EXPANDING THE SET OF KNOWN SUBSTRATES OF NUCLEAR RNASE P

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Biological Chemistry)
in The University of Michigan
2007

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To all who were there

when I needed them,

where I needed them, and

how I needed them.

Acknowledgements

Scientifically, I have benefited so much from my advisor and colleagues. David Engelke has trained me in many areas: scientific reasoning, oral presentation, and (hopefully even) dissertation writing. The core group of colleagues with me includes Becky Haeusler, Matt Pratt-Hyatt, Paul Good, Shaohua Xiao, May Tsoi, Scott Walker and Mike Marvin. I have never spent as much time with anyone that I was not related to before this fine group of folks. Additionally, Beth Goodwin, Felicia Scott, Becky Nugent, Chad Nihranz, E.J. Brace, Adam Avery and Leah Parkinson are wonderful people that deserve to be acknowledged.

Personally, I am far more blessed than I can possibly describe. My family has been loving and supportive throughout all my trials during graduate school. My mom, dad, sister, brother-in-law, grandma and grandpa specifically have helped motivate and support me far more than they can know. I have also had some wonderfully supportive friends. Adam Kuszak, Kelly Bosse, Mary Divin, Becky Haeusler and June Pais have been my friends, supporters, and commiserates from the beginning of graduate school. Chris Kateff and Dave LaGory have made sure I do not forget what life is like outside of the laboratory. And finally, although a late edition in my graduate career, my fiancé Tami Kaeb is an amazing person who provided the final push to get me through the struggles of my last few months of graduate school.

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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.