Resolving RAD51C function in late stages of homologous recombination
Shyam K Sharan* and Sergey G Kuznetsov

Address: Mouse Cancer Genetics Program, Center for Cancer Research, National Cancer Institute, Frederick, Maryland 21702, USA
Email: Shyam K Sharan* - ssharan@ncifcrf.gov; Sergey G Kuznetsov - skuznetsov@ncifcrf.gov
* Corresponding author

Abstract
DNA double strand breaks are efficiently repaired by homologous recombination. One of the last steps of this process is resolution of Holliday junctions that are formed at the sites of genetic exchange between homologous DNA. Although various resolvases with Holliday junctions processing activity have been identified in bacteriophages, bacteria and archeabacteria, eukaryotic resolvases have been elusive. Recent biochemical evidence has revealed that RAD51C and XRCC3, members of the RAD51-like protein family, are involved in Holliday junction resolution in mammalian cells. However, purified recombinant RAD51C and XRCC3 proteins have not shown any Holliday junction resolution activity. In addition, these proteins did not reveal the presence of a nuclease domain, which raises doubts about their ability to function as a resolvase. Furthermore, oocytes from infertile Rad51C mutant mice exhibit precocious separation of sister chromatids at metaphase II, a phenotype that reflects a defect in sister chromatid cohesion, not a lack of Holliday junction resolution. Here we discuss a model to explain how a Holliday junction resolution defect can lead to sister chromatid separation in mouse oocytes. We also describe other recent in vitro and in vivo evidence supporting a late role for RAD51C in homologous recombination in mammalian cells, which is likely to be resolution of the Holliday junction.

Background
Exchange of genetic material by homologous recombination during meiosis is essential for generating genetic diversity in living organisms. It also results in the formation of chiasmata, that are the cytological manifestation of crossovers and ensure proper chromosome segregation. Homologous recombination is important for error-free repair of damaged DNA as well [1]. One of the key steps of this process is the exchange of DNA strands between homologous chromosomes, which results in the formation of a cross-stranded DNA structure (Figure 1). More than 40 years ago, Robin Holliday proposed the formation of such an intermediate during DNA recombination [2]. It was hypothesized that such recombination intermediates, now known as Holliday junctions (HJs), can be resolved by the endonucleases capable of binding to such specialized DNA structures [3]. In 1982, the first resolvase was identified [4]. It was the bacteriophage T4 endonuclease VII, which was shown to cleave Holliday structures in vitro by nicking two strands of the same polarity near the branch point. The breaks generated as a result of this cleavage could be sealed by a DNA ligase, resulting in intact resolved products. A search for resolvases in other organisms resulted in the identification of one of the best-characterized resolvases, RuvC of E. coli [5,6], which resolves Holliday junctions by introducing concerted...
nicks across the junction branch point. Two additional proteins, RuvA and RuvB, have been shown to be an integral part of the resolvosome complex that facilitates the movement of HJ along the DNA in an ATP-dependent manner, in a process known as branch migration (Figure 1) [7]. Recently, resolvases that share some structural similarity with E. coli RuvC have been identified like CCE1 in yeast, RuvC in Lactococcus bacteriophage and A22R in Poxvirus [8-11]. Some other distinct resolvases have been identified in bacteriophages, such as the T7 endonuclease I, RusA and Rap [12,13]. In addition, Hjc and Hje proteins from archaeabacteria demonstrate HJ cleaving activity [14-16].

**Eukaryotic resolvases**

Although many resolvases have been identified in prokaryotes, few have been found in eukaryotes. The yeast CCE1 is encoded by a nuclear gene but functions as a resolvase in the mitochondria [9,17]. Identification of the Mus81-Eme1 endonuclease in Saccharomyces pombe raised hopes that the eukaryotic resolvase has been identified [18]. It has a substrate preference for nicked HJs and displacement loops (D-loops) [19]. Genetic studies showed that S. pombe mus81 mutants are infertile due to failed meiotic recombination, which could be rescued by the expression of bacterial RusA resolvase [18]. These studies demonstrated that Mus81 is indeed a eukaryotic resolvase. The homologs of fission yeast Mus81-Eme1 have been identified in other organisms, including Saccharomyces cerevisiae (Mus81-Mms4), Arabidopsis (AtMUS81/At4g30870) and humans (Mus81-Eme1 or Mms4) [20-26]. However, unlike the S. pombe mutants, S. cerevisiae mus81 mutants are partially fertile, and MUS81-deficient Arabidopsis plants and mice are fully fertile, with no defect in meiotic recombination [21-23,27,28]. These observations suggest the presence of other resolvases in these organisms. Also, it was shown that the HJ resolution activity in mammalian cells could be separated from MUS81, suggesting that MUS81 was not the mammalian HJ resolvase [29].

