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Epigenetics and Genomic Stability

Goal

To understand the role of chromatin and RNA surveillance mechanisms in genome organization and in the maintenance of genomic stability.

Background

Recent results have revealed another layer of complexity in the eukaryotic transcriptome. While previous expression analyses focused mostly on mRNA expression levels, new transcriptome profiling techniques provide unbiased, genome-wide information about transcription activity. These studies have revealed that genomic regions previously thought to be silent, such as heterochromatic regions, intergenic regions and antisense portions of the genome, are actually transcriptionally active and produce a significant amount of non-protein-coding RNA (ncRNA) transcripts. Although a growing number of examples show that some of these ncRNAs can affect key nuclear events, the majority of these transcripts, so-called cryptic unstable transcripts (CUTs) are rapidly degraded by the RNA surveillance machinery. The general function of this widespread, pervasive transcription is not understood, but its level is strictly controlled by the chromatin structure. Defects in this repressive chromatin structure or impairment of the RNA surveillance system can lead to strong accumulation of cryptic transcripts and to genomic instability.

The main focus of the research in our laboratory is:

(i) to understand the role of chromatin in the repression of pervasive transcription;

(ii) to reveal the molecular mechanisms responsible for the recognition and degradation of CUTs;

(iii) to understand the link between ncRNA accumulation and genomic instability and how it contributes to cancer development.

Research Highlights

We recently identified the multi-subunit MTREC complex in *S. pombe* that specifically binds to CUTs and unspliced pre-mRNAs and targets them to the nuclear exosome for degradation (Zhou *et al.*, 2015). Phylogenetic analysis of the subunits shows high similarity to subunits of the human Nuclear EXosome Targeting (NEXT) complex. Deletion or mutation of *S. pombe* MTREC complex subunits leads to strong accumulation of CUTs and unspliced or mis-spliced pre-mRNAs. MTREC complex physically interacts with the nuclear exosome, and with various RNA binding and processing complexes, including the cap-

binding, spliceosome, cleavage and poly-adenylation complexes. Our future goal is to further characterize the MTREC complex *in vitro* and *in vivo*, and to uncover the molecular mechanism by which this large, multi- subdomain complex is recruited to CUTs and aberrant mRNAs and targets them to the exosome (Figure 1).

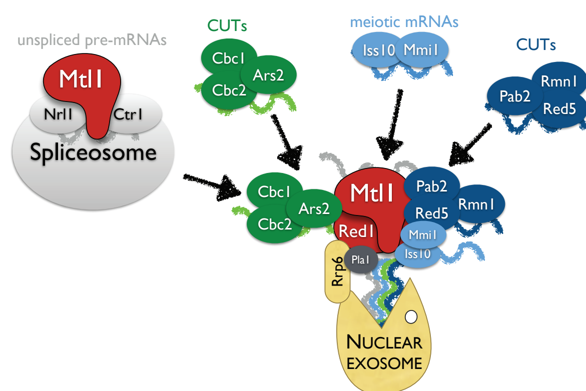

Figure 1. Proposed model for the role of MTREC complex in RNA surveillance. *Submodules of the MTREC complex, together with the Mtl1–Ctr1–Nrl1 complex, are recruited to different subsets of CUTs, meiotic mRNAs or unspliced pre-mRNA transcripts and deliver these RNAs to the MTREC complex. The RNAs are polyadenylated by the canonical poly(A) polymerase, Pla1. The RNA-loaded MTREC complex can dock to the nuclear exosome through the Red1–Rrp6 interaction. The helicase activity of the Mtl1 subunit then feeds the MTREC-bound RNAs into the exosome channel.*

Although excessive pervasive transcription can cause genomic instability, pervasive transcription in general might also have positive effects for the cell. We recently discovered that pervasive transcription and the resulting RNA-DNA hybrids are essential for the efficient repair of DNA double-strand breaks (DSBs). RNA-DNA hybrids are generated by RNA polymerase activation around DSB sites, and stabilization or destabilization of RNA-DNA hybrids strongly affects the length of strand resection during HR-mediated DSB repair (Ohle *et al.*, 2016). These observations strongly suggest that RNA polymerase transcription might have a direct role in strand resection, probably by opening the chromatin structure around the DSB site. This is a very exciting and novel development in the field of DNA-repair, and our mid-to long-term research goals will focus on the molecular mechanism behind these findings both in the fission yeast *S. pombe* and in mammalian cells (Figure 2).

