Regulation of pre-mRNA splicing and mRNA degradation in *Saccharomyces cerevisiae*

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt förvar i KBE303-Stora hörsalen, KBC-huset, Fredag den 22 September, kl. 09:00.
Avhandlingen kommer att försvaras på engelska.

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Abstract

Messenger RNAs are transcribed and co-transcriptionally processed in the nucleus, and transported to the cytoplasm. In the cytoplasm, mRNAs serve as the template for protein synthesis and are eventually degraded. The removal of intron sequences from a precursor mRNA is termed splicing and is carried out by the dynamic spliceosome. In this thesis, I describe the regulated splicing of two transcripts in *Saccharomyces cerevisiae*. I also describe a study where the mechanisms that control the expression of magnesium transporters are elucidated.

The pre-mRNA retention and splicing (RES) complex is a spliceosome-associated protein complex that promotes the splicing and nuclear retention of a subset of pre-mRNAs. The RES complex consists of three subunits, Bud13p, Snu17p and Pml1p. We show that the lack of RES factors causes a decrease in the formation of N4-acetylcytidine (ac4C) in tRNAs. This phenotype is caused by inefficient splicing of the pre-mRNA of the *TAN1* gene, which is required for the formation of ac4C in tRNAs. The RES mutants also show growth defects that are exacerbated at elevated temperatures. We show that the temperature sensitive phenotype of the bud13Δ and snu17Δ cells is caused by the inefficient splicing of the *MED20* pre-mRNA. The *MED20* gene encodes a subunit of the Mediator complex. Unspliced pre-mRNAs that enter the cytoplasm are usually degraded by the nonsense-mediated mRNA decay (NMD) pathway, which targets transcripts that contain premature translation termination codons. Consistent with the nuclear retention function of the RES complex, we find that NMD inactivation in the RES mutants leads to the accumulation of both *TAN1* and *MED20* pre-mRNAs. We also show that the cis-acting elements that promote RES-dependent splicing are different between the *TAN1* and *MED20* pre-mRNAs.

The NMD pathway also targets transcripts with upstream ORFs (uORFs) for degradation. The *ALR1* gene encodes the major magnesium importer in yeast, and its expression is controlled by the NMD pathway via a uORF in the 5' untranslated region. We show that the ribosome reaches the downstream main ORF by a translation reinitiation mechanism. The NMD pathway was shown to control cellular Mg2+ levels by regulating the expression of the *ALR1* gene. We further show that the NMD pathway targets the transcripts of the vacuolar Mg2+ exporter Mnri2p and the mitochondrial Mg2+ exporter Mme1p for degradation.

In summary, we conclude that the RES complex has a role in the splicing regulation of a subset of transcripts. We also suggest a regulatory role for the NMD pathway in maintaining the cellular Mg2+ concentration by controlling the expression of Mg2+ transporters.

Keywords

NMD, RES complex, pre-mRNA splicing, magnesium homeostasis