E. Coli Trp repressor

The tryptophan repressor proteins

The trp repressor proteins regulate transcription of several different operons located at separate sites on the E. coli chromosome. In addition to the trp operon, this repressor represses transcription of aroH, a single-gene operon that encodes an enzyme required for the synthesis of all aromatic amino acids, and trpR, another single-gene operon encoding the trp repressor itself. Such coordinately regulated operons constitute a regulon. Although all three operons in this regulon are repressed by the trp repressor, the extent of repression varies from about twofold for the aroH operon to seventyfold for the trp operon. This variation results from differences in the affinity of trp repressor for the specific operator in each of these operons, differences in the position of the operators relative to the −10 and −35 sequences of each promoter, and differences in the strengths of the three promoters. Thus the specific nucleotide sequences of the promoters and operators in a regulon allow the same repressor to differentially regulate transcription of the component operons.

The tryptophan biosynthetic pathway

Trp biosynthesis is a biologically expensive, complicated process. In fact, the products of four other pathways are essential contributors of carbon or nitrogen during tryptophan formation. Thus, the principal pathway precursor, chorismate, is also the precursor of the other aromatic amino acids, phenylalanine and tyrosine, as well as serving as the precursor of p-aminobenzoic acid and several other metabolites. In addition, glutamine, phosphoribosylpyrophosphate, and L-serine contribute nitrogen and/or carbon during tryptophan formation.
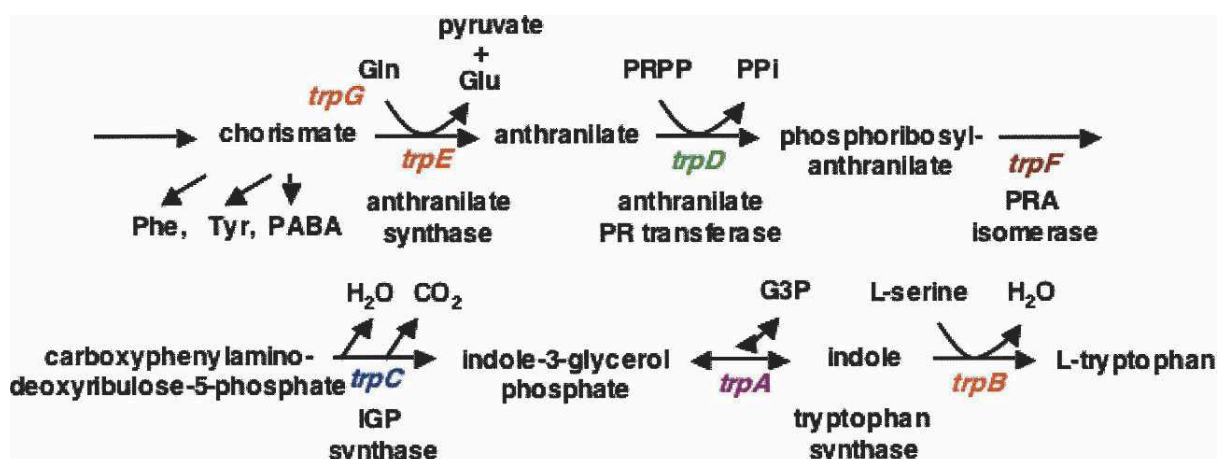

FIGURE 1. <u>The genes, enzymes, and reactions of the tryptophan biosynthetic pathway</u>. The seven genes, or genetic segments, seven enzymes, or enzyme domains, and seven reactions, involved in tryptophan formation are shown. Only one of the reactions is reversible. The products of four other pathways contribute carbon and/or nitrogen during tryptophan formation. Two of the tryptophan

pathway enzymes often function as polypeptide complexes: anthranilate synthase, consisting of the TrpG and TrpE polypeptides, and tryptophan synthase, consisting of the TrpB and TrpA polypeptides.