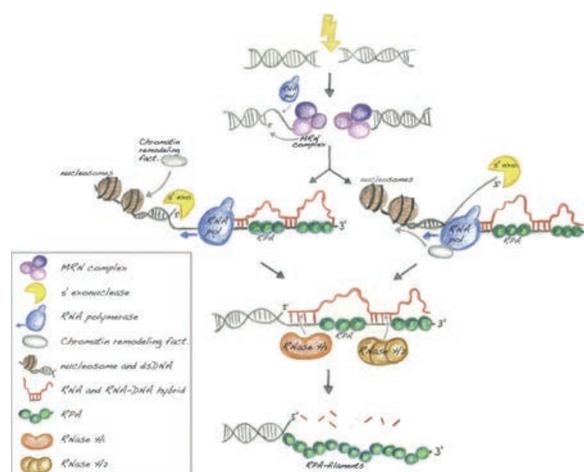

Figure 2. RNA-DNA Hybrids and RNase H Activity Are Required for Efficient DSB Repair. *Suggested model for the HR-mediated DSB repair pathway. Following the appearance of a DSB, the MRN complex is recruited to the broken DNA ends and, with the help of Sae2, initiates 5´ end resection. Pol II is recruited to the 3´ ssDNA overhangs and jump-starts transcription, without the requirement of PIC assembly or the recruitment of additional regulatory proteins. The nascent RNA transcripts are prone to re-hybridize with the ssDNA template strand and form RNA-DNA hybrids, which directly compete with the ssDNA-binding RPA complex. Subsequent, long-range resection of the 5´ strand is performed by the 5´-3´ exonuclease Exo1 or the Dna2-Sgs1/BLM complex. Additional chromatin remodelling activities likely facilitate the progression of nucleases through the chromatin environment. Pol II transcription either follows the strand resection process (left) or actively drives strand resection by opening the chromatin and the DNA helix ahead of the nucleases (right). RNA-DNA hybrids might play a role in controlling the speed and the length of the strand resection process by stalling or terminating Pol II transcription. RNA-DNA hybrids need to be degraded by RNase H enzymes in order to achieve full RPA loading on the ssDNA overhangs and to complete the DNA repair process.*

Selected Publications 2014 - 2016

Ohle, C.*, Tesorero, R.*, Schermann, G., Dobrev, N., Sinning, I., Fischer, T. Transient RNA-DNA Hybrids Are Required for Efficient Double-Strand Break Repair, Cell 167, 1001-1013 (2016). (*These authors contributed equally.)

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Tucker, J. F., Ohle, C., Schermann, G., Bendrin, K., Zhang, W., Fischer, T., Zhang, K. A Novel Epigenetic Silencing Pathway Involving the Highly Conserved 5'-3' Exoribonuclease Dhp1/Rat1/Xrn2 in Schizosaccharomyces pombe. PLoS Genet. 12, e1005873 (2016).

Zhou, Y.*, Zhu, J.*, Schermann, G., Ohle, C., Bendrin, K., Sugioka-Sugiyama, R., Sugiyama, T., and Fischer, T. The fission yeast MTREC complex targets CUTs and unspliced pre-mRNAs to the nuclear exosome. Nat. Commun. 7:7050 (2015). (*These authors contributed equally.)

Sancar, C., Ha, N., Yilmaz, R., Tesorero, R., Fischer, T., Brunner, M. and Sancar, G. Combinatorial control of light induced chromatin remodeling and gene activation in neurospora. PLoS Genet. 11, e1005105 (2015).

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