass the DNA damage postreplicatively [3,11••]. In addition, HR factors play roles in stabilizing and restarting stalled replication forks through fork remodeling [12].

Several steps are common to HR repair pathways and include: (1) resection of DSB ends or extension of the gaps before gap-filling; (2) assembly of the Rad51 helical filament known as the presynaptic complex; (3) homology search and strand invasion with the formation of a D-loop (Figure 1). In gene conversion, the D-loop is extended via DNA synthesis after which it can be disassembled by DNA helicases in a process known as synthesis-dependent strand annealing (SDSA) leading to noncrossover recombinants (Figure 2). Alternative to SDSA, D-loop extension can be combined with second end capture, leading to the formation of a double Holliday junction (dHJ) (Figure 2) or a pseudo-dHJ intermediate if the context is gap-filling. Subsequently, dHJs are either dissolved by a helicase-topoisomerase complex (Sgs1-Top3-Rmi1, known as STR in budding yeast, and BLM-TOPIIIα-RMI1-RMI2 in vertebrates) to noncrossovers or resolved by structure-specific endonucleases, with the potential of forming crossovers [13] (Figure 2). Importantly, most steps involved in HR repair rely on the action of DNA helicases (Figures 1 and 2).

Figure 1



Representation of critical steps in homologous recombination repair and regulatory DNA helicases. The model shows formation of the recombinogenic Rad51 filament following resection of double strand breaks, and includes also DNA helicases Chl1 and Pif1 implicated in the resection of stalled forks and daughter-strand gaps. The homology search or strand invasion step is facilitated by Rad54 and Rdh54 translocases and can be reversed by several DNA helicases, some of which can also disrupt Rad51 filaments.

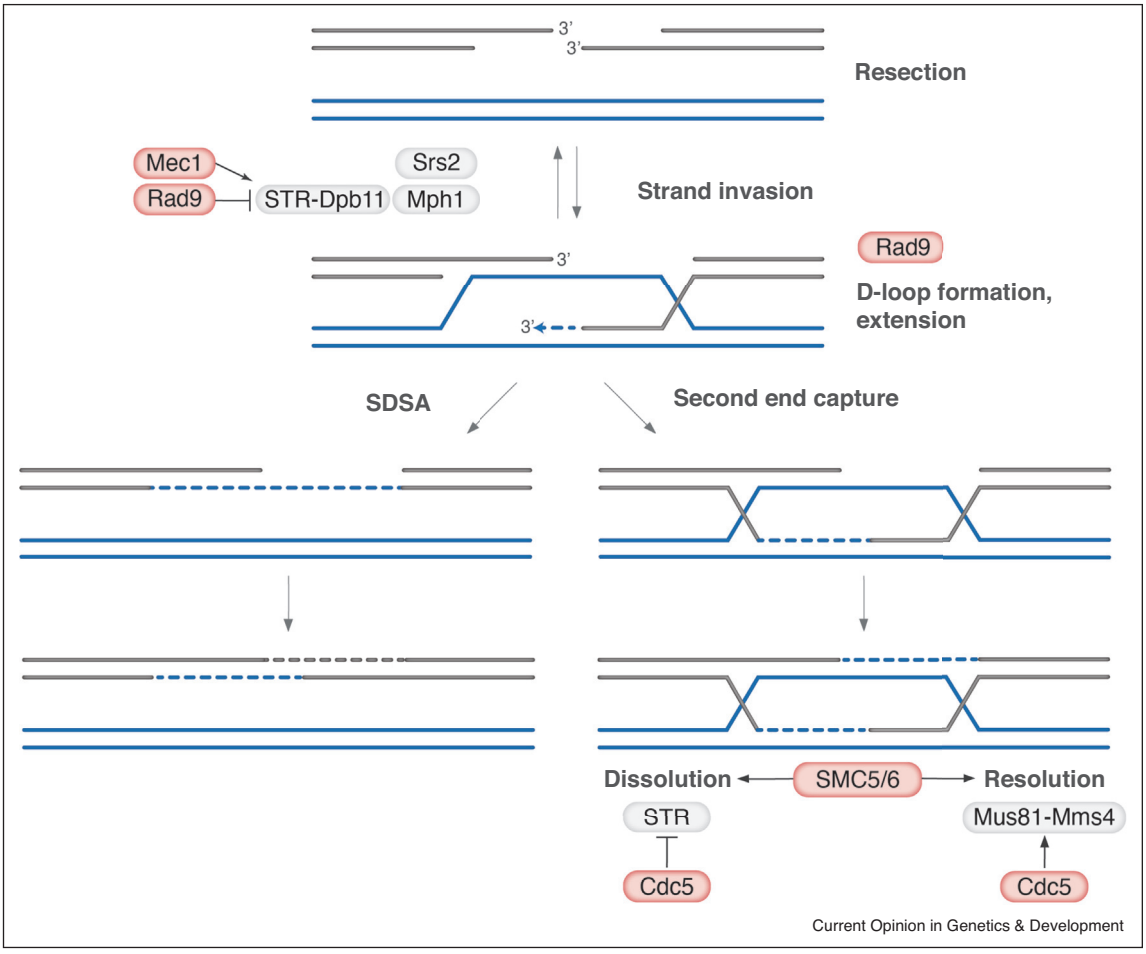
Basic functions of DNA helicase and how they affect various or specific HR steps have been recently described in a review [14]. Here, we focus on recent insights on how DNA helicases modulate HR, how their activity is influenced by posttranslational modifications and interaction with other proteins, and how in certain cases the helicase activity modulation is context or cell cycle specific. Although we occasionally refer to recently gained insights from other model systems, we largely focus on recent work in budding yeast related to helicase-mediated repair of DSBs and filling of daughter-strand gaps arising during replication as a consequence of repriming.

