Post-transcriptional Regulation

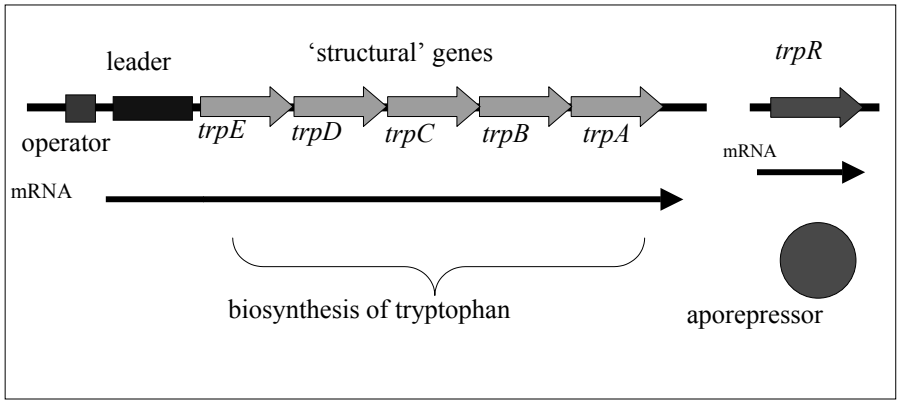
- gene expression can be controlled at stages following the initiation of RNA synthesis.
- rare in prokaryotes
- During transcription
  - anti-termination/attenuation
  - example: trp biosynthesis operon
- At initiation of translation
  - antisense RNA
  - example: *ompR* and *ompF*
- During translation
  - translational coupling
  - example: *ompR-envZ*

Tryptophan biosynthesis

- biosynthetic operon
- negatively regulated in response to presence of tryptophan
- operon regulated at initiation of transcription and during transcription
- TrpR repressor with tryptophan (trp) as co-repressor affect transcription initiation
- TrpR-trp negatively regulate by binding to operator
- when trp absent aporepressor (TrpR) not bind operator
- TrpR-trp negatively autoregulate unlinked *trpR* gene
  - why neg autoreg as need TrpR present when trp present?
  - quick response when trp appear?

first the transcriptional control……

No tryptophan



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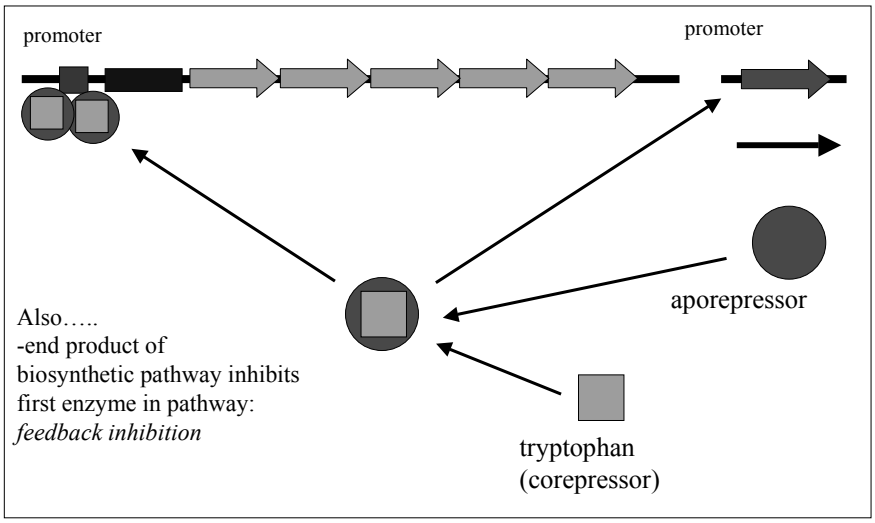
Tryptophan present