In many organisms tryptophan serves as the precursor of other biologically essential compounds, i.e., niacin in most eukaryotes, indoleacetic acid in most plants, and indole in many bacteria. Thus the regulatory strategies designed for the genes of tryptophan biosynthesis of each organism have had to be compatible with other metabolic objectives.

Regulatory mechanisms controlling transcription of the trp operon of E. coli

The genes required for tryptophan biosynthesis in Escherichia coli are organized as a single transcriptional unit, the trp operon. This operon has a single major promoter at which transcription initiation is regulated by a DNA–binding protein, the L–tryptophan– activated trp repressor. This repressor acts by binding at one or more of three operator sites located in the trp operon's promoter region.
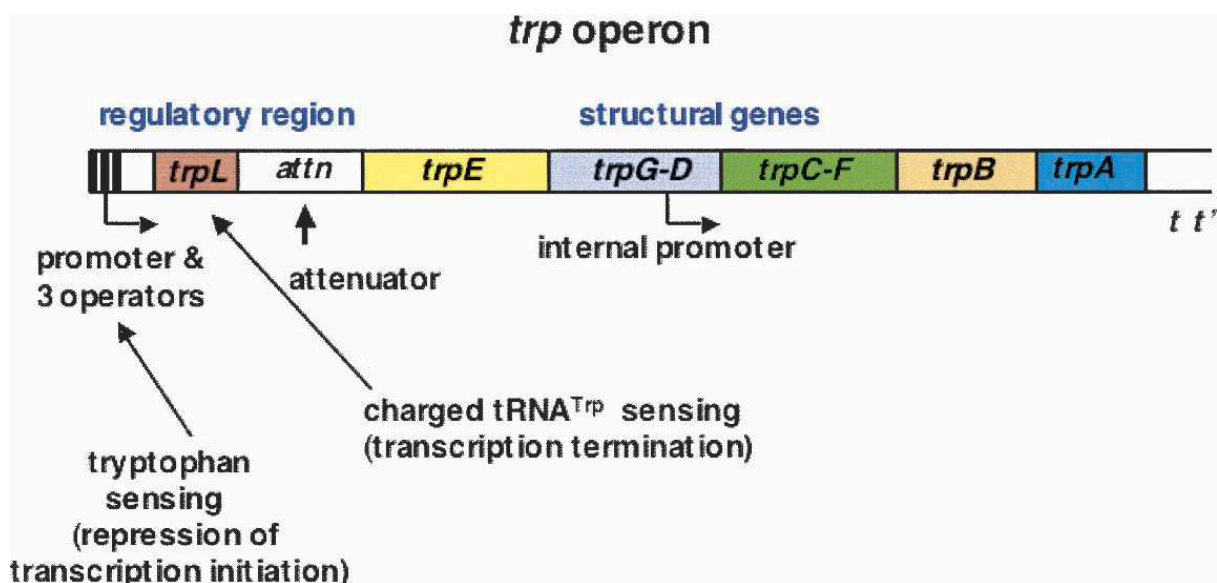

FIGURE 2. Organization of the trp operon of E. coli. The genes of E. coli required for tryptophan biosynthesis from chorismate are organized in a single operon, or transcriptional unit. Two pairs of genes are fused: trpG and trpD, and trpC and trpF. The structures of these two bifunctional polypeptides are known, and separate polypeptide segments are concerned with catalysis of each reaction. The relative order of the seven genetic segments, trpEGDFCBA, corresponds roughly to the relative order of the respective enzymatic reactions. The trp operon's regulatory region, located at the beginning of the operon, is designed to sense two signals, L–tryptophan, and charged vs. uncharged tRNATrp. Tryptophan, when in excess, activates the trp aporepressor, while charged and uncharged tRNATrp determine whether transcription will or will not be terminated in the operon's leader region. A poorly expressed internal promoter provides transcripts producing low levels of the

last few enzymes of the pathway. This promoter is useful when the principal promoter is turned off. (p = promoter; t = terminator).