Helicases facilitating DNA end resection

A common step in different HR pathways is the one of end resection, which generates extended 3′ single stranded DNA tails (Figure 1). Briefly, the resection process is initiated by the MRX complex (Mre11-Rad50-Xrs2 in budding yeast and MRE11-RAD50-NBS1 in mammalian cells) and the Sae2/CtIP nuclease, which together create an entry point for the Exo1 exonuclease, the Sgs1/BLM helicase and the Dna2 nuclease that mediate long resection [15]. Several recent findings showed that the activity of Sgs1 in end resection converges with the one of Dna2 nuclease, requires RPA, is facilitated by Top3 and Rmi1 interacting partners [16,17], and is restrained by Rad52 [18•]. Moreover, several other DNA helicases were recently shown to facilitate DNA resection. Specifically, the budding yeast Chl1 helicase acts at stalled replication forks jointly with Sgs1, in a process that leads to large ssDNA gaps necessary for cohesin recruitment [19•]. Upon genotoxic stress, cohesin recruitment to stalled forks facilitates fork restart [20] and mediates HR-mediated filling of daughter-strand gaps [9,21].

Longer gaps facilitate daughter-strand gap filling via HR [22,23] but the role of long resection in HR-mediated gap-

Figure 2



Representation of homologous recombination repair following strand invasion and D-loop formation. D-loop extension can be followed by synthesis dependent strand annealing or second end capture with the formation of double Holliday Junctions (dHJs). Negative and positive regulators of the STR activity in D-loop disruption and dissolution of dHJs are depicted.

filling remains to be elucidated as, so far, out of the factors implicated in DSB resection, only Exo1 was shown to positively influence replication-associated recombination [11••,23,24]. Exo1 was proposed to function complementarily with the Pif1 helicase at the different sides of the daughter strand gap, Exo1 at the 5′ side [24] and Pif1 at the 3′ end [23]. Paradoxically at the first sight, loss of Sgs1, which is critical for long range resection of DSBs, does not greatly affect the length of DNA gaps arising during HR-mediated gap-filling [22] or at stalled and converging forks [25••]. Rather, similarly with Top3 inactivation, Sgs1 loss causes the accumulation of DNA junctions with features of double Holliday Junctions [22] and, at stalled replication forks at replication termination regions, also of reversed forks [25••]. These results imply that the primary role of Sgs1 and the whole STR complex in the context of replication-associated recombination is the one of processing joint molecules. The less prominent role of Sgs1 in extending daughter-strand gaps may be due to the presence of Rad52, accumulating in postreplicative chromatin regions upon genotoxic stress [11••] and inhibiting Sgs1-mediated resection [18•]. For daughter-strand gap extension, Sgs1 loss may be compensated by other DNA helicases, such as Pif1 [23], potentially also synergizing with the Dna2 helicase/nuclease to achieve optimal resection and lagging strand DNA synthesis [26] (Figure 1). Chl1 family of helicases is also important for end resection, as supported by its role at stalled replication forks [19•], and by findings in mammalian cells, whereby its orthologs, FANCJ and DDX11 helicases facilitate DSB resection and RAD51 recruitment [27,28••] (Figure 1). FANCJ promotes recruitment of CtIP [27], whereas DDX11 mediates unwinding of secondary structures, such as G quadruplex [29•,30•] to facilitate loading of RPA, RAD51 and allow efficient HR repair [28••]. Along DDX11, other DNA helicases, including FANCJ, BLM