**RAD51 paralogs**

The idea that RAD51 paralogs may be involved in resolution of HJs came from the observation that the protein fraction with HJ resolution activity contained RAD51C and XRCC3, members of the RAD51-like protein family [30]. This observation was very exciting considering that members of this family were already known to play a role during the early stages of homologous recombination. In higher eukaryotes, including plants, chicken and mammals, there are six members of the RAD51-like protein family that show 20–30% sequence similarity to RAD51 [31,32]. These are RAD51B/RAD51L1, RAD51C/RAD51L2, RAD51D/RAD51L3, XRCC2, XRCC3 and DMC1. DMC1 shares about 50% sequence identity with RAD51 and is its structural and functional homolog that functions specifically in meiotic recombination [33]. The other five paralogs show 20–30% sequence similarity to RAD51 and have been shown to be part of at least two distinct protein complexes, namely, the BCDX2 and CX3 complexes [34]. The BCDX2 complex comprises RAD51B, RAD51C, RAD51D and XRCC2 proteins; the CX3 complex consists of RAD51C and XRCC3. All these RAD51 paralogs have been reported to be required for normal proliferation and play a role in RAD51-mediated homologous recombination [31].

Studies in chicken B-lymphocyte DT40 cells have shown that only RAD51 is essential for cell viability, while loss of other RAD51 paralogs does not affect cell survival [35]. Interestingly, although mutants lacking any of the RAD51 paralogs show sensitivity to DNA-damaging agents, their phenotypes are not identical, suggesting that their function is similar but not redundant [36]. This functional non-redundancy is corroborated by the loss-of-function studies in mice and Arabidopsis [37-40]. Depletion of RAD51C by siRNA in human cells also suggests that RAD51C plays a role in homologous recombination repair [41]. These findings are consistent with the idea that these paralogs play a role in early stages of homologous recombination. Recent work from Stephen West’s laboratory has suggested that RAD51C and XRCC3 may also play a role in the late stages of homologous recombination [30,42]. Here we discuss various findings that support the dual role of RAD51C.

**The role of RAD51C and XRCC3 in HJ resolution**

In a very significant study, Liu et al. demonstrated that RAD51C is required for the resolution of HJs in mammalian cells [30]. After an intricate series of HeLa cell nuclear extract fractionations, fractions with HJ resolution and branch migration activity were identified. These fractions lacked some of the possible candidates like Mus81, Flp endonuclease 1 (FEN1), RecQ DNA helicase (BLM) and Werner syndrome helicase (WRN). On the other hand, all the fractions with HJ resolution activity contained RAD51C. Immunodepletion using a RAD51C antibody resulted in the loss of the HJ processing function. This activity could be restored by adding purified RAD51C protein complexes containing RAD51C, but not those lacking it. These studies directly implicated RAD51C in the resolution process. Furthermore, the Chinese hamster ovary cell line irs3 defective in RAD51C was shown to lack the HJ processing function. Recently, mouse embryonic fibroblasts generated from Rad51c knockout embryos revealed marked reduction in HJ activity [43]. Interestingly, a hamster cell line with mutant XRCC3, irs15F, also showed a defect in HJ resolution activity, while mutation in another RAD51 paralog, XRCC2, had no effect on HJ resolution [30]. Although Liu et al. provided strong evi-
Schematic representation of the model showing repair of DSBs by homologous recombination. When a DSB occurs, it is processed to generate 3' single-stranded ends. RAD51 nucleoprotein microfilament is assembled on these ends, one of which invades the homologous DNA by strand displacement. This results in the formation of the D-loop. The invading strand primes DNA synthesis using the homologous DNA as a template. The second single-stranded DNA is also captured for priming DNA synthesis by using the displaced strand as a template. Extension of the 3' ends ultimately results in two cross-structures that hold the two homologs together, called Holliday junctions (HJs). Migration of HJs, called branch migration, results in the formation of heteroduplex regions. The double HJs are resolved by resolvases and, depending on the cleavage site, can either lead to crossover or non-crossover products. Resolution to yield crossover products requires symmetric cleavage of both HJs in opposite orientations.
evidence to show the involvement of both RAD51C as well as XRCC3 in the resolution of HJ, a direct cleavage of the HJ structure by purified recombinant proteins remains to be demonstrated. It is possible that these proteins undergo posttranslational modifications, which may be essential for the HJ processing function. This theory is supported by the observation that the mobility of the RAD51C present in the fractions of HeLa cell extract that possess HJ resolution function is different from the mobility of those present in unfraccionated whole cell extracts. Identifying the nature of this modification may elucidate the structural or conformational change in RAD51C that may be essential to its HJ resolution activity.

