Thiamine pyrophosphate riboswitches in *Bacteroides* species regulate transcription or translation of thiamine transport and biosynthesis genes

Zachary A. Costliow[a,*,$], Patrick H. Degnan[b,%], and Carin K. Vanderpool[a,$]

[a]Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

[b]Department of Microbiology and Plant Pathology, University of California-Riverside, Riverside, California, USA

*Present address: Broad Institute, Cambridge, Massachusetts, 02142

Short title: Regulation by TPP Riboswitches in *Bacteroides*

[$]Address correspondence to Zachary A. Costliow, zcostlio@broadinstitute.org, or Carin K. Vanderpool, cvanderp@illinois.edu

RNA-seq data generated for this project have been submitted to the NCBI SRA database as study SRP148918.

Abstract

Thiamine (vitamin B_1) and its phosphorylated precursors are necessary for decarboxylation reactions required in carbohydrate and branched chain amino acid metabolism. Due to its critical roles in central metabolism, thiamine is essential for human and animal hosts and their resident gut microbes. However, little is known about how thiamine availability shapes the composition of gut microbial communities and the physiology of individual species within those communities. Our previous work has implicated both thiamine biosynthesis and transport activities in the fitness of *Bacteroides* species. To better understand thiamine-dependent gene regulation in *Bacteroides*, we examined thiamine biosynthesis and transport genes in three representative species: *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*, and *Bacteroides vulgatus*. All three species possess thiamine biosynthetic operons controlled by highly conserved *cis*-acting thiamine pyrophosphate (TPP) riboswitches. *B. thetaiotaomicron* and *B. uniformis* have additional TPP riboswitch-controlled operons encoding thiamine transport functions. Transcriptome analyses showed that each *Bacteroides* species had a distinct transcriptional response to exogenous thiamine. Analysis of transcript levels and translational fusions demonstrated that in *B. thetaiotaomicron,* the TPP riboswitch upstream of biosynthesis genes acts at the level of transcription, while TPP riboswitches upstream of transport operons work at the level of translation. In *B. uniformis* and *B. vulgatus,* TPP riboswitches work at the transcriptional level to control downstream operons. The varying responses to exogenous thiamine and use of varied regulatory mechanisms may play an important role in niche establishment by the Bacteroidetes in the complex and constantly shifting gut environment.

40 **Importance**

41 *Bacteroides* species are important and abundant members of human gut microbiome
42 communities. Their activities in the gut are influenced by constant changes in nutrient
43 availability. In this study, we investigated the genetic basis of thiamine (Vitamin B_1) uptake and
44 biosynthesis in three representative *Bacteroides* species. We found species-specific differences
45 in the response to exogenous thiamine, and distinct mechanisms for regulation of uptake and
46 biosynthesis gene expression. Our work implies that gut *Bacteroides* have evolved distinct
47 strategies for making or acquiring an essential nutrient. These mechanisms may play an
48 important role in the success of *Bacteroides* in establishing a niche within complex gut
49 microbiome communities.
50

Introduction

Thiamine pyrophosphate (TPP) is the active cofactor form of thiamine (Vitamin B_1). Thiamine is required by all living organisms to perform a variety of metabolic activities. Thiamine-requiring pathways include central metabolism, branched chain amino acid biosynthesis, and nucleotide synthesis (1-4). Humans primarily acquire thiamine from their diet and convert it to the cofactor form, TPP (5, 6). In addition to dietary thiamine, there is some evidence that microbial communities in the gastrointestinal tract can provide thiamine to the host (7, 8). This thiamine may be supplied by resident gut microbes that possess the ability to synthesize thiamine and TPP *de novo* (9,10). A recent study suggested that certain pathogenic microbes can inhibit thiamine uptake by host intestinal epithelial cells (11). In addition, thiamine availability has been correlated with differences in composition of gut microbial communities (12). Thiamine excess and thiamine deficiency of the human host have been linked to different diseases, including Crohn's Disease, Irritable Bowel Disease, and Dementia, which are also associated with altered gut microbiome structure and function (6,13,14). While these studies reveal interesting correlations that suggest a role for thiamine at the host-microbe interface, very little is known about how dominant members of the gut microbiota sense and respond to thiamine availability.

Work to characterize and understand thiamine biosynthesis and transport has primarily been done in model organisms including *Escherichia coli, Salmonella enterica,* and *Bacillus subtilis* (10, 15-18). Thiamine biosynthesis is carried out by a complex suite of enzymes through a conserved bifurcated pathway. The first key intermediates in TPP synthesis are 4-amino-2-methyl-5-diphosphomethylpyrimidine (HMP-PP) and 2-(2-carboxy-4-methylthiazol- 5-yl) ethyl phosphate (cTHz-P). HMP-PP and cTHz-P are then condensed into thiamine monophosphate (TMP) and finally phosphorylated to make the active cofactor TPP (15). Thiamine biosynthesis activities are found in bacteria, yeast, and plants. Transport proteins specific for thiamine, thiamine monophosphate (TMP), TPP, and other thiamine precursors are broadly distributed

77 across all three domains of life (10, 19-23). Almost without exception, the genes and operons
78 encoding these diverse thiamine transport and biosynthesis pathways are regulated by highly
79 conserved TPP riboswitches.

80 Riboswitches are highly conserved *cis*-encoded and *cis*-acting regulatory RNAs found in
81 the untranslated regions of messenger RNAs. Riboswitches bind small molecules, changing
82 mRNA secondary structure and controlling expression outcomes at the level of transcription
83 elongation, translation initiation, or RNA splicing (24, 25). TPP riboswitches are unique in that
84 they are the only riboswitch found across all three domains of life (26). TPP riboswitches are so
85 highly conserved, they are now used to identify thiamine biosynthesis and transport genes in
86 diverse organisms (9). In bacteria, TPP riboswitches regulate biosynthesis and transport genes
87 at either the transcriptional or translational level (27). TPP-bound forms of riboswitches that
88 control transcription of downstream genes promote formation of a stem-loop that causes
89 premature transcription termination. TPP riboswitches that act at the level of translation promote
90 formation of a secondary structure that blocks ribosome binding when TPP is bound (28). TPP
91 riboswitches that act at the level of transcription have been found primarily in Gram-positive
92 bacteria, whereas Gram-negative bacteria tend to have TPP riboswitches that act at the level of
93 translation (29). It has been noted that TPP riboswitches in *E. coli* that repress translation (when
94 TPP is bound) also reduce mRNA stability, resulting in reduced steady-state levels of the
95 transcript (30). While TPP riboswitch structures and functions have been extensively studied in
96 the model organisms *E. coli* and *B. subtilis*, there has been little characterization of these
97 conserved regulatory elements in other microbes, including those belonging to the phylum
98 Bacteroidetes.

99 Computational analyses predicted that TPP riboswitches are present and regulate
100 putative thiamine biosynthesis and transport pathways across the Bacteroidetes (9, 22). TPP
101 riboswitch-controlled genes in *Bacteroides thetaiotaomicron,* a prominent member of the
102 Bacteroidetes, have recently been characterized (10, 20). These studies have shown that both

thiamine transport and biosynthesis are critical to the fitness of *B. thetaiotaomicron* under thiamine-limiting growth conditions. In this study, we build on previous work to investigate the global gene expression response of three representative *Bacteroides* species to exogenous thiamine and to determine the mechanisms of TPP riboswitch-dependent regulation of thiamine biosynthesis and transport genes. We found that each of the three species we studied had a distinct transcriptional response to exogenous thiamine. Investigation of the mechanisms of TPP riboswitch-mediated regulation of downstream genes and operons revealed that *B. thetaiotaomicron* uses both transcriptional- and translational-acting riboswitches, whereas *Bacteroides uniformis* and *Bacteroides vulgatus* use TPP riboswitches that act at the level of transcription. Identification of TPP riboswitches across the Bacteroidetes revealed a few different configurations of TPP riboswitch-controlled operons and suggested that both transcriptional and translational mechanistic classes are widely distributed.