and RTEL1 [31] mediate unwinding of G quadruplex structures during DNA repair or at specific genomic locations, such as telomeres.

RPA, RNA-DNA hybrids and Rad51 filament nucleation on resected ends

Following DNA end resection, RPA is generally thought to coat the exposed ssDNA until the Rad51 filament is assembled and HR can proceed [32]. Besides forming a barrier to the formation of the Rad51 nucleoprotein filament, RPA opens secondary structures in ssDNA [33], an activity which in certain cases is supported by specific DNA helicases, such as WRN in case of hairpins caused by TA-dinucleotide repeats [34,35] and DDX11 and possibly other helicases in case of G quadruplex structures [28••] (Figure 1). RPA binding to ssDNA limits spurious interactions with other DNA filaments [36], activates the checkpoint response, and facilitates resection by affecting the processivity and affinity of Sgs1 for DNA [16,17]. Moreover, in cases of hyper-resection, a Mec1-dependent branch of the checkpoint phosphorylates Sgs1 to suppress HR [37••] as discussed in more detail in the following section.

In spite of these various roles of RPA summarized above, recent work proposed that the protection of resected 3′-overhangs requires the transient formation of RNA-DNA hybrids by RNA polymerase III, which is recruited to DSBs by the MRN complex and is essential for HR repair in mammalian cells [38]. Controversially, RNA-DNA hybrids were also shown to inhibit resection and reduce HR repair fidelity [39,40,41••]. RNase H2A deficiency, causing accumulation of long RNA:DNA hybrids at DNA ends, impairs overall resection, and extended RNA:DNA hybrids inhibit both strand separation by BLM and resection by EXO1 [41••]. In the same vein, Sen1 helicase and its ortholog Senataxin were shown to support HR and DNA end-resection by facilitating resolution of DNA-RNA hybrids [39,40]. Recent work revealed that RNA-DNA hybrids and RNA:DNA triplexes represent a prominent mode of RNA-mediated chromatin interactions that can affect the torsional stress on chromosomes with impact on HR [42]. Thus, more studies will be needed to elucidate how the levels and distribution of RNA-DNA hybrids affect HR at resected ends or in other biological contexts.

The dynamic Rad51 nucleoprotein filaments and D-loop structures

The Rad51 nucleofilament assembled on ssDNA is a dynamic structure subjected to competing activities that promote its stabilization or disassembly. Rad51 paralogues promote the stability of the Rad51 filament and can restrain its disassembly mediated by certain DNA helicases [2]. The budding yeast Srs2 helicase has been amply characterized in this regard, but several other RecQ helicases, including mammalian RECQL5 and budding yeast Sgs1 [43] possess Rad51 removal activity. Matching these biochemical activities with the observed phenotypes of the corresponding mutants, it emerges that this quality control step optimizes the efficiency of HR and restricts Rad51 function to appropriate DNA substrates. This latter function is enforced and often coupled, as in the case of Srs2 and Sgs1 [44•], with a second activity, the one of disrupting D-loops formed by the invasion of the Rad51 filament into a duplex DNA (Figure 1). The invasion leads to the transient formation of a three-stranded intermediate that precedes the formation of heteroduplex DNA composed of the invading strand and the complementary strand of the invaded molecule [5,14]. Several DNA helicases are now known to promote D-loop disassembly. Recent studies that used a proximity ligation assay to detect early chromosome associations during DSB repair found evidence that this signal was increased in *sgs1*, *mph1* and *srs2* mutants [44•]. The D-loop disruption activities could be grouped into two main pathways, one defined by Srs2, the other by Mph1 and the STR complex, without major overlap between them [44•]. Similar with the situation in budding yeast, mammalian RTEL1, FANCM, BLM and FANCJ can also disrupt D-loop structures (reviewed in [5]). The main scopes of the D-loop disruption activity are to prevent recombination to ectopic substrates, counteract dHJ formation that can lead to crossover (see Figure 2), and limit the extent of gene conversion that can be mutagenic [5,45].

