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# The different roles of tryptophan transfer RNA in regulating *trp* operon expression in *E. coli* versus *B. subtilis*

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***Escherichia coli*** **and** ***Bacillus subtilis*** **use different mechanisms of sensing and responding to tryptophan and uncharged tRNA$^{Trp}$ as regulatory signals. In** ***E. coli,*** **tryptophan activates a repressor that binds to the** ***trp*** **promoter- operator, inhibiting transcription initiation. In** ***B. subtilis,*** **tryptophan activates an RNA-binding protein, TRAP, which binds to the** ***trp*** **operon leader RNA, causing transcription termination. In** ***E. coli*** **uncharged tRNA$^{Trp}$ accumulation stalls the ribosome attempting translation of tandem Trp codons in the leader-peptide coding region of the operon. This stalling permits the formation of an RNA antiterminator structure, preventing transcription termination. In** ***B. subtilis*** **uncharged tRNA$^{Trp}$ accumulation activates transcription and translation of the** ***at*** **operon. AT protein inhibits tryptophan-activated TRAP, thereby preventing TRAP-mediated transcription termination. These differences might reflect the unique organizational features of the respective** ***trp*** **operons and their ancestry.**

Protein synthesis in all organisms is dependent on the availability of all 20 amino acids, which are charged to their respective tRNAs. Amino acids are provided by biosynthesis, transport from the environment and/or degradation of existing proteins. Charging of their respective tRNAs is catalyzed by tRNA synthetases, whose properties and cellular levels determine their ability to provide sufficient levels of all the charged amino acids for protein synthesis. The biosynthesis of amino acids is energetically costly, particularly the biosynthesis of tryptophan, therefore, the synthetic processes providing amino acids are generally highly regulated. The mechanisms used are designed to respond to deficiencies of the amino acids and/or their charged tRNAs. Selective pressures were undoubtedly applied during the evolution of each organism, demanding development of strategies that would sense the availability of each amino acid and/or its corresponding charged tRNAs, and enable an appropriate response that would adjust their rate of synthesis. Often both an amino acid and its charged or uncharged tRNA serve as regulatory signals. This double sensing is essential because the concentration of each signal might be influenced by independent events. For example, of the two organisms compared in this article, *Escherichia coli* can degrade tryptophan to indole, pyruvate and ammonia, whereas *Bacillus subtilis* can not. Situations undoubtedly arose in nature where separate events affected the levels of tryptophan and charged tRNA$^{Trp}$, therefore, it was necessary to design strategies that would sense both, particularly when protein synthesis was the primary objective.

The Gram-negative enteric bacterium *E. coli* and the Gram-positive sporulating soil bacterium *B. subtilis,* like most organisms that are capable of synthesizing tryptophan, use essentially the same sequence of biosynthetic reactions and the same seven catalytic enzyme domains [1]. The tryptophan pathway therefore probably evolved just once and the *trp* genes of all organisms with this capability were derived from a common ancestor [1]. Despite this conservation of reactions and enzymes, the chromosomal organization of the *trp* genes of *E. coli* and *B. subtilis* differs appreciably [1]. Accompanying these organizational differences, the regulatory strategies that are employed to sense and respond to free tryptophan and charged and uncharged tRNA$^{Trp}$ also differ [2–4]. In this article, I will describe the regulatory mechanisms that are used by *E. coli* and *B. subtilis* to sense and respond to tryptophan and uncharged tRNA$^{Trp}$ as metabolic signals. I will focus on the recently discovered difference in the response of these two organisms to the accumulation of uncharged tRNA$^{Trp}$ [5–9].

## Organization of the *trp* operons of *E. coli* and *B. subtilis*

In *E. coli,* five genes encode the seven protein catalytic domains that are responsible for tryptophan biosynthesis from chorismate, the common aromatic precursor (Figure 1) [2]. These five genes are organized in a single transcriptional unit, the *trp* operon. Two of the genes, *trpG-D* and *trpC-F,* consist of gene fusions (i.e. each of their specified polypeptides is bifunctional). The five structural genes are preceded by a complex transcription regulatory region, which is designed to sense both tryptophan and charged and uncharged tRNA$^{Trp}$ [2]. A single promoter is used to initiate transcription of the entire operon; initiation at this promoter–operator is highly regulated by the tryptophan-activated *trp* repressor [2,10,11]. Transcription beyond the leader region into the structural genes of the operon is regulated by transcription attenuation [2].

