

EXPANDING THE SET OF KNOWN SUBSTRATES OF NUCLEAR RNASE P

by

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To all who were there
when I needed them,
where I needed them, and
how I needed them.

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Table of Contents

Dedication	ii
Acknowledgements	iii
List of Figures	vi
List of Appendices	vii
List of Abbreviations	viii
Abstract	ix
Chapter	
I. Introduction	1
Bacterial RNase P	1
Bacterial RNase P Substrates	1
Eukaryotic RNase P	5
Yeast RNase P Substrates	8
Mammalian RNase P Substrates	10
Acknowledgements	13
II. Genome-wide search reveals nuclear RNase P is involved in maturation of intron-encoded box C/D small nucleolar RNAs	14
Introduction	14
Identifying RNAs that copurify with RNase P	17
Identifying RNAs that accumulate in temperature sensitive mutants	19
Intron-encoded snoRNAs	20
Box C/D intron encoded snoRNAs accumulate known processing intermediate in an RNase P temperature sensitive mutant	20
Discussion	26
Methods	31
Acknowledgements	35
III. Prediction and verification of mouse tRNA gene families identifies intron-containing families with structures analogous those in yeast ..	36
Abstract	36
Introduction	37
Methods	40
Scanning the mouse genome for predicted tRNA genes	43
Removing SINEs from predicted tRNA genes	43
Assigning tRNA genes into families	45
Comparisons between mouse and human tRNA gene families	47
Expression of predicted tRNAs	49
Intron-containing tRNA genes confirmed by northern blot	53

Discussion.....	63
Acknowledgements.....	69
IV. Conclusion	70
Discussion of yeast non-tRNA RNase P substrates.....	70
Discussion of mouse tRNA genes.....	73
Appendices.....	76
Bibliography.....	180

List of Figures

Figure

1.1	Representative secondary structure of a pre-tRNA	3
1.2	Three dimensional structure of a pre-tRNA.....	4
1.3	Comparison of RNase P holoenzyme from bacteria and yeast.....	7
2.1	Multipronged approach to identify additional RNase P substrates.....	16
2.2	Box C/D intron encoded snoRNAs accumulate processing intermediate in temperature sensitive RNase P strain.....	23
2.3	Primer extension of <i>in vitro</i> cleavage of 5' extended pre-snoRNAs by RNase P.....	25
2.4	Adding wild type RNase P to extract made from RNase P mutant strain does not convert 5' extended pre-snoRNA	27
2.5	Intron-encoded snoRNA processing pathways.....	29
3.1	Different tRNAs and SINEs are identified by both scanning programs.....	44
3.2	Northern blot confirmation of predicted tRNA gene families	52
3.3	Northern blot confirmation of intron-containing tRNA genes	55
3.4	Sequence alignment of intron-containing tRNA genes	57
3.5	Predicted structure of intron-containing tRNA genes.....	60
3.6	Predicted structures of the single-copy “orphan” pre-tRNAs that contain introns.....	62
3.7	Map of tRNA gene locations in the mouse genome	65
3.8	Mouse orphan tRNA ^{Tyr} gene corresponds to a multi-gene tRNA ^{Tyr} family in humans.....	68

List of Appendices

Appendix

A.....	77
B.....	93
C.....	95
D.....	102
E.....	104
F.....	122
G.....	146
H.....	179

List of Abbreviations

RNase P	Ribonuclease P
tRNA	Transfer RNA
pre-tRNA	Precursor tRNA
snoRNA	Small nucleolar RNA
snRNA	Small nuclear RNA
mRNA	Messenger RNA

Abstract

Ribonuclease P (RNase P) is an essential endonuclease found in all living organisms and is responsible for the removal of the 5' leader sequence from precursor tRNAs (pre-tRNAs). In bacteria, RNase P is known to process many different RNAs in addition to pre-tRNAs. However, eukaryotic RNase P is only known to process pre-tRNAs. In the first part of this work we take a multipronged approach to identify novel substrates for yeast nuclear RNase P. RNAs that copurify with affinity purified RNase P and RNAs that accumulate in RNase P mutant strains were identified as putative substrates. In addition to a potential role for RNase P in the regulation of translation-related mRNAs, we identified a novel class of non-coding RNAs, the intron-encoded box C/D snoRNAs, that copurify with RNase P and accumulate aberrant processing intermediates in RNase P mutant strains. RNase P appears to be essential for one of the intron-encoded snoRNA biogenesis pathways.

In mammalian systems, the set of pre-tRNA substrates is surprisingly not known, as only 11 tRNA sequences have been confirmed in mouse. However, with the mouse genome completely sequenced and *in silico* tRNA gene prediction programs available, it is possible to assemble a more complete set of mouse tRNA genes. First, a list of predicted genes was generated using two different gene prediction tools, and then the tRNAs were experimentally verified by both microarray and northern blot analysis. Over 80% of the original output of the tRNA scanning programs was actually tRNA-derived SINE elements, repetitive sequences found abundantly in mammalian genomes. However, after removing the SINEs, we predicted and verified the expression of tRNA families

corresponding to 446 genes, of which 423 were sorted into 35 tRNA gene families based on sequence homology. The expression of all 35 tRNA gene families was confirmed by both microarray and northern blot analysis, and thus is expected to represent the first comprehensive index of expressed mouse tRNA genes. The gene families are expected to be broadly applicable since all are conserved in the human genome and expressed.