This important quality control step is likely to undergo strict regulation. Recent work highlighted an intricate link between the extent of resection and D-loop disruption activities. The DNA damage checkpoint mediator Rad9, ortholog of 53BP1 and limiting DSB resection and the length of ssDNA filament needed for recombination, was shown to promote annealing between the invading filament and the donor template, thus preventing D-loop disruption by STR and the Mph1 helicase [46•] (Figure 2). In this manner, Rad9 favors long tract gene conversion often associated with second-end capture and crossover outcome. This final effect observed in *rad9* mutants may have another component, mediated by the activation of a Mec1 checkpoint kinase cascade in *rad9* cells [37••]. In the context of hyper-resection, Mec1 signaling phosphorylates the Sgs1 helicase, promoting an interaction between STR and the DNA repair scaffold Dpb11 to discourage HR repair [37••]. Although Sgs1 is able to disrupt Rad51-ssDNA filaments [43], the newly uncovered Mec1-signaling cascade facilitates STR stabilization at DNA lesions, which is then capable of rejecting recombination intermediates by disrupting D-loops [37••].

D-loop unwinding versus dHJ dissolution

While D-loop disassembly promotes noncrossover and prevents dHJ formation, it can redirect events to different recombination pathways as this early intermediate can undergo additional rounds of invasion and disassembly. On the other hand, when dHJ formation is entailed by

dissolution by the STR complex, this process not only leads exclusively to noncrossover but also terminates the recombination process (Figure 2). The Sgs1/BLM helicase is unique in its ability to function with Top3 to facilitate dHJ dissolution, a process that is relevant not only for DSB repair [47,48] but also for the removal of recombination intermediates arising during DNA damage tolerance and at converging forks during replication termination [22,25••]. Both D-loop disruption and dHJ dissolution activities reported for Sgs1 crucially rely on Top3 [49,50]. Very recently, several other regulators of Sgs1 and STR emerged, in addition to the one mediated by Mec1-mediated phosphorylation of Sgs1 discussed above [37••] (Figure 2). One is related to phosphorylation of Sgs1 by S-CDK and Cdc5 kinases [51•]. This phosphorylation enhances the *in vitro* helicase activity of Sgs1, which may cause increased processing of joint molecules. Interestingly, subsequent Cdc5 hyperphosphorylation of Sgs1 may reduce its activity, while Cdc5 activates the Mus81-Mms4 nuclease to resolve persistent intermediates before chromosome segregation [51•] (Figure 2). The activity window of Mus81-Mms4 is then additionally enforced via its turnover in mitosis, a process relying on the proteasomal degradation of SUMO-chains-modified and ubiquitin-modified Mms4 in mitosis, to prevent the unscheduled action of the Mus81-Mms4 endonuclease action during chromosome replication [52].

SUMOylation and the structural maintenance of chromosome complex, Smc5/6, are also important regulators of the STR complex in HR repair and processing of endogenous DNA damage [25••,53] (Figure 2). Smc5/6 enhances STR joint molecule processing activity via SUMOylation events mediated by the SUMO ligase activity of its Mms21/Nse2 component [54,55]. In normal conditions of replication, Smc5/6 facilitates the recruitment of Top3 and STR to genomic regions prone to accumulate torsional stress and recombination structures, which subsequently they jointly process [25••]. In addition, Smc5/6 coordinates other DNA crossed-strand processing enzymes to facilitate removal of joint molecules [25••], one of which is likely the Mus81-Mms4 endonuclease (Figure 2). Thus, Smc5/6 and Cdc5 regulators may potentially couple DNA recombination intermediate processing with local genome compaction for chromosome segregation.

