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Edited by Torben Heick Jensen

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PREFACE

The diversity of RNAs inside living cells is amazing. We have known of the more "classic" RNA species: mRNA, tRNA, rRNA, snRNA and snoRNA for some time now, but in a steady stream new types of molecules are being described as it is becoming clear that most of the genomic information of cells ends up in RNA. To deal with the enormous load of resulting RNA processing and degradation reactions, cells need adequate and efficient molecular machines. The RNA exosome is arising as a major facilitator to this effect. Structural and functional data gathered over the last decade have illustrated the biochemical importance of this multimeric complex and its many co-factors, revealing its enormous regulatory power. By gathering some of the most prominent researchers in the exosome field, it is the aim of this volume to introduce this fascinating protein complex as well as to give a timely and rich account of its many functions.

The exosome was discovered more than a decade ago by Phil Mitchell and David Tollervey by its ability to trim the 3'end of yeast, *S. cerevisiae*, 5.8S rRNA. In a historic account they laid out the events surrounding this identification and the subsequent birth of the research field. In the chapter by Kurt Januszyk and Christopher Lima the structural organization of eukaryotic exosomes and their evolutionary counterparts in bacteria and archaea are discussed in large part through presentation of structures. The functional implications of many of these are discussed in subsequent chapters dealing with the organizations and utilities of archea (Elena Evguenieva-Hackenberg), protist (Christine Clayton and Antonio Estevez) and plant (Heike Lange and Dominique Gagliardi) exosomes.

Exosomes gain their functional properties by associating with exo- and endo-nucleolytic subunits as well as with additional enzymes like RNA helicases and poly(A) polymerases. Collectively, this results in a flexible molecular machine capable of dealing with a multitude of cellular RNA substrates of both nuclear and cytoplasmic origin. Interestingly, prokaryotes employ a basic set of enzymes and therefore may illustrate the evolutionary origin of the eukaryotic system. These issues are discussed in three chapters: by Aleksander Chlebowski, Rafal Tomecki, Maria Eugenia Gas Lopez, Bertrand Seraphin, and Andrzej Dziembowski; by Daneen Schaeffer, Amanda Clark, Alejandra Klauer, Borislava Tsanova, and Ambro van Hoof as well as by Scott Butler and Phil

Mitchell. Recently discovered roles in the elimination of transcriptional noise and in heterochromatization underscore the tremendous flexibility of the RNA exosome. The chapters by Coy and Vasiljeva and Rougemaille and Libri deal with these effects of the exosome on gene silencing.

Finally, given the essential nature of the complex, it may come as no surprise that it is implicated with different human disease states. Even before exosome function was described in *S. cerevisiae*, sera of certain autoimmune patients identified a protein complex which later turned out to be the human exosome. The chapter by Staals and Pruijn gives a historic perspective on this parallel discovery of exosome and discusses the occurrence of autoantibodies to exosome components in autoimmune diseases and the connection of the exosome with cancer.

In closing, I wish to thank all contributing authors for doing a fantastic job of portraying our current structural and mechanistic knowledge about the RNA exosome.

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ABOUT THE EDITOR...

TORBEN HEICK JENSEN is a Professor at the Department of Molecular Biology at Aarhus University, Denmark. He is directing the Centre for mRNP Biogenesis and Metabolism established by the Danish National Research Foundation and employing a total of ~30 RNA researchers at all levels. Main research interests include the coupling between transcription and RNA biogenesis, quality control of RNA and the occurrence and potential function of pervasive transcription of eukaryotic genomes.

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CONTENTS

CHAPTER 1

FINDING THE EXOSOME

Phil Mitchell*,[1] and David Tollervey*,[2]

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Abstract: We describe the events surrounding the identification of the exosome complex and the subsequent early development of the field. Like many scientific discoveries, the initial identification and characterization of the exosome was based on a combination of skill, good fortune—and the availability of cutting edge technology.