Results

Global transcriptional responses to exogenous thiamine vary between Bacteroides *species.*

Our previous study identified putative thiamine biosynthesis and transport genes in 114 *Bacteroides* genomes (10). We found biosynthesis and transport genes in 107 of 114 genomes, and a few genomes that appeared to contain either biosynthesis (2 of 114) or transport (5 of 114) genes. To further understand the diversity of thiamine biosynthesis and transport functions and regulation in *Bacteroides*, we chose three representative species to further characterize in this study. In *B. thetaiotaomicron*, we identified and characterized three gene clusters (Fig. 1A, 1C, 1E) involved in thiamine biosynthesis (*thiSEGCHF*$_{Bt}$-*tenI*$_{Bt}$), outer membrane thiamine transport (*OMthi*$_{Bt}$) and cytoplasmic membrane transport (*pnuT*$_{Bt}$-*tnr3*$_{Bt}$) in a previous study (10). Each of these three gene clusters is preceded by a putative TPP riboswitch (Fig. 1A). In *B. uniformis,* we identified two putative TPP riboswitch-controlled gene clusters: for biosynthesis (*thiSEGCHF*$_{Bun}$-*tenI*$_{Bun}$) and transport (*OMthi*$_{Bun}$-*pnuT*$_{Bun}$-*tnr3*$_{Bun}$) (Fig. 1C). *B. vulgatus* has only a

129 single putative TPP riboswitch dependent operon for thiamine biosynthesis (*thiSEGCHF*$_{Bv}$-
130 *tenI*$_{Bv}$) and apparently lacks thiamine transport genes (Fig. 1E).
131 Our previous study showed that *B. thetaiotaomicron* differentially expresses genes
132 comprising a number of pathways, including thiamine biosynthesis and transport, in the
133 presence versus absence of exogenous thiamine (10). To determine if the transcriptional
134 response to thiamine limitation is consistent across *Bacteroides* species we analyzed
135 transcriptomes of *B. uniformis* and *B. vulgatus* in minimal medium with and without exogenous
136 thiamine (as described in Materials and Methods and (10)). A global view of differential gene
137 expression in the absence versus presence of exogenous thiamine is shown for *B.*
138 *thetaiotaomicron* (Fig. 1B and (10)), *B. uniformis* (Fig. 1D) and *B. vulgatus* (Fig. 1F). Whole
139 transcriptome responses to exogenous thiamine were markedly different among these three
140 species. *B. thetaiotaomicron* had 151 genes significantly differentially expressed by ≥ 2-fold in
141 the presence of exogenous thiamine compared to its absence (Fig. 1B). Genes that showed
142 reduced expression in the presence vs. absence of exogenous thiamine in *B. thetaiotaomicron*
143 encode functions belonging to a few pathways, including thiamine biosynthesis, amino acid
144 biosynthesis, TCA cycle enzymes, purine, and pyruvate metabolism (Table S1 and 10). In
145 contrast, minimal transcriptome responses to exogenous thiamine were observed in *B.*
146 *uniformis* (Fig. 1D) and *B. vulgatus* (Fig. 1F). *B. uniformis* responded to exogenous thiamine
147 with reduced expression of the genes in the TPP riboswitch-controlled thiamine biosynthesis
148 cluster (*thiSEGCHF*$_{Bun}$-*tenI*$_{Bun}$, Fig. 1C, 1D). There were no significant differences in the gene
149 expression profile of *B. vulgatus* in the absence vs. presence of exogenous thiamine (Fig. 1F).
150 Our previous study revealed that *B. vulgatus* lacks an *OMthi* homolog found in many other
151 *Bacteroides* species (10), suggesting that *B. vulgatus* may not be able to recognize or take up
152 exogenous thiamine. In addition, while *B. vulgatus* does have a *pnuT* homolog, this gene is not
153 preceded by a TPP riboswitch and is not clustered with other putative thiamine transport or
154 biosynthesis genes, suggesting that this PnuT-like protein may have a different substrate

specificity compared to the *B. thetaiotaomicron* and *B. uniformis* PnuT proteins (20, 31). Together, these data suggest that *B. vulgatus* does not respond transcriptionally to exogenous thiamine because it is not recognized or transported.

TPP riboswitches have been found to control either transcription elongation or translation initiation, depending on the organism (27). We noted that in *B. thetaiotaomicron* and *B. uniformis,* TPP riboswitch-controlled operons for biosynthesis and transport had different responses to exogenous thiamine at the level of mRNA abundance based on RNA-seq experiments. We hypothesized that this might reflect differences in TPP riboswitch-mediated mechanisms of regulation of these different operons. In *B. thetaiotaomicron,* only the biosynthesis operon mRNAs were differentially regulated in response to exogenous thiamine (Fig. 1A, (10)). The abundance of $OMthi_{Bt}$ and $pnuT_{Bt}$-$tnr3_{Bt}$ mRNAs encoding transport functions in *B. thetaiotaomicron* was not significantly different in the presence or absence of exogenous thiamine (Fig. 1A, (10)), suggesting that the TPP riboswitches controlling *B. thetaiotaomicron* thiamine transport genes might act at the level of translation. The levels of TPP riboswitch-controlled biosynthesis and transport operon mRNAs in *B. uniformis* were reduced in the presence of exogenous thiamine (Fig. 1C). To confirm the results of RNA-seq with respect to thiamine-induced changes in mRNA levels, RT-qPCR was performed. Primers were designed to measure levels of biosynthesis (*thiC*), outer membrane TonB-dependent transporter (*OMthi*), and inner membrane transporter (*pnuT*) mRNAs in each organism. The mRNA abundance trends observed in all three organisms in RNA-seq were replicated in RT-qPCR experiments. In *B. thetaiotaomicron*, $thiC_{Bt}$ mRNA levels were 60-fold higher in cells grown without thiamine compared to cells grown in the presence of 10 μM thiamine (Fig. 2A). The abundance of $OMthi_{Bt}$ and $pnuT_{Bt}$ mRNAs was equivalent in cells grown with and without thiamine. In *B. uniformis,* $thiC_{Bun}$ mRNA levels were 2-fold higher in cells grown without thiamine compared to cells grown with thiamine (Fig. 2B). In contrast to *B. thetaiotaomicron*, *B. uniformis* transporter mRNA ($OMthi_{Bun}$ $pnuT_{Bun}$) levels also responded to exogenous thiamine with ~3-fold

181 higher levels in cells grown without thiamine (Fig. 2B). Transcript levels of the *B. vulgatus* TPP
182 riboswitch-controlled biosynthesis operon did not respond to exogenous thiamine (Fig. 2C), also
183 consistent with RNA-seq results. These data suggest that in *Bacteroides* organisms that have
184 thiamine transport functions, exogenous thiamine affects transcript levels of thiamine
185 biosynthesis genes. The transcriptional response to thiamine varied between species for
186 mRNAs encoding transport functions.
187
188 *Translational regulation of TPP-controlled operons in* Bacteroides

189 We next investigated if TPP riboswitch-controlled operons that did not show a
190 transcriptional response to exogenous thiamine were instead regulated at the level of
191 translation. We constructed nanoluciferase translational fusions to the start codon of the first
192 gene in TPP riboswitch-controlled operons. All nanoluciferase (NanoLuc) fusions are controlled
193 by native promoters and *cis*-acting TPP riboswitches. It is important to note that these
194 translational fusions would capture thiamine-dependent transcriptional regulation caused by
195 riboswitch-mediated premature transcription termination and translational regulation, *e.g.,*
196 riboswitch-mediated changes in ribosome binding site accessibility. Fusions for the three
197 predicted TPP riboswitches (numbered as indicated in Fig. 1A) in *B. thetaiotaomicron* are called
198 pBtTPP1, pBtTPP2 and pBtTPP3 (Fig. 3A). *B. uniformis* fusions were pBunTPP1 and
199 pBunTPP2 (Fig. 3B), and the *B. vulgatus* fusion was called pBvTPP1 (Fig. 3C). Strains
200 containing each fusion were grown in media containing a range of exogenous thiamine
201 concentrations (from 0 to 10,000 nM). Promoterless NanoLuc controls were tested in each
202 strain background without and with 10,000 nM thiamine. These controls showed that thiamine
203 did not significantly alter luminescence (RLU/OD_{630}), as indicated by dashed lines in Fig. 3A-D.

204 In *B. thetaiotaomicron,* the BtTPP1 reporter fusion was repressed as exogenous
205 thiamine concentrations increased, with statistically significant differences beginning at 10 nM
206 thiamine (Fig. 3A). This result, combined with the reduced levels of $thiSEGCHF_{Bt}$-$tenI_{Bt}$ mRNA

207 observed in RNA-seq (Fig. 1B) and RT-qPCR (Fig. 2A), suggests that BtTPP1 riboswitch acts at
208 the level of transcription. The *B. thetaiotaomicron* BtTPP2 and BtTPP3 riboswitches control
209 thiamine transport $OMthi_{Bt}$ and $pnuT_{Bt}$-$tnr3_{Bt}$ genes, and the levels of these mRNAs were not
210 affected by exogenous thiamine (Figs. 1B and 2A). In contrast, the BtTPP2 and BtTPP3
211 reporters were repressed in the presence of exogenous thiamine, starting at 100 nM (Fig. 3A).
212 These data suggest that the *B. thetaiotaomicron* riboswitches controlling transport operons
213 regulate at the level of translation.
214 The reporters fused to *B. uniformis* riboswitches controlling biosynthesis (BunTPP1) and
215 transport (BunTPP2) operons followed a pattern similar to that observed in *B. thetaiotaomicron.*
216 The biosynthesis riboswitch reporter BunTPP1 was repressed by concentrations of exogenous
217 thiamine at or above 10 nM, while the transport riboswitch reporter BunTPP2 required
218 concentrations of 100 nM or greater for repression (Fig. 3B). These data, along with RNA-seq
219 (Table S1) and RT-qPCR (Fig. 2B) are consistent with the model that both TPP riboswitches in
220 *B. uniformis* act at the level of transcription.
221 In *B. vulgatus,* the riboswitch reporter controlling biosynthesis genes (BvTPP1) did not
222 respond to exogenous thiamine (Fig. 3C). However, since it appears that *B. vulgatus* lacks a
223 thiamine transport system, we reasoned that the lack of a response could be due to failure to
224 take up and accumulate the thiamine provided exogenously. To test whether the BvTPP1
225 riboswitch could respond to thiamine in an organism competent for thiamine transport, we
226 moved BvTPP1-NanoLuc into *B. thetaiotaomicron* (*Bt* pBvTPP1-NanoLuc, Fig. 3D). In a *B.*
227 *thetaiotaomicron* background, the *B. vulgatus* BvTPP1 biosynthesis riboswitch was repressed at
228 concentrations at or above 10 nM exogenous thiamine (Fig. 3D). This response is similar to
229 both the *B. thetaiotaomicron* and *B. uniformis* thiamine biosynthesis regulating TPP riboswitches
230 (Fig. 3A, B). The response to exogenous thiamine observed for the *B. vulgatus* TPP1 riboswitch
231 reporter in *B. thetaiotaomicron* shows that the biosynthesis regulating riboswitch of *B. vulgatus*
232 is functional. To determine whether the *B. vulgatus* TPP1 riboswitch controls transcription or