Concluding remarks and open questions

The HR repair regulation by DNA helicases is an intricate process with effects on genome alterations and integrity, frequency of crossovers and chromosome structure. Some of the DNA helicases discussed here are regulated via posttranslational modifications events that affect their processivity or activity, binding affinity to genomic regions, DNA substrates and interacting partners. Future work will be needed to reveal whether noncrossover and crossover pathways of HR repair are sequentially activated in mitotically dividing cells and the principles that orchestrate such concerted mechanisms. The process of recombination repair is in crosstalk with the DNA damage checkpoint, which affects the temporal window of activation of cell cycle kinases, the functionality of DNA crossed-strand processing enzymes and possibly chromosome structure. As we have only begun to gain information on the latter processes, an important topic for the future will be to reveal how these regulations are interconnected to ensure the stable propagation of the newly replicated genome.

Conflict of interest statement

Nothing declared.

Acknowledgements

We apologize to our colleagues whose contributions are not cited due to space limitations. DB acknowledges the Italian Association for Cancer Research (AIRC IG 23710) and the European Research Council (Consolidator grant 682190) for funding.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Moynahan ME, Jasin M: **Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis**. *Nat Rev Mol Cell Biol* 2010, **11**:196-207.

2. Bonilla B, Hengel SR, Grundy MK, Bernstein KA: **RAD51 gene family structure and function**. *Annu Rev Genet* 2020, **54**:25-46.

3. Branzei D, Szakal B: **DNA damage tolerance by recombination: molecular pathways and DNA structures**. *DNA Repair* 2016, **44**:68-75 http://dx.doi.org/10.1016/j.dnarep.2016.05.008.

4. Panzarino NJ, Krais JJ, Cong K, Peng M, Mosqueda M, Nayak SU, Bond SM, Calvo JA, Doshi MB, Bere M *et al.*: **Replication gaps underlie BRCA deficiency and therapy response**. *Cancer Res* 2021, **81**:1388-1397.

5. Scully R, Panday A, Elango R, Willis NA: **DNA double-strand break repair-pathway choice in somatic mammalian cells**. *Nat Rev Mol Cell Biol* 2019, **20**:698-714.

6. Lu H, Davis AJ: **Human RecQ helicases in DNA double-strand break repair**. *Front Cell Dev Biol* 2021, **9**:640755.

7. Niu H, Klein HL: **Multifunctional roles of Saccharomyces cerevisiae Srs2 protein in replication, recombination and repair**. *FEMS Yeast Res* 2017, **17**.

8. Kockler ZW, Osia B, Lee R, Musmaker K, Malkova A: **Repair of DNA breaks by break-induced replication**. *Annu Rev Biochem* 2021, **90**:165-191.

9. Fumasoni M, Zwicky K, Vanoli F, Lopes M, Branzei D: **Error-free DNA damage tolerance and sister chromatid proximity during DNA replication rely on the Polalpha/Primase/Ctf4 Complex**. *Mol Cell* 2015, **57**:812-823.

10. Bainbridge LJ, Teague R, Doherty AJ: **Repriming DNA synthesis: an intrinsic restart pathway that maintains efficient genome replication**. *Nucleic Acids Res* 2021, **49**:4831-4847.

11. •• Wong RP, Garcia-Rodriguez N, Zilio N, Hanulova M, Ulrich HD: **Processing of DNA polymerase-blocking lesions during genome replication is spatially and temporally segregated from replication forks**. *Mol Cell* 2020, **77**:3-16.e14
This paper visualizes sites of postreplicative DNA damage bypass involving translesion synthesis and homologous recombination in a different compartment from that of the stalled replisome.

12. Berti M, Cortez D, Lopes M: **The plasticity of DNA replication forks in response to clinically relevant genotoxic stress**. *Nat Rev Mol Cell Biol* 2020, **21**:633-651.

13. Matos J, West SC: **Holliday junction resolution: regulation in space and time**. *DNA Repair (Amst)* 2014, **19**:176-181.

14. Crickard JB, Greene EC: **Helicase mechanisms during homologous recombination in *Saccharomyces cerevisiae***. *Annu Rev Biophys* 2019, **48**:255-273.

