EXPANDING THE SET OF KNOWN SUBSTRATES OF NUCLEAR RNASE P

by

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To all who were there
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where I needed them, and
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<tr>
<td>RNase P</td>
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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.