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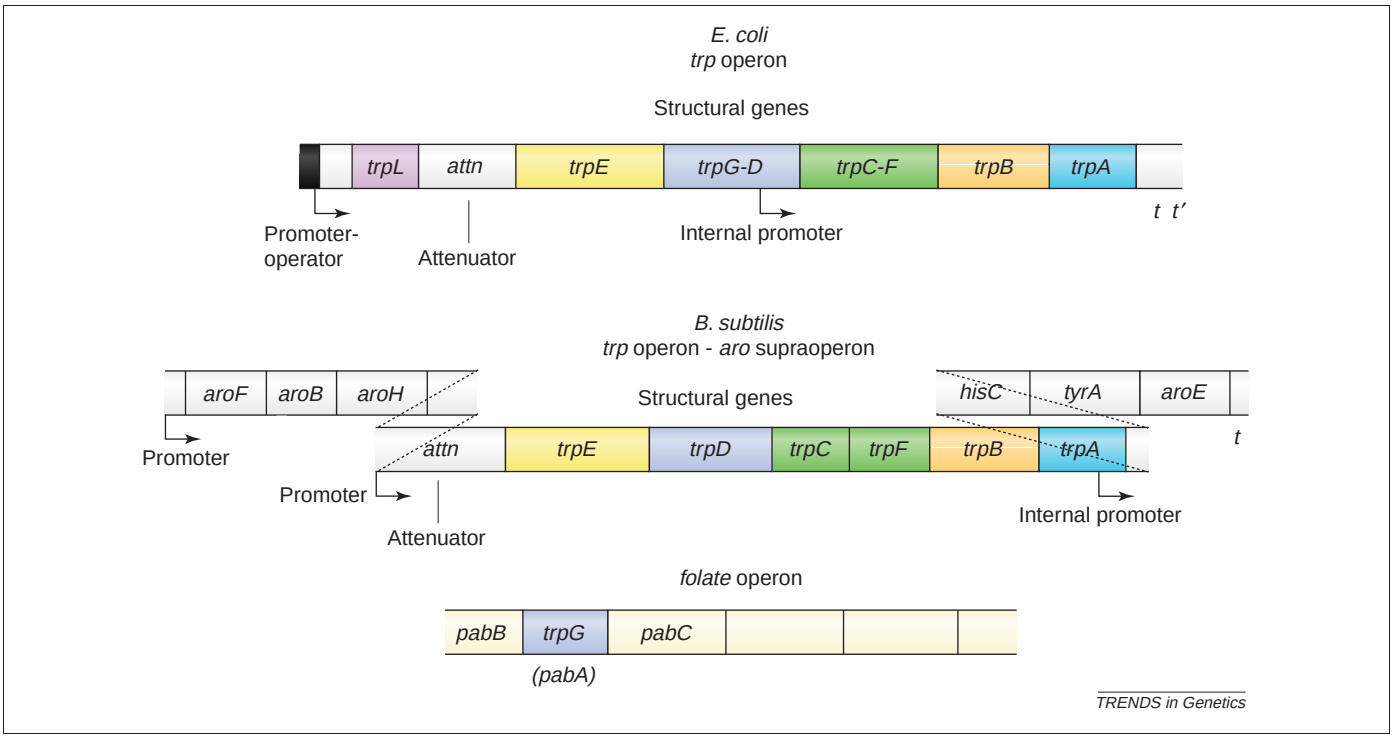



**Figure 1**. The organization of the *trp* operon of *Escherichia coli* and of *Bacillus subtilis*. The *trp* operon of *E. coli* is a single transcriptional unit, whereas the *trp* operon of *B. subtilis* is a segment of a supraoperon. In *B. subtilis,* one of the *trp* genes, *trpG(pabA),* is in a separate transcriptional unit, the *folate* operon. The *trp* operon regulatory region in *E. coli* contains a promoter–operator and an attenuator. This operon also has an unregulated internal promoter that provides the TrpC-F, TrpB and TrpA polypeptides when the operon is repressed maximally. Tandem terminators are located at the end of the operon. Two promoters drive transcription of the *trp* operon segment of the *aro* supraoperon of *B. subtilis*. Transcription initiated at each is regulated at a single attenuator, in the leader region of the *trp* operon. Regulation of transcription initiation at the upstream *aroF* promoter is not understood. An internal promoter is located within *trpA*; this promoter is used to transcribe the last three genes of the supraoperon; its use is important when transcription of the upstream regions of the supraoperon is inhibited. Termination at the end of the supraoperon has not been studied. There are additional genes (not shown) in the *folate* operon.

Depending on charged $tRNA^{Trp}$ availability, transcription is either terminated in the leader region or allowed to continue into the structural genes of the operon [2].

