UNIVERSITY OF
GLOUCESTERSHIRE

This is a peer-reviewed, post-print (final draft post-refereeing) version of the following published document and is licensed under Creative Commons: Attribution-Noncommercial-No Derivative Works 4.0 license:

Rein, Hayley L, Bernstein, Kara A and Baldock, Robbie ORCID logoORCID: https://orcid.org/0000-0002-4649-2966 (2021) RAD51 paralog function in replicative DNA damage and tolerance. Current Opinion in Genetics and Development, 71. pp. 86-91. doi:10.1016/j.gde.2021.06.010

Official URL: https://doi.org/10.1016/j.gde.2021.06.010
DOI: http://dx.doi.org/10.1016/j.gde.2021.06.010
EPrint URI: https://eprints.glos.ac.uk/id/eprint/10049

Disclaimer

The University of Gloucestershire has obtained warranties from all depositors as to their title in the material deposited and as to their right to deposit such material.

The University of Gloucestershire makes no representation or warranties of commercial utility, title, or fitness for a particular purpose or any other warranty, express or implied in respect of any material deposited.

The University of Gloucestershire makes no representation that the use of the materials will not infringe any patent, copyright, trademark or other property or proprietary rights.

The University of Gloucestershire accepts no liability for any infringement of intellectual property rights in any material deposited but will remove such material from public view pending investigation in the event of an allegation of any such infringement.

PLEASE SCROLL DOWN FOR TEXT.

Title: RAD51 paralog function in replicative DNA damage and tolerance

Hayley L. Rein[1], Kara A. Bernstein[1], Robert A. Baldock[2±]

[1]University of Pittsburgh School of Medicine, Department of Pharmacology and Chemical Biology, Pittsburgh, PA, USA.

[2]School of Natural and Social Sciences, University of Gloucestershire, Cheltenham, UK.

[±]Corresponding Author, Robert A. Baldock, rbaldock@glos.ac.uk.

Corresponding author full postal address:

QU116, The Quad, Francis Close Hall, Swindon Road, University of Gloucestershire, UK, GL50 4AZ.

Email addresses of co-authors:

Hayley L. Rein, HLR42@pitt.edu

Kara A. Bernstein, KARAB@pitt.edu

Abstract

RAD51 paralog gene mutations are observed in both hereditary breast and ovarian cancers. Classically, defects in RAD51 paralog function are associated with homologous recombination (HR) deficiency and increased genomic instability. Several recent investigative advances have enabled characterization of non-canonical RAD51 paralog function during DNA replication. Here we discuss the role of the RAD51 paralogs and their associated complexes in integrating a robust response to DNA replication stress. We highlight recent discoveries suggesting that the RAD51 paralogs complexes mediate lesion-specific tolerance of replicative stress following exposure to alkylating agents and the requirement for the Shu complex in fork restart upon fork stalling by dNTP depletion. In addition, we describe the role of the BCDX2 complex in restraining and promoting fork remodeling in response to fluctuating dNTP pools. Finally, we highlight recent work demonstrating a requirement for RAD51C in recognizing and tolerating methyl-adducts. In each scenario, RAD51 paralog complexes play a central role in lesion recognition and bypass in a replicative context. Future studies will determine how these critical functions for RAD51 paralog complexes contribute to tumorigenesis.

Introduction

The RAD51 paralogs regulate RAD51 filament formation, an essential step in DNA double-strand break (DSB) repair and in tolerance of DNA damage that arises during replication. RAD51 and its regulators principally function in the homologous recombination (HR) pathway to facilitate error-free DSB repair [1]. However, recent

evidence has provided additional functions for RAD51, and its paralogs, in repair of replicative damage independent from their canonical HR functions and will be the focus of this review.

There are six RAD51 paralogs in mammalian cells including RAD51B, RAD51C, RAD51D, XRCC2, XRCC3, and the recently identified, SWSAP1 (**Fig. 1**;[2-7]). The RAD51 paralogs share between 20-30% sequence identity with RAD51, except for SWSAP1, which shares an equivalent proportion of amino acid sequence identity with the archaeal recombinase, RadA [7, 8]. The majority of this sequence identity is at the conserved Walker A and Walker B motifs, which fold into domains that enable ATP-binding and hydrolysis [9]. In RAD51C, the ATP-binding activity is critical for function as demonstrated by increased sensitivity to the crosslinking agent, mitomycin C, in cells with a mutated Walker A motif [10].

The RAD51 paralogs were initially shown to form two distinct complexes with RAD51B-RAD51C-RAD51D-XRCC2 forming the BCDX2 complex and RAD51C-XRCC3 forming a separate CX3 complex (**Fig. 1**; [7, 11-14]). The BCDX2 complex preferentially binds single-stranded DNA (ssDNA), which also stimulates its ATPase activity [15]. Similar DNA-binding activity was also observed for the CX3 complex and is ATP independent [15]. Uniquely, RAD51C is common to both the BCDX2 and CX3 complexes, and also associates with a third complex containing PALB2, RAD51, and BRCA2 (**Fig. 1**;[16]).

