Taking *Drosophila* Rad51 for a SPiN

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A recent paper reports that Spn-A is the Rad51 ortholog in *Drosophila melanogaster*. This and other findings in this study open the door to genetic analysis of Rad51 functions in a model metazoan.

Genomic integrity is essential to the survival of any organism, and DNA double-strand breaks (DSBs) are a dangerous threat to this integrity. Although it is well known that exogenous agents such as ionizing radiation can induce DSBs, problems encountered during replication are probably the most significant source of this type of damage. In addition, some DSBs are generated enzymatically during meiotic recombination. Among the multiple systems for repairing double strand breaks are several homologous recombination pathways[1]. An important feature of these pathways is that they are typically error-free, and so act to maintain genome stability.

A key step in homologous recombination is the DNA pairing and strand exchange reaction. In this process, one end of the DSB is used to find a homologous duplex; subsequently, this end invades the duplex, displacing one strand to form a 'D-loop' (**Fig. 1a**). Base pairing with the complementary strand allows the end to prime repair DNA synthesis using the homologous sequence as a template. In eukaryotes, the strand exchange reaction is catalyzed by Rad51, a homolog of the *Escherichia coli* RecA protein[2]. Extensive work on *Saccharomyces cerevisiae* has shown that *rad51* mutants are viable. However, these mutants are sensitive to ionizing radiation and defective in mitotic and meiotic recombination[3]. In contrast, genetic analysis of *rad51* in vertebrates has not been feasible because Rad51 is essential for cell proliferation, making *rad51*$^{-/-}$ cells inviable[4].

A *Drosophila* gene encoding a putative Rad51 ortholog has been previously identified on the basis of sequence similarity[5,6]. In a recent report, Staeva-Vieira *et al.*[7] show that this gene corresponds to *spindle-A* (*spn-A*), and that the activity of Spn-A is required for repair of DNA double-strand breaks. Notably, although *spn-A* mutants confer female sterility, they appear to have no effect on viability.

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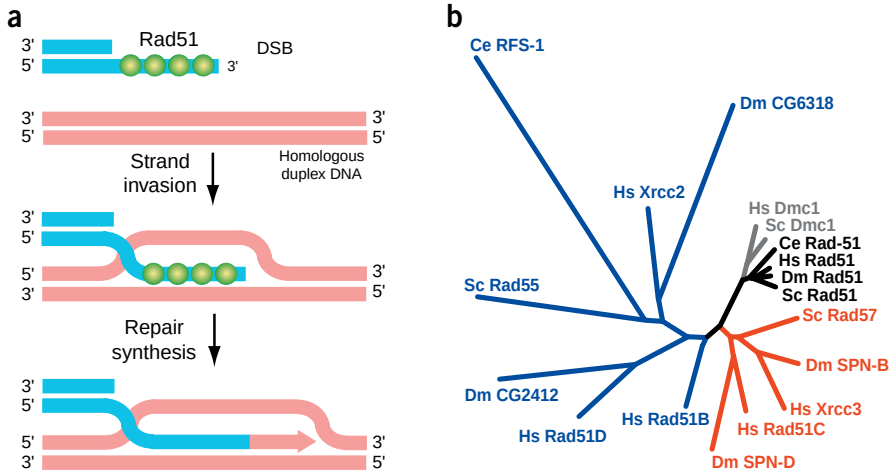

Figure 1 Homologous recombination and the Rad51 family. (**a**) The end of a double-strand break (DSB) is resected to leave a 3′ overhang, which is then coated with Rad51. After finding a homologous duplex DNA sequence, Rad51 carries out a strand invasion reaction in which one strand of the target is displaced to form a D-loop. The invading strand base-pairs to the homologous target strand and is then extended by repair synthesis, using the homologous target as a template. Several other proteins, including the Rad51 paralogs, are involved in each step. For details, see ref. 2. (**b**) The dendrogram illustrates the sequence relationships among all Rad51-related proteins in *S. cerevisiae* (Sc), *C. elegans* (Ce), *D. melanogaster* (Dm) and humans (Hs). Rad51 and Dmc1 orthologs are black and gray, respectively. The more divergent paralogs fall onto two major branches, colored blue and red. For this analysis, the core-conserved sequences were aligned using ClustalX 1.8 (ref. 19), and the result of 1,000 bootstrapped N-J trees was displayed in Treeview 1.6.5 (ref. 20).