More recently, in a follow-up study, Liu et al. provide new biochemical evidence to link RAD51C and XRCC3 to the HJ processing function [42]. Using affinity chromatography, they showed a direct link between RAD51C and HJ activity. In a nickel column loaded with recombinant His-tagged RAD51C protein, HJ activity present in HeLa cell extracts was shown to bind to the column, which could subsequently be eluted. XRCC3 present in the extract was also shown to bind to the column and elute with HJ activity. Using gel filtration, the HJ resolvase activity was found to elute with an average molecular mass of 80–90 kDa, similar to the sum of the molecular masses of RAD51C (∼42 kDa) and XRCC3 (∼38 kDa). This finding suggests that the RAD51C-XRCC3 heterodimer should have the HJ activity. Because efforts to demonstrate this activity using purified recombinant proteins have failed, it has been speculated that the resolvosome complex may contain an additional component, such as a small nuclease, which may be essential for the HJ resolution function of the RAD51C-XRCC3 dimer. This suggestion may also explain the absence of any apparent nuclease domain in RAD51C present in the fractions of HeLa cell extract that possess HJ resolution function. Future studies aimed at determining whether the HJ resolvosome complex involves other component(s) in addition to RAD51C and XRCC3 are important.

Localization of RAD51C on meiotic chromosomes

In addition to the biochemical studies, Liu et al. have presented interesting immunofluorescence data showing the localization of RAD51C on meiotic chromosomes, which further supports its role in late stages of homologous recombination [42]. Since various stages of meiotic prophase I coincide well with different stages of double-strand-break (DSB) repair by homologous recombination, it is possible to associate the function of a protein based on its localization on meiotic chromosomes at any given prophase I stage. Interestingly, RAD51C was first detected at the pachytene stage, when it was observed as one or two distinct foci associated with each synapsed bivalent. This pattern of expression is similar to the foci formed by the mismatch repair protein MLH1 [44]. These foci are believed to represent the sites of crossovers, formed as a result of recombination between homologous chromosomes. In addition, spermatocytes from Mlh1-deficient mice, which have a severely reduced number of crossover sites, also showed a marked reduction in RAD51C foci. This observation strengthens the notion that RAD51C foci formation during pachytene depends on the generation of crossovers, which is one of the last steps of homologous recombination. Surprisingly, co-localization of MLH1 and RAD51C foci on the bivalents was not observed. Also, XRCC3 foci were not detected on these bivalents. These observations raise concerns and are currently difficult to explain. The lack of co-localization of RAD51C and MLH1 may be due to temporal differences in their localization at crossover sites. It is interesting that, although no XRCC3 foci were observed on autosomal bivalents, XRCC3 foci, along with RAD51C and MLH1 foci, were present in the pseudoautosomal region of the sex chromosomes, a region on X and Y chromosomes that undergoes an obligatory crossover event. These foci in the pseudoautosomal region were not detected in Mlh1-deficient spermatocytes, supporting their dependence on a crossover event.

It was surprising that Liu et al. did not detect RAD51C foci during the leptotene and zygotene stages of prophase I, where it is expected to play an important role based on its known function in the early stages of homologous recombination. However, failure to observe RAD51C foci does not rule out its functional importance at this stage. Different antibodies and more sensitive imaging methods may help resolve this discrepancy. In the meantime, it may be interesting to examine the consequence of the loss of these proteins in an in vivo model system. In Drosophila, the loss of RAD51C-like protein encoded by spn-D gene results in a meiosis-specific defect and may play a role similar to DMC1 [45]. Recent studies in Arabidopsis revealed that RAD51C is essential for repair of Spo11-induced DSBs during prophase I of meiosis [40,46]. Homologous chromosomes in Rad51c-deficient plant meiocytes fail to synapse and become severely fragmented. These results support a role for RAD51C early in meiotic recombination but do not shed light on its role later in the process.

