SFB 960-/BZR – Kolloquium

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H 53

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CRACing odd transcription – RNA polymerases I and III at nucleotide resolution

RNA polymerase I and III (RNAPI and RNAPIII) synthesize a range of highly abundant stable RNAs. RNAPI transcribes a single long transcript that is processed to the mature ribosomal RNAs (rRNA). RNAPIII transcribes a range of short RNAs, principally pre-tRNAs. Due to the high accumulation of these mature RNAs, stringent purification is needed to reliably identify association with the nascent transcripts in genome-wide analyses.
Mapping of RNAPIII at nucleotide resolution using UV cross-linking and analysis of cDNA (CRAC) revealed strikingly uneven polymerase distributions across short transcription units, generally with a predominant 5´ peak. This peak was relatively higher for more heavily transcribed genes, suggesting that initiation site clearance is rate limiting during RNAPIII transcription. Many tRNA genes were found to generate long, 3´-extended forms due to read-through of the canonical poly(U) terminators. The degree of read-through was anti-correlated with the density of T-residues in the coding strand, and multiple, functional terminators were identified that are located far downstream. The steady-state levels of 3´-extended pre-tRNA transcripts are low, apparently due to targeting by the nuclear surveillance machinery; especially the RNA-binding protein Nab2, cofactors for the nuclear exosome and the 5´-exonuclease Rat1.
CRAC similarly revealed the distribution of transcribing RNAPI across the pre-rRNA transcription unit with nucleotide resolution. A strong 5’ bias was observed both by CRAC and high-resolution ChIP-seq. Moreover, the distribution of RNAPI was strikingly uneven, specifically across the first 1000 nucleotides (nt) of the transcription unit, with peak spacing of 80-90 nt. Sequence analysis in this region suggested that GC-content may play a role in establishing the spacing between polymerases. A similar profile was for nuclear RNA surveillance factors of TRAMP and exosome complexes, indicating that nascent transcripts are subject to a degree of premature transcription termination and degradation. Prominent pause sites were also located within internal transcribed spacer 1 (ITS1) region, which is essential for pre-rRNA processing. This pausing is influenced by the level of RNAPI ubiquitination (RNAPI-Ub), involving the ubiquitin ligase Rsp5 and the RNA-binding, ubiquitin protease complex Bre5-Ubp3, as well as the ribosome assembly factor Rrp5. Lack of Rrp5 or increased RNAPI-Ub (in strains lacking Bre5 or Ubp3) resulted in decreased pausing, whereas decreased RNAPI-Ub resulted in extended pausing.

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