The existence of viable mutants lacking Rad51 facilitates further genetic investigation of homologous recombination in mitosis and meiosis in a model metazoan.

The *spn-A* mutations, which have existed in the stock collection of Christiane Nüsslein-Volhard for at least 15 years, were originally identified as members of a class of mutations that cause eggshell patterning defects and female sterility. The *spindle* name comes not from any relationship to the microtubule-based spindle involved in chromosome segregation, but because the mispatterned eggs laid by mutant females are shaped like the spindles of spinning wheels.

The intriguing question here is thus: why does loss of Spn-A (Rad51) result in eggshell patterning defects? This patterning involves communication between the oocyte nucleus and the overlying follicle cells that secrete the eggshell[8]. It is believed that defects in processing DSBs that are used to initiate meiotic recombination leads to activation of a checkpoint that arrests the oocyte cell cycle, and this in turn disrupts oocyte-to-follicle-cell signaling[9]. Consistent with this model, Staeva-Vieira *et al.*[7] show that mutations in *mei-W68*, which encodes the enzyme believed to make the DSBs that initiate meiotic recombination, rescue the eggshell-patterning defect of *spn-A* mutants. Furthermore, mutations that inactivate the G2-M DNA damage checkpoint also suppress this *spn-A* mutant phenotype. These observations suggest that the defects associated with *spn-A* mutants are due to activation of a cell cycle checkpoint when the DSBs were not repaired.

Similar experiments have been performed previously for other genes in this class, including those encoding two Rad51 paralogs (see below) and the gene encoding the Rad54 ortholog[10,11]. The study of Staeva-Vieira *et al.*[7] goes one step further, showing that staining with an antibody to the phosphory-

lated form of H2AX (a histone variant that becomes phosphorylated at sites of DSBs) persists much later in oogenesis in *spn-A* mutants than in wild-type females. A reasonable interpretation of this observation is that unrepaired meiotic DSBs persist in *spn-A* mutants. Furthermore, H2AX staining is not localized into foci, as observed in wild-type cells, but instead is extensive along the DNA. These data indicate that DSBs are not repaired and accumulate in *spn-A* mutants, suggesting a role for this Rad51 ortholog in DNA repair.

Most eukaryotic genomes encode a family of Rad51-related proteins[12]. For example, *S. cerevisiae* has, in addition to Rad51, the related proteins Dmc1, Rad55, and Rad57, whereas vertebrate genomes encode six related proteins (Dmc1, Rad51B, Rad51C, Rad51D, Xrcc2, and Xrcc3), and the *Drosophila* genome encodes four (SPN-B, SPN-D, CG2412, and CG6318). A dendrogram of eukaryotic Rad51 paralogs reveals some interesting and perhaps informative patterns (**Fig. 1b**). The Rad51 orthologs cluster very tightly with the Dmc1 orthologs. Dmc1 functions exclusively in meiotic recombination in fungi, vertebrates, and plants[13], but it is notably absent from the *Drosophila* genome[14].

Reasoning that one or more of the other Rad51 paralogs may functionally substitute for Dmc1, Staeva-Vieira *et al.*[7] examined the expression patterns of each of the Rad51 family members in *Drosophila*. They found that *spn-D* and *CG6318* are expressed exclusively in the female germline, suggesting meiosis-specific expression. Although mutations in *CG6318* have not been reported, *spn-D* mutants do have a meiotic phenotype similar to that of *spn-A* mutants[11]. Based on sequence comparison and functional considerations, however, it seems unlikely that SPN-D and CG6318 can substitute for Dmc1: Dmc1 retains some strand exchange activity *in vitro*, consistent with its high degree of sequence similarity to Rad51 (ref. 15). In contrast, the more distantly related paralogs appear to have lost strand exchange activity, but instead function as heterodimers or larger complexes to facilitate Rad51-mediated strand exchange[2]. SPN-D and CG6318 clearly fall on these more divergent branches and therefore probably lack the strand exchange activity of Dmc1. Nevertheless, there have been no biochemical studies of *Drosophila* Rad51 paralogs to address this issue.

It is possible that meiotic recombination in *Drosophila* does not require a Dmc1 function. In *S. cerevisiae* and vertebrates, one function of meiotic recombination is to promote synapsis of homologous chromosomes[16,17]. In *Drosophila*, however, synapsis occurs in the absence of recombination[18]. This is also the case in *Caenorhabditis elegans*, which also lacks a Dmc1 ortholog. Perhaps the ability to complete synapsis in the absence of recombination alleviates the need for Dmc1.

