Describe the mechanisms that regulate the tryptophan operon in bacteria.

Gene expression is highly regulated, and not all bacterial genes are expressed at the same time or to the same level. This is because this is wasteful and slows down bacterial replication, as the energy is spent on expression, transcription and translation instead. Transcription is a major control point in gene expression, and gene expression may be positively or negatively controlled. A key example of negative control is the tryptophan operon. Tryptophan biosynthesis must be regulated, as it is a highly complex and energetically expensive process. In the presence of tryptophan, the repressor binds the operator, and expression of the tryptophan operon is repressed. The tryptophan biosynthesis gene is only expressed if tryptophan is absent from the growth medium. If the operator is not bound to anything it is turned "on," and transcription can occur.

There are five *E. coli* genes which encode the three enzymes involved in tryptophan biosynthesis. These five genes form an operon, and so are co-regulated and co-expressed. All of the genes in the

polycistronic

operon are transcribed as one piece of mRNA, and their expression is controlled by an operator, which is specific DNA sequence found at the promoter region. The repressor binds at one or more of three operator sites. When protein is ingested and metabolised, tryptophan enters the bacterial cell. In this case, the cell does not need to synthesise tryptophan, so the genes for tryptophan biosynthesis are not expressed. The tryptophan repressor protein recognises the DNA sequence of the operator, and the repressor binds the operator DNA. This stops RNA transcription, as RNA polymerase cannot gain access to the promoter, because the binding of the repressor to the operon in the presence of tryptophan physically blocks the movement of RNA polymerase.

X-ray crystallography has revealed that the tryptophan repressor is a dimer of two symmetrical helix-turn-helix "DNA reading heads." Each head is bound to tryptophan, and recognises a specific nucleotide sequence in each operator. When tryptophan is bound to the repressor, the repressors are forced into the exact position required for their interaction with the operator sequences, as the spacing between the DNA binding sites in the repressors is altered. This allosteric reorientation enables the repressor to bind the operator. This increases the extent of repression of the tryptophan biosynthesis gene, as the interactions between the repressors at adjacent tryptophan operator sites stabilise the binding of the repressor to the operator, enabling the repressor to remain bound. Tryptophan also forms a hydrogen bond to a DNA phosphate group, which strengthens the association between the repressor and the operator.

In order for the repressor to bind the operator DNA, it must have two tryptophan molecules, which act as co-repressors, bound to it. The binding of tryptophan causes a conformational change, which in turn causes the helix-turn-helix motif of the repressor to tilt. When tryptophan is absent, the motif

swings inwards, thereby preventing the repressor protein from binding the operator. This means that RNA polymerase is free to transcribe the genes for tryptophan biosynthesis.

Tryptophan biosynthesis is regulated by end-product inhibition, in which tryptophan, the end produce, binds to the tryptophan repressor. The resulting complex binds specifically to the tryptophan operator, thereby reducing the rate of transcription.

The expression of the operon involved in tryptophan regulation is also controlled by attenuation, a process which depends on the availability of tryptophan-charged tRNA ($tRNA^{Trp}$). Attenuation occurs when tryptophan is in abundance. Tryptophan mRNA consists of 6720 nucleotides, and includes the Trp-L sequence, which is the element controls tryptophan transcription. The entire tryptophan sequence is transcribed when tryptophan levels are low. The rate of tryptophan transcription decreases as levels of tryptophan increase, due to the increasing amount of repressor - co-repressor complexes formed. As the levels of tryptophan increase, the level of attenuation increases. This is when an increasing amount of transcribed tryptophan mRNA is incomplete, and only consists of the element which corresponds to the 5' end of Trp-L, because an increase in tryptophan levels also causes transcription of the tryptophan operon to terminate prematurely. Attenuation is dependent on the 5' end of the mRNA product. A fourteen amino acid leader open reading frame is found upstream of the coding region for the tryptophan biosynthesis enzymes. The attenuator, which follows this short open reading frame, can have one of three different hairpin structures. Because transcription and translation are tightly coupled in bacteria, translation of the tryptophan mRNA starts very quickly after synthesis of the ribosome binding site. When enough tryptophan is available, the ribosome can translate the leader region of the mRNA product. Translation is rapid, and results in the formation of a stem-loop hairpin structure in the attenuator region. The hairpin structure causes the release of RNA polymerase from the DNA, and hence terminates transcription. The fourteen amino acid leader has two adjacent tryptophan residues. There is little tryptophanyl-tRNA present when less tryptophan is available, which causes the ribosome to stall when it encounters the tandem tryptophan UGG codons. This causes the downstream mRNA to be exposed as transcription proceeds. Transcription is able to continue through the coding regions for the enzymes, as an RNA structure is formed which does not act as a terminator, so the enzymes necessary for tryptophan synthesis can still be synthesised.