translation of biosynthesis genes, we performed RT-qPCR on *B. thetaiotaomicron* strains carrying reporter fusions to detect changes in NanoLuc fusion mRNAs in the absence and presence of thiamine (Fig. 3E). As controls we also monitored levels of fusion mRNAs for the *B. thetaiotaomicron* transcriptional riboswitch (BtTPP1) and the translational riboswitches (BtTPP2 and BtTPP3). The results for controls were as expected – levels of the biosynthesis BtTPP1 reporter mRNA were reduced in the presence of thiamine, and levels of the transporter BtTPP2 and BtTPP3 fusion mRNAs were unchanged. The levels of *B. vulgatus* biosynthesis BvTPP1 reporter mRNA were also reduced in response to exogenous thiamine in the *B. thetaiotaomicron* strain background (Fig. 3E) suggesting that the *B. vulgatus* TPP riboswitch acts at the level of transcription.

Collectively, these data suggest *Bacteroides* species utilize riboswitches that act at either the transcriptional or the translational level. The riboswitches controlling biosynthesis operons are more sensitive than transport operon riboswitches, suggesting a hierarchical response to thiamine concentrations in the range of 10 to 100 nM.

Distances of TPP Riboswitches Correspond to Regulatory Mechanism

Our results so far suggest that several TPP riboswitches – BtTPP1, BunTPP1, BunTPP2, and BvTPP1 – in *B. thetaiotaomicron*, *B. uniformis*, and *B. vulgatus* regulate downstream gene expression at the level of transcription. In contrast, BtTPP2 and BtTPP3 regulation was only observed at the level of translation. We observed that the transcriptionally acting riboswitches in *B. thetaiotaomicron, B. uniformis, and B. vulgatus* were located further upstream from the first gene in the operon compared to the translational-acting riboswitches in *B. thetaiotaomicron.* The differences in distance between the TPP riboswitch and the downstream gene that correlate with regulatory mechanism are consistent with previously characterized TPP riboswitches in model organisms (25, 29, 30). Transcriptional-acting riboswitches commonly found in Gram-positive bacteria are typically located >40-nt upstream of

the start codon of the adjacent gene, whereas translational-acting riboswitches often found in Gram-negative bacteria are usually <30-nt from the downstream gene. These trends that are consistent with our observations in *Bacteroides* led us look more globally at the distance between TPP riboswitches and downstream genes across the Bacteroidetes.

We analyzed 219 TPP riboswitches in 114 Bacteroidetes genomes. The riboswitches were grouped and categorized according to the genes that they regulate (Fig 4A, Table 2). Among the 219 riboswitches identified, 122 were predicted to regulate thiamine biosynthetic operons. *Bacteroides* species have biosynthetic operons in which critical genes for thiazole synthesis, pyrimidine synthesis, and thiamine condensation are in the same TPP riboswitch-controlled operon (Fig. 4A). More distantly related Bacteroidetes, e.g., *Parabacteroides* and *Paraprevotella* species appear to have split biosynthesis operons, with one TPP-controlled gene cluster for thiazole synthesis and a second for HMP synthesis and TMP condensation (Fig. 4A). A total of 97 TPP riboswitches were found upstream of predicted thiamine transport genes, primarily operons with *OMthi* (85 of 97) or *pnuT* (12 of 97) as the riboswitch-proximal gene (Fig. 4A, Table 2). In contrast to the diversity observed among TPP riboswitch controlled thiamine biosynthesis operons; thiamine transport operon organization is highly conserved. TPP riboswitches regulate both the inner and outer membrane transporters in addition to a thiamine pyrophosphokinase (*tnr3*) (Fig. 4A).

For the transcriptional-acting TPP riboswitches in *B. uniformis, B. vulgatus,* and *B. thetaiotaomicron* (biosynthetic operon only), we observed a >50-nt distance between the TPP riboswitch and the start codon of the first gene in the operon. In contrast, for the *B. thetaiotaomicron* thiamine transport operons, the TPP riboswitch was <5-nt from the first gene in each operon. We next analyzed the distances between TPP riboswitches and downstream genes more broadly across the Bacteroidetes (Table 2). While there is a range, the average distance between TPP riboswitches and downstream thiamine biosynthesis genes is 60 nt (± 34 nt). The average distance between TPP riboswitches and downstream thiamine transport genes

is shorter at 21 nt (± 20 nt) (Table 2). We plotted the number of occurrences of riboswitches at specific distances from downstream genes, classified by the predicted function of the downstream operon (Fig. 4B). This analysis illustrated the overall pattern we observed, where TPP riboswitches upstream of biosynthesis genes were located further from the start codon and TPP riboswitches upstream of transporter genes were located closer to the start codon. Based on our experimental data from *B. thetaiotaomicron, B. uniformis,* and *B. vulgatus* and the observed TPP riboswitch distances across other gut Bacteroidetes, we predict that the TPP riboswitches located <20 nt from the start codon regulate at the level of translation while riboswitches >40 nt from the start codon regulate at the level of transcription.

Discussion

Thiamine plays an essential role in both host and microbial metabolism, so thiamine biosynthesis and transport may play a key role in modulating the dynamics of host-microbe interactions. It is thought that most of the thiamine in the gastrointestinal tract is derived from the host's diet (5, 23), and that microbial community composition may be influenced by competition for this key nutrient (10). In this study, we characterized variable TPP riboswitch-dependent regulation of thiamine biosynthesis and transport genes in three representative *Bacteroides* species. In all three organisms, the thiamine biosynthesis genes were controlled at the level of transcription by a TPP riboswitch located ~50-nt upstream from *thiS*, the first gene in the biosynthesis operon. In *B. thetaiotaomicron*, the thiamine transport genes were controlled at the level of translation, by TPP riboswitches encoded immediately upstream of the start codons of transport genes. When we examined gut Bacteroidetes more broadly, we found that the distances between TPP riboswitches and downstream start codons were consistently correlated with the functional category, with longer distances for biosynthesis genes and shorter distances for transport genes. We postulate that in the Bacteroidetes, TPP riboswitches upstream of

biosynthesis genes commonly control expression at the level of transcription elongation whereas riboswitches upstream of transport genes frequently control translation initiation.

Historically, it was thought that mechanisms of regulation by TPP-dependent riboswitches were divided between Gram-negative bacteria, where regulation of translation was common, and Gram-positive bacteria, where transcription attenuation was typical (29, 32, 33). Recent studies have demonstrated that these distinctions are not so clear. For example, the *lysC* riboswitch in *E. coli* is a dual-acting riboswitch. Ligand binding promotes both translational repression and RNase E-dependent cleavage and subsequent mRNA decay (30). Chauvier, *et* al., recently found a role for transcriptional pausing in modulation of TPP binding and subsequent regulatory events for the *E. coli thiC* riboswitch (34, 35). Pausing of the transcription complex at a regulatory site within the *thiC* riboswitch inhibits TPP binding and allows transcription elongation and translation. If TPP binds co-transcriptionally, translation is inhibited and Rho-dependent termination occurs. Our results further emphasize that diverse organisms use both transcriptional and translational strategies for regulation mediated by TPP-dependent riboswitches.

Trends in distances between TPP riboswitches and downstream genes in *B. thetaiotaomicron*, *B. uniformis* and *B. vulgatus* suggest that riboswitches located further upstream from the adjacent start codon control downstream gene expression at the level of transcription elongation. These more distant riboswitches were found upstream of thiamine biosynthesis genes in all three organisms and thiamine transport genes in *B. uniformis,* and all promoted a thiamine-dependent reduction in the respective mRNAs. It is possible that one or more of these riboswitches could act at both transcriptional and translational levels, or that translational regulation could be coupled to nuclease-dependent degradation. The riboswitches upstream of thiamine transport genes in *B. thetaiotaomicron* were directly adjacent to the downstream start codons, and these riboswitches clearly regulated translation in response to thiamine, but had no effect on transcript levels. The molecular details of TPP riboswitch-

dependent regulation in *Bacteroides* species will require additional work. In these organisms, much remains to be discovered even in terms of basic mechanisms of regulation of transcription, translation, and mRNA processing and decay.

