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γH2AX spreading linked to homology search

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DNA double-strand breaks (DSBs) are one of the most toxic types of DNA damage, as their inaccurate repair results in mutations and chromosomal rearrangements.[1] One of the earliest responses to a DSB is the formation of γH2AX around the break, a highly conserved process by which canonical yeast histone 2A (H2A) or the mammalian histone variant H2A.X are phosphorylated by DNA damage response kinases.[2] Cells expressing the respective phosphorylation-defective H2A/H2A.X mutant are sensitive to DSB-inducing agents, and mice bearing the phosphorylation-defective H2A.X mutant exhibit chromosomal instability.[3] These phenotypes have been mainly connected to γH2A serving as a recruitment scaffold for proteins assisting in repair or to a role of γH2A in the DNA damage checkpoint.[2] Notably, γH2A can spread over very large regions surrounding the DSB encompassing hundreds of kilobases in yeast[4] and up to megabases in mammals.[5] However, the underlying causes and consequences of this broad distribution of γH2A remained enigmatic.[2]

We recently demonstrated that the distribution of γH2A does not simply reflect linear spreading of this modification to both sides of the DSB, but follows the pattern of homology search during homologous recombination.[6] Homologous recombination relies on the usage of an intact homologous donor sequence to repair a DSB. How homology search, the exploration of the genome for intact homologous donor sequences, operates in the context of a crowded nuclear environment was puzzling for a long time.[7] However, using time-resolved chromatin immunoprecipitation (ChIP) of the recombinase Rad51 in *S. cerevisiae*, we noticed that the distribution pattern of Rad51 on chromatin is a direct result of homology probing on chromosomes after DSB induction.[6] Monitoring homology search by this approach in vivo, we found that the repair complex (presynaptic nucleoprotein filament) preferentially samples on the broken chromosome around the DSB, but also on regions of other chromosomes if they are in close proximity due to nuclear organization.[6]

In addition to these general findings regarding homology search, we discovered that the distribution of γH2A ChIP signals closely parallels the distribution of the homology search-linked Rad51 ChIP signals.[6] When a DSB was induced at the *S. cerevisiae* mating-type (*MAT*) locus, homology search and Rad51 ChIP signals preferentially targeted the left chromosomal arm (*HML*) in *MATa* cells, and the right chromosomal arm (*HMR*) in *MAT*α cells, according to the known donor preferences for repair of the 2 different mating types.[6] Notably, the γH2A ChIP signal profiles almost precisely matched those for Rad51 in the 2 strains upon DSB induction at *MAT*,[6] indicating that both Rad51 and γH2A ChIP signal distributions are direct consequences of homology search. Since the observed distribution of γH2A also paralleled the homology search in cases when a single DSB was induced on other chromosomes, γH2A signal spreading and homology search are apparently generally directly linked.

Interestingly, when a DSB was induced close to the centromere of a chromosome, homology search monitored by Rad51 ChIP signals was also detectable around centromeres of all other chromosomes.[6] This is most likely due to the fact that yeast centromeres cluster in the nucleus, thereby potentially facilitating proximity-guided homology search from the broken chromosome to centromeric regions of other chromosomes. Notably, γH2A ChIP signals also appeared at centromeric regions analogous to Rad51,[6] indicating again that the observed ChIP signals for Rad51 and γH2A are both direct consequences of homology search. This finding demonstrates that γH2A formation does not necessarily occur only in cis on the broken chromosome, but also in trans on other chromosomes. An important corollary of this finding is that the widely used DSB marker γH2A does not exclusively label areas of DSBs.

Based on these findings, we would like to propose that the broad chromosomal distribution of γH2A previously observed in yeast and mammalian cells[4,5,8] are, in large part, consequences of DSB-triggered homology search. A plausible scenario is that checkpoint kinases travel along with the presynaptic filament during homology search, thereby phosphorylating histones encountered during the search process. If true, this model would predict that factors reported to impact γH2A distribution, like cohesion and transcription,[5,8] might influence homology search as well. Despite these findings, one pressing question remains: what might be the function of γH2A during the homology-probing process? Hypothetically, γH2A might render the chromatin environment more accessible for homology probing or might recruit factors that facilitate the search. However, the finding that a phosphorylation-defective mutant variant of H2A had apparently no measurable deleterious effect on homology search in yeast[6] may argue against this possibility. Alternatively, γH2A could mark the chromosomal sites

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that have already been sampled (and render them inaccessible) or amplify the checkpoint response until homology has been found. In whatever way, the finding that γH2A follows homology search adds an important new twist to DNA repair and suggests that studies in yeast and mammalian cells relying on γH2A foci and signals should consider homology search, and not only the DSBs per se, as a potential source for γH2A signals. (**Fig. 1**)

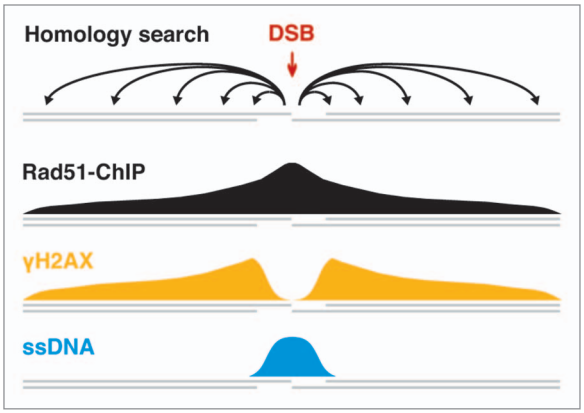

Figure 1. γH2AX follows homology search. Homology search (depicted as black arrows) during homologous recombination can be monitored in vivo by Rad51-directed chromatin immunoprecipitations (ChIP). The profiles of ChIP signal distributions for γH2A (orange) and Rad51 (black) both follow homology search and only differ near the DSB because of histone eviction linked to resection (blue, ssDNA).

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