CHAPTER 8

Rrp6, Rrp47 AND COFACTORS
OF THE NUCLEAR EXOSOME

J. Scott Butler*1 and Phil Mitchell*2
1Departments of Microbiology and Immunology, and Biochemistry and Biophysics, and Center for RNA Biology: from Genome to Medicine, University of Rochester Medical Center, Rochester, New York, USA; 2Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, UK.
*Correspondence—Emails: scott_butler@urmc.rochester.edu, p.j.mitchell@shef.ac.uk

Abstract: This chapter reviews the present state of knowledge on the activity of enzymes that function with the RNA exosome in the nucleus. In this compartment, the exosome interacts physically and functionally with the exoribonuclease Rrp6 and several cofactors, most prominently Rrp47 and the TRAMP complex. These interactions decide the fate of RNA precursors from transcription through the formation of mature ribonucleoprotein particles (RNPs) and the export of the RNPs to the cytoplasm. The nuclear exosome catalyzes the formation of the mature 3' ends of many of these RNAs, but in other cases degrades the RNAs to mononucleotides. Cofactors such as Mpp6, TRAMP and the Nrd1/Nab3 complex play important roles in determining the outcome of the interaction of RNPs with the nuclear exosome. The details that govern the specificity of these decisions remain a rich source for future investigation.

INTRODUCTION

The RNA exosome plays an essential role in the processing and degradation of RNAs in eukaryotic organisms. In the nucleus and the cytoplasm the nine-subunit exosome core, Exo9, and the ribonuclease Dis3/Rrp44 function as a unit, designated Exo10. In Saccharomyces cerevisiae this complex interacts physically and functionally with a nucleus specific enzyme, Rrp6, to form the nuclear exosome, Exo11. While the majority of Rrp6 resides in the nucleus in S. cerevisiae, evidence suggests its presence in the cytoplasm in humans, T. brucei and A. thaliana.1-3 In S. cerevisiae, where Rrp6
has been studied most extensively, deletion of the sole copy of its gene (RRP6) causes a slow growth phenotype at 30°C and extremely poor growth at 37°C. Nevertheless, the fact that deletion of any of the other exosome genes causes lethality has made the use of rrp6-Δ strains a valuable tool for the study of exosome defects in nuclear RNA processing. These studies revealed a critical role for Rrp6 in maturation and degradation pathways that include all known classes of RNA. The targeting of Rrp6 and Exo10 to these different RNA processing pathways is specified by interactions with protein co-factors such as Rrp47, Mpp6 and the TRAMP complex. Moreover, studies in rrp6 mutants, or cells depleted of Rrp6, uncovered the existence of RNA polymerase II transcripts from virtually every part of the genomes of organisms as divergent as yeast, plants and humans. These revelations along with evidence that Rrp6 regulates the levels of specific mRNAs indicate that the nuclear exosome and its co-factors may have key functions in the control of gene expression and organismal development.

STRUCTURE AND ACTIVITY OF Rrp6

Rrp6 belongs to the RNaseD family of the DEDD superfamily of exoribonucleases, which use a two-metal ion mechanism for RNA hydrolysis (Fig. 1). Structure-function studies of Rrp6 proteins with point mutations in the exonuclease domain confirmed the two-metal ion mechanism and suggested that, like the exonuclease domains of DNA polymerases, Rrp6 utilizes a phenylalanine to stabilize the hydroxyl anion intermediate activated for phosphodiester bond cleavage. Unlike Dis3/Rrp44, whose activity is attenuated by interaction with Exo9, Rrp6 retains its characteristic properties in the Exo11 complex. Rrp6 contains two HRDC (Helicase RNaseD C-terminal) domains, only one of which was predicted by sequence homology. The Rrp6 HRDC1 domain folds into a characteristic 5-helix structure nearly identical to the homologous portion of E. coli RNaseD (Fig. 1). Surprisingly, a second HRDC domain appears directly after this in

![Structure of Rrp6](image)

**Figure 1.** Structure of Rrp6. The top diagram illustrates the polypeptide structure of Rrp6. The bottom panels compare the crystal structures of E. coli RNaseD and a N-terminal and C-terminal truncated version of Rrp6 from S. cerevisiae. The molecules in the panels were derived from the Protein Data Base using PyMol.