RAD51C function in meiosis

Because RAD51C is predicted to have a dual function, an early and a late role in homologous recombination, it may be difficult to demonstrate the latter function in vivo, as a defect in the former may result in the arrest of meiocytes. Because RAD51C-deficient mice die during embryogenesis and no suitable meiosis-specific Cre transgenic mouse line is available yet, it is a challenge to generate a suitable mouse model to study the meiotic functions of RAD51C. We recently reported the generation of a hypomorphic...
allele of Rad51c that results in a reduction of the protein level due to aberrant splicing [43]. This aberrant splicing is caused by the presence of the neomycin resistance gene in one of the introns. Mice that are homozygous for the hypomorphic allele and have about 60% reduction in RAD51C protein level are viable and fertile. However, mice with only one copy of the hypomorphic allele (while the other allele is a null) are viable, but 35% of males and 12% of females are infertile. The infertile males and females provide an ideal in vivo model system to study the role of RAD51C in meiotic recombination. In addition, the meiotic phenotype associated with the loss of RAD51C function is sexually dimorphic, showing an early meiotic defect in males and a late defect in females, which provides a unique opportunity to study the dual function of RAD51C in mouse meiosis.

Infertile males revealed an early role for RAD51C in meiosis, marked by the spermatocyte arrest at leptotene and early zygote stages, reduction of RAD51 foci at leptotene, and persistence of DNA breaks and unsynapsed chromosomes at pachytene. This finding is consistent with the known function of RAD51C in DSB repair but does not provide any evidence to implicate RAD51C in the late steps of the recombination.

Histological examination of the ovaries of mutant females revealed a defect in ovulation, which could be partially rectified by hormonal treatment. The number of oocytes was markedly reduced, and the embryos that developed from these oocytes suffered from severe developmental defects. The reduced number of oocytes suggests that some oocytes may have been lost due to a defect in the early meiotic prophase I. While all oocytes obtained after superovulation progressed normally to metaphase I, they displayed a number of abnormal features at metaphase II. These abnormalities included aneuploidy and broken chromosomes, but most strikingly, precocious separation of sister chromatids (PSSC; Figure 2). The observation that the oocytes showed a defect after metaphase I supports the notion that RAD51C plays a role during late stages of meiotic recombination. Oocytes with a defect early in the DSB repair process show an aberrant phenotype at metaphase I. However, the sister chromatid cohesion defects in mutant spermatocytes yielded no supporting data (Kuznetsov and Sharan, unpublished observation).

Next, the option that a defect in sister chromatid cohesion could be the consequence of unresolved HJs persisting on meiotic chromosomes during anaphase I was explored [43]. It was proposed that the increased physical tension on sister centromeres at the onset of anaphase I may somehow disrupt their cohesion (Figure 3). This physical tension may arise if the homologs are unable to separate from each other when the HJs are not resolved. The homologs normally align at the metaphase plate, and the kinetochores are attached to the spindle, allowing them to be pulled to the opposite poles. This action ensures proper segregation of each homolog to one of the daughter cells at the end of meiosis I. Sister chromatid cohesion is established during the S-phase and lost between the sister chromatid arms prior to metaphase I [51]. However, the sister chromatids do not dissociate as they remain attached by their centromeres. The centromeric cohesion is protected from degradation by a conserved family of centromere-associated protein called shugoshin (Sgo) [52-54]. In meiosis, during anaphase II, when the sister chromatids separate from each other and move to opposite poles, shugoshin is degraded, exposing the centromeric cohesion complex to separate, leading to a loss of centromeric cohesion. Recent studies have shown that, in addition to the main function of protecting centromeric cohesion, shugoshin is involved in sensing tension at the kinetochore [55,56]. This tension-sensing mechanism is essential for activation of the spindle checkpoint. It has been demonstrated that a lack of tension between achiasmatic
univalents activates the spindle checkpoint [57]. It is postulated that if the bivalents remain attached in Rad51c mutant oocytes due to unresolved HJs, an increase in tension is likely to occur at the kinetochore, which then may either physically disrupt the cohesion complex or may somehow activate the degradation of shugoshin, exposing the centromeric cohesion complex to separase (Figure 3).