Our translational fusions revealed that TPP riboswitches regulating biosynthesis genes were responsive to thiamine at lower concentrations compared to riboswitches controlling transport genes. Biosynthesis genes were significantly downregulated in the presence of 10 nM thiamine, while transport genes responded only to concentrations at or above 100 nM. These concentrations are similar to the concentrations at which *E. coli* TPP riboswitches regulate cognate genes (33), suggesting that internal thiamine requirements may be similar across gut microbes. In addition, the 10 to 100 nM thiamine concentration range corresponds to the expected concentration of thiamine in the gut (20 to 2000 nM) (5). These data suggest that TPP riboswitch affinities among the Bacteroidetes and other gut bacteria may have evolved to operate at levels common to the human intestinal tract and to build a hierarchy of regulation between thiamine biosynthesis and transport to deal with rapidly changing nutrient availability in the gut. Such regulatory systems are not uncommon in the Bacteroidetes as a similar hierarchical regulatory phenomenon was observed for vitamin B_{12} riboswitches which respond to different concentrations of B_{12} to differentially control when transporters of varying affinities are expressed (36). It is worth noting that despite the similarities between thiamine and B_{12} riboswitches in hierarchical responses to concentrations of the cognate vitamin, the B_{12} riboswitches found in Bacteroidetes have only been observed to act at the level of translation (37).

We inspected the publicly available genomes to identify TPP riboswitches in 114 Bacteroidetes genomes and found that TPP riboswitches were commonly found upstream of putative thiamine biosynthetic and transport operons. These predicted riboswitches appear to follow the distance trends observed in *B. thetaiotaomicron, B. uniformis,* and *B. vulgatus.* If distances between the TPP riboswitch and downstream start codon are indicative of regulatory

mechanism, as suggested by our results, then Bacteroidetes commonly use both transcriptional and translational acting TPP riboswitches to maintain internal thiamine homeostasis in the gut. While regulatory thresholds of Bacteroidetes TPP riboswitches may be similar across species and genera, global responses to thiamine are distinct. We have shown that *B. thetaiotaomicron* has a large suite of genes that are differentially regulated in the presence of exogenous thiamine, while closely related *B. vulgatus* and *B. uniformis* have no or a much narrower response. Such regulon differences have been observed in *E. coli* and *Salmonella enterica* where thiamine metabolism in the absence of thiamine show strikingly different global effects. In *E. coli,* branched-chain amino acid catabolism is involved while *S. enterica* leverages purine metabolism to produce precursors for thiamine synthesis (16-18).

Bacteroidetes TPP riboswitch structures are highly conserved across the species investigated we have investigated here, but we have shown that at least *B. thetaiotaomicron* utilizes both transcriptional and translational acting TPP riboswitches. While a single organism using different mechanisms for TPP riboswitch-dependent regulation is rare, it is not unheard of for a single riboswitch to transcriptionally regulate genes for biosynthesis of its ligand while regulating its transporter at the level of translation (25, 30). While the structure of the adaptor domain that binds TPP is highly conserved, the expression platform evolves much more quickly and likely has the largest impact on the mechanism of regulation mediated by each riboswitch (38). When riboswitches first evolved in the RNA world, it is believed that transcriptional control came first and allowed riboswitches to control the function of ribozymes (39). The regulation of translation likely evolved later and was retained because it is advantageous in some cases to have a reversible form of regulation to respond to rapidly changing environmental concentrations of the riboswitch ligand (38, 40). In the context of Bacteroidetes in the gut it may be beneficial to have transporters regulated at the level of translation as it is a reversible and fast-acting mechanism of regulation that could respond to rapidly changing thiamine concentrations in the gut. Conversely, the tighter control of thiamine biosynthesis in the

388 Bacteroidetes via transcriptional regulation may help save the energy of producing multiple
389 biosynthetic enzymes unless they are strictly necessary under very limiting thiamine
390 concentrations. The existence of these regulatory hierarchies between transcriptional and
391 translational acting riboswitches are uncommon in model organisms studied so far. Expansion
392 of model systems to include a greater diversity of bacterial phyla may reveal new examples that
393 regulate responses to vitamins, amino acids, or other nutrients involved in core metabolic
394 pathways (24, 36, 27).

395 The study of how thiamine impacts microbial metabolism, global gene expression and
396 microbial physiology may help us understand nutrition-dependent mechanisms responsible for
397 modulating microbial community dynamics in the gastrointestinal tract. Our work demonstrates
398 that *B. vulgatus* is effectively blind to exogenous thiamine, suggesting that it will not compete for
399 available thiamine with the host or other gut microbes. Reliance on biosynthesis for production
400 of essential vitamins may be a strategy used by certain species to establish a foothold in
401 complex communities where availability of these molecules varies depending on diet and
402 competition. The lack of thiamine transport capacity of *B. vulgatus* was not obvious based on
403 existing genome sequence annotation of open reading frames, highlighting the importance of
404 functional genomics and accurate annotation of cis-acting regulatory elements like riboswitches.
405 Further characterization of mechanisms regulating thiamine acquisition and biosynthesis might
406 lead to development of new strategies to modulate microbial community composition and
407 metabolic function through targeted approaches controlling the activities of vitamin-dependent
408 riboswitches (10, 41). These strategies could have a variety of exciting therapeutic applications
409 that leverage the beneficial roles of microbial communities in human health and nutrition.
410

411 **Materials and Methods**

412 *Bacterial Strains and Plasmids, Culturing and Genetic Manipulation*

413 Bacterial strains and plasmids and oligonucleotides used for strain construction are
414 described in Table S2.
415 Culturing of *Bacteroides* strains *B. thetaiotaomicron* VPI-5482, *B. uniformis* ATCC 8492,
416 and *B. vulgatus* ATCC 8482 occurred anaerobically at 37°C in liquid tryptone yeast extract
417 glucose (TYG) medium (42) or a modified minimal medium with 0 to 10 μM (final concentration)
418 of thiamine hydrochloride as described previously (10, 43, 44) or Difco brain heart infusion (BHI)
419 agar with the addition of 10% defibrinated horse blood (QuadFive, Ryegate, MT, USA). Cultures
420 were grown in a vinyl anaerobic chamber with an input gas mix consisting of 70% nitrogen, 20%
421 carbon dioxide, and 10% hydrogen (Coy Laboratory Products, Grass Lake, MI, USA). *E. coli*
422 S17-1 λ *pir* strains used for cloning and conjugation of reporter vectors were grown in LB
423 medium at 37°C aerobically. Antibiotics were added to the media when appropriate at final
424 concentrations as follows: ampicillin 100 μg/mL, gentamicin 200 μg/mL, and erythromycin 25
425 μg/mL.
426 Nanoluciferase reporter vectors were constructed via traditional cloning of the
427 nanoluciferase gene (45, 46) into the vector pNBU2 (36, 47). DNA fragments encompassing
428 TPP riboswitches and the first 5 codons of the downstream gene were amplified from genomic
429 DNA by PCR using HiFi Taq MasterMix (KAPA Biosystems, Wilmington, MA, USA) and inserted
430 in frame preceding the nanoluciferase gene in the pNBU2 vector. The constructs were
431 transformed into conjugation donor *E. coli* S17-1 λ *pir*, and confirmed via PCR amplicon size
432 and sequencing. After confirmation, vectors were then conjugated into *B. thetaiotaomicron* VPI-
433 5482, *B. uniformis* ATCC 8492, and *B. vulgatus* ATCC 8482 making a single insertion into the
434 genome of each strain (36, 47). A complete list of primers and plasmids used in this study are
435 provided in Table 3.
436
437 *Expression analysis*

Replicate cultures of wild-type *B. uniformis* and *B. vulgatus* were grown overnight in 5 mL minimal medium supplemented with a final concentration of 10 µM thiamine HCl (THI, > 99% pure) (Sigma Aldrich, St. Louis, MO, USA). Aliquots of each culture were pelleted by centrifugation (1 min at 13,300 x g), culture supernatants were decanted and the cells were washed 4 times in minimal medium with 0 nM thiamine. Cells were subcultured at a dilution of 1:2000 into 10 mL of minimal medium with either 0 or 15 µM (final concentration) thiamine HCl in two biological replicates. Cell growth was monitored and cells were harvested between OD_{600} 0.4 and 0.6 on a UV spectrophotometer. Total RNA was extracted using the Qiagen RNeasy kit and stored at -80°C. Total RNA was DNAse treated with DNase I (Thermo Fisher, Waltham, MA, USA) and NEB DNase treatment protocol. DNAse treated RNAs were re-purified using the Qiagen RNeasy kit (Hilden, Germany), quantitated using a Qubit 2.0 (Life Technologies, Carlsbad, CA, USA), and stored at -80°C. This was the same method used for a previously published RNA-seq experiment for *B. thetaiotaomicron* (10). RNA was submitted for integrity analysis, ribosomal RNA depletion, and library construction at the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign. RNA sequencing was performed using an Illumina 2500 HiSeq generating 100 nucleotide paired end reads for all samples.

