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Phosphorylation and the creation of interhomolog bias during meiosis in yeast

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One of the fundamental differences between mitotic and meiotic cells is the source of homology used to repair double strand breaks (DSBs). Recombination in vegetative cells occurs preferentially between sister chromatids to repair unexpected DNA damage.[1] In contrast, repair of programmed DSBs in meiotic cells occurs primarily between non-sister chromatids of homologous chromosomes.[2] The resulting crossovers generate physical connections that promote accurate segregation at the first meiotic division. Mistakes in meiotic chromosome segregation in humans lead to infertility and birth defects. An important question, therefore, is how the bias for recombination between homologs is established during meiosis.

The decision of whether to use sister chromatids or homologs as templates for repair is made at the time of strand invasion. Strand invasion is mediated by recombinases, RecA-like proteins that bind to the single stranded ends of resected DSBs to form filaments.[3] The major recombinase in vegetative yeast cells is Rad51. Rad51 activity requires interaction with Rad54, a member of the Swi/Snf family of chromatin remodeling proteins. Rad54 helps to clear off nucleosomes, denature the recipient DNA duplex to promote strand invasion and remove Rad51 after strand invasion is complete.[4] In meiotic cells, a meiosis-specific recombinase called Dmc1 is also present. Dmc1 functions with a paralog of Rad54 called Rdh54/Tid1. Although both *RAD51* and *DMC1* are required for interhomolog recombination, a variety of data suggests that Rad51 and Dmc1 are utilized primarily for inter-sister and inter-homolog recombination, respectively.[3] Recent work by Niu et al.[5] has helped clarify the different roles these two recombinases play during meiosis.

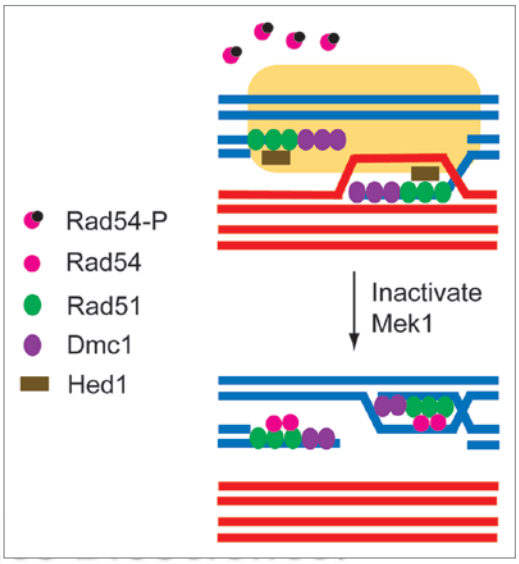

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Figure 1. Mek1 is a dynamic switch controlling inter-sister DSB repair during budding yeast meiosis. The yellow box indicates the "barrier to sister chromatid repair" (BSCR) created by Mek1 phosphorylation of an as yet unidentified protein. Inactivation of Mek1 serves two purposes—it allows Rad51/Rad54 complex formation through de-phosphorylation of Rad54 and removal of the BSCR, thereby allowing strand invasion of sister chromatids even in the presence of Dmc1.

The activity of a meiosis-specific kinase called Mek1/Mre4 is necessary to prevent Rad51 from invading sister chromatids, but the substrates of Mek1 responsible for suppressing inter-sister DSB repair were unknown.[6] Niu et al. screened various purified proteins involved in Rad51-mediated recombination for phosphorylation by Mek1 and found that Rad54 is phosphorylated on threonine 132 both in vitro and in meiotic cells. The negative charge conferred by phosphorylation reduces the affinity of Rad54 for Rad51 and reduces Rad54 stimulation of Rad51's activity. In vivo, the inability to phosphorylate Rad54 suppresses the interhomolog recombination defect of *dmc1*Δ. This suppression, however, is dependent upon Mek1, indicating that Mek1 performs two functions during meiosis: (1) it inhibits Rad51 recombinase activity by decreasing Rad51/Rad54 complex formation through phosphorylation of Rad54 and (2) it prevents filaments containing Rad51/Rad54 from invading sister chromatids by phosphorylation of a second, as yet unknown, protein (**Fig. 1**). The Mek1-dependent mechanism for inhibiting Rad51/Rad54 complex formation acts in parallel with Hed1, a meiosis-specific protein that binds to Rad51, thereby excluding Rad54.[7]

This work indicates that interhomolog bias is created by (1) suppression of Rad51 recombinase activity through the combined actions of Hed1 and Rad54 phosphorylation, (2) the Mek1 phosphorylated

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substrate that prevents Rad51/Rad54 containing filaments from invading sister chromatids and (3) promotion of interhomolog strand invasion by Dmc1 (**Fig. 1**). The fact that *RAD51* is necessary for interhomolog recombination despite being inactive, suggests that it is the presence of Rad51, not its recombinase activity, that is required, perhaps to properly load/position Dmc1 onto the filament. *RAD54* is not required for interhomolog recombination yet *rad54*Δ exhibits reduced sporulation and spore viability, indicating that DSB repair is faulty.[8] We propose that Mek1 phosphorylation is a dynamic switch that can be used to turn off Rad51/Rad54-mediated intersister DSB repair until every chromosome has at least one interhomolog crossover. Inactivation of Mek1 then allows repair of these residual DSBs by Rad51/Rad54 using sister chromatids (**Fig. 1**). In the absence of *RAD54*, leftover DSBs may persist, resulting in spore death. Having two phases of meiotic recombination, one between homologs and one between sister chromatids, may be evolutionarily conserved, although the mechanisms for achieving this goal may differ. In nematodes, for example, Rad51 is loaded onto chromosomes in two temporally distinct waves.[9] Rad51 loaded early is competent for interhomolog recombination, while Rad51 loaded later in meiosis is not.

Acknowledgements

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