15. Gobbini E, Casari E, Colombo CV, Bonetti D, Longhese MP: **The 9-1-1 complex controls Mre11 nuclease and checkpoint activation during short-range resection of DNA double-strand breaks**. *Cell Rep* 2020, **33**:108287.

16. Kasaciunaite K, Fettes F, Levikova M, Daldrop P, Anand R, Cejka P, Seidel R: **Competing interaction partners modulate the activity of Sgs1 helicase during DNA end resection**. *EMBO J* 2019, **38**:e101516.

17. Xue C, Wang W, Crickard JB, Moevus CJ, Kwon Y, Sung P, Greene EC: **Regulatory control of Sgs1 and Dna2 during eukaryotic DNA end resection**. *Proc Natl Acad Sci U S A* 2019, **116**:6091-6100.

18. • Yan Z, Xue C, Kumar S, Crickard JB, Yu Y, Wang W, Pham N, Li Y, Niu H, Sung P *et al.*: **Rad52 restrains resection at DNA double-strand break ends in yeast**. *Mol Cell* 2019, **76**:699-711.e6

This paper elucidates negative regulations imposed by Rad52 on a key resection factor, Sgs1.

19. • Delamarre A, Barthe A, de la Roche Saint-Andre C, Luciano P, Forey R, Padioleau I, Skrzypczak M, Ginalski K, Geli V, Pasero P *et al.*: **MRX increases chromatin accessibility at stalled replication forks to promote nascent DNA resection and cohesin loading**. *Mol Cell* 2019, **77**:395-410

This study starts to elucidate the complex phenomenon of stalled fork resection and highlights that one of the functions of this controversial process is related to efficient cohesin loading.

20. Tittel-Elmer M, Lengronne A, Davidson MB, Bacal J, Francois P, Hohl M, Petrini JH, Pasero P, Cobb JA: **Cohesin association to replication sites depends on rad50 and promotes fork restart**. *Mol Cell* 2012, **48**:98-108.

21. Litwin I, Bakowski T, Szakal B, Pilarczyk E, Maciaszczyk-Dziubinska E, Branzei D, Wysocki R: **Error-free DNA damage tolerance pathway is facilitated by the Irc5 translocase through cohesin**. *EMBO J* 2018, **37**.

22. Giannattasio M, Zwicky K, Follonier C, Foiani M, Lopes M, Branzei D: **Visualization of recombination-mediated damage bypass by template switching**. *Nat Struct Mol Biol* 2014, **21**:884-892.

23. Garcia-Rodriguez N, Wong RP, Ulrich HD: **The helicase Pif1 functions in the template switching pathway of DNA damage bypass**. *Nucleic Acids Res* 2018, **46**:8347-8356.

24. Karras GI, Fumasoni M, Sienski G, Vanoli F, Branzei D, Jentsch S: **Noncanonical role of the 9-1-1 clamp in the error-free DNA damage tolerance pathway**. *Mol Cell* 2013, **49**:536-546.

25. •• Agashe S, Joseph CR, Reyes TAC, Menolfi D, Giannattasio M, Waizenegger A, Szakal B, Branzei D: **Smc5/6 functions with Sgs1-Top3-Rmi1 to complete chromosome replication at natural pause sites**. *Nat Commun* 2021, **12**:2111

This study visualizes recombination structures forming at stalled replication forks and shows Smc5/6 as a critical factor in facilitating the functionality of the STR complex and coordinating other processes of joint molecules-removal.

26. Rossi SE, Foiani M, Giannattasio M: **Dna2 processes behind the fork long ssDNA flaps generated by Pif1 and replication-dependent strand displacement**. *Nat Commun* 2018, **9**:4830.

27. Nath S, Nagaraju G: **FANCJ helicase promotes DNA end resection by facilitating CtIP recruitment to DNA double-strand breaks**. *PLoS Genet* 2020, **16**:e1008701.

28. •• Jegadesan NK, Branzei D: **DDX11 loss causes replication stress and pharmacologically exploitable DNA repair defects**. *Proc Natl Acad Sci U S A* 2021, **118**

This study highlights that DDX11 helicase is critical for survival of BRCA1 and BRCA2 defective tumors by facilitating upstream DNA repair steps related to efficient unwinding and resection of a subset of genomic regions.