For targeted gene expression analysis, total RNA was purified as described above and used to generate cDNA libraries using the first strand cDNA synthesis kit (Thermo Fisher, Waltham, MA, USA). RT-qPCR was performed on a Biorad CFX connect instrument (Biorad, Hercules, CA, USA) and SYBR Fast MasterMix 2x Universal (Kapa Biosystems, Wilmington, MA, USA) following the manufacturer's instructions for triplicate biological samples with three technical replicates. Five probe sets were used in three strains to amplify products specific to: 16S ribosomal RNA, *thiC, thiS*, *OMthi*, and *pnuT.* 16S rRNA primers were used as the control (36) and novel primers for *thiC, thiS*, *OMthi,* and *pnuT* for *B. thetaiotaomicron* (10), *B. uniformis*, and *B. vulgatus* were designed using Primer3 (48). Standard curves were used to evaluate the

464 efficiency of the amplification, all 5 genes had $R^2 \geq 0.98$ and slope between -3.30 and -3.40.
465 Relative expression changes were calculated using the $\Delta\Delta C_q$ method (49).
466
467 *Nanoluciferase Reporter Fusion Assays*
468 Reporter assays were performed through a modified and combined protocol based on
469 Lim *et al.* 2017 and Mimee *et al.* 2015. *Bacteroides* strains with integrated pNBU2 TPP
470 riboswitch nanoluciferase reporters were grown in a modified minimal medium and then washed
471 and back diluted to an OD_{600} of 0.004 in 1.5 mL Axygen deep 96-well plates (Corning, Inc.,
472 Corning, NY) over a gradient of thiamine HCl (Sigma-Aldrich, St. Louis, MO). Growth was
473 monitored by via a BioTek Synergy HTX Multi-Mode microplate reader (BioTek, Winooski, VT)
474 (10). When cells reached mid-log phase growth (0.35-0.58 OD_{600}) growth was stopped. 1 mL of
475 mid log phase culture was spun down in the Axygen deep well plate at 3000 x g for 10 minutes
476 and medium was poured off the pellets. Cell pellets were then resuspended in 50 µL of 10X
477 BugBuster protein extraction reagent (MilliporeSigma, Darmstadt, Germany). Cell suspensions
478 were then incubated at room temperature with gentle shaking for 10 minutes. Nanoluciferase
479 extracts were mixed 1:1 (10 µL:10 µL) with Nano-Glo Luciferase mixture. Mixtures were then
480 incubated for 5 minutes at room temperature. Luminescence was read on a BioTek Synergy
481 HTX Multi-Mode microplate reader (BioTek, Winooski, VT). Relative Luminescence Units were
482 normalized to the OD_{600} of cell cultures. Comparisons were made across thiamine concentrations
483 using pairwise Student's *t* tests in GraphPad Prism v6, and *P* values of ≤0.05 were considered
484 significant.
485 In Silico *identification of TPP riboswitches in* Bacteroides *species*
486 Detection of TPP riboswitches among Bacteroidetes was done as described in (10).
487 Infernal 2.0 was used to identify riboswitches using the covariance model of TPP riboswitches in
488 RFAM (RF00059) (50, 51) in 114 Bacteroidetes genomes available in RefSeq from the Human
489 Microbiome Project (52, 53). To confirm that Infernal correctly predicted TPP riboswitches,

TIGRFAM, PFAM, and Hidden Markov Models with HMMR were used to identify thiamine pyrophosphate biosynthesis and transport genes among the same 114 RefSeq Bacteroidetes genomes (53-55).

To analyze the trend in distances between riboswitches and downstream genes, nucleotide distances between coordinates identified with Infernal 2.0 and TIGRFAM, PFAM, and HMMR were taken and subtracted from one another (51, 54-57). Annotations and genes were then manually inspected utilizing Artemis to confirm that genes and riboswitches were called correctly and that distances were accurate and agreed with previous predictions.

ACCESSION NUMBER

RNAseq data generated for this project have been submitted to the NCBI SRA database as study SRP148918

FUNDING INFORMATION

This work was supported by an investigator award from the Roy J. Carver Charitable Trust to PHD (#15-4501), and University of Illinois at Urbana-Champaign start-up funds to PHD as well as NIH grant R01 GM092830 to CKV. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

ACKNOWLEDGEMENTS

We thank members of the Degnan and Vanderpool labs at the University of Illinois at Urbana-Champaign for comments on the manuscript. In addition, we thank Dr. Andrew Goodman and Dr. Bentley Lim from Yale University for protocols and plasmids associated with the NanoLuciferase reporter system. We also thank Alvaro Hernandez from the W.M. Keck Center for Comparative and Functional and Genomics at the University of Illinois at Urbana-Champaign for their technical services.

References

1. Stanger O. 2011. Water Soluble Vitamins: Clinical Research and Future Application. Springer Science & Business Media. 1:199-227.

2. Preedy VR. 2012. B Vitamins and Folate: Chemistry, Analysis, Function and Effects. Royal Society of Chemistry. 1:71-92.

3. Lonsdale D. 2006. A review of the biochemistry, metabolism and clinical benefits of thiamin(e) and its derivatives. Evid Based Complement Alternat Med 3:49–59.

4. Donnino M. 2004. Gastrointestinal Beriberi: A Previously Unrecognized Syndrome. Ann Intern Med 141:898.

5. Said HM. 2011. Intestinal absorption of water-soluble vitamins in health and disease. Biochem J 437:357–372.

6. Hoffman R. 2016. Thiamine deficiency in the Western diet and dementia risk. Br J Nutr 116:188–189.

7. Ward R, Sabin AB, Najjar VA, Holt LE. 1943. Thiamin Excretion Tests in Children with Paralytic Poliomyelitis. Exp Biol Med 52:5–7.

8. Sannino DR, Dobson AJ, Edwards K, Angert ER, Buchon N. 2018. The *Drosophila melanogaster* Gut Microbiota Provisions Thiamine to Its Host. MBio 9:e00155–18.

9. Rodionov DA, Vitreschak AG, Mironov AA, Gelfand MS. 2002. Comparative genomics of thiamin biosynthesis in procaryotes. New genes and regulatory mechanisms. J Biol Chem 277:48949–48959.

10. Costliow ZA, Degnan PH. 2017. Thiamine Acquisition Strategies Impact Metabolism and Competition in the Gut Microbe. mSystems 2 (5) e00116-17.

11. Ashokkumar B, Kumar JS, Hecht GA, Said HM. 2009. Enteropathogenic Escherichia coli inhibits intestinal vitamin B_1 (thiamin) uptake: studies with human-derived intestinal epithelial Caco-2 cells. Am J Physiol Gastrointest Liver Physiol 297:G825–33.

12. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI. 2012. Human gut microbiome viewed across age and geography. Nature 486:222–227.

13. Quince C, Ijaz UZ, Loman N, Eren AM, Saulnier D, Russell J, Haig SJ, Calus ST, Quick J, Barclay A, Bertz M, Blaut M, Hansen R, McGrogan P, Russell RK, Edwards CA, Gerasimidis K. 2015. Extensive Modulation of the Fecal Metagenome in Children With Crohn's Disease During Exclusive Enteral Nutrition. Am J Gastroenterol 110:1718–29; quiz 1730.

14. Knecht C, Fretter C, Rosenstiel P, Krawczak M, Hütt M-T. 2016. Distinct metabolic network states manifest in the gene expression profiles of pediatric inflammatory bowel disease patients and controls. Sci Rep 6:32584.

15. Begley TP, Downs DM, Ealick SE, McLafferty FW, Van Loon APGM, Taylor S, Campobasso N, Chiu H-J, Kinsland C, Reddick JJ, Xi J. 1999. Thiamine biosynthesis in prokaryotes. Arch Microbiol 171:293–300.

16. Bazurto JV, Farley KR, Downs DM. 2016. An Unexpected Route to an Essential Cofactor: *Escherichia coli* Relies on Threonine for Thiamine Biosynthesis. MBio 7:e01840–15.

17. Palmer LD, Leung MH, Downs DM. 2014. The cysteine desulfhydrase CdsH is conditionally

required for sulfur mobilization to the thiamine thiazole in *Salmonella enterica*. J Bacteriol 196:3964–3970.

18. Bazurto JV, Dearth SP, Tague ED, Campagna SR, Downs DM. 2017. Untargeted metabolomics confirms and extends the understanding of the impact of aminoimidazole carboxamide ribotide (AICAR) in the metabolic network of *Salmonella enterica*. Microb Cell Fact 5:74–87.

19. Webb E, Claas K, Downs D. 1998. *thiBPQ* encodes an ABC transporter required for transport of thiamine and thiamine pyrophosphate in *Salmonella typhimurium*. J Biol Chem 273:8946–8950.

20. Genee HJ, Bali AP, Petersen SD, Siedler S, Bonde MT, Gronenberg LS, Kristensen M, Harrison SJ, Sommer MOA. 2016. Functional mining of transporters using synthetic selections. Nat Chem Biol 12:1015–1022.

21. Rodionov DA, Leyn SA, Li X, Rodionova IA. 2017. A Novel Transcriptional Regulator Related to Thiamine Phosphate Synthase Controls Thiamine Metabolism Genes in Archaea. J Bacteriol 199.