The newest RAD51 paralog member, SWSAP1, forms a complex with the SWIM domain-containing protein, SWS1, and is referred to as the Shu complex, based upon its yeast orthologs (**Fig. 1**;[7, 17, 18]). Recent investigation of the human Shu complex revealed additional associated factors to include SPIDR and PDS5B [19, 20]. SPIDR is a scaffolding protein known to bind RAD51 and the ATPase, FIGNL1 [21-23]. Interestingly, PDS5B also binds RAD51, PALB2 and BRCA2 [24]. Together, these four RAD51 paralog containing complexes have unique and important functions in repair and tolerance of replication associated DNA damage, replication fork restart, and/or replication fork protection.

Role of the RAD51 paralogs in tolerance and bypass of specific replicative DNA lesions

When a replication fork encounters a fork blocking lesion, such as an abasic site or DNA crosslink, the fork can stall or collapse [25, 26]. Fork stalling enables time and space for the lesion to be repaired and/or bypassed, whereas fork collapse creates a toxic DNA DSB. These lesions are bypassed through several error-prone pathways, such as translesion synthesis (TLS), but emerging evidence has revealed a novel role for RAD51, and its paralogs, in error-free bypass of replication fork blocking lesions (**Fig. 2**). As there are a wide array of fork blocking lesions, recent studies suggest that recognition of these lesions is specific to unique RAD51 paralog complexes and maybe the reason why so many RAD51 paralogs are needed to modulate RAD51 function (**Fig. 2**).

Suggesting that the RAD51 paralogs aid in the repair and tolerance of specific replication associated DNA damage, the specialized functions of the RAD51 paralog complexes stems from their unique DNA damage sensitivity. In budding yeast, Shu complex mutants are primarily sensitive to the alkylating agent, methyl methanesulfonate [18, 27]. DNA alkylation damage is primarily repaired by the base excision repair (BER) pathway [28]. However, if DNA alkylation or their BER repair intermediates persist into S phase, these aberrant structures can cause replication fork stalling, collapse, and eventually DSB formation [25]. Thus, the Shu complex may play a role in the tolerance of specific MMS-induced DNA lesions during DNA replication. For example, Shu complex mutants have exquisite MMS sensitivity when combined with specific BER mutants such as the DNA glycosylase, *mag1Δ,* or the AP endonucleases/lyases, which generate and process abasic sites, respectively [29]. In contrast, Shu complex mutants are not sensitive to ultra-violet light, ionizing radiation, bleomycin, hydroxyurea, hydrogen peroxide further indicating lesion specificity of the Shu complex [29]. Work from Rosenbaum et al., (2019) identified preferential binding of the Shu complex to double-flap substrates *in vitro* and with highest affinity for substrates containing an abasic sites analog, tetrahydrofuran, at the fork junction [30, 31]. While at the fork, the Shu complex protects the lesion by inhibiting the activity of BER enzymes, such as the AP endonucleases. AP endonucleases would normally process the abasic site by creating a ssDNA nick in the dsDNA template that would subsequently become a DSB as the fork progressed. Alternatively, AP endonuclease cleavage in ssDNA at a fork junction would directly result in a DSB. Note that DSBs formed as a result of replication fork collapse are unique from those processed during canonical HR because

they are single-ended breaks rather than double-ended [25]. By promoting tolerance of these lesions, the Shu complex provides time for later processing of the damage, post-replication.

RAD51 permits tolerance of DNA damage by switching replication templates to the available sister chromatid DNA such that the lesion is bypassed and left for later processing (**Fig. 3**). Work in budding yeast and mice has shown that during meiosis, the Shu complex plays an important role in homolog bias and; therefore, is likely important for enabling recombination with a sister chromatid, rather than a homologous chromosome, in this context as well [32, 33]. Like the yeast Shu complex, the human Shu complex is also sensitive to alkylating agents (**Fig. 1**). Consistent with a conserved role for the human Shu complex in tolerance of MMS-induced DNA damage, Martino et al., (2019) showed that the human Shu complex aids in RAD51 recruitment to DNA damage sites [19]. Thus, the human Shu complex may similarly aid RAD51 in the bypassing DNA alkylation damage during replication.

In addition to the Shu complex, RAD51C, may also play a role in the recognition and tolerance of specific types of DNA damage during replication. A recent paper by Mohan et al., (2019) identified an interaction between RAD51C and ALKBH3 [34], an alpha-ketoglutarate-dependent dioxygenase that demethylates and repairs bulky adducts, N1-methyladenine (1meA) and N3-methylcytosine (3meC) (**Fig. 2**). These methylated DNA lesions can form predominately in single-stranded DNA upon MMS exposure. It was suggested that RAD51C is required for ALKBH3-mediated repair of 3meC and that

RAD51C loss resulted in accumulation of 3meC in DNA. Thus, RAD51C may play a role in tolerance and repair of 3meC during DNA replication outside its canonical role in DSB repair. It remains unknown whether RAD51C function with ALKBH3 occurs independently or in the context of the BCDX2, CX3, or RAD51C-PALB2-RAD51-BRCA2 complexes. Suggesting a conserved function for processing of 3meC, a recent study demonstrated that the yeast Shu complex may similarly recognize and bypass fork-like substrates containing 3meC [35].

