Construction and Mutational Analysis of a Heavy Metal Biosensor in E. coli

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The flow of genetic information in living cells involves the transcription of DNA into mRNA and the translation of mRNA into protein. Transcription is initiated when an RNA polymerase binds to a sequence of DNA called a promoter. In bacteria, the RNA polymerase produces RNA by sliding along the DNA template until it encounters a transcriptional terminator. Naturally occurring terminators effect termination by encoding a hairpin loop in the RNA that is thought to exert strain on the RNA/DNA base pairing required for continued transcription. Riboswitches couple bacterial gene expression to the concentration of ligands involved in metabolism or external stimuli. Many riboswitches function by using a ligand to control the formation of the hairpin loop of a transcriptional terminator. A new riboswitch was discovered in Clostridium scindens that allows these bacteria to respond to heavy metal ions. The current project was to clone the riboswitch into Escherichia coli as part of a heavy metal biosensor and investigate its structure and function with mutational analysis. The results showed that the biosensor functioned and was more sensitive to cobalt (II) ions than to other heavy metal ions. Three riboswitches in tandem increased the sensitivity of the biosensor. A library of mutations of bases important for the binding of cobalt (II) ions was produced and explored. Twenty-nine mutants were isolated, sequenced, and tested for riboswitch function. One of the mutants chosen showed a 2-fold increase in induction by cobalt ions over the original NiCo riboswitch. Combined with RNA folding analyses of the mutant riboswitches, the results provide an improved understanding of the function of bacterial riboswitches and transcriptional terminators.

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