Although these possibilities remain to be tested, a genetic evidence reported earlier was used to support this hypothesis [43]. Koehler et al. have shown that, in mouse oocytes, segregation of dicentric chromosomes very frequently (>90%) results in PSSC [58]. They proposed that the physical strain exerted on the homologous centromeres of the dicentric chromatin by the poleward microtubules can result in the PSSC cohesion. Kuznetsov et al. have suggested that unresolved chromosomes and dicentric chromosomes are likely to experience a similar mechanical stress at the centromere during anaphase I and therefore have a similar fate [43]. The processing of dicentric chromosomes is sexually dimorphic and so far has been reported only in mouse and human oocytes. In other organisms, such as maize and flies, such dicentric chromosomes are known to undergo a “bridge-fusion-breakage” cycle [59,60]. Why dicentric chromosomes have a different fate in mice and human oocytes is currently not understood. Interestingly, some of the spermatocytes from Rad51c mutant infertile males that progressed to metaphase II exhibited chromosomes with broken centromeres, but none showed any sister chromatid cohesion defect, which is consistent with the sexually dimorphic behavior of dicentric chromosomes.

In general, resolution of recombination intermediates in meiosis appears to be tightly linked to sister chromatid cohesion. The condensin-dependent removal of cohesion from the chromosome arms is required for efficient homolog separation in meiosis [61]. At the same time, the condensin – polo-like kinase axis is dispensable for cohesion removal in mitosis [62]. It is not clear how RAD51C might be involved in this particular process and whether it could explain the PSSC phenotype of the RAD51C-deficient mouse oocytes. However, it is intriguing that cohesins and RAD51C are now associated with the resolution of recombination intermediates after previously being independently implicated in the homologous recombination process [63].

Conclusion
Three years since the initial report showing a role for RAD51C in HJ resolution in mammalian cells, are we any closer to resolving the biological function of RAD51C? In spite of all the biochemical experiments and examination of loss-of-function mutations in Drosophila, Arabidopsis, and mice, the late function in HJ resolution remains to be
Proposed connection between HJ resolution defect and abnormalities in Rad51c mutant oocytes at metaphase II. During meiosis, HJs are established between homologous chromosomes by the pachytene stage of prophase I using the homologous recombination machinery. At metaphase I, bivalents are pulled in opposite directions by microtubules attached to kinetochores of sister chromatids that are oriented toward the same pole. While centromeric cohesion is protected by Shugoshin to ensure that sister chromatids stay together during the reductional division, cohesion is released along the chromosome arms. During anaphase I, homologous chromosomes segregate to separate cells. In the absence of chiasmata, homologous chromosomes do not align properly at the metaphase plate, and this activates a spindle checkpoint resulting in metaphase I arrest. In Rad51c-deficient oocytes, meiosis progresses normally until anaphase I. However, due to accumulation of recombination intermediates, such as double Holliday junctions (dHJs), which hold the homologous chromosomes together, there is an increase in tension at the centromere due to the persistence of unresolved dHJs. The increased tension is thought to disrupt the sister chromatid cohesion at the centromere, resulting in the PSSC phenotype and fragmented chromosomes. Homologous chromosomes are shown in red and green; REC8 is shown in yellow; shugoshin is orange; and centromeres are shown in purple. (Reproduced from The Journal of Cell Biology, 2007, 176:581–592, Copyright 2007 The Rockefeller University Press.)
unequivocally demonstrated. The model to explain the phenotype of Rad51c mutant oocytes at metaphase II is intriguing but needs to be validated. It will be fascinating to directly observe the oocytes undergoing in vitro maturation by time lapse imaging to visualize the bivalents being pulled to opposite poles but remaining attached at the site of the crossover by chiasmata-like structures during anaphase I. Also, it will be interesting to examine the fate of shugoshin and cohesins on the centromeres that have undergone precocious separation. An alternative approach may be to bypass the early meiotic arrest during male meiosis by using a conditional Rad51c allele and generating appropriate meiosis-specific Cre transgenic lines. This approach may provide a more convincing phenotype and help explain the late role of RAD51C in male meiosis by using a conditional Rad51c allele and generating appropriate meiosis-specific Cre transgenic lines. This approach may provide a more convincing phenotype and help explain the late role of RAD51C in homologous recombination. Similar studies on XRCC3 in meiotic recombination may also provide valuable clues.

Competing interests
The author(s) declare that they have no competing interests.

Acknowledgements
The authors thank Drs. Stephen West and Yilun Liu for critical review of the manuscript and Allen Kane of the Publication Department for the illustrations.