The role of the RAD51 paralog complexes in fork protection and restart

In DNA replication lesion bypass, two carefully orchestrated processes enable cells to avoid generating toxic DNA breaks that would arise from the uncontrolled collapse of stalled DNA replication forks, fork protection and restart. These mechanisms rely on either protecting stalled forks from degradation, thereby providing time to remodel the fork structure to bypass the lesion, or alternatively, by triggering the controlled collapse and restart of the fork. In both cases, RAD51 plays an important role, albeit at distinct stages of these mechanisms (**Fig. 3**). Crucially, this raises significant questions as to the requirement for RAD51 paralog proteins, or specific complexes, in mediating these processes. Several recent studies have highlighted the non-canonical functions of these proteins in tolerating DNA replicative lesions – further exemplifying the conservation between yeast and humans.

Indicating a role for the RAD51 paralogs in replication fork progression, Henry-Mowatt et al identified a role for XRCC3 and RAD51 in chicken DT40 cells [36]. More recently,

the RAD51 paralogs have been implicated in fork protection in mammalian cells, where Somyajit et al., (2015) demonstrated increase fork collapse in RAD51 paralog knock-out Chinese Hamster Ovary (CHO) cells treated with HU [37]. By introducing RAD51C and XRCC3 Walker A mutants, Somyajit et al., (2015) showed that CX3 is specifically required for the efficient restart of stalled forks. Replication fork restart and stability was assessed using DNA fiber spreading, where replication forks are labeled before or after fork stalling by hydroxyurea using thymidine analogues, such as IdU and CldU. While fork restart required both RAD51C and XRCC3 ATP-binding and hydrolysis, fork stability only required ATP-binding activity. Reciprocally, work from Saxena et al., (2018) also confirmed a non-canonical role for XRCC2 and RAD51D in sensing changes in dNTP pools [38]. In this context, XRCC2 loss led to unrestrained DNA synthesis despite decreased dNTP pools, thereby further demonstrating the disparate functions of the distinct RAD51 paralog complexes in replication fork stability.

Crucially, restraining DNA synthesis and maintaining fork stability enables remodeling to take place prior to restart. Fork reversal generates a ‘chicken foot-like’ structure that uses the newly synthesized leading strand to read through the lesion by synthesizing the complementary strand (depicted in **Fig. 3**). Recent research from Berti et al., (2020) highlighted the role of the BCDX2 complex in promoting fork reversal also by preventing unrestrained fork progression [39]. Members of the BCDX2 complex were identified by screening an siRNA library and looking for factors that, when depleted, led to a decrease in RAD51 foci formation following treatment with a topoisomerase inhibitor, camptothecin (CPT), as a marker of reversed forks. The authors note that the

characteristic fork collapse observed in *BRCA2* deficient cells, appears to be preceded by the activity of the BCDX2 complex. Though the exact mechanism of this remains enigmatic, it may utilize the dNTP sensing activity of XRCC2 through RRM2 [38]. XRCC3, the unique member of the CX3 complex, showed only mild impact on RAD51 foci formation when depleted, suggesting its role is again distinct from BCDX2 complex function. In support of this, the CX3 complex is dispensable for stalled fork reversal but is required for efficient restart [39]. The notion of non-canonical functions of HR mediators in response to different replicative stresses was further demonstrated by Rickman et al., (2020) who showed that mutation of the DNA-binding domain of BRCA2 produced a separation-of-function mutation that conferred sensitivity to interstrand crosslinking agents, but not HU induced replicative stress [40]. This provides evidence to suggest there are diverse mechanistic responses, initiated by common mediators, to tolerate either direct damage to DNA or sense and tolerate fluctuating dNTP pools.

Martino et al., (2019) provided further evidence for conservation of function between yeast and humans Shu complex in which cells lacking Shu complex components, SWS1 and SWSAP1, are unable to efficiently restart stalled forks; however, fork protection is unabated [19]. This suggests that the human Shu complex also acts at a later stage in lesion bypass, potentially by modulating the flexibility of RAD51 filaments formed [41]. Ultimately, further work is required to tease out the intricate specificities provided by the RAD51 paralogs, but it is clear the modularity of these proteins provides a basis for mechanistically diverse responses.

