Wide-spread Antisense RNAs in Bacteria: Where Are They, What Do They Do, and How Are They Controlled?

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Abstract

Antisense transcription is widespread in bacterial genome and plasmid, but the functional significance of this phenomenon was not well understood. By base pairing with overlapping sense RNAs, antisense RNAs (asRNA) can form long double-stranded RNAs (dsRNA), which are cleaved by RNase III, a dsRNA endoribonuclease. Ectopic expression of plant tombusvirus p19 in E. coli stabilizes ~21 bp dsRNA RNase III decay intermediates, which enabled us to characterize otherwise highly unstable asRNAs by deep sequencing of p19-captured dsRNAs. Analysis on p19-captured dsRNA sequences revealed distinctive features of RNase III cleavage sites on perfectly paired dsRNAs and could explain how 21-22 bp dsRNAs are produced by RNase III. The most abundant dsRNA clusters were mostly formed by divergent transcription of sense and antisense transcripts overlapping at their 5'-ends. As certain loci, asRNA and RNase III are regulating the expression of the sense gene. dsRNAs accumulated in bacterial cells lacking RNase III, increasing in stationary phase, and correlated with increased cell death in RNase III mutant bacteria in late stationary phase, which suggests dsRNA decay by RNase III might be important for bacterial physiology. Ectopic expression of p19 is a sensitive method for identifying antisense transcripts and RNase III cleavage sites in bacteria.