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Global Analysis of RNA Stability in Bacteria

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The rate of mRNA degradation plays an important role in the regulation of gene expression in both prokaryotic and eukaryotic cells. mRNA decay has been studied in a range of organisms, and much has been learned about the substrate features and ribonucleolytic enzymes that influence mRNA stability. However, much of the information available about RNA decay has been derived from study of RNAs encoded by a very limited number of genes. To gain a broader understanding of the fate of mRNAs in bacteria, we adapted DNA microarray methodology to measure the abundance of each chromosomally encoded mRNA simultaneously.

DNA microarrays containing sequences derived from thousands of individual genes enable mRNA abundance to be measured in parallel on a genome-wide scale. Chromosomal DNA sequences are displayed on a solid substrate for hybridization with labeled cDNA probes corresponding to the collective complement of mRNA transcripts. One commonly used approach for microarray analysis employs PCR products spotted on a polylysine-coated glass microscope slide. mRNA hybridization is quantitated by comparing signals generated during concurrent hybridization of two differentially labeled mRNA pools with the target DNAs arrayed on the surface of the slide. This strategy has been widely used to compare steady-state levels of RNAs present in experimental and control samples and to evaluate events and conditions that may perturb these levels.

In *Escherichia coli*, the 118 KDa protein ribonuclease E (RNase E) has a key role in mRNA degradation and the processing of catalytic and structural RNAs (for reviews, see 1 and 2). Homologs of RNase E or of its close relative, RNase G, have been identified in, or inferred to exist from, DNA sequence analysis of more than 50 bacterial species, as well as Archae and plants (3). The *Escherichia coli* endoribonucleases RNase E (Rne) and RNase G (Rng) have sequence similarity and broadly similar sequence specificity. Whereas the absence of Rne normally is lethal, we show here that *E. coli* bacteria that lack the *rne* gene can be made viable by overexpression of Rng. Rng-complemented cells accumulated precursors of 5S ribosomal RNA (rRNA) and the RNA component of RNase P (i.e. M1 RNA), indicating that normal processing of these Rne-cleaved RNAs was not restored by RNase G; additionally, neither 5S rRNA nor M1 RNA was generated from precursors by RNase G cleavage *in vitro*. Using DNA microarrays containing 4405 *Escherichia coli* open reading frames (ORFs), we identified mRNAs whose steady-state level was affected by Rne, Rng or the N-terminal catalytic domain of RNase E. Most transcript species affected by

RNase E deficiency were also elevated in an *rne* deletion mutant complemented by Rng. However, approximately 100 mRNAs that accumulated in Rne-deficient cells were decreased by *rng*-complemention, thus identifying targets whose processing or degradation may be the basis for RNase E essentiality. Remarkably prominent in this group were mRNAs implicated in energy-generating pathways or in the synthesis or degradation of macromolecules.

The processing of RNAs by RNase E is known to be affected by anaerobiosis during *E. coli* cell growth (4) and also to occur prominently in transcripts that encode proteins involved in energy-generating pathways (5). While these findings suggest that RNase E activity may be affected by cell physiology, little is known about cellular mechanisms that may modulate the degradation of RNA by RNase E. During experiments aimed at identifying genes whose overexpression enhances disulfide isomerase activity in *E. coli*, we identified trans-active regulators of RNase E activity, which we name RraA and B (6). We show that RraA and B which, like RNase E, are widely distributed among bacteria and plants and increase disulfide isomerase production in *E. coli* by increasing the stability of mRNA encoding the disulfide isomerase, DsbC. We further show that these regulators interact with RNase E at different sites to inhibit its endoribonuclease action and that the effects of the regulators on RNase E lead to global changes in RNA abundance, as well as to functionally altered gene expression.

Previous work has characterized a functional ortholog of *E. coli* RNase E in S. *coelicolor* (Rns) that shows shuffling of catalytic and polynucleotide phosphorylase-binding domains (3). Rns is not essential, unlike *E. coli* Rne, which has a major role in mRNA decay and the processing of catalytic and structural RNAs. However, global analysis of mRNA transcript profile using DNA microarrys containing 97% *S. coelicolor* ORFs showed that *S. coelicolor* bacteria that lack *rns* gene globally accumulate mRNA species encoding mainly house-keeping gene products and these global changes become reversed in *S. coelicolor* bacteria that overexpress Rns. Co-precipitation studies identified two RNA species precipitated with Rns protein; 23S ribosomal RNA and *hrdB* mRNA encoding a principal sigma factor that is known to be responsible for expressing house-keeping genes in *S. coelicolor*. *In vitro* cleavage assay further confirmed that *hrdB* transcript is cleaved at several sites exclusively in its 5' untranslated region (UTR) by RNase ES, which has a broad sequence specificity similar to that of *E. coli* RNase E that cleaves AU rich, single stranded regions of RNA. These data indicate that RNase ES controls expression of house-keeping genes by a previously unrecoganized mechanism that posttranscriptionally modulates expression of the principal sigma factor in *S. coelicolor*, a high GC gram positive microoganism which has a developmentally complex life cycle.

References

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