Future Challenges

Several challenges remain in studying the function of the RAD51 paralogs, primarily pertaining to their properties – low *in vitro* solubility, cellular abundance, and embryonic lethality in mouse knock-out models [11, 33, 37, 42-44]. Despite strong associations with breast and ovarian cancer susceptibility and their inclusion on hereditary breast/ovarian cancer screening panels, very few missense variants in the *RAD51* paralog genes have any defined impact and are therefore, so called “variants of uncertain significance” (VUS). This presents a significant challenge within the field to classify genetic changes based on whether they are pathogenic or benign. Previous attempts to experimentally validate the pathogenicity of *RAD51D* mutants has identified specific missense mutations adjacent to the Walker A motif that led to impaired interaction with its binding partner, XRCC2 [45]. A new tool developed by Garcin et al., (2019) helped address this shortfall, in which human *RAD51* paralog knock-out cell lines were generated [46]. This feat was achieved in multiple cell lines including MCF10A, HEK293 and U2OS. These cell lines complement the RAD51 paralog knockouts generated in Chinese Hamster Ovary cells such as CL-V4B$^{(RAD51C-/-)}$, irs1$^{(XRCC2-/-)}$ and irs1-SF$^{(XRCC3-/-)}$ used by Somyajit et al to study RAD51 paralog cancer variants [2, 37, 47]. Importantly, these cells recapitulated the decreased RAD51 stability and displayed sensitivity to the crosslinking agent, mitomycin C, and to Olaparib (conferred by HR-deficiency). Furthermore, the inclusion of direct repeat and sister-chromatid exchange green fluorescent protein reporter constructs (DR-GFP and SCR-GFP) enabled direct assaying of recombination proficiency. Crucially, only knock-out cells complemented with the wild-type cDNA of the missing *RAD51* paralog restored recombination and

RAD51 focus formation. The authors were then able to introduce specific point mutations to demonstrate the functional requirement of the Walker A and B motifs in RAD51B. Using these cellular models, it is now possible to address whether cancer-associated variants impact unique functions of the RAD51 paralogs in replication associated processes [37].

Excitingly, the development of Poly-ADP Ribosyl Polymerase (PARP) inhibitors has enabled the synthetic lethal relationship between PARP and HR-deficiency to be exploited to selectively kill cancerous cells (Reviewed in [48]). PARP inhibition results in DSB formation by trapping PARP1 on ssDNA breaks that are subsequently converted to DSBs upon replication fork progression, which would require HR for repair. Taken together, the novel *RAD51* paralog knock-out cell lines along with the ability to screen recombination proficiency with specific missense mutations will elucidate the impact of the many VUS, which in turn will help drive-forward precision medicine approaches for treating cancer patients. Synonymous with the *BRCA2* separation-of-function mutation identified by Rickman et al., (2020) in tolerating different replicative stress, characterization of RAD51 paralog VUS has the potential to delineate these non-canonical replicative functions for the RAD51 paralogs in cancer [40]. Furthermore, this may help to reconcile the diverse lesion specificity in relation to each of the RAD51 paralogs and complexes formed. Excitingly, recent development of an inhibitor that disrupts the protein-protein interaction between BRCA2 and RAD51, known as CAM833, demonstrated synergy with the PARP inhibitor, Olaparib [49]. Inhibiting the formation of RAD51 filaments may enable lowering the effective dose of

chemotherapeutics required for targeted cellular killing or may even help to combat resistance to PARP inhibitors [50].

Acknowledgements:

K.A.B. is supported by National Institutes of Health grants ES030335 and ES031796. R.A.B is supported by Royal Society grant RGS\R2\192126.

Conflict of interest:

The authors declare no conflict of interest.

References:

Papers of particular interest have been highlighted:

*special interest

**outstanding interest

1. Roy, U., et al., *The Rad51 paralog complex Rad55-Rad57 acts as a molecular chaperone during homologous recombination.* Mol Cell, 2021. **81**(5): p. 1043-1057 e8.
2. Jones, N.J., R. Cox, and J. Thacker, *Isolation and cross-sensitivity of X-ray-sensitive mutants of V79-4 hamster cells.* Mutat Res, 1987. **183**(3): p. 279-86.
3. Cartwright, R., et al., *The XRCC2 DNA repair gene from human and mouse encodes a novel member of the recA/RAD51 family.* Nucleic Acids Res, 1998. **26**(13): p. 3084-9.
4. Fuller, L.F. and R.B. Painter, *A Chinese hamster ovary cell line hypersensitive to ionizing radiation and deficient in repair replication.* Mutat Res, 1988. **193**(2): p. 109-21.
5. Albala, J.S., et al., *Identification of a novel human RAD51 homolog, RAD51B.* Genomics, 1997. **46**(3): p. 476-9.
6. Pittman, D.L., L.R. Weinberg, and J.C. Schimenti, *Identification, Characterization, and Genetic Mapping ofRad51d,a New Mouse and HumanRAD51/RecA-Related Gene.* Genomics, 1998. **49**(1): p. 103-111.
7. Liu, T., et al., *hSWS1.SWSAP1 is an evolutionarily conserved complex required for efficient homologous recombination repair.* J Biol Chem, 2011. **286**(48): p. 41758-66.
8. Thacker, J., *A surfeit of RAD51-like genes?* Trends Genet, 1999. **15**(5): p. 166-8.
9. Walker, J.E., et al., *Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold.* EMBO J, 1982. **1**(8): p. 945-51.