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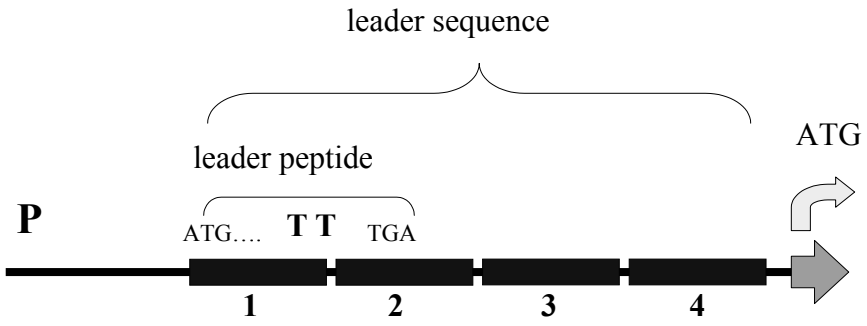
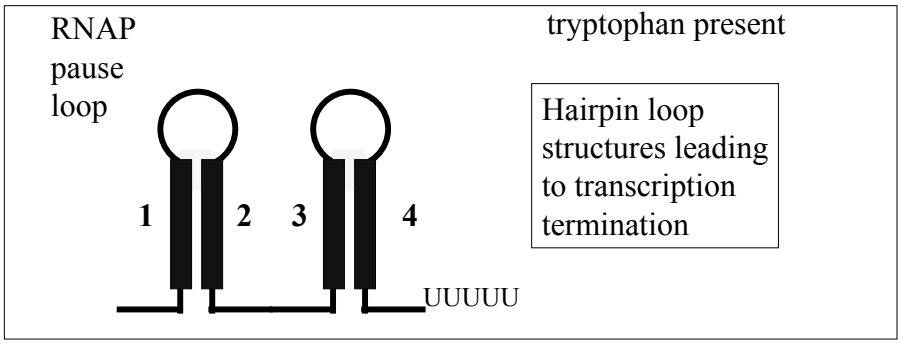
transcriptional attenuation

- transcription of structural genes (*trpA-E*) also controlled by attenuation
- after initiation of transcription from promoter
- termination of transcription at leader sequence
- level of tryptophan available to ribosome determines if transcription termination loop formed
- several other *Ec* biosynthetic operons controlled this way (*his*, *leu*, *thr*, *phe*….) also tRNA synthetase in *B. subtilis*.

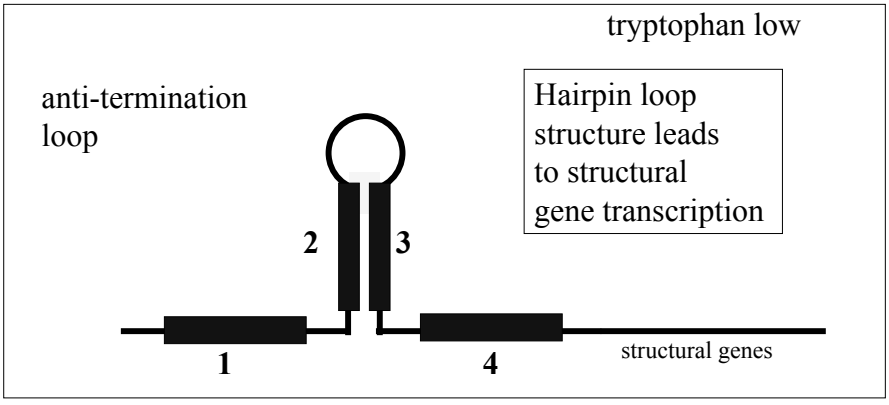
Stem loop structures



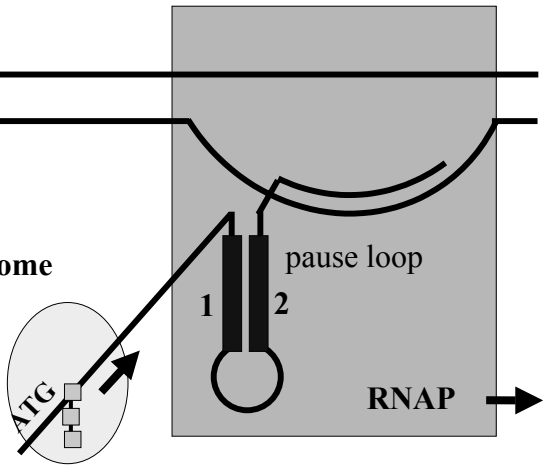
Hairpin loops with tryptophan present



Loops when tryptophan levels are low….