29. • van Schie JJM, Faramarz A, Balk JA, Stewart GS, Cantelli E, Oostra AB, Rooimans MA, Parish JL, de Almeida Esteves C, Dumic K *et al.*: **Warsaw breakage syndrome associated DDX11 helicase resolves G-quadruplex structures to support sister chromatid cohesion**. *Nat Commun* 2020, **11**:4287

Together with Ref. [30], this paper uncovers a critical role for DDX11 helicase in processing G-quadruplex structures and suggests that this activity is critical for sister chromatid cohesion.

30. • Lerner LK, Holzer S, Kilkenny ML, Svikovic S, Murat P, Schiavone D, Eldridge CB, Bittleston A, Maman JD, Branzei D *et al.*: **Timeless couples G-quadruplex detection with processing by DDX11 helicase during DNA replication**. *EMBO J* 2020, **29**:e104185 http://dx.doi.org/10.15252/embj.2019104185

Together with Ref. [29], this paper uncovers a critical role for DDX11 helicase along with Timeless in processing G-quadruplex structures to maintain epigenetic integrity.

31. Lansdorp P, van Wietmarschen N: **Helicases FANCJ, RTEL1 and BLM Act on guanine quadruplex DNA in vivo**. *Genes (Basel)* 2019, **10**.

32. San Filippo J, Sung P, Klein H: **Mechanism of eukaryotic homologous recombination**. *Annu Rev Biochem* 2008, **77**:229-257.

33. Chen H, Lisby M, Symington LS: **RPA coordinates DNA end resection and prevents formation of DNA hairpins**. *Mol Cell* 2013, **50**:589-600.

34. Chan EM, Shibue T, McFarland JM, Gaeta B, Ghandi M, Dumont N, Gonzalez A, McPartlan JS, Li T, Zhang Y *et al.*: **WRN helicase is a synthetic lethal target in microsatellite unstable cancers**. *Nature* 2019, **568**:551-556.

35. van Wietmarschen N, Sridharan S, Nathan WJ, Tubbs A, Chan EM, Callen E, Wu W, Belinky F, Tripathi V, Wong N *et al.*: **Repeat expansions confer WRN dependence in microsatellite-unstable cancers**. *Nature* 2020, **586**:292-298.

36. Deng SK, Chen H, Symington LS: **Replication protein A prevents promiscuous annealing between short sequence homologies: implications for genome integrity**. *Bioessays* 2015, **37**:305-313.

37. •• Sanford EJ, Comstock WJ, Faca VM, Vega SC, Gnugge R, Symington LS, Smolka MB: **Phosphoproteomics reveals a distinctive Mec1/ATR signaling response upon DNA end hyper-resection**. *EMBO J* 2021, **40**:e104566 http://dx.doi.org/10.15252/embj.2019104185

This study uncovers that hyper-resection in the absence of the checkpoint adaptor Rad9 activates a Mec1 checkpoint branch that phosphorylates Sgs1 to increase its D-loop disruption activity.

38. Liu S, Hua Y, Wang J, Li L, Yuan J, Zhang B, Wang Z, Ji J, Kong D: **RNA polymerase III is required for the repair of DNA double-strand breaks by homologous recombination**. *Cell* 2021, **184**:1314-1329.e10.

39. Cohen S, Puget N, Lin YL, Clouaire T, Aguirrebengoa M, Rocher V, Pasero P, Canitrot Y, Legube G: **Senataxin resolves RNA:DNA hybrids forming at DNA double-strand breaks to prevent translocations**. *Nat Commun* 2018, **9**:533.

40. Rawal CC, Zardoni L, Di Terlizzi M, Galati E, Brambati A, Lazzaro F, Liberi G, Pellicioli A: **Senataxin ortholog Sen1 limits DNA:RNA hybrid accumulation at DNA double-strand breaks to control end resection and repair fidelity**. *Cell Rep* 2020, **31**:107603.

41. •• Daley JM, Tomimatsu N, Hooks G, Wang W, Miller AS, Xue X, Nguyen KA, Kaur H, Williamson E, Mukherjee B *et al.*: **Specificity of end resection pathways for double-strand break regions containing ribonucleotides and base lesions**. *Nat Commun* 2020, **11**:3088

This study reports the effects of ribonucleotides, RNA-DNA hybrids and damaged bases on the resection nucleases and BLM activity.