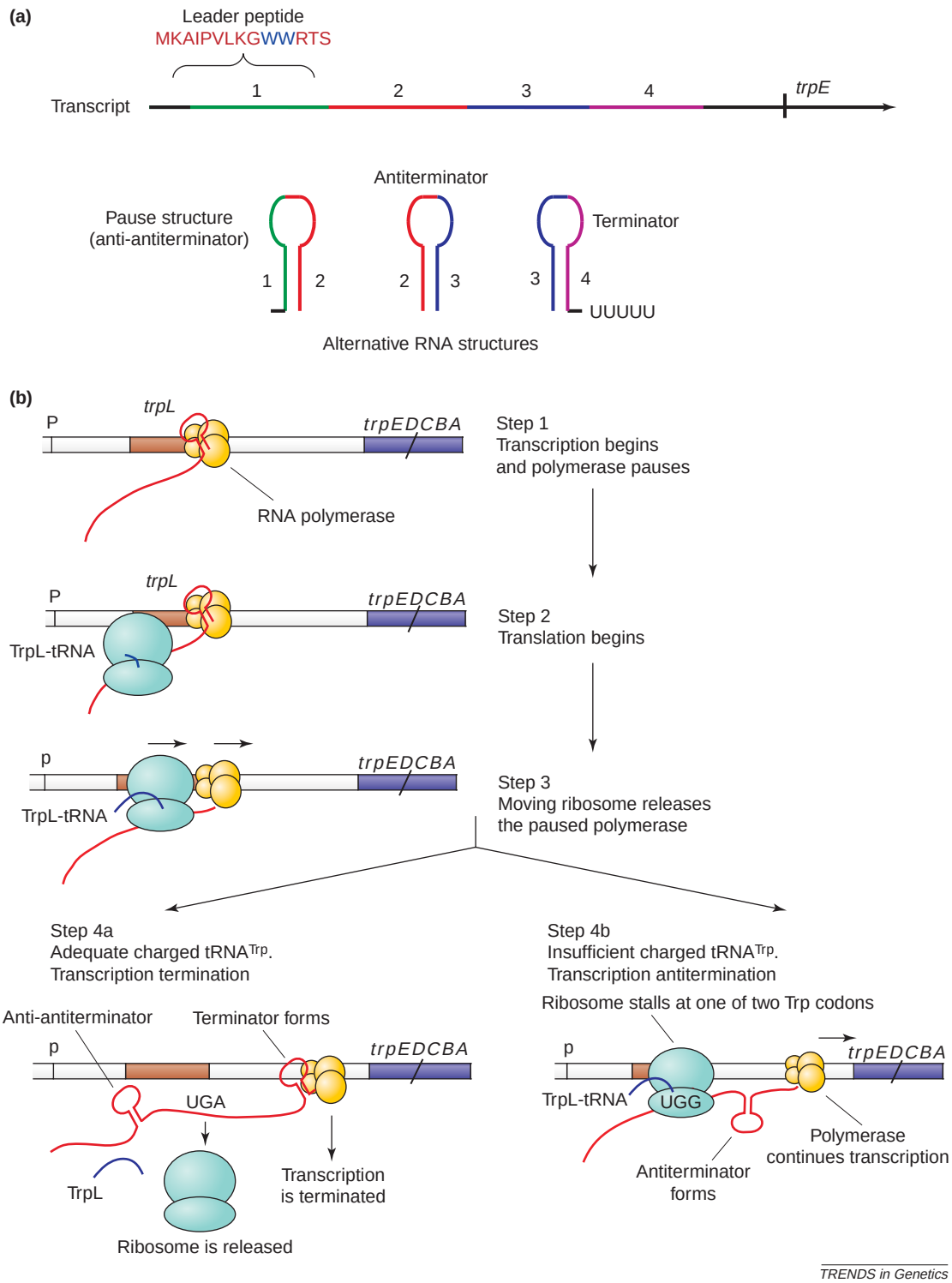
The *trp* operon of *B. subtilis* is organized differently (Figure 1). It consists of six genes that are located within a 12-gene aromatic (*aro*) supraoperon, which has three genes upstream and three genes downstream of the *trp* operon region [3,12–14]. These additional genes are concerned with related biosynthetic pathways, mostly providing chorismate and the other aromatic amino acids [3,12–14]. The seventh *trp* gene required for tryptophan biosynthesis, *trpG*(*pabA*), is located in the *folate* operon (Figure 1) [15]. This TrpG-PabA polypeptide, a glutamine amidotransferase, serves as a component of two enzyme complexes, one catalyzing the first reaction in the tryptophan pathway, and the second performing a related reaction in the folate pathway [15].

Two promoters are used to transcribe the *trp* operon region of the *aro* supraoperon, one preceding the upstream *aroF* gene and the second preceding *trpE*, the first gene in the *trp* operon region [3,12–14]. RNA polymerase initiating at either of these promoters is subject to a single regulatory decision in the leader region of the *trp* operon: whether to terminate transcription or allow it to continue into the structural genes of the operon. This regulatory decision is based on the availability of both tryptophan and charged $tRNA^{Trp}$. Tryptophan activates the TRAP regulatory protein, and active TRAP can bind to leader RNA and promote formation of an RNA terminator structure that causes transcription termination. Uncharged $tRNA^{Trp}$ accumulation leads to inactivation of the TRAP protein.

## Tryptophan regulation of *trp* operon expression in *E. coli* and *B. subtilis*

In both organisms tryptophan accumulation is the principal event resulting in downregulation of transcription of the structural genes of the *trp* operon. Each organism synthesizes a tryptophan-activated, regulation-dedicated protein. Whenever tryptophan accumulates in a growing cell, this regulatory protein is activated and transcription of the structural genes of the respective operon is inhibited. In *E. coli*, tryptophan activates a *trp* repressor that downregulates transcription initiation by binding to one or more of three operator sites located in the

**Figure 2**. The organization and regulatory functions of the *trp* operon leader region of *Escherichia coli*. **(a)** Features of the regulatory leader transcript of the *E. coli trp* operon. The leader transcript segment extending from the start site to *trpE*, is 162 nucleotides in length. This transcript can form three alternative RNA secondary structures: 1:2, the pause or anti-antiterminator structure; 2:3, the antiterminator structure; and 3:4, the terminator structure. These numbers refer to sequential linear segments of the *trp* operon leader transcript. Structure 3:4, the terminator, functions as an intrinsic terminator. When formed, it directs the transcribing RNA polymerase to terminate transcription at a specific site preceding *trpE*. The earlier, competing RNA structure, 2:3, the antiterminator, is formed whenever the ribosome translating the leader peptide




