Nucleolar accumulation of the RNA-binding protein NONO promotes DNA repair

Kaspar Burger

Biocenter of the Julius Maximilian University Wuerzburg, Department of Biochemistry & Molecular Biology, Am Hubland, 97074 Wuerzburg, Germany

DNA double-strand breaks (DSBs) are highly toxic lesions that threaten genome stability and lead to cancer, if left unrepaired. The DNA damage response (DDR) counteracts the accumulation of DSBs and requires long non-coding (lnc)RNA and RNA-binding proteins (RBPs) for efficient DSB repair (DSBR). We could previously show that RNA polymerase II (RNAPII) and the RNAi factor Dicer promote DSBR. RNAPII that is specifically phosphorylated at CTD tyrosine-1 residues accumulates at promoter-associated DSBs in a c-Abl kinase-dependent manner and produces damage-induced lncRNA. Such transcripts form double-stranded (ds)RNA intermediates and undergo processing by nuclear phosphorylated Dicer to facilitate the recruitment of repair factors like 53BP1 to DSBs.

However, the regulatory principles that integrate the RNA metabolism with DSBR remain poorly understood. We currently investigate the role of nuclear bodies for genome stability and are particularly interested in paraspeckles. Paraspeckles function as RNA metabolic hubs to regulate gene expression by retention of a subset of mRNA. The structural lncRNA NEAT1 and the multifunctional RBP NONO are core components of paraspeckles and frequently upregulated in tumours. NONO and NEAT1 are required for the efficient response to DSBs. The depletion of NONO or NEAT1 in human U2OS cells delays DDR signaling and increases persistent DNA damage. We observe that a subset of NONO accumulates on chromatin and in the nucleolus upon incubation with Etoposide, while NEAT1 levels are specifically elevated in the nucleoplasm of damaged cells. To investigate the molecular principles that link NONO and NEAT1 to genome stability we combined mass spectrometry, CLIP-seq, and site-directed mutagenesis to determine damage-induced changes in the NONO interactome. Our preliminary data suggest that the DDR redistributes a subset of NONO to the nucleolus in an RNA-dependent manner possibly to mitigate the accumulation of aberrant transcripts on damaged chromatin and promote DSBR pathway choice.