Title:	Studies on RNase E variants of Escherichia coli
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Abstract:	The endoribonuclease RNase E of Escherichia coli is involved in mRNA degradation as well as processing and maturation of rRNA and tRNAs and is essential for bacterial viability. It is the product of gene rne, which encodes a 1061 amino – acid long polypeptide whose N-terminal half (NTH, residues 1-529) bears the catalytic domain while the C-terminal half (CTH) is dispensable for viability. X-ray crystallographic studies on the NTH of RNase E have established its structure to be that of a homo-tetramer (dimer of dimers); each of the subunits is comprised of a “large domain” (residues 1 to 400, which includes the essential catalytic active site residues D303 and D346) and a small domain (residues 415 to 529) which participates in the assembly of pair of dimers to form the tetramer. The expression of RNase E in vivo is also autoregulated since the enzymes cleave the 5’-untranslated region (UTR) of the rne transcript in a negative feedback loop to homeostatically maintain the intracellular activity of the enzyme. Previous studies from this laboratory had identified a novel genetic phenomenon of “ recessive resurrection”, where it was shown that a full-length hetero – tetramer of RNase E composed of a mixture of wild-type and recessive lethal active site - mutant subunits with D303A or D346A substitutions, exhibit identical activity in vivo as the wild type homo-tetramer itself. In this study, altered RNase E polypeptides were sought to be generated to test whether recessive resurrection can occur in a dimeric enzyme. Since it is the small domain of the NTH that is responsible for tetramerization, DNA constructs were generated encoding deletion of (residues 438 to 529) the small domain (Δ SD) both in the wild type polypeptide as well as in the active site mutant versions D303A and D346A, and they were verified by DNA sequencing. The Δ SD variants of wild type RNase E conferred viability to a Δ rne mutant and exploiting the feature of RNase E autoregulation, it was also shown that their specific activity in vivo is lower than that of wild type RNase E. The objective of future studies would be to determine the oligomerization status of the RNase E Δ SD variants (whether they are dimeric) and whether the phenomenon of recessive resurrection is observed also with the Δ SD variants.
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