STRINGENCY OF ANTISENSE REGULATION VARIES BASED ON VOLATILITY OF mRNA TARGET REGION

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Bacteria can regulate gene expression by transcribing antisense RNA to interfere with protein translation. Antisense has been shown to control a wide variety of prokaryotic proteins, including membrane proteins, protein toxins, and proteins involved in transport and metabolism. This type of regulation can be used in the production of biologics to optimize the health of the culture and maximize production of the desired product. We examined naturally occurring antisense to enhance design principles for product optimization. We found that the level of secondary structure fluctuation of the antisense binding site varied depending on the function of the target. We hypothesized that stringency of regulation by naturally evolved antisense was driven by the impact of the target molecule on cellular survival. Specifically, high stringency was important for toxin-antitoxin systems where survival depended on high levels of control. Toxin-antitoxin systems rely on effective antisense to prevent the translation of self-damaging proteins. Antisense-based systems regulating transport and metabolism potentially benefited from less stringent antisense control. Basal levels of antisense-regulated proteins involved in metabolic processes could allow for quick adaptation to changing nutrient conditions. More than fifty naturally occurring sense/antisense pairs were analyzed to demonstrate that antisense binding sites correlate to the level of stringency needed in regulating the target protein.

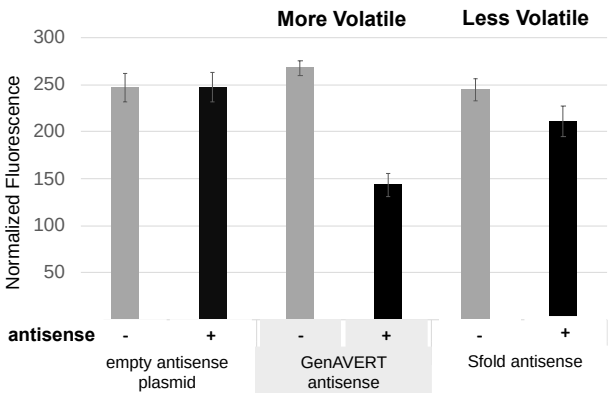

Figure 1 – GFP was expressed in all conditions. Fluorescence normalized by OD_{595} was down regulated more when antisense targeting a more volatile region was induced.

We postulated mRNA secondary structure to be an ensemble of conformations sampling different possible low Gibbs energy states around the global minima. Certain regions break and form hydrogen bonds more frequently, making them more volatile and available for antisense molecules to bind. Less volatile regions resulted in more stable hydrogen bonded secondary structures making accessibility by antisense less likely. By applying an algorithm developed by our lab, GenAVERT, to predict volatile regions of mRNA(1), we were able to examine antisense volatility. Antisense binding regions for targets that encoded toxins were more likely to align with high volatility predictions than other targets. Targeting high volatility regions of toxin mRNAs likely maximized antisense efficacy where stringent control was critical for survival. Less stringent control of metabolic targets could also provide an evolutionary benefit. Analogous to leaky promoter systems, such as the *lac* operon, a basal level of metabolic proteins available when nutrient conditions change would also serve as an evolutionary benefit. A random forest classification was performed to orthogonally verify the results. With 94% accuracy, the random forest was able to correctly predict whether or not an antisense binding region would result in stringent or astringent regulation.

Antisense was also tested in *Escherichia coli* to assess the efficacy of artificial antisense. Antisense sequences designed using the GenAVERT and Sfold algorithms were expressed targeting green fluorescent protein (GFP). GFP fluorescence was downregulated 46% when the more volatile region was targeted (GenAVERT) compared to a 14% decrease when a less volatile region was targeted (Sfold). However, neither sequence resulted in stringent down regulation of GFP fluorescence. The random forest correctly classified both antisense molecules as astringent.

These efforts provided new insight into how bacteria have evolved elaborate regulatory mechanisms. Antisense can regulate its target in a very specific manner based on the volatility of the target region. Our work in understanding antisense has the potential to provide a regulation tool that can be controlled based on expression level needs.

1. Johnson E, Srivastava R. 2012. *Nucleic Acids Res.* 41(3):10