coding region stalls at one of its two Trp codons. Antiterminator formation prevents formation of the terminator. The third alternative RNA structure, 1:2, serves three roles. As a transcription pause structure it is essential for the coupling of transcription and translation in the leader region. As an anti-antiterminator, it prevents formation of the antiterminator. In addition, RNA segment 1 encodes a 14-residue leader peptide, which has two adjacent Trp residues. The ability to translate the codons for these Trp residues is used to sense the presence or absence of tryptophan-charged $tRNA^{Trp}$. Whenever the ribosome translating the leader peptide-coding region stalls at either of these Trp codons the RNA antiterminator structure forms. This prevents terminator formation, enabling the transcribing RNA polymerase to continue transcription into the five structural genes. When $tRNA^{Trp}$ is plentiful, the leader peptide is synthesized, the translating ribosome is released, the terminator forms and transcription is terminated. **(b)** Transcription attenuation regulation of the *trp* operon of *E. coli*. The sequential events that can occur during transcription of the leader region of the *trp* operon are pictured, with the alternatives shown. Which RNA structures form depends on ribosome position on the message and ribosome release from the leader peptide-coding region. The termination decision is based on charged $tRNA^{Trp}$ availability. When the operon is transcribed, the transcribing polymerase stalls following synthesis of a transcription pause structure (step 1). Translation then begins (step 2) and the translating ribosome releases the paused polymerase (step 3). When the cell has adequate levels of charged $tRNA^{Trp}$ the translating ribosome reaches the leader peptide stop codon and is released. The anti-antiterminator and terminator then form, and transcription is terminated (step 4a.) When the cell is deficient in charged $tRNA^{Trp}$ the ribosome synthesizing the leader peptide stalls at a Trp codon. This enables the antiterminator to form, the antiterminator prevents the terminator from forming, and transcription continues into the structural genes of the operon (step 4b). The vertical slash through the coding region indicates that this region is much larger than shown.

*trp* promoter region [2]. This repressor also downregulates transcription initiation in operons involved in tryptophan transport and chorismate biosynthesis, and it also autoregulates its own synthesis [2]. Whenever a cell lacks sufficient tryptophan to activate the *trp* repressor, repression is relieved. Complete relief results in an 80-fold increase in the rate of initiation of transcription of the *trp* operon [16]. The regulatory region of the *trp* operon of *E. coli* contains a second regulatory checkpoint, downstream from the promoter, in the leader region of the operon. Here the cell assesses the availability of charged $tRNA^{Trp}$ for leader peptide synthesis [2]. The details of this regulatory mechanism will be described in the following sections and compared with that of *B. subtilis*.

In *B. subtilis,* the regulatory response to tryptophan is more complex because the *trp* operon is a sub-operon of a 12-gene aromatic (*aro*) supraoperon (Figure 1) [3,12,17]. Two promoters serve as the sites of transcription initiation of the *trp* operon region of this *aro* supraoperon. Transcription initiation at the upstream *aroF* promoter is subject to regulation by aromatic amino acids (C. Yanofsky *et al*., unpublished), however, the extent and mechanisms are unknown. Regulation of transcription of the *trp* operon segment is well understood [3,7,12–14]. Every RNA polymerase molecule initiating transcription at either the *aroF* promoter or *trp* operon promoter, when it begins to transcribe the *trp* operon leader region, is subjected to a transcription termination decision. If tryptophan is plentiful, it activates an 11-subunit RNA-binding protein, TRAP, and TRAP binds to NAG (GAG > UAG > AAG > CAG) repeats in *trp* operon leader RNA. TRAP binding prevents formation of an antiterminator structure. This enables formation of an overlapping intrinsic terminator and transcription is therefore terminated [3,12,13,14,18–20]. An additional hairpin structure that forms in the 5′ segment of the *trp* operon leader RNA appears to contribute to TRAP binding [21].

TRAP binding can downregulate transcription of the structural gene region of *trp* operon, reducing it by ~200 times [22]. TRAP binding also regulates tryptophan synthesis in a second way: it promotes formation of an additional RNA hairpin structure in the small fraction of leader transcripts that are not terminated at the attenuator. This hairpin structure inhibits *trpE* translation, further reducing the ability of the organism to synthesize tryptophan [22,23]. Tryptophan-activated TRAP also inhibits the initiation of translation of *trpG*, the single *trp* gene that is not in the *trp* operon [24,25]. Uncharged $tRNA^{Trp}$ accumulation also increases *trp* operon expression. The features of $tRNA^{Trp}$ sensing and action in *B. subtilis* will be described in a later section and will be compared with those of *E. coli*.

## Features of $tRNA^{Trp}$ sensing and regulation in *E. coli*

Whenever there is sufficient charged $tRNA^{Trp}$ to maintain rapid protein synthesis, transcription is terminated in the leader region of the *trp* operon [2,4]. Transcription termination reduces transcription of the structural genes of the operon about six-fold. However, if a culture is shifted from a medium containing tryptophan to a medium lacking this amino acid, there will be a temporary tryptophan deficiency. Repression will generally be relieved but not transcription termination; repression relief will provide sufficient charged $tRNA^{Trp}$ for continued protein synthesis. Under extreme starvation conditions, when neither tryptophan nor charged $tRNA^{Trp}$ is available for protein synthesis, both repression and transcription termination will be relieved, and transcription of the *trp* operon structural genes will be increased ~500-fold over the level that is attained in the presence of excess tryptophan and charged $tRNA^{Trp}$. Figure 2a presents the organization and functions of the segments of the *trp* leader transcript that are responsible for attenuation control. Figure 2b summarizes the regulatory events that occur when cultures either have or do not have sufficient charged $tRNA^{Trp}$ to maintain protein synthesis.