10. French, C.A., C.E. Tambini, and J. Thacker, *Identification of functional domains in the RAD51L2 (RAD51C) protein and its requirement for gene conversion.* J Biol Chem, 2003. **278**(46): p. 45445-50.
11. Masson, J.-Y., et al., *Identification and purification of two distinct complexes containing the five RAD51 paralogs.* Genes & Development, 2001. **15**(24): p. 3296-3307.
12. Schild, D., et al., *Evidence for simultaneous protein interactions between human Rad51 paralogs.* J Biol Chem, 2000. **275**(22): p. 16443-9.
13. Miller, K.A., et al., *RAD51C interacts with RAD51B and is central to a larger protein complex in vivo exclusive of RAD51.* J Biol Chem, 2002. **277**(10): p. 8406-11.
14. Wiese, C., et al., *Interactions involving the Rad51 paralogs Rad51C and XRCC3 in human cells.* Nucleic Acids Res, 2002. **30**(4): p. 1001-8.
15. Masson, J.Y., et al., *Complex formation by the human RAD51C and XRCC3 recombination repair proteins.* Proc Natl Acad Sci U S A, 2001. **98**(15): p. 8440-6.
16. Park, J.Y., et al., *Breast cancer-associated missense mutants of the PALB2 WD40 domain, which directly binds RAD51C, RAD51 and BRCA2, disrupt DNA repair.* Oncogene, 2014. **33**(40): p. 4803-12.
17. Godin, S.K., et al., *Evolutionary and functional analysis of the invariant SWIM domain in the conserved Shu2/SWS1 protein family from Saccharomyces cerevisiae to Homo sapiens.* Genetics, 2015. **199**(4): p. 1023-33.
18. Shor, E., J. Weinstein, and R. Rothstein, *A genetic screen for top3 suppressors in Saccharomyces cerevisiae identifies SHU1, SHU2, PSY3 and CSM2: four genes involved in error-free DNA repair.* Genetics, 2005. **169**(3): p. 1275-89.
19. Martino, J., et al., *The human Shu complex functions with PDS5b and SPIDR to promote homologous recombination.* Nucleic Acids Research, 2019. **47(19): p. 10151-10165**.
20. Prakash, R., et al., *Distinct pathways of homologous recombination controlled by the SWS1-SWSAP1-SPIDR complex.* Under Review at Nature Communications.
21. Wan, L., et al., *Scaffolding protein SPIDR/KIAA0146 connects the Bloom syndrome helicase with homologous recombination repair.* Proc Natl Acad Sci U S A, 2013. **110**(26): p. 10646-51.
22. Matsuzaki, K., et al., *Human RAD51 paralogue SWSAP1 fosters RAD51 filament by regulating the anti-recombinase FIGNL1 AAA+ ATPase.* Nat Commun, 2019. **10**(1): p. 1407.
23. Yuan, J. and J. Chen, *FIGNL1-containing protein complex is required for efficient homologous recombination repair.* Proc Natl Acad Sci U S A, 2013. **110**(26): p. 10640-5.
24. Couturier, A.M., et al., *Roles for APRIN (PDS5B) in homologous recombination and in ovarian cancer prediction.* Nucleic Acids Res, 2016. **44**(22): p. 10879-10897.
25. Cortez, D., *Replication-Coupled DNA Repair.* Mol Cell, 2019. **74**(5): p. 866-876.
26. Bhat, K.P. and D. Cortez, *RPA and RAD51: fork reversal, fork protection, and genome stability.* Nat Struct Mol Biol, 2018. **25**(6): p. 446-453.
27. Mankouri, H.W., H.P. Ngo, and I.D. Hickson, *Shu proteins promote the formation of homologous recombination intermediates that are processed by Sgs1-Rmi1-Top3.* Mol Biol Cell, 2007. **18**(10): p. 4062-73.
28. Memisoglu, A. and L. Samson, *Base excision repair in yeast and mammals.* Mutat Res, 2000. **451**(1-2): p. 39-51.
29. Godin, S.K., et al., *The Shu complex promotes error-free tolerance of alkylation-induced base excision repair products.* Nucleic Acids Res, 2016. **44**(17): p. 8199-215.
30. Godin, S., et al., *The Shu complex interacts with Rad51 through the Rad51 paralogues Rad55-Rad57 to mediate error-free recombination.* Nucleic Acids Res, 2013. **41**(8): p. 4525-34.
31. Rosenbaum, J.C., et al., *The Rad51 paralogs facilitate a novel DNA strand specific damage tolerance pathway.* Nat Commun, 2019. **10**(1): p. 3515.