The structures of the inactive trp aporepressor, the tryptophan–activated trp repressor, and the trp repressor–operator complex have all been determined, The aporepressor and repressor are dimers composed of identical helix–turn–helix monomers. Each repressor half–molecule has a bound tryptophan, and it recognizes an identical nucleotide sequence in each operator. Bound tryptophan alters the spacing between the DNA binding sites in each repressor molecule, positioning them for precise interaction with their operator sequences. Interactions between separate repressor molecules bound at adjacent trp operator sequences in the trp promoter increase the likelihood that each repressor molecule will remain bound at an operator, thereby increasing the degree of repression. The trp repressor also autoregulates its own synthesis, though weakly. It binds at an operator site located in its promoter, inhibiting initiation of transcription. Repressor autoregulation permits E. coli to synthesize increased levels of the aporepressor in preparation for a potential subsequent event—response to the appearance of excess tryptophan.

The availability of tryptophan–charged tRNATrp is also sensed as a regulatory signal in controlling trp operon transcription—by a transcription attenuation mechanism.
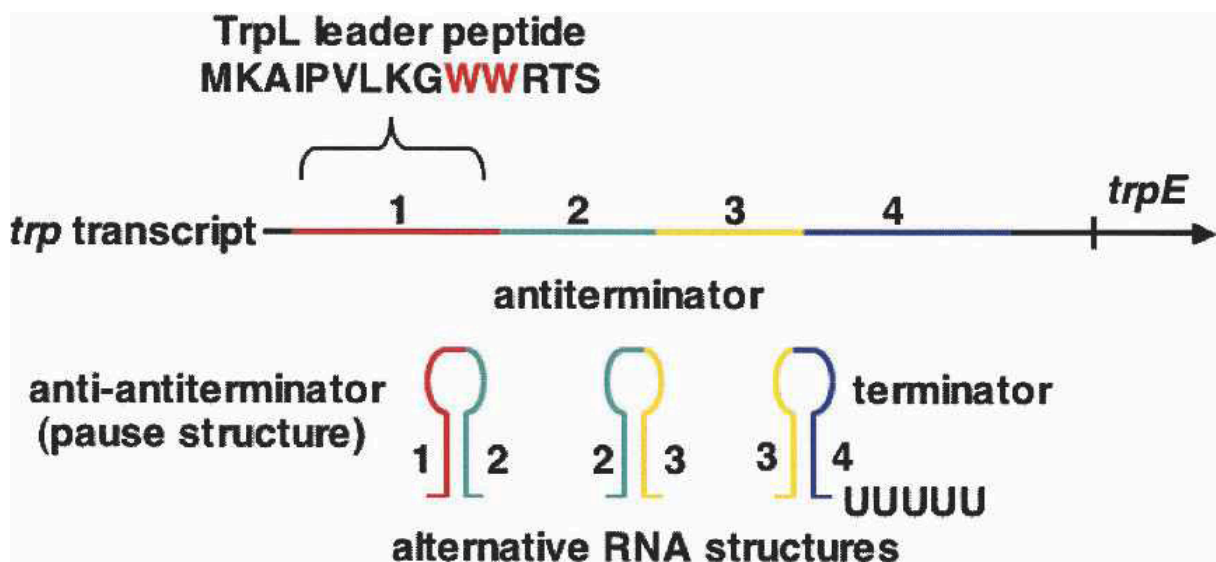