INTRODUCTION

The early 1990s were in some respects a frustrating time for ribosome research. On the one hand, established plasmid-based rDNA systems enabled the precise mapping and mutagenesis analysis of processing sites within the pre-rRNA sequence and a steady flow of processing factors were being identified by genetic, biochemical and bioinformatic approaches. However, genetic depletion of the identified trans-acting factors typically caused a common set of defects in pre-rRNA processing that led to a general loss of rRNA levels and it was therefore impossible to identify the specific molecular function of the protein. We therefore decided to concentrate on trying to identify enzymes that participate in the pre-rRNA processing reactions. The identification of these factors promised opportunities for more insightful experiments such as in vitro biochemical analyses, targeted mutagenesis studies and studies on the regulation of the pathway.

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FROM NORTHERNS TO NUCLEASES

We were therefore interested in screening banks of conditional yeast mutants for those showing blocks in specific steps in the pre-rRNA processing pathway, rather than just the loss of the pre-rRNAs. The problem was that generating and testing banks of mutants required and still requires, a great deal of work. Happily, at about this time Zoe Lygerou, in the group of Bertrand Séraphin also working at EMBL, was attempting to identify mutants defective in the interaction between the U4 and U6 small nuclear RNAs. To this end they had generated a collection of around 250 temperature-sensitive (ts) lethal yeast strains, extracted RNA from each and resolved the RNA on nondenaturing gels suitable for separating low molecular weight RNAs. The gels were far from ideal for the analysis of pre-rRNA processing defects and carried RNA from only around 250 strains, not all of which had given usable separation (see Fig. 1). The blots were, however, at hand and so we screened them by northern hybridization for defects in processing of the similarly sized 5.8S rRNA.

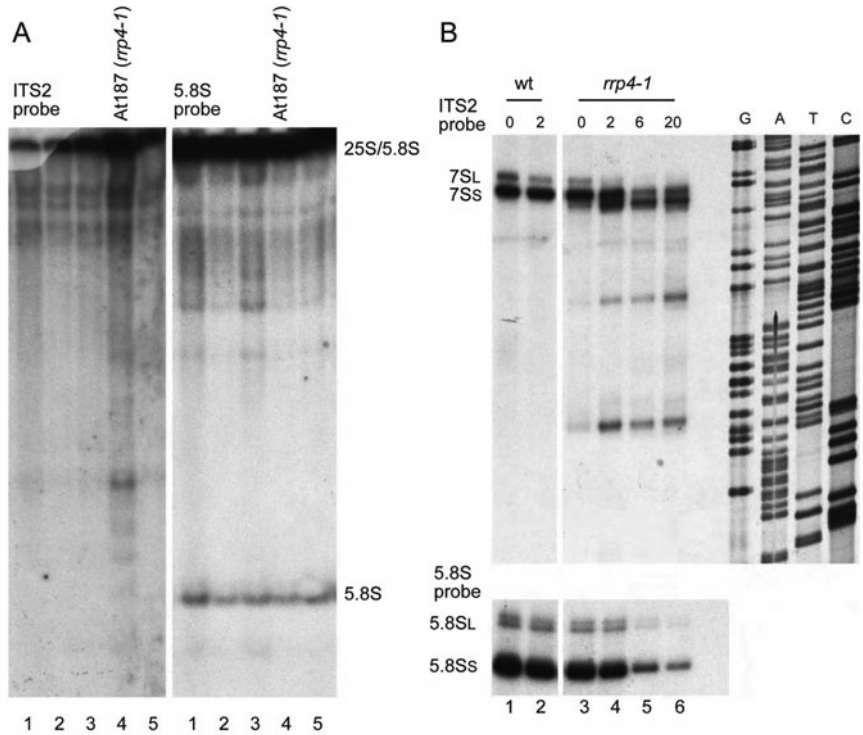

Figure 1. Identification of strain At187 harboring the *rrp4–1* mutation. A) RNA was extracted under nondenaturing conditions from a collection of temperature-sensitive mutant yeast strains, separated by native gel electrophoresis and analyzed by northern hybridization. Due to the native gel conditions 5.8S rRNA largely remains associated with the 25S rRNA, but the ITS2 probe in strain At187 lit bands above the 5.8S rRNA. B: Subsequent analyses on denaturing gels revealed that these represented the accumulation of 3′ extended species. A sequencing ladder was run on the same gel and transferred to the northern filter as a size marker. Following back-crossing, the *RRP4* gene was cloned by complementation of the ts-phenotype of the *rrp4–1* mutation that is carried by this strain.