References
1. Brugmans L, Kanaar R, Essers J: Analysis of DNA double-strand break repair pathways in mice. Mutat Res 2007, 614:95-108.
2. Holliday R: A mechanism for gene conversion in fungi. Genet Res 1964, 5:282-304.
3. Liu Y, West SC: Happy Hollidays: 40th anniversary of the Holliday junction. Nat Rev Mol Cell Biol 2004, 5:937-944.
4. Mizuuchi K, Kemper B, Hays J, Weisberg RA: T4 endonuclease VII cleaves Holliday structures. Cell 1982, 29:357-365.
5. Dunderdale HJ, Benson FE, Parsons CA, Sharless GJ, Lloyd RG, West SC: Formation and resolution of recombination intermediates by E. coli RecA and RuvC proteins. Nature 1991, 354:506-510.
6. Iwasaki T, Takahashi M, Shiba T, Nakata A, Shinagawa H: Escherichia coli RuvC protein is an endonuclease that resolves the Holliday structure. Embryo J 1991, 10:4381-4389.
7. West SC: Processing of recombination intermediates by the RuvABC proteins. Annu Rev Genet 1997, 31:213-244.
8. Sharless GJ: The X philes: structure-specific endonucleases that resolve Holliday junctions. Mol Microbiol 2001, 39:823-834.
9. Kleff S, Kemper B, Stegmann R: Identification and characterization of yeast mutants and the gene for a cruciform cutting endonuclease. Embryo J 1992, 11:699-704.
10. Bidnerko E, Ehrlich SD, Chopin MC: Lactococcus lactis phage operon coding for an endonuclease homologous to RuvC. Mol Microbiol 1998, 28:823-834.
11. Garcia AD, Aravind L, Koonin EV, Moss B: Bacterial-type DNA Holliday junction resolvest in eukaryotic viruses. Proc Natl Acad Sci U S A 2000, 97:8926-8931.
12. Dickie P, McFadden G, Morgan AR: The site-specific cleavage of synthetic Holliday junction analogs and related branched DNA structures by bacteriophage T7 endonuclease I. J Biol Chem 1987, 262:14826-14836.
13. Mahdi AA, Sharless GJ, Mandal TN, Lloyd RG: Holliday junction resolvest encoded by homologous rusA genes in Escherichia coli K-12 and phage 82. J Mol Biol 1996, 257:561-573.
14. Komori K, Sakae S, Shinagawa H, Morikawa K, Ishino Y: A Holliday junction resolvest from Pyrococcus furiosus: functional sim-
ilarity to Escherichia coli RuvC provides evidence for conserved mechanism of homologous recombination in Bacteria, Eukarya, and Archaea. Proc Natl Acad Sci U S A 1999, 96:8873-8878.
15. Kvaratskhelia M, White MF: An archaeal Holliday junction resolving enzyme from Sulfolobus solfataricus exhibits unique properties. J Mol Biol 2000, 295:193-202.
16. Kvaratskhelia M, White MF: Two Holliday junction resolving enzymes in Sulfolobus solfataricus. J Mol Biol 2000, 297:923-932.
17. Lockshon D, Zweifel SG, Freeman-Cook LL, Lorimer HE, Brewer BJ, Fangman WL: A role for recombination junctions in the segregation of mitochondrial DNA in yeast. Cell 1995, 81:947-953.
18. Boddy MN, Gaillard PH, McDonald WH, Shanahan P, Yates JR 3rd, Russell P: Mus81-Eme1 are essential components of a Holliday junction resolvest. Cell 2001, 107:537-548.
19. Osman F, Dixon J, Doe CL, Whitty MC: Generating crossovers by Holliday junction resolvest: a role for Mus81-Eme1 in meiosis. Mol Cell 2003, 12:761-774.
20. Interseth H, Heyer WD: MUS81 encodes a novel helix-hairpin-helix protein involved in the response to UV- and methyla-
tion-induced DNA damage in Saccharomyces cerevisiae. Mol Cell Genet 2000, 23:857-866.
21. de los Santos T, Hunter N, Lee C, Larkin B, Loidl J, Hollingsworth NM: The Mus81/Mms4 endonuclease acts independently of a Holliday junction resolution to promote a distinct subset of crossovers during meiosis in budding yeast. Genetics 2003, 164:81-94.
22. de los Santos T, Loidl J, Larkin B, Hollingsworth NM: A role for MMS4 in the processing of recombination intermediates during meiosis in Saccharomyces cerevisiae. Genetics 2001, 159:1511-1525.
23. Hartung F, Suer S, Bergmann T, Puchta H: The role of AtMus81 in DNA repair and its genetic interaction with the helicase AtRecQ4A. Nucleic Acids Res 2006, 34:4438-4448.
24. Chen XB, Melchionna R, Denis CM, Gaillard PH, Blasina A, Van de Weyer I, Boddy MN, Russell P, Vialard J, McGowan CH: Human Mus81-associated endonuclease cleaves Holliday junctions in vitro. Mol Cell 2001, 8:1117-1127.
25. Ciccia A, Constantinou A, West SC: Identification and characterization of the human mus81-eme1 endonuclease. J Biol Chem 2003, 278:25172-25177.
26. Haber JE, Heyer WD: The fuss about Mus81. Cell 2001, 107:551-554.
27. McPherson JP, Lemmers B, Chalwain R, Pamidi A, Migon E, Matsyiak-Zablocki E, Ploymaneh ME, Essers J, Harada K, Poonepalli A, Sanchez-Swannoua Q, Khoka R, Kanaar R, Jasin M, Hande MP, Hakem R: Involvement of mammalian Mus81 in genome integrity and tumor suppression. Science 2004, 304:1822-1826.
28. Dendouga N, Gao H, Mochers MR, Janicot M, Vialard J, McGowan CH: Disruption of murine Mus81 increases genomic instability and DNA damage sensitivity but does not promote tumori-
genesis. Mol Cell Biol 2005, 25:7569-7579.
29. Constantinou A, Chen XB, McGowan CH, West SC: Holliday junction resolvest in human cells: two junction endonuclease with distinct substrate specificities. Embo J 2002, 21:5577-5585.
30. Liu Y, Masson JY, Shah R, O’Regan P, West SC: RAD51C is required for Holliday junction processing in mammalian cells. Science 2004, 303:243-246.
31. Kawabata M, Kawabata T, Nishibori M: Role of recA/RAD51 family proteins in mammals. Acta Med Okayama 2005, 59:1-9.
32. Miller KA, Sawicka D, Barsky D, Abulaj M: Domain mapping of the Rad51 paralog protein complexes. Nucleic Acids Res 2004, 32:169-178.
33. Shinozaki A, Shinozaki M: Roles of RecA homologues Rad51 and Dmc1 during meiotic recombination. Cytogenet Genome Res 2004, 107:201-207.
34. Masson JY, Tarsounas MC, Stasiak AZ, Stasiak A, Shah R, McIlwraith MJ, Benson FE, West SC: Identification and purification of two distinct complexes containing the five RAD51 paralogues. Genes Dev 2001, 15:3296-3307.
35. Takata M, Sasaki MS, Tachibana S, Fukushima T, Sonoda E, Schuld D, Thompson LH, Takeda S: Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogues. Mol Cell Biol 2001, 21:2858-2866.
36. Yonetani Y, Hochegger H, Sonoda E, Shinya S, Yoshikawa H, Takeda S, Yamazoe M: Differential and collaborative actions of Rad51-paralog proteins in cellular response to DNA damage. Nucleic Acids Res 2005, 33:4544-4552.