The length of the leader transcript, from its 5′ end to the *trpE* start codon, is 162 nucleotides. This transcript can form three alternative RNA secondary structures: 1:2, a pause or anti-antiterminator; 2:3, an antiterminator; and 3:4, a terminator [2]. These numbers refer to sequential segments of the *trp* operon leader transcript (Figure 2a). Structure 3:4 is an intrinsic terminator. Whenever this structure forms it directs the transcribing RNA polymerase to terminate transcription at a specific site immediately preceding the *trpE* coding region (Figure 2b). The competing RNA structure, 2:3, the antiterminator, when formed, prevents folding of the terminator, thereby preventing transcription termination. The third alternative RNA structure, 1:2, serves two roles. As a transcription pause structure it delays RNA polymerase movement until it is released by the translating ribosome. This coupling is crucial because transcription antitermination is dependent on attempted translation of the 14-residue leader-peptide coding region. Structure 1:2 also acts as an anti-antiterminator. Thus, whenever the ribosome translating the leader peptide-coding region dissociates, formation of structure 1:2 prevents formation of structure 2:3, thereby enabling the formation structure 3:4, the terminator. RNA segment 1 also contains the coding region for a 14-residue leader peptide containing two adjacent Trp codons. It is the act of attempted translation of these two Trp codons that is used to sense the availability of tryptophan-charged $tRNA^{Trp}$. Whenever there is a charged $tRNA^{Trp}$ deficiency the ribosome translating this peptide-coding region stalls at either of these Trp codons (Figure 2b). This stalling enables formation of the RNA antiterminator, which prevents formation of the more distal terminator. Whenever the terminator cannot be formed, RNA polymerase continues transcription into the remainder of the operon. If sufficient tryptophan-charged $tRNA^{Trp}$ is available for completion of synthesis of the leader peptide, formation of the antiterminator is prevented. The terminator structure then forms and transcription is terminated in the leader region. Depending on charged $tRNA^{Trp}$ availability appropriate RNA structures form in the *trp* operon leader transcript and these structures determine whether transcription will continue into the structural genes of the operon [2] (Figure 2b).