FIGURE 4. The trp operon leader transcript and its functions. The initial 141 nt of the trp operon transcript can fold and form three alternative RNA hairpin structures: an anti–antiterminator (1:2), an antiterminator (2:3), and a terminator (3:4). The anti–antiterminator structure (1:2) also serves as a transcription pause structure. The terminator structure is a typical intrinsic terminator which, when formed, directs the transcribing RNA polymerase to terminate transcription. However, whenever the preceding antiterminator structure has formed and persists, it prevents formation of the terminator. Hence transcription is not terminated. The initial leader RNA sequence has an

additional role: It encodes a 14 nt leader peptide, TrpL. Translation of the two Trp codons of trpL is used to sense the availability of charged tRNATrp. Whenever the charged tRNATrp level is adequate for rapid translation of the two Trp codons of trpL, translation of trpL is completed, the translating ribosome dissociates, and the anti-antiterminator (1:2) and terminator (3:4) structures form. This results in transcription termination in the leader region of the operon. When the tRNATrp in the cell is largely uncharged, the translating ribosome stalls at one of the trpL Trp codons. The antiterminator RNA structure (2:3) then forms, preventing terminator formation and transcription termination. Pausing following formation of the anti-antiterminator structure is essential for the coupling of translation of trpL mRNA with transcription of the leader region. The anti-antiterminator, if allowed to form and persist, would prevent formation of the antiterminator, thus the transcription terminator would form.

Premature Termination by Attenuation Helps Regulate Expression of Some Bacterial Operons

Transcription attenuation mechanisms generally involve several sequential stages or events, described in the following figure.
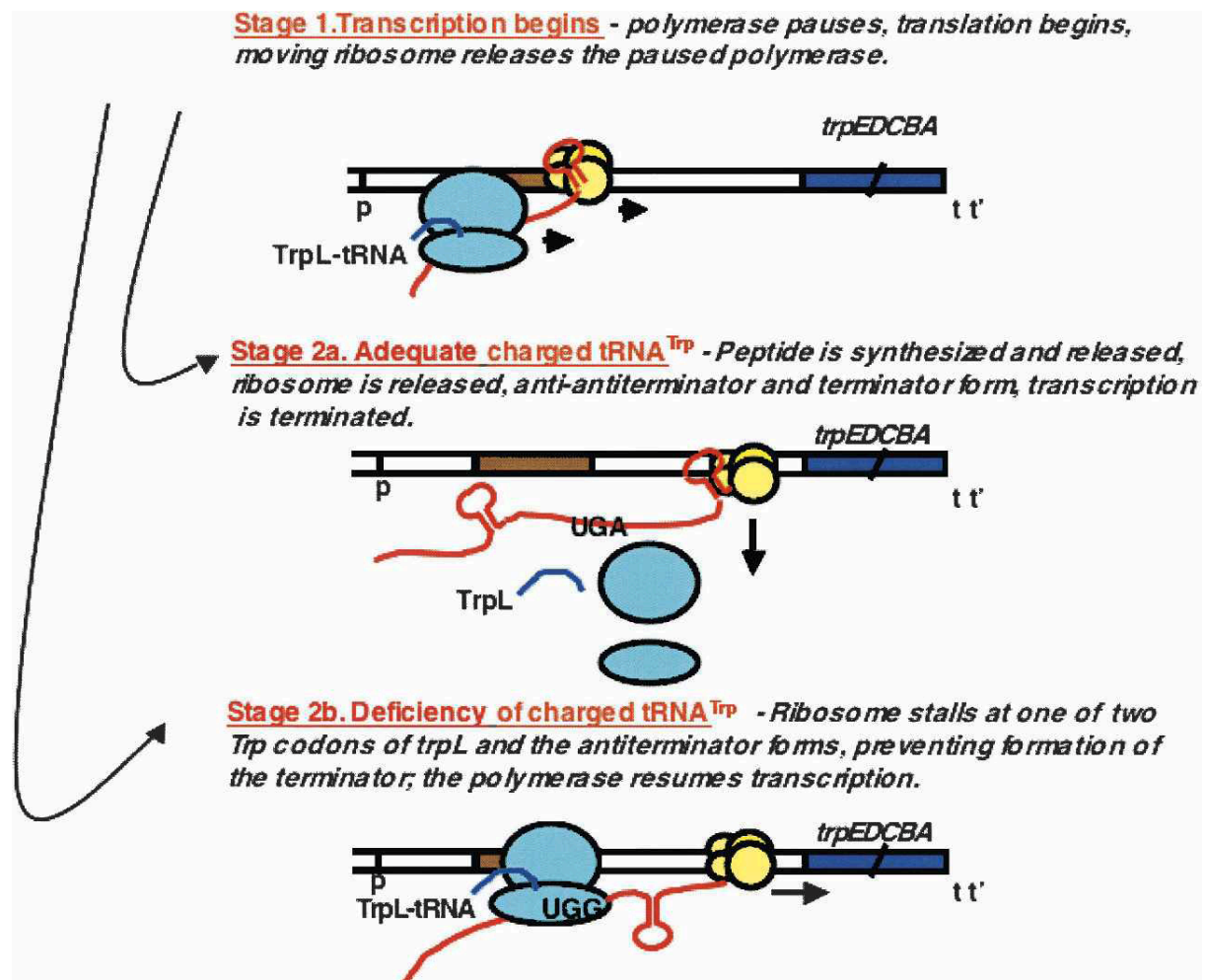
Stage 1. Transcription begins - *polymerase pauses, translation begins, moving ribosome releases the paused polymerase.*

