

## §S12. Two Predicted TPP Riboswitches

Based on conservation, we identified three TPP binding riboswitches<sup>1</sup>, one of which has not been described in *Aspergillus*. Riboswitches are regulatory elements found in untranslated regions of mRNA which fold to bind target metabolites leading to altered gene expression<sup>1-3</sup>. A TPP riboswitch, which binds thiamin pyrophosphate and regulates genes involved in thiamine biosynthesis, was previously identified in the 5' UTR of the *A. oryzae thi4*<sup>1</sup>. We find this element conserved upstream of *thi4* in *A. fumigatus* and *A. nidulans*. We also find an element recently shown to be present upstream of orthologous NMT1 thiamine biosynthesis genes (AN8009, 53.m03858, AO70322000058)<sup>4</sup>, corresponding to the known *N. crassa* NMT1 riboswitch<sup>1</sup>. A third element was found conserved upstream of orthologous purine-cytosine permease genes (AN4526, 53.m05980, AO70321000134). This element has not been previously characterized in any organism, although evidence linking purine influx to thiamine biosynthesis has been reported<sup>5,6</sup>.

## References

1. Sudarsan, N., Barrick, J. E. & Breaker, R. R. Metabolite-binding RNA domains are present in the genes of eukaryotes. *Rna* 9, 644-7 (2003).
2. Vitreschak, A. G., Rodionov, D. A., Mironov, A. A. & Gelfand, M. S. Riboswitches: the oldest mechanism for the regulation of gene expression? *Trends Genet* 20, 44-50 (2004).
3. Abreu-Goodger, C., Ontiveros-Palacios, N., Ciria, R. & Merino, E. Conserved regulatory motifs in bacteria: riboswitches and beyond. *Trends Genet* 20, 475-9 (2004).
4. Kubodera, T. et al. Thiamine-regulated gene expression of *Aspergillus oryzae thiA* requires splicing of the intron containing a riboswitch-like domain in the 5'-UTR. *FEBS Lett* 555, 516-20 (2003).
5. Frodyma, M., Rubio, A. & Downs, D. M. Reduced flux through the purine biosynthetic pathway results in an increased requirement for coenzyme A in thiamine synthesis in *Salmonella enterica* serovar typhimurium. *J Bacteriol* 182, 236-40 (2000).
6. Allen, S., Zilles, J. L. & Downs, D. M. Metabolic flux in both the purine mononucleotide and histidine biosynthetic pathways can influence synthesis of the hydroxymethyl pyrimidine moiety of thiamine in *Salmonella enterica*. *J Bacteriol* 184, 6130-7 (2002).