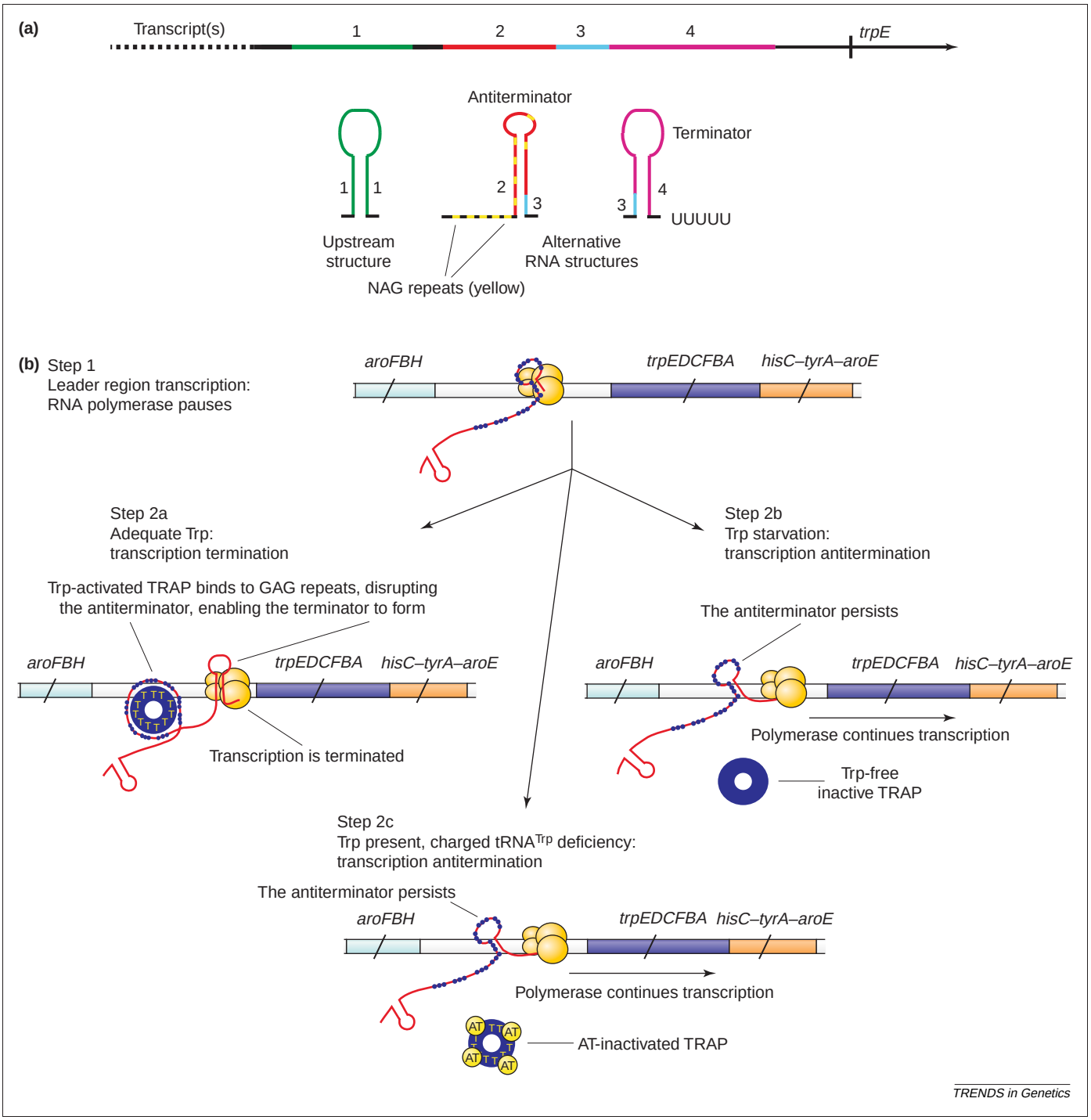



**Figure 3**. Organization and regulatory functions of the *trp* operon leader region of *Bacillus subtilis*. **(a)**. Features of the *B. subtilis trp* operon leader transcript. Transcription initiated at either the upstream *aroF* promoter or the *trp* operon promoter enters the *trp* operon leader region where the decision is made whether to terminate transcription. The leader transcript segment is 203 nt in length and contains the linear segments shown. These segments form two alternative RNA structures, 2:3, an antiterminator structure, and 3:4, a more distal, alternative, intrinsic terminator structure. Four nucleotides at the base of the 3′ strand of structure 2:3 (in light blue) are essential for formation of the terminator structure 3:4. Thus, as in *Escherichia coli*, formation of the antiterminator precludes formation of the terminator. The RNA segment immediately preceding structure 2:3, as well as the 5′ strand of structure 2:3, contains multiple NAG triplets (yellow), generally separated by two nucleotides; these serve as the binding site for the TRAP regulatory protein. When TRAP binds at this site it leads to formation of the terminator, resulting in transcription termination. Tryptophan activates TRAP; therefore, tryptophan availability determines whether TRAP will promote termination in the leader region of the operon. An additional upstream structure, 1:1, appears to increase TRAP function. **(b)** Sequential events in tryptophan-mediated and uncharged $tRNA^{Trp}$ -mediated regulation of transcription attenuation in the *trp* operon of *B. subtilis*. The alternative events occurring in the leader region of the *trp* operon are shown; these depend on whether TRAP is activated by tryptophan and whether TRAP is inhibited by the Anti-TRAP protein, AT. Each RNA polymerase molecule entering the leader region of the *trp* operon segment of the supraoperon pauses following formation of a pause structure (step 1). If there is sufficient tryptophan to activate TRAP, TRAP binds to leader RNA and prevents or disrupts formation of the antiterminator. This enables the terminator to form, and terminate transcription (step 2a). When a cell is starved of tryptophan, TRAP is inactive, the antiterminator therefore persists, and transcription continues into the remainder of the operon (step 2b). When cells are deficient in charged $tRNA^{Trp}$, AT is synthesized. AT binds to tryptophan-activated TRAP, inactivating TRAP, therefore, transcription continues into the structural genes of the operon (step 2c). A vertical slash through a coding region indicates that this region is much larger than shown.

### Features of tRNA$^{Trp}$ sensing and regulation in *B. subtilis*

The organization and functions of the segments of the *trp* leader transcript of *B. subtilis* that are responsible for attenuation regulation are illustrated in Figure 3a, and the events that occur when tryptophan and/or charged or uncharged tRNA$^{Trp}$ is in excess, are summarized in Figure 3b. Early studies performed by others and confirmed in my laboratory established that *B. subtilis* responds to uncharged tRNA$^{Trp}$ as well as tryptophan as a regulatory signal [5,6,26,27]. Our analyses showed that the accumulation of uncharged tRNA$^{Trp}$ led to the inhibition of the ability of TRAP to promote transcription termination in the *trp* operon leader region [5]. Exploring this observation further, we identified the operon responsible for tRNA$^{Trp}$ sensing; it was an operon of previously unknown function. We named it the *at* operon because one of its products is the Anti-TRAP protein, AT [6,8]. AT can bind to tryptophan-activated TRAP and inhibit the ability of TRAP to bind to leader RNA [6].

The features of the *at* operon and its regulation are summarized in Figure 4. The operon is designed to respond to the accumulation of uncharged tRNA$^{Trp}$. Expression of the operon is regulated by two sequential events, the first transcriptional and the second translational [7]. The leader transcript of this operon can fold to form alternative antitermination-termination structures, which are common to the many tRNA synthetase and biosynthetic operons of *B. subtilis* that are regulated by the T box transcription termination-antitermination mechanism [12,28–32]. This mechanism involves uncharged tRNA$^{Trp}$ binding to leader RNA, stabilizing an antiterminator structure, which prevents formation of the terminator [5,6], enabling transcription to continue into the structural genes of the operon. Immediately downstream of the T box leader RNA there is a coding region, *rtpLP*, for a ten-residue leader peptide. This coding region contains three consecutive Trp codons [7] and its stop codon is located only six nucleotides upstream from the Shine-Dalgarno region of *rtpA*, the AT structural gene. This proximity suggests that the ribosome completing translation of *rtpLP* could inhibit initiation of *rtpA* translation, limiting AT synthesis [7,9]. However, if there were a deficiency of charged tRNA$^{Trp}$, the ribosome translating *rtpLP* would stall at one of its three Trp codons, exposing the *rtpA* Shine-Dalgarno-start codon region, which would permit efficient AT synthesis. The AT produced would then bind to tryptophan-activated TRAP, inhibiting TRAP function. Transcription of the *trp* operon would then proceed. The features of the *at* operon are designed to assess the steady state levels of charged and uncharged tRNA$^{Trp}$, and to enable an appropriate regulatory response. The consequences of the presence of different levels of AT on TRAP action and *trp* operon expression have yet to be fully analyzed. It is already apparent, however, that both transcription-termination relief and uncharged tRNA$^{Trp}$-induced stalling of the ribosome translating *rtpLP* are necessary for sufficient AT to be produced to severely inhibit TRAP action [7,9].