22. Rodionov DA, Hebbeln P, Eudes A, ter Beek J, Rodionova IA, Erkens GB, Slotboom DJ, Gelfand MS, Osterman AL, Hanson AD, Eitinger T. 2009. A novel class of modular transporters for vitamins in prokaryotes. J Bacteriol 191:42–51.

23. Sassoon CS, Zhu E, Fang L, Subramanian VS, Said HM. 2016. Inhibition of Intestinal Thiamin Transport in Rat Model of Sepsis. Crit Care Med 44:e875–81.

24. McCown PJ, Corbino KA, Stav S, Sherlock ME, Breaker RR. 2017. Riboswitch diversity and distribution. RNA 23:995–1011.

582 25. Winkler W, Nahvi A, Breaker RR. 2002. Thiamine derivatives bind messenger RNAs
583 directly to regulate bacterial gene expression. Nature 419:952–956.

584 26. Sudarsan N. 2003. Metabolite-binding RNA domains are present in the genes of
585 eukaryotes. RNA 9:644–647.

586 27. Pavlova N, Penchovsky R. 2019. Genome-wide bioinformatics analysis of FMN, SAM-I,
587 *glmS*, TPP, lysine, purine, cobalamin, and SAH riboswitches for their applications as
588 allosteric antibacterial drug targets in human pathogenic bacteria. Expert Opin Ther Targets
589 23:631–643.

590 28. Cheah MT, Wachter A, Sudarsan N, Breaker RR. 2007. Control of alternative RNA splicing
591 and gene expression by eukaryotic riboswitches. Nature 447:497–500.

592 29. Nudler E, Mironov AS. 2004. The riboswitch control of bacterial metabolism. Trends
593 Biochem Sci 29:11–17.

594 30. Caron M-P, Bastet L, Lussier A, Simoneau-Roy M, Massé E, Lafontaine DA. 2012. Dual-
595 acting riboswitch control of translation initiation and mRNA decay. Proc Natl Acad Sci U S A
596 109:E3444–53.

597 31. Jaehme M, Guskov A, Slotboom DJ. 2014. Crystal structure of the vitamin B_3 transporter
598 PnuC, a full-length SWEET homolog. Nat Struct Mol Biol 21:1013–1015.

599 32. Sudarson J, Ramalingam S, Kishorekumar P, Venkatesan K. 2014. Expeditious
600 quantification of lignocellulolytic enzymes from indigenous wood rot and litter degrading
601 fungi from tropical dry evergreen forests of Tamil Nadu. Biotechnol Res Int 2014:127848.

602 33. Guedich S, Puffer-Enders B, Baltzinger M, Hoffmann G, Da Veiga C, Jossinet F, Thore S,
603 Bec G, Ennifar E, Burnouf D, Dumas P. 2016. Quantitative and predictive model of kinetic

604 regulation by *E. coli* TPP riboswitches. RNA Biol 13:373–390.

605 34. Chauvier A, Picard-Jean F, Berger-Dancause J-C, Bastet L, Naghdi MR, Dubé A, Turcotte
606 P, Perreault J, Lafontaine DA. 2017. Transcriptional pausing at the translation start site
607 operates as a critical checkpoint for riboswitch regulation. Nat Commun 8:13892.

608 35. Chauvier A, Nadon J-F, Grondin JP, Lamontagne A-M, Lafontaine DA. 2019. Role of a
609 hairpin-stabilized pause in the *Escherichia coli thiC* riboswitch function. RNA Biol 16:1066–
610 1073.

611 36. Degnan PH, Barry NA, Mok KC, Taga ME, Goodman AL. 2014. Human Gut Microbes Use
612 Multiple Transporters to Distinguish Vitamin B_{12} Analogs and Compete in the Gut. Cell Host
613 & Microbe.

614 37. Vitreschak AG, Rodionov DA, Mironov AA, Gelfand MS. 2003. Regulation of the vitamin B_{12}
615 metabolism and transport in bacteria by a conserved RNA structural element. RNA 9:1084–
616 1097.

617 38. Arnvig KB. 2019. Riboswitches: choosing the best platform. Biochem Soc Trans 47:1091–
618 1099.

619 39. Breaker RR. 2012. Riboswitches and the RNA world. Cold Spring Harb Perspect Biol 4.

620 40. Lemay J-F, Desnoyers G, Blouin S, Heppell B, Bastet L, St-Pierre P, Massé E, Lafontaine
621 DA. 2011. Comparative Study between Transcriptionally- and Translationally-Acting
622 Adenine Riboswitches Reveals Key Differences in Riboswitch Regulatory Mechanisms.
623 PLoS Genetics. 7:e1001278.

624 41. Portari GV, Ovidio PP, Deminice R, Jordão AA Jr. 2016. Protective effect of treatment with
625 thiamine or benfotiamine on liver oxidative damage in rat model of acute ethanol

626 intoxication. Life Sci 162:21–24.

627 42. Moore WEC, Cato EP, Holdeman LV. 1978. Some current concepts in intestinal
628 bacteriology. Am J Clin Nutr 31:S33–S42.

629 43. Scholle RR, Steffen HE, Goodman HJ, Woods DR. 1990. Expression and regulation of a
630 Bacteroides fragilis sucrose utilization system cloned in *Escherichia coli*. Appl Environ
631 Microbiol 56:1944–1948.

632 44. Varel VH, Bryant MP. 1974. Nutritional features of *Bacteroides fragilis* subsp. *fragilis*. Appl
633 Microbiol 28:251–257.

634 45. Mimee M, Tucker AC, Voigt CA, Lu TK. 2015. Programming a Human Commensal
635 Bacterium to Sense and Respond to Stimuli in the Murine Gut Microbiota. Cell Syst 1:62–
636 71.

637 46. Lim B, Zimmermann M, Barry NA, Goodman AL. 2017. Engineered Regulatory Systems
638 Modulate Gene Expression of Human Commensals in the Gut. Cell 169:547–558.e15.

639 47. Martens EC, Chiang HC, Gordon JI. 2008. Mucosal glycan foraging enhances fitness and
640 transmission of a saccharolytic human gut bacterial symbiont. Cell Host Microbe 4:447–
641 457.

642 48. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. 2012.
643 Primer3--new capabilities and interfaces. Nucleic Acids Res 40:e115.

644 49. Bookout AL, Cummins CL, Mangelsdorf DJ, Pesola JM, Kramer MF. 2006. High-throughput
645 real-time quantitative reverse transcription PCR. Curr Protoc Mol Biol Chapter 15:Unit 15.8.

646 50. Griffiths-Jones S, Bateman A, Marshall M, Khanna A, Eddy SR. 2003. Rfam: an RNA family
647 database. Nucleic Acids Res 31:439–441.

51. Nawrocki EP, Eddy SR. 2013. Infernal 1.1: 100-fold faster RNA homology searches. Bioinformatics 29:2933–2935.

52. NIH HMP Working Group, Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, Bonazzi V, McEwen JE, Wetterstrand KA, Deal C, Baker CC, Di Francesco V, Howcroft TK, Karp RW, Lunsford RD, Wellington CR, Belachew T, Wright M, Giblin C, David H, Mills M, Salomon R, Mullins C, Akolkar B, Begg L, Davis C, Grandison L, Humble M, Khalsa J, Little AR, Peavy H, Pontzer C, Portnoy M, Sayre MH, Starke-Reed P, Zakhari S, Read J, Watson B, Guyer M. 2009. The NIH Human Microbiome Project. Genome Res 19:2317–2323.

53. O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, Astashyn A, Badretdin A, Bao Y, Blinkova O, Brover V, Chetvernin V, Choi J, Cox E, Ermolaeva O, Farrell CM, Goldfarb T, Gupta T, Haft D, Hatcher E, Hlavina W, Joardar VS, Kodali VK, Li W, Maglott D, Masterson P, McGarvey KM, Murphy MR, O'Neill K, Pujar S, Rangwala SH, Rausch D, Riddick LD, Schoch C, Shkeda A, Storz SS, Sun H, Thibaud-Nissen F, Tolstoy I, Tully RE, Vatsan AR, Wallin C, Webb D, Wu W, Landrum MJ, Kimchi A, Tatusova T, DiCuccio M, Kitts P, Murphy TD, Pruitt KD. 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44:D733–45.

54. Selengut JD, Haft DH, Davidsen T, Ganapathy A, Gwinn-Giglio M, Nelson WC, Richter AR, White O. 2007. TIGRFAMs and Genome Properties: tools for the assignment of molecular function and biological process in prokaryotic genomes. Nucleic Acids Res 35:D260–4.

55. Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J, Sonnhammer ELL, Tate J, Punta M. 2014. Pfam: the protein families database. Nucleic Acids Res 42:D222–30.

672 56. Finn R, Griffiths-Jones S, Bateman A. 2003. Identifying protein domains with the Pfam
673 database. Curr Protoc Bioinformatics Chapter 2:Unit 2.5.

674 57. Griffiths-Jones S. 2003. Rfam: an RNA family database. Nucleic Acids Res 31:439–441.