32. Schwacha, A. and N. Kleckner, *Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway.* Cell, 1997. **90**(6): p. 1123-35.
33. Abreu, C.M., et al., *Shu complex SWS1-SWSAP1 promotes early steps in mouse meiotic recombination.* Nat Commun, 2018. **9**(1): p. 3961.
34. Mohan, M., et al., *Human RAD51 paralogue RAD51C fosters repair of alkylated DNA by interacting with the ALKBH3 demethylase.* Nucleic Acids Res, 2019. **47**(22): p. 11729-11745.
35. Bonilla, B., et al., *The Shu Complex Prevents Mutagenesis and Cytotoxicity of Single-Strand Specific Alkylation Lesions.* bioRxiv, 2021: p. 2021.04.09.439200.
36. Henry-Mowatt, J., et al., *XRCC3 and Rad51 modulate replication fork progression on damaged vertebrate chromosomes.* Mol Cell, 2003. **11**(4): p. 1109-17.
37. Somyajit, K., et al., *Mammalian RAD51 paralogs protect nascent DNA at stalled forks and mediate replication restart.* Nucleic Acids Res, 2015. **43**(20): p. 9835-55.
38. Saxena, S., et al., *ATR Signaling Uncouples the Role of RAD51 Paralogs in Homologous Recombination and Replication Stress Response.* Cell Rep, 2019. **29**(3): p. 551-559 e4.
39. Berti, M., et al., *Sequential role of RAD51 paralog complexes in replication fork remodeling and restart.* Nat Commun, 2020. **11**(1): p. 3531.
40. Rickman, K.A., et al., *Distinct roles of BRCA2 in replication fork protection in response to hydroxyurea and DNA interstrand cross-links.* Genes Dev, 2020. **34**(11-12): p. 832-846.
41. Taylor, M.R.G., et al., *Rad51 Paralogs Remodel Pre-synaptic Rad51 Filaments to Stimulate Homologous Recombination.* Cell, 2015. **162**(2): p. 271-286.
42. Shu, Z., et al., *Disruption of muREC2/RAD51L1 in mice results in early embryonic lethality which can Be partially rescued in a p53(-/-) background.* Mol Cell Biol, 1999. **19**(12): p. 8686-93.
43. Deans, B., et al., *Xrcc2 is required for genetic stability, embryonic neurogenesis and viability in mice.* EMBO J, 2000. **19**(24): p. 6675-85.
44. Pittman, D.L. and J.C. Schimenti, *Midgestation lethality in mice deficient for the RecA-related gene, Rad51d/Rad51l3.* Genesis, 2000. **26**(3): p. 167-73.
45. Baldock, R.A., et al., *RAD51D splice variants and cancer-associated mutations reveal XRCC2 interaction to be critical for homologous recombination.* DNA Repair (Amst), 2019. **76**: p. 99-107.
46. Garcin, E.B., et al., *Differential Requirements for the RAD51 Paralogs in Genome Repair and Maintenance in Human Cells.* PLoS Genet, 2019. **15**(10): p. e1008355.
47. Godthelp, B.C., et al., *Mammalian Rad51C contributes to DNA cross-link resistance, sister chromatid cohesion and genomic stability.* Nucleic Acids Res, 2002. **30**(10): p. 2172-82.
48. Janysek, D.C., et al., *Clinical use and mechanisms of resistance for PARP inhibitors in homologous recombination-deficient cancers.* Transl Oncol, 2021. **14**(3): p. 101012.
49. Scott, D.E., et al., *A small-molecule inhibitor of the BRCA2-RAD51 interaction modulates RAD51 assembly and potentiates DNA damage-induced cell death.* Cell Chem Biol, 2021.
50. Kondrashova, O., et al., *Secondary Somatic Mutations Restoring RAD51C and RAD51D Associated with Acquired Resistance to the PARP Inhibitor Rucaparib in High-Grade Ovarian Carcinoma.* Cancer Discov, 2017. **7**(9): p. 984-998.

Annotated References and Recommended Reading:

****Garcin et al., PLoS Genetics, 2019. Ref 46**

Garcin et al. generated RAD51 paralog knock-out U2OS, HEK293, and MCF10A cell lines. These cells demonstrated sensitivity to mitomycin C and Olaparib as well as reduced RAD51 foci formation. The knock-out cells are a novel and powerful tool for future investigation of RAD51 paralog function.

****Rosenbaum et al., Nature Communications, 2019. Ref 31**

Rosenbaum et al. discover that the Rad51 paralog-containing complex, the yeast Shu complex, directly binds a fork-like DNA structure *in vitro* and that the Shu complex enables cells to tolerate abasic sites, generated by lesion processing, on the lagging DNA strand. This publication demonstrates the capability of lesion specificity by Rad51 paralog containing complexes.

