The Escherichia coli thiB Thiamine Pyrophosphate Riboswitch Uses Strand Invasion as a Primary Mechanism of Gene Regulation

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Riboswitches are RNA elements that regulate gene expression by changing their structure upon binding small molecule ligands in their environment. Development of synthetic riboswitches for use as molecular biosensors remains an active area of research [1]. However, such investigation requires that the relationship between ligand binding and gene regulatory function in riboswitches is more clearly defined. Contemporary understanding of riboswitch function highlights the complex mechanisms by which they regulate gene expression. Some mechanisms, such as those elucidated for the *Escherichia coli lysC* riboswitch, are dual-acting to prevent translation initiation and trigger mRNA decay [2]. Others, like those of the *N. crassa* NMT1 riboswitch, use alternative splicing to regulate the development of mature mRNA [3]. This project focuses on the *E. coli thiB* TPP riboswitch, one of five thiamine pyrophosphate (TPP) riboswitches in the *E. coli* genome, which represses gene expression when exposed to high concentrations of TPP. We investigate the hypothesis that the *thiB* riboswitch functions through an RNA strand invasion mechanism. This was tested through site-directed mutagenesis of functionally critical parts of the *thiB* riboswitch, such as the ligand binding aptamer region and the invading strand sequence. Following mutagenesis, in-vivo gene expression assays were utilized to determine the minimal structures necessary for the riboswitch to conduct efficient regulation. It was found that in absence of TPP, the strand invasion mechanism occurs. Here, the invading sequence base pairs with nucleotides in the aptamer region, which opens the aptamer and prevents sequestering of the ribosome binding site (RBS) to allow gene expression. In high concentrations of TPP, the *thiB* riboswitch adopts a stable conformation which blocks strand invasion, thereby inhibiting translation initiation. With further understanding of their common regulatory pathways, we believe the engineering of synthetic riboswitches as molecular biosensors for clinical or field-testing applications will be much more reasonable.

References

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