675

676

677

678

679 **Tables:**

Table 1. Reads of quality trimmed and mapped RNA sequencing

Species	Treatment	Replicate	SRA Accession	Raw Reads	Final Processed Reads	Mapped Reads	Percent Mapped
B. thetaiotaomicron	–Thi	1	SRX3130807*	9,170,438	8,231,596	7,712,374	93.7%
		2	*SRX3130806**	8,258,740	7,454,637	6,830,124	91.6%
	+Thi	1	*SRX3130804**	9,017,267	8,169,029	7,783,046	95.3%
		2	*SRX3130805**	8,444,554	7,652,018	7,144,413	93.4%
B. uniformis	–Thi	1	*SRX4123144*	15,534,762	13,334,281	13,036,981	97.8%
		2	*SRX4123145*	15,576,078	13,358,338	12,771,513	95.6%
	+Thi	1	*SRX4123142*	17,382,438	14,900,079	14,437,339	96.9%
		2	*SRX4123143*	15,505,061	13,290,981	12,756,005	96.0%
B. vulgatus	–Thi	1	*SRX4123140*	19,391,595	13,334,281	13,036,981	97.8%
		2	*SRX4123141*	18,821,066	13,358,338	12,771,513	95.6%

	+Thi	1	*SRX4123138*	18,447,248	14,901,073	14,437,549	96.9%
		2	*SRX4123139*	18,300,462	13,748,120	13,183,146	95.9%

***denotes results previously published in Costliow and Degnan 2017**

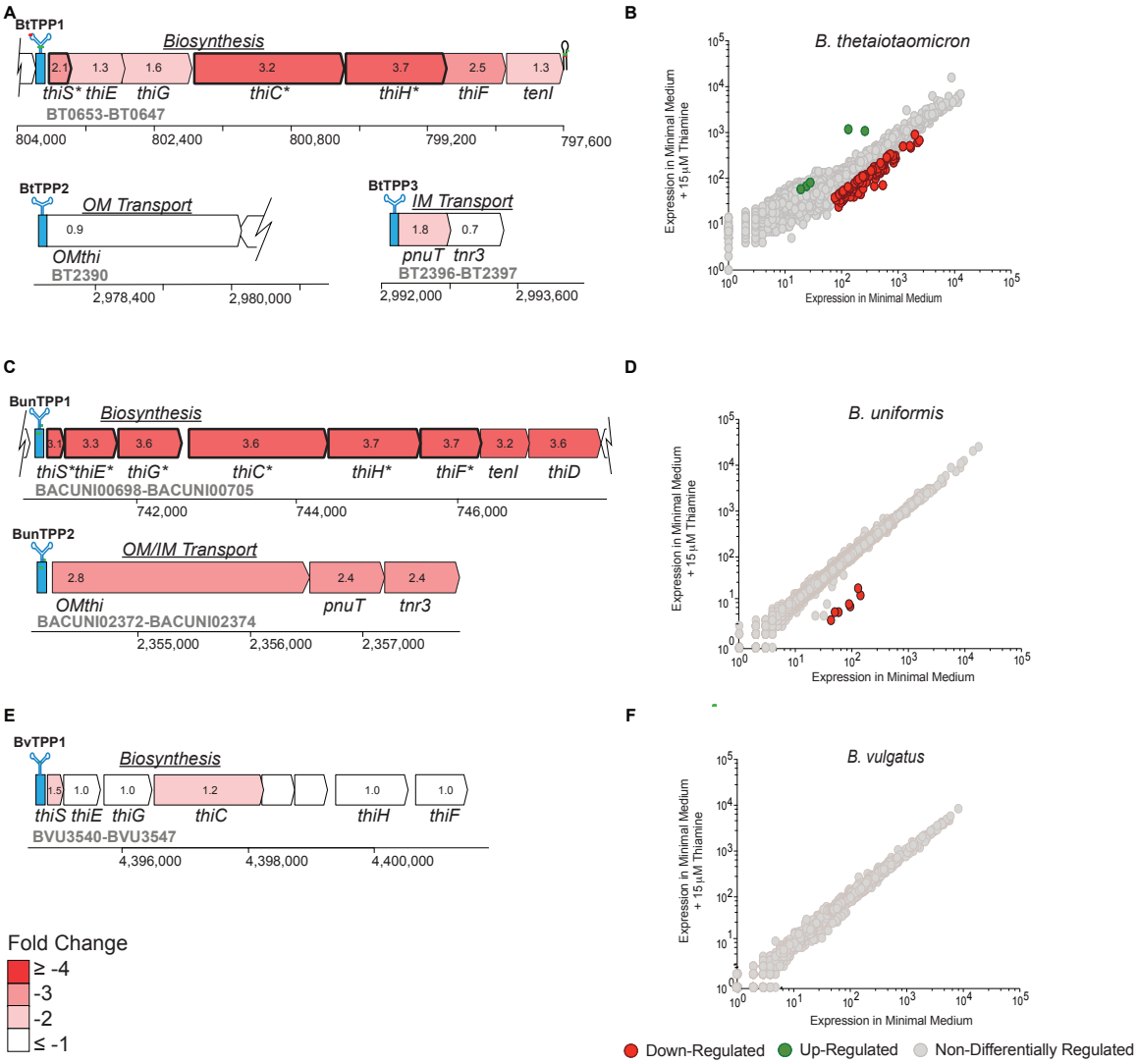
Table 2. Average distance distributions of TPP riboswitches from the first gene of their regulon

Gene Function	Gene	TIGRFAM / PFAM	TPP Riboswitches	Riboswitch Distance (Avg. ± St. Dev.)
Biosynthesis	*thiS*	TIGR01683 / PF02597	77	64 ± 14
	thiD	TIGR00097 / PF08543	17	24 ± 34
	thi4	TIGR00292 / PF01946	16	55 ± 19
	thiE	TIGR00693 / PF02581	9	106 ± 82
	thiC	TIGR00190 / PF05690	3	132 ± 0
		Total:	122	60 ± 34
Transport	*OMthi*	N.A. / PF00593, PF07715, PF14905	85	23 ±23
	pnuT	PF04973 / TIGR01528	12	3 ± 0
		Total	97	21 ± 23

681

682

683 **Figures:**



684

FIG 1. Transcriptional response to exogenous thiamine

Operons controlled by TPP riboswitches are shown for (A) *B. thetaiotaomicron,* (C) *B. uniformis,* and (E) *B. vulgatus.* Expression changes across operons are visualized in a schematic of each operon and color coded according to fold-change values comparing RNA-seq reads for cells grown with and without added thiamine. Negative fold-change values indicate that reads were reduced in cells grown in the presence of thiamine. Asterisks (*) and bold numbers indicate significance expression differences (*q-value* ≤ 0.05 and a change in expression ≥ 2-fold). All operons and distances are represented to horizontal scale. RNA-seq data from (B) *B. thetaiotaomicron* (previously in Costliow and Degnan 2017), (D) *B. uniformis,* and (F) *B. vulgatus* grown in a defined minimal medium without thiamine (0 nM thiamine added) and with thiamine (15 μM thiamine added). Grey circles indicate genes that are not differentially regulated. Green circles indicate genes that are significantly up regulated in the presence of thiamine. Red circles indicate genes that are significantly down regulated in the presence of exogenous thiamine. Data were analyzed using Rockhopper. The experiment was carried out in biological duplicate.

FIG 2. RT-qPCR confirmation of transcript level responses to exogenous thiamine

Transcript abundance of TPP riboswitch-regulated biosynthesis and transport genes was measured by RT-qPCR in (A) *B. thetaiotaomicron,* (B) *B. uniformis,* and (C) *B. vulgatus*. Primer sets were specific for genes indicated below bar graphs. Transcript levels of cells grown in the absence of thiamine (-) were normalized to levels in cells grown in the presence of 10,000 nM thiamine. Error bars represent the standard deviation (S.D.) for each experiment carried out in biological triplicate. Statistical significance for the differential abundance of gene transcripts were determined via a Student's t-test. Changes in expression were considered significant when the fold change was ≥ 2-fold with a *p*-value ≤ 0.05.