In *E. coli,* transcription may be paused by the anti-antiterminator structure. Transcription can then be reinitiated by the binding of the ribosome at the Trp-L mRNA start codon. The movement of the ribosome interferes with the RNA pause hairpin, thereby causing the release of RNA polymerase, which is now free to continue movement along the DNA. If the majority of $tRNA^{Trp}$ is uncharged, it

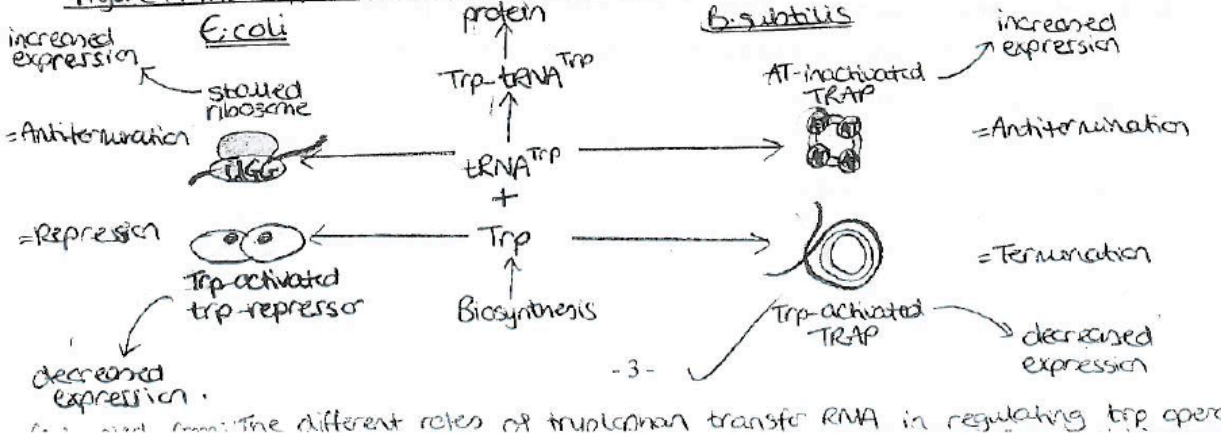
is more difficult for the adjacent tryptophan codons to be translated, which causes the ribosome to stall. This results in the formation of the antiterminator structure, which in turn prevents the terminator structure from forming. Therefore transcription is able to proceed, and the tryptophan gene is expressed. When the levels of charged $tRNA^{Trp}$ are higher, there is complete translation ofTrp-L. The ribosome subsequently dissociates when it reaches the Trp-L stop codon, which enables the formation of the anli-antiterminator and terminator hairpin structures from the leader transcript, resulting in transcription termination, and repression of the gene.