***Matsuzaki et al., Nature Communications, 2019. Ref 22**

Matsuzaki et al. identify that the FxxA motif in the human Shu complex protein, SWSAP1, is critical for RAD51 binding and foci formation after treatment with camptothecin. In addition, the authors demonstrate the requirement role of interacting partner, FIGNL1, in suppressing RAD51 foci formation in SWSAP1-depeleted cells.

***Martino et al., Nucleic Acids Research, 2019. Ref 19**

Martino et al. uncovered a role of the human Shu complex proteins in tolerating MMS and mitomycin C-induced damage by generating SWSAP1-/- and SWS1-/- RPE1 knock-out cell lines. Furthermore, the authors discover additional interactions with SPIDR and PDS5B; further demonstrating the modularity of these proteins in regulating RAD51 foci formation and replication fork restart.

****Saxena., Cell Reports, 2019. Ref 38**

Saxena et al. demonstrate a non-canonical role for BCDX2 complex member, XRCC2, in dNTP sensing and preventing unrestrained replication fork progression following treatment with HU. The authors uncover a mechanistic switch revealing that this function relies on phosphorylation of XRCC2 by ATR.

***Baldock et al., DNA Repair, 2019. Ref 45**

Baldock et al. characterized the effect of specific cancer-associated missense mutations in RAD51D on HR proficiency and the ability to interact with binding partner, XRCC2. This publication identifies two residues (G107V and G96C) close to the Walker A motif that are required for its protein interactions and homologous recombination function. Furthermore, Baldock et al showed that isoform 1 of RAD51D is the only isoform proficient for homologous recombination.

****Berti et al 2020. Nature Communications, 2020. Ref 39**

Berti et al. analyzed fork progression in camptothecin treated cells and demonstrated the requirement for the BCDX2 complex, but not CX3 complex, in fork slowing and remodeling. Furthermore, the authors demonstrated that the BCDX2 complex precedes fork collapse in BRCA2-deficient cells.

****Mohan et al., Nucleic Acids Research, 2019. Ref 34**

Mohan et al. uncovered the interaction of RAD51C with ALKBH3 in recognition and processing of specific DNA methyl-adducts, 3-methyl-cytosine; further demonstrating modular lesion specificity provided by RAD51 paralog complexes.

***Rickman et al., Genes and Development, 2020. Ref 40**

Rickman et al. identified a separation-of-function mutation in the DNA binding domain of BRCA2 that confers cellular sensitivity to ICL agents, but not HU induced damage; indicating different lesion-specific functions.

Figure Legends:

Figure 1. The human RAD51 paralog containing complexes. From top left to bottom right; the CX3 complex (RAD51C, XRCC3), the human Shu complex (SWS1, SWSAP1, SPIDR, PDS5B, and possibly FIGNL1), the BCDX2 complex (RAD51B, RAD51C, RAD51D, XRCC2) and the PALB2-RAD51-RAD51C-BRCA2 complex. Note that FIGNL1 may be a Shu complex accessory protein since it directly interacts with SPIDR. The RAD51 paralogs in blue ovals whereas other proteins are indicated with purple ovals.

Figure 2. RAD51 paralog function in fork-blocking lesion bypass and recognition. When the replication fork encounters a fork-blocking lesion that stalls the fork, these lesions can be bypassed through recombination-dependent or recombination-independent mechanisms such as translesion synthesis (TLS). The RAD51 paralogs aid in recombination-dependent bypass by recognizing the context of the stalled forks. For example, recent studies demonstrate that RAD51C in combination with ALKBH3 recognizes 3meC lesions, the BCDX2 complex recognizes low dNTP pools, and the yeast Shu complex recognizes abasic sites. Red lines show parent strands, black-dashed arrowed lines newly synthesized DNA as well as direction of synthesis and black box represents a DNA lesion.

Figure 3 – Summary of RAD51 paralog function recombination-dependent lesion bypass mechanisms in DNA replication. Schematic depicts a DNA replication fork stalled by a DNA lesion (red lines show parent strands, black-dashed arrowed lines newly synthesized DNA as well as direction of synthesis; 5’ to 3’, black box represents a DNA lesion and purple circles denotes the presence of RAD51). When a DNA replication fork encounters a DNA lesion, cells can use recombination-based mechanisms to bypass the DNA lesion initiated by either fork reversal (i) or by triggering fork collapse and restart (ii) (shaded-blue regions and solid black arrows show the direction of pathways for each type of repair). (i) Fork reversal requires regression of the replication fork by annealing of the two newly synthesized daughter strands (black-dashed lines), DNA synthesis away from the fork creates a four-way junction that, when resected, can yield an overhang capable of strand-invading ahead of the DNA lesion. The newly displaced strand (red D-loop) can be synthesized back to fill in the remaining sequence to bypass the lesion. The BCDX2 complex, highlighted in red text, functions during fork protection by restraining fork progression. (ii) Persistent stalling of a replication fork may result in its collapse, generating a single-ended DNA double-strand break. Replication can be restarted by strand-invasion and recombination with the unbroken strand. Both the CX3 and Shu complex function during fork restart and are highlighted in red text.