Two other *Drosophila* Rad51 paralogous genes, *spn-B* and *CG2412*, appear to be expressed ubiquitously. Mutations in *spn-B* have a meiotic phenotype identical to that of *spn-A* and *spn-D* mutants[10,11], and Staeva-Vieira *et al.*[7] have now demonstrated that *spn-B* mutants are mildly sensitive to ionizing radiation, suggesting a function in DNA repair in somatic cells. No mutations in *CG2412* have been reported thus far, but on the basis of map position and the sequence of a mutant allele, *CG2412* probably corresponds to *rad201* (J.J.S., unpublished data). Mutations in *rad201* confer hypersensitivity to ionizing radiation but no meiotic defects.

Biochemical studies of the Rad51 paralogs (other than Rad51 and Dmc1) indicate that they function as heterodimers or larger complexes. The only two such paralogs in *S. cerevisiae*, Rad55 and Rad57, are believed to function as a heterodimer in both mitotic and meiotic DSB repair[3]. Similarly, vertebrate Xrcc3 and Rad51C are believed to function as a heterodimer[2]. Mutations in the *Drosophila* orthologs of these proteins, SPN-B and SPN-D, have identical meiotic phenotypes[10,11], suggesting they may also function together in meiotic recombination. However, only SPN-B is expressed in somatic cells[7], so this relationship cannot extend to mitotic DSB repair. Clearly, biochemical studies of the *Drosophila* paralogs will be essential in determining the various functional relationships.

In closing, the identification of 22 missense alleles of *spn-A*, spanning most of the protein coding sequence, make possible a detailed structure-function study of the *Drosophila* Rad51 protein[7]. Future genetic and biochemical analyses of the entire Rad51 family in *Drosophila* may shed light on the various functions of these proteins in homologous recombination and DNA repair pathways.

1. Haber, J.E. *Trends Genet.* **16**, 259–264 (2000).
2. Sung, P., Krejci, L., Van Komen, S. & Sehorn, M.G. *J. Biol. Chem.* **278**, 42729–42732 (2003).
3. Symington, L.S. *Micro. Mol. Biol. Rev.* **66**, 630–670 (2002).
4. Tsuzuki, T. *et al. Proc. Natl. Acad. Sci. USA* **93**, 6236–6240 (1996).
5. McKee, B.D., Ren, X. & Hong, C.A. *Chromosoma* **104**, 479–488 (1996).
6. Akaboshi, E., Inoue, Y. & Ryo, H. *Jap. J. Genet.* **69**, 663–670 (1994).
7. Staeva-Vieira, E., Yoo, S. & Lehmann, R. *EMBO J.* **22**, 5863–5874 (2003).
8. Dobens, L.L. & Raftery, L.A. *Dev. Dyn.* **218**, 80–93 (2000).
9. Morris, J. & Lehmann, R. *Curr. Biol.* **9**, R55–58 (1999).
10. Ghabrial, A., Ray, R.P. & Schüpbach, T. *Genes Dev.* **12**, 2711–2723 (1998).
11. Abdu, U., Gonzalez-Reyes, A., Ghabrial, A. & Schupbach, T. *Genetics* **165**, 197–204 (2003).
12. Thacker, J. *Trends Genet.* **15**, 166–168 (1999).
13. Masson, J.Y. & West, S.C. *Trends Biochem. Sci.* **26**, 131–136 (2001).
14. Sekelsky, J.J., Brodsky, M.H. & Burtis, K.C. *J. Cell Biol.* **150**, F31–F36 (2000).
15. Li, Z., Golub, E.I., Gupta, R. & Radding, C.M. *Proc. Natl. Acad. Sci. USA* **94**, 11221–11226 (1997).
16. Bishop, D.K., Park, D., Xu, L. & Kleckner, N. *Cell* **69**, 439–456 (1992).
17. Yoshida, K. *et al. Mol. Cell* **1**, 707–718 (1998).
18. Hunter, N. *Mol. Cell* **12**, 533–535 (2003).
19. Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., & Higgins, D.G. *Nucleic Acids Res.* **25**, 4876–4882 (1997).
20. Page, R.D.M. *Comp. Appl. Biosci.* **12**, 357–358 (1996).