Transcriptional repression accounts for RNA:DNA hybrid accumulation at DNA Double Strand breaks

Emma Lesage, Florian Saur, Sarah Collins, Anne-Laure Finoux, Benjamin Lebozec, Vincent Rocher, Marie Couralet, Melanie Petiot, Thomas Clouaire, Aline Marnef and Gaëlle Legube

MCD, Centre de Biologie Intégrative (CBI), CNRS, Université de Toulouse, UT3

Presenting author: Aline Marnef: aline.marnef@univ-tlse3.fr

RNA:DNA hybrids accumulate at the vicinity of DNA double-strand breaks (DSBs) and were shown to regulate homologous recombination repair. The mechanism responsible for the formation of these non-canonical RNA:DNA structures remains unclear although they were proposed to arise consequently to RNA Polymerase II or III loading followed by DSB-induced *de novo* transcription at the break site. Here, we found no evidence of RNA polymerases recruitment at DSBs. Rather, strand-specific R-loop mapping revealed that RNA:DNA hybrids are mainly generated at DSB occurring in transcribing loci, from the hybridization of pre-existing RNA to the 3' overhang left by DNA end resection. We further identified the H3K4me3 reader Spindlin 1 as promoting RNA:DNA hybrid accumulation at DSBs, through its role in mediating transcriptional repression *in cis* to DSBs. Altogether, we provide evidence that RNA:DNA hybrids accumulate at DSBs occurring in transcribing loci as a result of DSB-induced transcriptional shut-down.