# RNA polymerase senses DNA damage and makes R-loop for repair

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The R-loops forming around DNA double-strand breaks (DSBs) within actively transcribed genes play a critical role in the DSB repair process. However, the mechanisms underlying R-loop formation at DSBs remain poorly understood, with diverse proposed models involving protein factors associated with RNA polymerase (RNAP) loading, pausing/backtracking or preexisting transcript RNA invasion. We developed single-molecule fluorescence assays to study co-transcriptional R-loop formation in real time, and discovered that transcribing RNAP alone acts as a highly effective DSB sensor, responsible for generation of R-loops upon encountering downstream DSBs, without requiring any additional factors. The R-loop formation efficiency is greatly influenced by DNA end structures, ranging here from 2.8% to 73%, and notably higher on sticky ends with 3' or 5' single-stranded overhangs compared to blunt ends without any overhangs. The R-loops extend unidirectionally upstream from the DSB sites and can reach the transcription start site, interfering with ongoing-round transcription. Furthermore, the extended R-loops can persist and maintain their structures, effectively preventing the efficient initiation of subsequent transcription rounds. Our results are consistent with the bubble extension model rather than the 5'-end invasion model or the middle insertion model.