37. Deans B, Griffin CS, Maconochie M, Thacker J: Xrcc2 is required for genetic stability, embryonic neurogenesis and viability in mice. Embryo J 2000, 19:6675-6685.

38. Preman DL, Schimenti JC: Midgestation lethality in mice deficient for the RecA-related gene, Rad51d/Rad51l3. Genesis 2000, 26:167-173.

39. Shu Z, Smith S, Wang L, Rice MC, Kmiec EB: Disruption of murRec2/Rad51L1 in mice results in early embryonic lethality which can be partially rescued in a p53(-/-) background. Mol Cell Biol 1999, 19:8686-8693.

40. Bleuyard JY, Gallego ME, Savigny F, White CI: Differing requirements for the Arabidopsis Rad51 paralogs in meiosis and DNA repair. Plant J 2005, 41:533-545.

41. Liu Y, Tarsounas M, O'Regan P, West SC: Role of RAD51C and XRC3 in genetic recombination and DNA repair. J Biol Chem 2007, 282:1973-1979.

42. Kuznetsov S, Pellegrini M, Shuda K, Fernandez-Capetillo O, Liu Y, Liu Y, Tarsounas M, O'Regan P, West SC: Disruption of murRec2/Rad51L1 in mice results in early prophase I arrest in males and sister chromatid separation at metaphase II in females. J Cell Biol 2007, 176:581-592.

43. Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yaco X, Christie DM, Monell C, Arnhem N, Bradley A, Ashley T, Liskay RM: Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. Nat Genet 1996, 13:336-342.

44. Moll MS, Schupbach T: The Drosophila spn-D gene encodes a RAD51C-like protein that is essential for genetic stability, sister chromatid cohesion along arms and at centromeres. Trends Genet 2005, 21:405-412.