There is a different organisation of genes in the tryptophan operon in *B. subtilis* than in *E. coli.* In *B. subtilis,* there are seven tryptophan genes, six of which form the tryptophan suboperon, which lies within an aromatic supraoperon. There are two promoters, one at the start of the aromatic supraoperon and one just before Trp-E, which are involved in the initiation of transcription of the tryptophan suboperon. Transcription is regulated by attenuation by the tryptophan-activated RNA-binding attenuator protein, TRAP. Transcription of the section before Trp-E can result in the formation of either an antiterminator or a terminator hairpin structure. The structure formed is determined by TRAP. If TRAP has been activated by tryptophan, it prevents the formation of the antiterminator structure by binding to the RNA segment that forms the antiterminator. Therefore the terminator structure forms, and hence transcription is terminated. If tryptophan is available. But TRAP has not terminated transcription, the transcription ofTrp-E can be prevented by the formation of a hairpin structure. This results in a decrease in the levels of production of anthramlate synthase, which is the enzyme which catalyses the first reaction in the tryptophan biosynthetic pathway. The *at* operon detects low levels of charged tRNATrp. When there is an increase in levels of uncharged $tRNA^{Trp}$, an anti terminator forms, which prevents the formation of the terminator, so transcription is able to proceed. Anti-TRAP protein is the product of the *at* operon, and binds specifically to tryptophan-activated TRAP, If the anti-TRAP protein is bound at TRAP, TRAP is not able to bind its target RNA, so there is less termination of transcription in the leader region of the tryptophan operon. Figure 1 summarises the different methods of tryptophan expression in *E. coli* and *B. subtilis*.

Extra!

and B. subtilis.

figure 1: The different methods of tryptophan expression in E.coli and B. subtilis



The different roles of tryptophan transfer RNA in regulating trp operon

In conclusion, the tryptophan operon is negatively controlled, and the genes for tryptophan biosynthesis are not expressed when the cell has tryptophan available for use. In this instance, a repressor binds the operon, which blocks DNA transcription, because RNA polymerase cannot access the promoter, and it is not free to move along the template DNA. The DNA reading head structures on the repressor allosterically reorientate in response to tryptophan binding, which enables the operator to bind to the repressor, thereby repressing tryptophan biosynthesis. Furthermore, hydrogen bonds formed between tryptophan and the DNA phosphate group strengthen the interaction between the repressor and operator, so increase the extent of repression.
Attenuation is an additional mechanism by which tryptophan biosynthesis is regulated. When tryptophan levels are higher, transcription of the operon is prematurely terminated. When there is less tryptophan available, there is less tryptophanyl-tRNA. so the ribosome stalls when it reaches tandem tryptophan codons, which exposes downstream mRNA, so trancription can continue. There are different mechanisms of attenuation in *E. coli* and *B. sublilis.* In *E. coli,* gene repression occurs by the formation of terminator and anli-antiterminator hairpin structures, which both terminate transcription. On the other hand, In *B. subtilis,* TRAP, which is activated by tryptophan, prevents formation of the antiterminator, so the terminator forms, thereby terminating transcription. However, both mechanisms result in the negative control of the tryptophan operon, a process which is crucial in ensuring that the genes for tryptophan biosynthesis are not unnecessarily expressed when tryptophan is already available, thereby conserving energy for other bacterial processes.

References

Pollard, T., Eamshaw, *W. ,* Lippincott-Schwartz, J. (2004) *Cell Biology.* 2nd Edition. Philadelphia, Saunders Elsevier

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Waiter, P. (2002) *Molecular Biology of the Cell,* Fourth edition. London, Garland Science.

Yanofsky. C (2007) RNA-based regulation of genes of tryptophan synthesis and degradation, in bacteria. *RNA.* [Online] 13 (8), 11 41-1154. Available form: Web of Knowledge [Accessed 6th April 2008]

Yanofsky, C (2004) The different roles of tryptophan transfer RNA in regulating trp operon expression in E-coli versus B-subtilis. *Trends in Genetics.* [Online] 20 (8) 367-374. Availables from. Web of Knowledge [Accessed 6th April 2008]