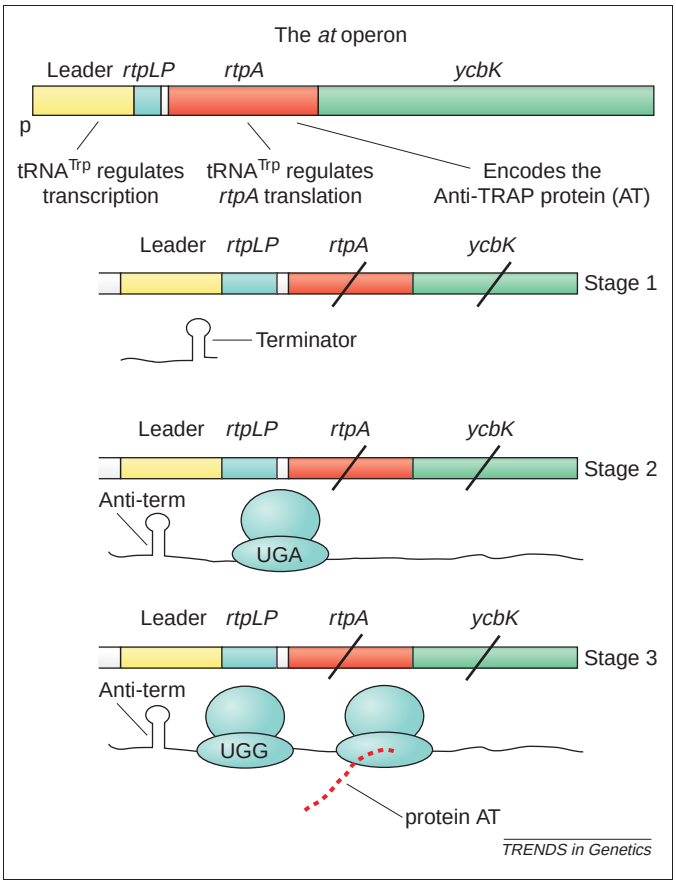



**Figure 4**. Organization of the *at* operon of *Bacillus subtilis*, and tRNA$^{Trp}$ regulation of *at* operon expression. The *at* operon is designed to sense uncharged tRNA$^{Trp}$ using both transcriptional and translational sensing mechanisms. The leader transcript of the operon can fold to form either an antiterminator or terminator structure. When tRNA$^{Trp}$ is mostly charged, the terminator forms and transcription is terminated (stage 1). When uncharged tRNA$^{Trp}$ accumulates, it pairs with *at* leader RNA and stabilizes the antiterminator; this prevents terminator formation and enables transcription to proceed into the *at* operon structural genes (stage 2). If there is sufficient charged tRNA$^{Trp}$ for translation of the leader peptide *rtpLP* to be completed, the translating ribosome reaches the *rtpLP* stop codon where it inhibits *rtpA* translation initiation. Therefore, AT protein is not produced (stage 2). When a cell is severely deficient in charged tRNA$^{Trp}$ the ribosome translating *rtpLP* stalls at one of its three Trp codons (stage 3). This frees the Shine-Dalgarno region of *rtpA*, enabling efficient AT synthesis. Elevated AT levels then inhibit tryptophan-activated TRAP. A vertical slash through the coding region indicates that this region is much larger than shown.

### Comparison of regulatory strategies

The regulatory strategies described in this article, used by *E. coli* and *B. subtilis* to sense and respond to tryptophan and uncharged tRNA$^{Trp}$, are compared in Figure 5. We would of course like to know why these regulatory differences exist, and when they were adopted. As mentioned previously, the enzymes and pathway of tryptophan biosynthesis are identical in the two species, thus gene, enzyme and pathway differences could not have triggered development of their respective regulatory strategies. However, operon organization in the two species clearly differs and these differences might have been responsible in part for the strategies in use today. Ancestry might also have contributed to the different regulatory strategies that these organisms use. In addition, one of the *trp* genes of *B. subtilis*, *trpG*, is not in the *trp* operon, it resides in a separate *folate* operon and specifies a bifunctional polypeptide that participates in both tryptophan and folate biosynthesis. It is possible that in an ancestor of *E. coli*, *trpG* was duplicated and one copy was fused to *trpD* and was dedicated to tryptophan biosynthesis, whereas the second copy, *pabA*, remained