Stage 2a. Adequate charged tRNA^{Trp} - *Peptide is synthesized and released, ribosome is released, anti-antiterminator and terminator form, transcription is terminated.*

Stage 2b. Deficiency of charged tRNA^{Trp} - *Ribosome stalls at one of two Trp codons of trpL and the antiterminator forms, preventing formation of the terminator; the polymerase resumes transcription.*

FIGURE 5. The sequential, alternative events regulating transcription termination in the leader region of the trp operon of E. coli. Stage 1: The RNA polymerase molecule that initiates transcription of the trp operon pauses after synthesizing the initial segment of the transcript—the segment that forms the anti–antiterminator pause structure. While the polymerase is paused, a ribosome binds at the trpL mRNA start codon and initiates synthesis of the leader peptide. This translating ribosome then disrupts the anti–antiterminator pause structure, releasing the paused polymerase and allowing it to resume transcription. Stage 2a: When there is sufficient charged tRNATrp in the cell to allow rapid completion of synthesis of the leader peptide, the translating ribosome is released. The anti–antiterminator and terminator structures then form, promoting transcription termination. Stage 2b: When there is a deficiency of charged tRNATrp, the ribosome translating trpL mRNA stalls at one of its two Trp codons. This permits the RNA antiterminator structure to form, which prevents formation of the terminator. Transcription then continues into the operon's structural genes.

An attenuator site, in effect, is a DNA sequence where a choice is made by RNA polymerase between continued transcription and termination. When tryptophan is abundant, little initiation of transcription takes place, and virtually all of the transcripts that are initiated terminate at the attenuator. In contrast, when tryptophan is scarce, initiation occurs at a high rate and many RNA polymerase molecules continue transcribing past the attenuator.

Attenuation requires a particular stem–loop structure in the mRNA leader sequence. Formation of this structure depends on the rate of ribosomal translation of the leader sequence, which is engaged by a ribosome soon after it is synthesized. The rate of translation of the leader sequence depends, in turn, on the supply of aminoacyl–tRNAs charged with the amino acids encoded by the sequence.

An essential feature of this attenuation mechanism is the synchronization of translation of a 14–residue leader peptide coding region, trpL, with transcription of the operon's leader region. Synchronization is achieved by exploiting features of the initial segment of the leader transcript, the segment overlapping trpL. This segment can form an RNA hairpin structure, designated hairpin 1–2, called the anti–antiterminator. Hairpin 1:2 also serves as a transcription pause signal. Transcriptional pausing is relieved when a ribosome binds at the trpL mRNA start codon and initiates synthesis of the TrpL leader peptide. The moving ribosome appears to disrupt the RNA pause hairpin, releasing the paused RNA Polymerase. Subsequently, either of two events occurs, depending on the availability of uncharged versus charged tRNATrp. When most of the cellular tRNATrp is uncharged, difficulty in translating the two Trp codons of trpL mRNA results in ribosome stalling at one of these Trp codons (Figs. 4, 5, Stage 2b). This allows the

antiterminator structure, hairpin 2–3, to form (Fig. 4), which prevents formation of the terminator structure.