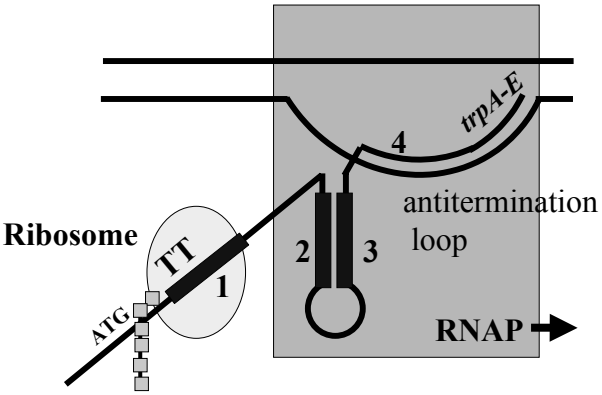


Transcription and translation start…



- Transcription starts
- RNAP makes mRNA
- Ribosome begins translation
- Pause loop forms
- RNAP pauses
- Ribosome 'catches up'

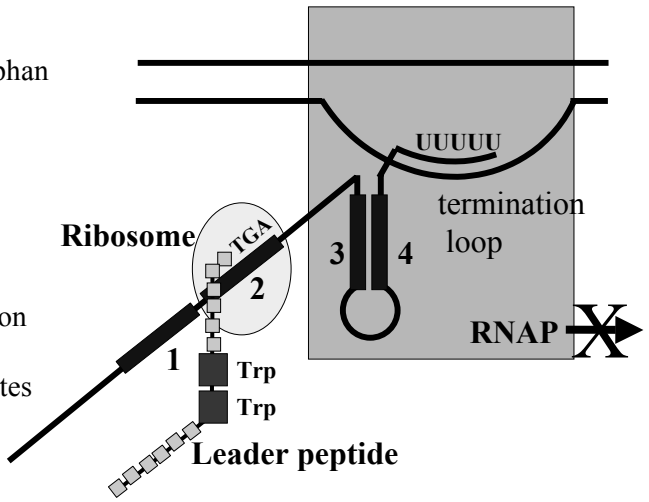
When levels of tryptophan are low……



- Ribosome encounters codons encoding tryptophan
- low levels of tryptophan means low levels of tryptophanyl-tRNA
- Ribosome temporarily stalls
- Ribosome not open 2-3 hairpin loop
- RNAP progresses thru past stem 4 and into structural genes

When levels of tryptophan are high……

- Ribosome encounters codons encoding tryptophan
- high levels of tryptophan means high levels of tryptophanyl-tRNA
- Ribosome quickly moves thru codons
- Ribosome opens 2-3 hairpin loop
- 3-4 hairpin termination loop forms
- transcription terminates



Antisense RNA

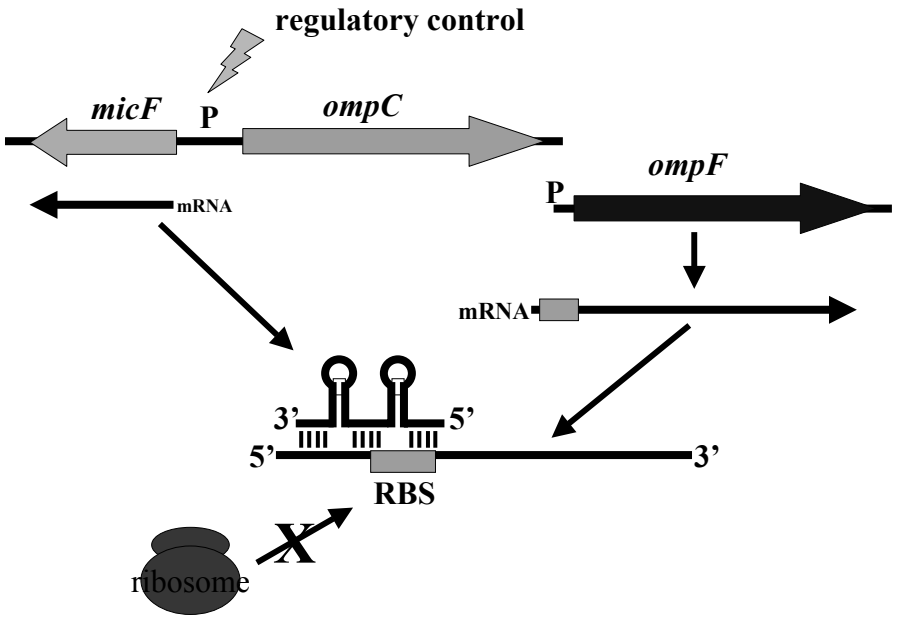
- Copy number control in plasmids!
- Control of transposition in Tn10
- Also examples in gene expression
  - lambda late gene expression
  - control of *ompF* expression
- Antisense RNA thought to provide another point where other regulators can affect porin expression.
- temperature host *vs* environment
- Larger porin size make small molecules that are detrimental to cell more likely to enter
- Not necessarily change osmolarity and be detected by EnvZ
- examples –antibiotics, bile salts, salicylate, oxygen stress, EtOH