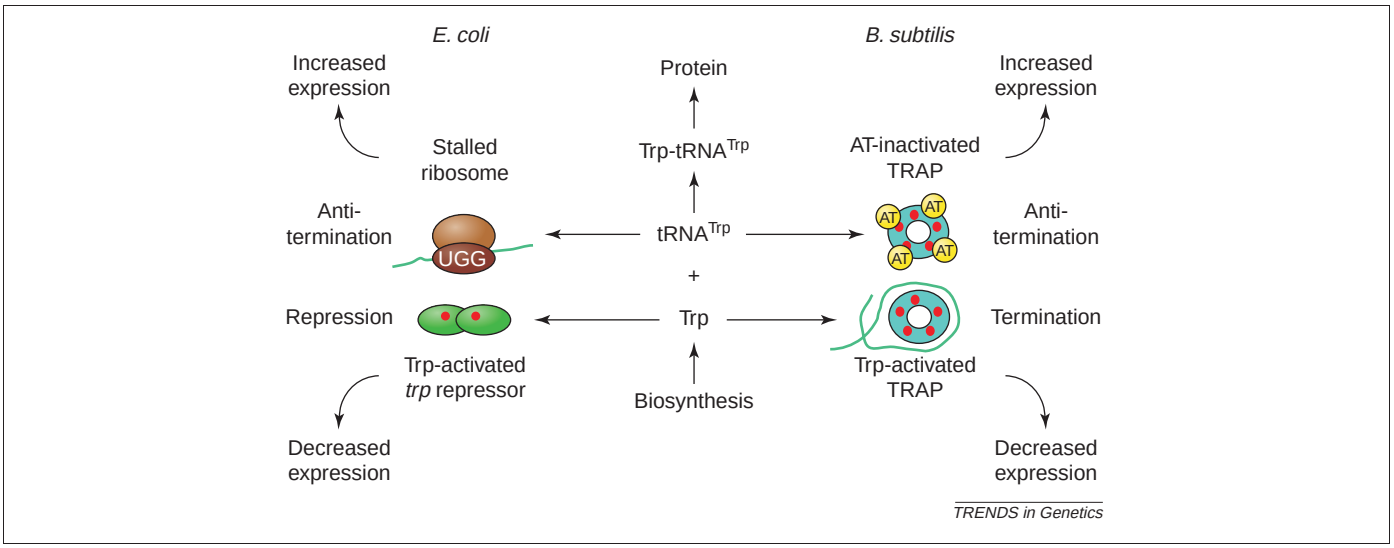



**Figure 5**. Comparison of the regulatory strategies used by *Escherichia coli* with those used by *Bacillus subtilis* to sense and respond to tryptophan and uncharged $tRNA^{Trp}$. In *E. coli* tryptophan activates the *trp* aporepressor. The *trp* repressor binds at the *trp* operator region and inhibits transcription initiation at the *trp* operon promoter. In *B. subtilis* tryptophan activates the TRAP protein. Active TRAP binds to *trp* operon leader RNA, causing transcription termination. When uncharged $tRNA^{Trp}$ accumulates in *E. coli*, the ribosome attempting translation of the leader peptide-coding region of the *trp* operon stalls over one of its two tryptophan codons. This stalling enables the antiterminator structure to form, preventing transcription termination. Transcription of the *trp* operon structural genes therefore continues. In *B. subtilis* uncharged $tRNA^{Trp}$ accumulation activates transcription antitermination in the *at* operon leader region, permitting transcription of the structural genes of the *at* operon. Uncharged $tRNA^{Trp}$ accumulation also prevents translation of the tryptophan codons of the leader peptide-coding region, *rtpLP*. This ribosome stalling exposes the *rtpA* (AT) Shine-Dalgarno region, enabling efficient *rtpA* translation and AT synthesis. The AT produced binds to tryptophan-activated TRAP, preventing TRAP from binding at its RNA binding sites, thereby increasing the rate of transcription of the *trp* operon and the rate of translation of *trpG*.

in a separate operon and was dedicated to folate biosynthesis. The significant difference in *trp* gene organization in the two organisms is that the *trp* operon of *B. subtilis* resides within a 12-gene supraoperon. Thus, an upstream *aroF* promoter and the *trp* operon promoter drive the transcription of the structural genes of the operon. Although a tryptophan-activated repressor could regulate transcription of the *trp* operon region, it probably could not regulate transcription initiation at the *aroF* promoter. Obviously, a mechanism that would regulate transcription initiated at either promoter would be most desirable. An additional significant difference between the two organisms is the mechanisms they use to regulate expression of *trpS*, the tryptophanyl-tRNA synthetase structural gene. In *B. subtilis* uncharged $tRNA^{Trp}$ is sensed as the regulatory signal that promotes *trpS* transcription, whereas in *E. coli trpS* expression is growth-rate regulated and is not $tRNA^{Trp}$ dependent. Having its *trpS* subject to $tRNA^{Trp}$ regulation introduces other considerations for *B. subtilis* when it experiences growth conditions where charged $tRNA^{Trp}$ is growth-limiting. An additional relevant consideration is that the T Box mechanism [28–31] used by *B. subtilis* to sense uncharged $tRNA^{Trp}$ is a common mechanism in this organism but is non-existent in *E. coli* [33]. An additional ancestral difference is that ribosome-mediated transcription attenuation is a common mechanism used to regulate amino acid biosynthetic operons in the enterobacteria but not in *B. subtilis*. Evolutionary accommodation by adopting a regulatory mechanism currently in use in an organism was certainly an attractive option.

## Concluding remarks

Why unrelated species develop or adopt different regulatory strategies for essentially the same purpose is a difficult question to answer. Current studies are establishing the widespread use of multiple regulatory mechanisms in every species. Perhaps when we know more about *trp* gene organization and regulation in many organisms, and their evolutionary history [1], it will be possible to provide logical explanations for the differences illustrated by the examples described in this article.

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