Prevention of terminator formation allows transcription to continue into the structural genes of the operon (Fig. 5, Stage 2b). When charged tRNATrp is plentiful, however, translation of trpL is completed, and the translating ribosome dissociates at the trpL stop codon. This permits the leader transcript to fold and form the anti–antiterminator and terminator structures, 1:2 and 3:4 (Fig. 4), promoting transcription termination (Fig. 5, Stage 2a). Thus, depending on the availability of charged tRNATrp during translation of trpL, transcription of the structural gene region of the trp operon will or will not proceed.

The activity of the first enzyme of the tryptophan biosynthetic pathway, anthranilate synthase, is subject to feedback inhibition by Ltryptophan. This is typical of the first enzyme of many biosynthetic pathways. Feedback inhibition is a physiologically advantageous process because it allows an instantaneous—and readily reversible—reduction in the flow of carbon and nitrogen into a pathway.
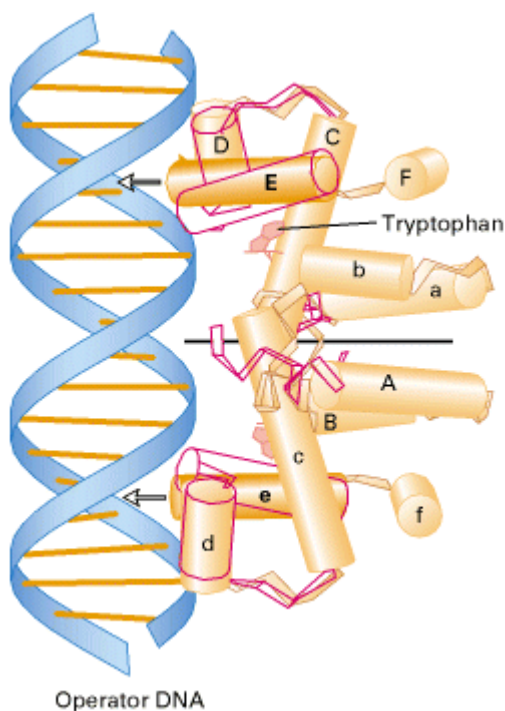

Operator DNA

FIGURE 6. Conformational change in the trp aporepressor caused by the binding of tryptophan. The α helices in the dimeric protein, represented as cylinders, are identified by upper-case letters in one monomer and lower-case letters in the other. The recognition helices (E and e) in the aporepressor (transparent with red holding line) are too close together to fit into adjacent major grooves in the operator DNA. When the aporepressor binds tryptophan, the N-terminal ends of helices E and e move apart by 8 Å. As a result, helices E and e in the repressor with bound tryptophan (orange) fit neatly into the operator DNA.

This inhibition, however, is not complete, and is supplemented by attenuation, which provides an additional level for regulating tryptophan synthesis according to the requirements for protein synthesis.

In essence, attenuation results from the premature termination of transcription at an **attenuator site** located just downstream from the promoter–operator region and before the coding sequences for the trp enzymes. E. coli mutants with small deletions in this region of the trp operon manufacture greater-than-normal amounts of tryptophan-synthesizing enzymes in the presence and absence of tryptophan. Such deletions cause increased synthesis of trp mRNA.

Sources:

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RNA-based regulation of genes of tryptophan synthesis and degradation, in bacteria, Yanofsky Charles, Department of Biological Sciences, Stanford University Stanford, California 94305, USA, Published in Advance June 29, 2007, doi: 10.1261/rna.620507 RNA 2007. 13: 1141–1154