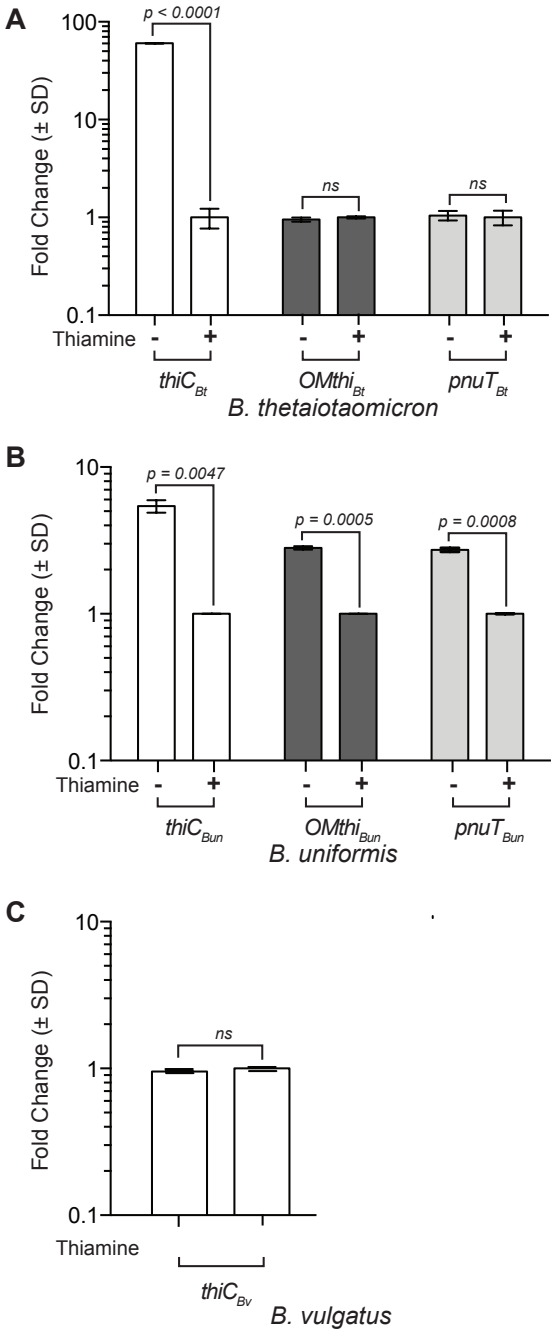


Fig. 2

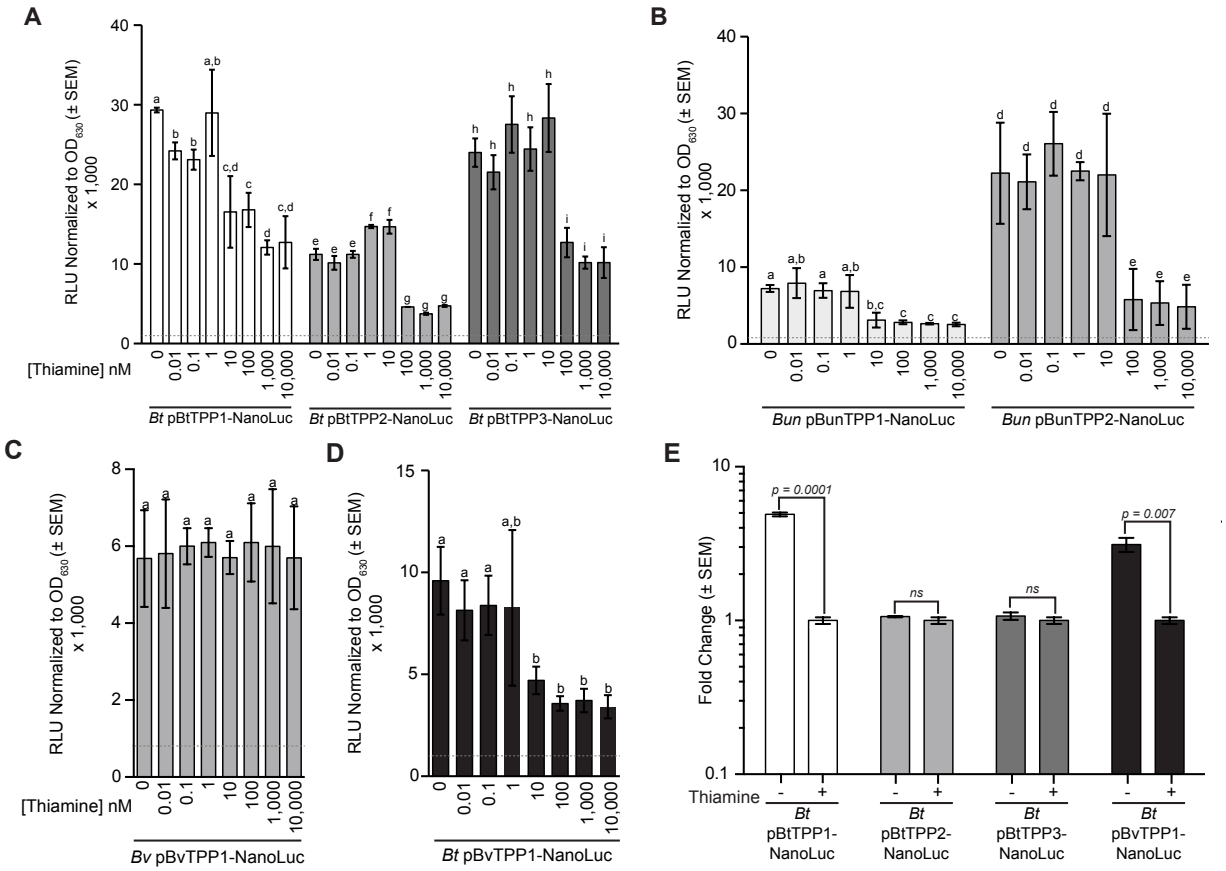


Fig. 3

724

725

FIG 3. Nanoluciferase assays of *Bacteroides* TPP riboswitches

Nanoluciferase translational fusions to genes downstream of all six TPP riboswitches found in (A) *B. thetaiotaomicron,* (B) *B. uniformis,* and (C) *B. vulgatus* were tested. The *B. vulgatus* reporter fusion was integrated into the *B. thetaiotaomicron* chromosome (D) to test the mechanism of regulation in response to thiamine because *B. vulgatus* does not respond to exogenous thiamine. RT-qPCR was carried out to measure reporter fusion mRNAs in *B. thetaiotaomicron* with thiamine (+) or without thiamine (-) to confirm the mechanism of regulation by predicted riboswitches from *B. thetaiotaomicron* and *B. vulgatus* (E). Relative light units (RLU) were normalized to the OD_{630} of the bacterial cultures before cell lysis and activation of the nanoluciferase reaction. Cells were harvested in mid-log phase for all samples (OD_{630} between 0.4-0.6). The activity of each fusion was represented as the averaged RLU/ OD_{630} x 1000 from six independent biological replicates. Error bars represent the standard error of the mean (SEM). Letters (a, b, c, etc.) indicate significant differences based on a *p-value* ≤ 0.05 using a Student's t-test. Grey dashed lines in A, B, C, and D indicate average RLU/ OD_{630} x 1000 from 3x biological replicates of a promoter-less nanoluciferase vector control for each experiment.

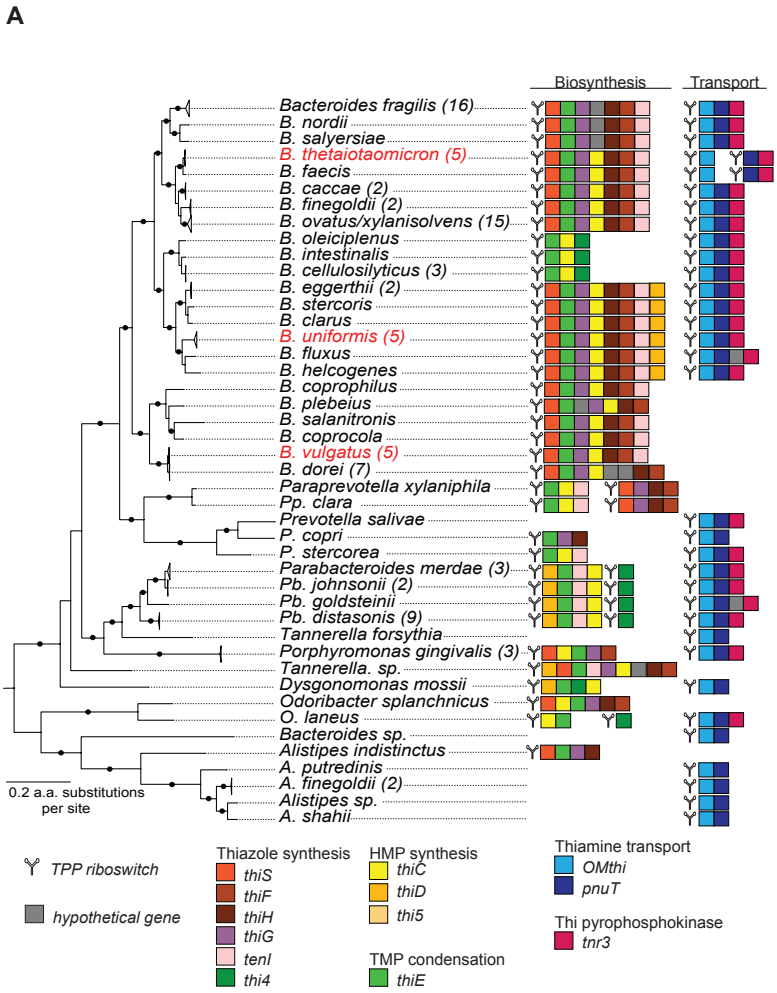


Fig. 4

FIG 4. TPP riboswitches throughout the Bacteroidetes

Infernal and the RFAM 00059 covariance model were used to identify TPP riboswitches across the Bacteroidetes. (A) ORFs downstream were inspected to determine the operons directly preceded by putative TPP riboswitches. Function and syntenic context were assigned to all predicted riboswitch regulated operons. (B) The distance of each TPP riboswitch from its predicted operon was recorded and plotted and divided into the subcategories based on functional class of genes regulated by the riboswitch - thiamine biosynthesis (red) or thiamine transport (blue).