Big data analysis of differential production within toxin-antitoxin systems

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Short Abstract — **Toxin-antitoxin systems are key regulators of bacterial persistence, a multidrug tolerant state found in bacterial species. Type II toxin-antitoxin systems consist of two proteins, a toxin and an antitoxin, which together form a complex to neutralize the toxin. The ratio of antitoxin to toxin is significantly greater than 1.0 in the normal, non-persister state, but this ratio is expected to decrease during persistence. Analysis of multiple public datasets (RNA-seq, ribosomal profiling), and results from translation rate calculators reveal multiple mechanisms that ensure a high antitoxin to toxin ratio in the normal, non-persister state.**

Keywords* — toxin-antitoxin systems, bioinformatics, bacterial persistence, *Escherichia coli

Full Abstract

A major contributing factor to the growing antibiotic resistance health crisis is a multidrug tolerant subpopulation of persister cells found in many bacterial species [1]. Bacterial persistence arises from a bet-hedging strategy, where a small population of cells (approximately 1 out of 1,000,000) enter a metabolically dormant state to enable survival of a range of environmental stresses, including antibiotic treatment. One mechanism that triggers the persistent state is a network of toxin-antitoxin (TA) systems, bicistronic operons made up of a toxin and its cognate antitoxin [2]. Type II TA systems produce two proteins, which form a protein-protein complex to neutralize the toxin. The antitoxin protein is rapidly degraded by proteases and is produced at a higher rate than the toxin to effectively prevent a buildup of free toxin [3]. Differential protein production within operons can occur at both the transcriptional level and the translational level [4]. Both mechanisms have been suggested for type II TA systems, but often in the literature only transcriptional regulation is explored. This study examines both possibilities by using big data analysis (e.g. multiple data sets from different groups) of type II TA systems in *Escherichia coli*.

RNA-seq datasets for *E. coli* from multiple studies and growth conditions provide the expression levels of genes at the mRNA level. TA systems with different expression levels for the toxin and antitoxin open reading frames (ORF's) likely use mRNA transcript concentration to regulate increased antitoxin protein concentration. TA systems with statistically different mRNA expression are classified based on which gene (toxin or antitoxin) is first (upstream). The toxin first class uses an internal promoter to increase antitoxin concentration, whereas the toxin second class has some other mechanism. However, most TA systems do not have a difference in mRNA concentration between toxin and antitoxin, and therefore the difference in protein concentration is likely due to translational regulation. Data from a ribosomal profiling study of *E. coli* supports this fact by determining that the several TA systems with similar expression levels have antitoxins with higher translation rates than toxins [5]. The higher translation rate of antitoxin is further supported by results derived from three different methods to calculate the translation initiation rate of each gene. We find that at least two out of the three of these translation rate estimation methods agree that antitoxin translation rates are higher than the toxin in TA systems without transcriptional regulation.

In summary, both transcriptional and translation regulation play important roles in the maintenance of TA system ratios. RNA-seq analysis reveals TA systems that have different mRNA expression levels and are dependent upon both the organization of the TA system and the mRNA transcript concentration. The remaining systems require different translation rates to create the necessary toxin-antitoxin ratio, as can be seen in ribosomal profiling analysis and various translation rate calculators. Further applications of these methods can be used to expand the knowledge of TA systems in other organisms and lead to a more developed understanding of the role that toxin-antitoxin modules play in bacterial persistence.

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