Genetic evidence of post transcriptional control

- increase in *micF* expression reduces *ompF* expression
- but deletion of *micF* not greatly affect *ompF* expression
- DNA sequence and position of *micF* gives clue to function

micF

- *micF* gene transcribed divergently from *ompC*
- 93bp mRNA
- ~70% complementry to RBS of *ompF* mRNA
- controls translation initiation
- responds to regulators: SoxRS, MarA and also OmpR



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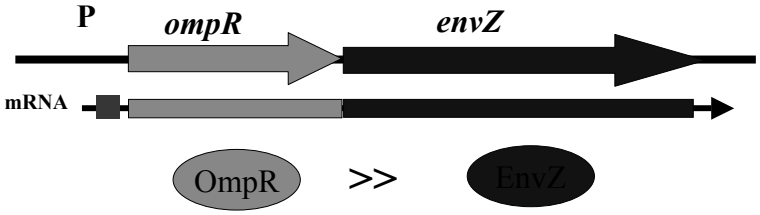
translational coupling



- In OmpR-EnvZ system:
  - 1000 OmpR molecules/cell
  - 10 EnvZ molecules/cell
  - co-transcribed from one promoter
  - one transcript
- Evidence links to translation control
  - EnvZ translation requires translation of OmpR
  - OmpR-LacZ & EnvZ-LacZ translational fusions show OmpR>EnvZ by 8x

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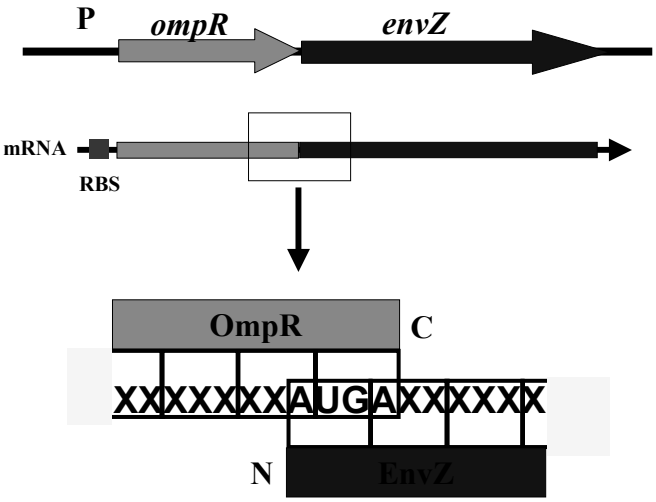
ORFs of OmpR and EnvZ overlap..



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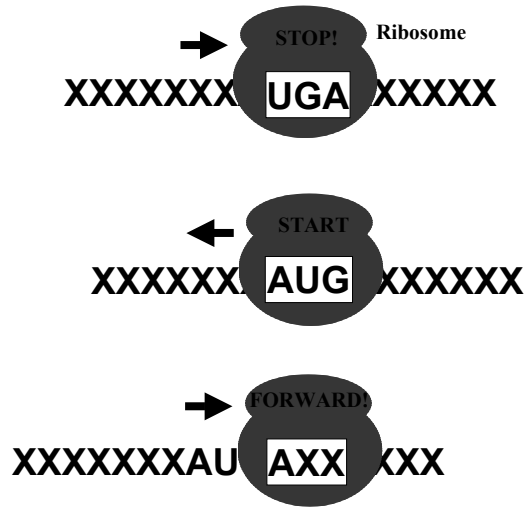
One step forward, one step back…..



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translational coupling (cont.)

- poor re-initiation of translation leads to polar effect on expression
- fine control of expression levels but fixed
- other examples: trp operon, gal operon in Ec
- additional internal RBS can be used to increase transl of downstream genes

To summarise…

- examples of how bacterial gene expression can be controlled following initiation of transcription
  - Attenuation
  - Antisense RNA
  - Translational coupling
- Attenuation/Antisense additional mechanisms bacteria can use to respond to environ. change
- In contrast to Eukaryotes, post-transcriptional control uncommon in bacteria