Figure 1

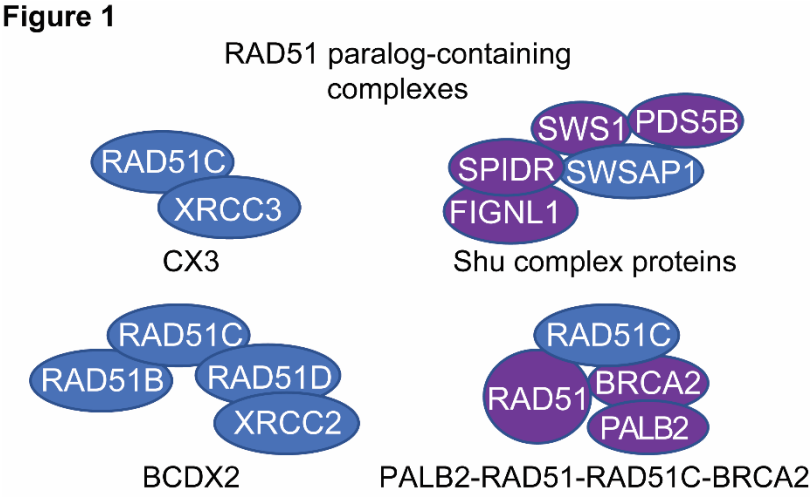


Figure 2

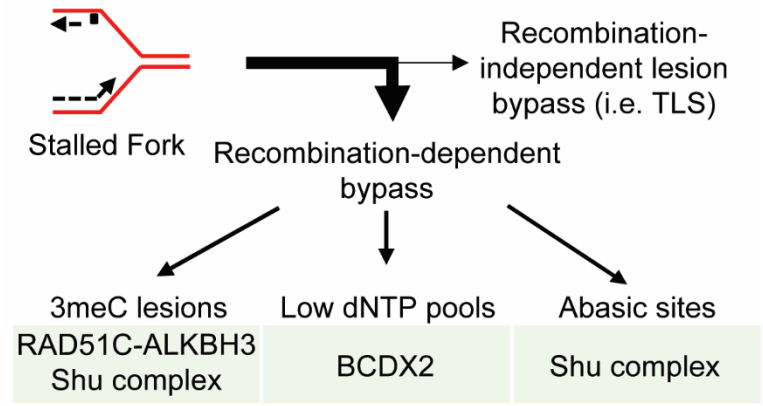
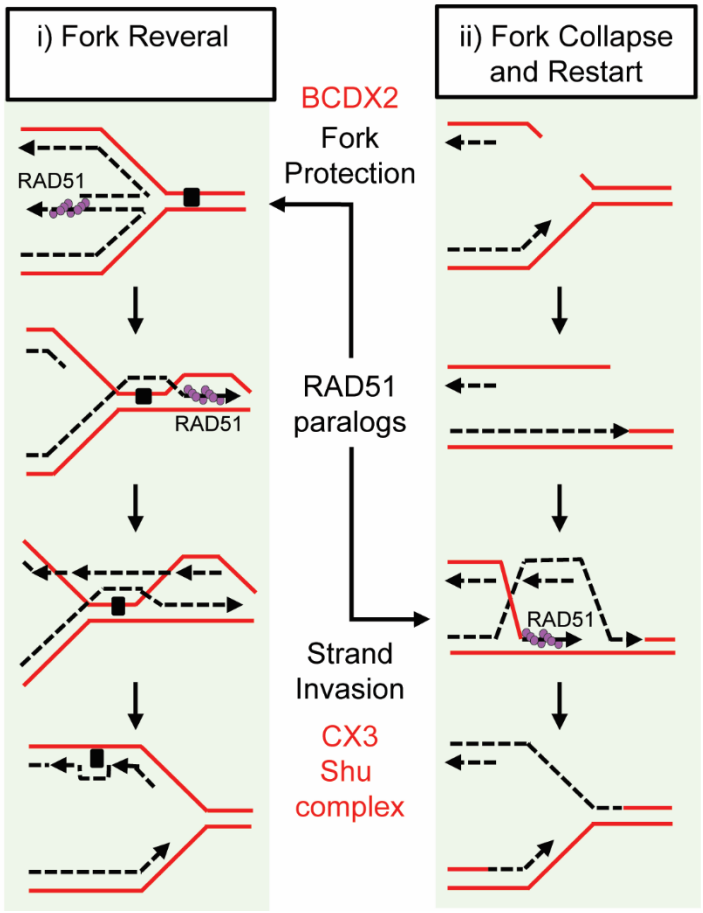


Figure 3


i) Fork Reveral
ii) Fork Collapse and Restart
BCDX2
Fork Protection
RAD51
RAD51
RAD51 paralogs
RAD51
Strand Invasion
CX3 Shu complex