42. Marnef A, Legube G: **R-loops as Janus-faced modulators of DNA repair**. *Nat Cell Biol* 2021, **23**:305-313.

43. Crickard JB, Xue C, Wang W, Kwon Y, Sung P, Greene EC: **The RecQ helicase Sgs1 drives ATP-dependent disruption of Rad51 filaments**. *Nucleic Acids Res* 2019, **47**:4694-4706.

44. • Piazza A, Shah SS, Wright WD, Gore SK, Koszul R, Heyer WD: **Dynamic processing of displacement loops during recombinational DNA repair**. *Mol Cell* 2019, **73**:1255-1266.e4
This study reports the development of a novel assay that can detect the formation of D-loops *in vivo* and separates known D-loop processing helicases into two pathways delineated by Srs2 and STR, Mph1, respectively.

45. Nath S, Somyajit K, Mishra A, Scully R, Nagaraju G: **FANCJ helicase controls the balance between short- and long-tract gene conversions between sister chromatids**. *Nucleic Acids Res* 2017, **45**:8886-8900.

46. • Ferrari M, Rawal CC, Lodovichi S, Vietri MY, Pellicioli A: **Rad9/53BP1 promotes DNA repair via crossover recombination by limiting the Sgs1 and Mph1 helicases**. *Nat Commun* 2020, **11**:3181
This work provides a molecular mechanism on Rad9 functioning as a critical regulator of long tract gene conversion and crossover by limiting strand rejection mediated by STR and Mph1.

47. Kaur H, Gn K, Lichten M: **Unresolved recombination intermediates cause a RAD9-dependent cell cycle arrest in *Saccharomyces cerevisiae***. *Genetics* 2019, **213**:805-818.

48. Ira G, Malkova A, Liberi G, Foiani M, Haber JE: **Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast**. *Cell* 2003, **115**:401-411.

49. Fasching CL, Cejka P, Kowalczykowski SC, Heyer WD: **Top3-Rmi1 dissolve Rad51-mediated D loops by a topoisomerase-based mechanism**. *Mol Cell* 2015, **57**:595-606.

50. Wu L, Hickson ID: **The Bloom's syndrome helicase suppresses crossing over during homologous recombination**. *Nature* 2003, **426**:870-874.

51. • Grigaitis R, Ranjha L, Wild P, Kasaciunaite K, Ceppi I, Kissling V, Henggeler A, Susperregui A, Peter M, Seidel R *et al.*: **Phosphorylation of the RecQ helicase Sgs1/BLM controls its DNA unwinding activity during meiosis and mitosis**. *Dev Cell* 2020, **53**:706-723.e5
This work uncovers that Sgs1 is phosphorylated by S-CDK and Cdc5 with effects on its DNA helicase activity.

52. Waizenegger A, Urulangodi M, Lehmann CP, Reyes TAC, Saugar I, Tercero JA, Szakal B, Branzei D: **Mus81-Mms4 endonuclease is an Esc2-STUbL-Cullin8 mitotic substrate impacting on genome integrity**. *Nat Commun* 2020, **11**:5746.

53. Menolfi D, Delamarre A, Lengronne A, Pasero P, Branzei D: **Essential roles of the Smc5/6 complex in replication through natural pausing sites and endogenous DNA damage tolerance**. *Mol Cell* 2015, **60**:835-846.

54. Bermudez-Lopez M, Villoria MT, Esteras M, Jarmuz A, Torres-Rosell J, Clemente-Blanco A, Aragon L: **Sgs1's roles in DNA end resection, HJ dissolution, and crossover suppression require a two-step SUMO regulation dependent on Smc5/6**. *Genes Dev* 2016, **30**:1339-1356.

55. Bonner JN, Choi K, Xue X, Torres NP, Szakal B, Wei L, Wan B, Arter M, Matos J, Sung P *et al.*: **Smc5/6 mediated sumoylation of the Sgs1-Top3-Rmi1 complex promotes removal of recombination intermediates**. *Cell Rep* 2016, **16**:368-378.