PRINCIPE FELIPE
CENTRO DE INVESTIGACION

Doctoral Thesis

Identification of RNA structures modulating the expression of the mRNA biogenesis factor SUS1

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Abstract: Sus1 is a conserved protein involved in chromatin remodeling and mRNA biogenesis. The SUS1 gene of Saccharomyces cerevisiae is unusual, as it contains two introns and is alternatively spliced, retaining one or both introns in response to changes in environmental conditions. SUS1 splicing may allow the cell to control Sus1 expression, but the mechanisms that regulate this process remain unknown. In this thesis project, we have investigated whether the structure adopted by SUS1 RNA sequences contributes to regulate the splicing of this gene. Using in silico analyses together with NMR spectroscopy, gel electrophoresis and UV thermal denaturation experiments, we first show that the downstream intron (I2) of SUS1 forms a weakly-stable, 37-nucleotide stem-loop structure containing the branch site near its apical loop and the 3' splice site after the stem terminus. A cellular assay revealed that two of four mutants containing altered I2 structures had significantly impaired SUS1 expression. Semi-quantitative RT-PCR experiments indicated that all mutants accumulated unspliced SUS1 pre-mRNA and/or induced distorted levels of fully spliced mRNA relative to wild-type. Concomitantly, Sus1 cellular functions in histone H2B deubiquitination and mRNA export were affected in I2 hairpin mutants that inhibited splicing. The second part of the thesis project focuses on the exon located between the two introns of the SUS1 gene. This middle exon (E2) can be skipped during splicing, is generated in circular form, and has been found to influence the splicing of the flanking introns, an unusual situation in budding yeast where splicing mainly relies on intron recognition. Using NMR spectroscopy, gel electrophoresis, UV thermal denaturation and ribose 2'-OH modification experiments combined with computational predictions, we show that E2 of SUS1 comprises a conserved double-helical stem topped by a three-way junction. One of the hairpins emerging from the junction exhibited significant thermal stability and was closed by an unusually structured purine-rich loop. This loop contained two consecutive sheared G:A base pairs and was structurally related to the substrate loop of the VS ribozyme. Cellular assays revealed that three mutants containing altered E2 structures had impaired SUS1 expression and that a compensatory mutation restoring the conserved stem recovered expression to wild-type levels. Semi-quantitative RT-PCR experiments indicated that all mutants were capable of altering the quantities of unspliced and/or fully-spliced SUS1 RNA transcripts relative to wild-type. Overall, the results gathered in this thesis project indicate that RNA structures formed by the middle exon and the second intron of the S. cerevisiae SUS1 gene are relevant for splicing and also influence other processes of SUS1 mRNA biogenesis.

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