NUCLEOSOMES DYNAMICS AT DNA DOUBLE STRAND BREAKS

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Background: DNA double-strand breaks (DSBs) are highly toxic lesions that, if not correctly repaired, can have detrimental consequences on genome integrity and cell survival. Repair of DSBs by homologous recombination (HR) requires the processing of DSB ends during DNA end resection, which, through the degradation of 5'-terminated strands, generates a long stretch of single-stranded DNA (ssDNA). Therefore, resection divides the chromatin surrounding a DSB in distinct ssDNA and dsDNA domains. The molecular composition of these domains is crucial for HR repair as well as for DNA damage signaling and checkpoint activation. However, it was unclear whether nucleosomes, the fundamental unit of chromatin, could be found in the ssDNA domain as well.

Aim: Our aim was to analyze the *in vivo* DNA binding mode of key DSB repair proteins as well as nucleosomes.

Methods: Here we combined site-specific induction of DSBs in yeast by HO and AsiSI nucleases, followed by chromatin immunoprecipitation (ChIP), strand-specific library preparation and next-generation sequencing to discriminate between double-stranded and single-stranded DNA binding of proteins *in vivo*.

Results: In proof-of-principle experiments, strand-specific ChIP-sequencing recapitulated the characteristic binding pattern of RPA and Rad51 to ssDNA at resected DSBs. Using this technique, we were also able to detect Rad51 binding to dsDNA during homology search. The 9-1-1 signaling platform was suggested to bind at the ss-dsDNA junction at resected DSBs. We observed that, *in vivo*, 9-1-1 associates with the dsDNA compartment and locates at the leading edge of resection. Furthermore, we did not find evidence of the presence of nucleosomes on ssDNA but, in contrast, we observed that nucleosomes become fully evicted in concomitance with resection. In addition, we found that the chromatin remodelers RSC and SWI/SNF play a crucial role in promoting such nucleosome eviction.

Conclusions: Taken together, our study revealed that: 1) nucleosomes are not a major, persistent species in the ssDNA compartment; 2) nucleosome eviction and resection are intrinsically coupled; 3) nucleosome eviction and, consequently resection, require the remodeling activity of RSC and SWI/SNF.