45. Wang X, Dai W: Shugoshin, a guardian for sister chromatid segregation. Exp Cell Res 2005, 310:1-9.

46. Lio YC, Schild D, Brenneman MA, Redpath JL, Chen DJ: Human Rad51C deficiency destabilizes XRC3C, impairs recombination, and radiosensitizes S/G2-phase cells. J Biol Chem 2004, 279:42313-42320.

47. Libby BJ, De La Fonte R, O'Brien MJ, Wigginsworth K, Cobb J, Inselman A, Eaker S, Handel MA, Eppig JJ, Schimenti JC: The mouse meiotic mutation mei disrupts chromosome synapsis with sexually dimorphic consequences for meiotic progression. Dev Biol 2002, 242:174-187.

48. Sharan SK, Pyle A, Cottola V, Babus J, Swaminathan S, Benedic J, Swing D, Martin BK, Tesserollo L, Evans JP, Flaws JA, Handel MA: BRCA2 deficiency in mice leads to meiotic impairment and infertility. Development 2004, 131:131-142.

49. Ryvkinova E, Elige M, Heyting C, Hodges CA, Hunt PA, Liebe B, Scherthan H, Jessberger R: Cohesin SMC1 beta is required for meiotic chromosome dynamics, sister chromatid cohesion and DNA recombination. Nat Cell Biol 2004, 6:553-562.

50. Godthelp BC, Wiegant WW, van Duin-Poehl, A, Scherer OD, van Buul PP, Kanaar R, Zdanowicz MZ: Mammalian Rad51C contributes to DNA cross-link resistance, sister chromatid cohesion and genomic stability. Nucleic Acids Res 2002, 30:2172-2182.

51. Marston AL, Amon A: Meiosis: cell-cycle controls shuffle and deal. Nat Rev Mol Cell Biol 2004, 5:893-907.

52. Watanabe Y: Shugoshin: guardian spirit in the centromere. Curr Opin Cell Biol 2005, 17:590-595.

53. Watanabe Y: Sister chromatid cohesion along arms and at centromeres. Trends Genet 2005, 21:405-412.

54. Wang X, Dai W: Shugoshin, a guardian for sister chromatid segregation. Exp Cell Res 2005, 310:1-9.

55. Hsu A, Lio YC, Schild D, Brenneman MA, Redpath JL, Chen DJ: Human Rad51C deficiency destabilizes XRC3C, impairs recombination, and radiosensitizes S/G2-phase cells. J Biol Chem 2004, 279:42313-42320.

56. Li W, Yang X, Lin Z, Timofejeva L, Xiao R, Makaroff CA, Ma H: The AtRad51C Gene Is Required for Normal Meiotic Chromosome Synapse and Double-Stranded Break Repair in Arabidopsis. Plant Physiol 2005, 138:965-976.

57. Libby BJ, De La Fonte R, O’Brien MJ, Wigginsworth K, Cobb J, Inselman A, Eaker S, Handel MA, Eppig JJ, Schimenti JC: The mouse meiotic mutation mei disrupts chromosome synapsis with sexually dimorphic consequences for meiotic progression. Dev Biol 2002, 242:174-187.

58. Koehler KE, Millie EA, Cherry JP, Burgoyne PS, Evans EP, Hunt PA, Hassold TJ: Sex-specific differences in meiotic chromosome segregation revealed by dicentric bridge resolution in mice. Genetics 2002, 162:1367-1379.

59. Novitski E: Genetic measures of centromere activity in Drosophila melanogaster. J Cell Physiol Suppl 1955, 45:151-169.

60. McClintock B: The Fusion of Broken Ends of Chromosomes Following Nuclear Fusion. Proc Natl Acad Sci U S A 1942, 28:458-463.

61. Yu HG, Koshland D: Chromosome morphogenesis: condensin-dependent cohesin removal during meiosis. Cell 2005, 123:397-407.

62. Hsu A, Lio YC, Schild D, Brenneman MA, Redpath JL, Chen DJ: Human Rad51C deficiency destabilizes XRC3C, impairs recombination, and radiosensitizes S/G2-phase cells. J Biol Chem 2004, 279:42313-42320.

63. Kim JS, Krasieva TB, LaMorte V, Taylor AM, Yokomori K: Specific recruitment of human cohesin to laser-induced DNA damage. J Biol Chem 2002, 277:45149-45153.

Publish with BioMed Central and every scientist can read your work free of charge

*BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime.*

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp