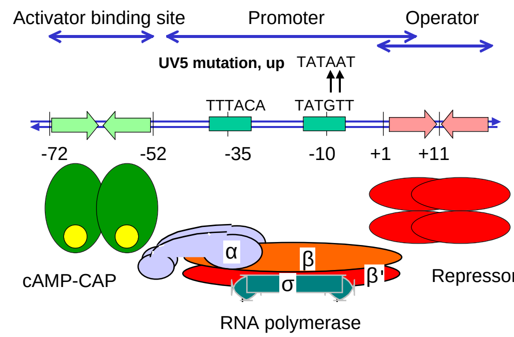
Regulation after initiation

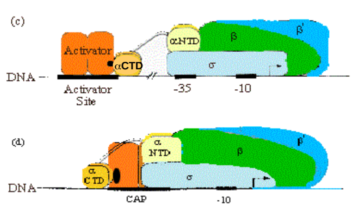
Antitermination of transcription: λ

Attenuation in biosynthetic operons: *trp*

lac regulatory region



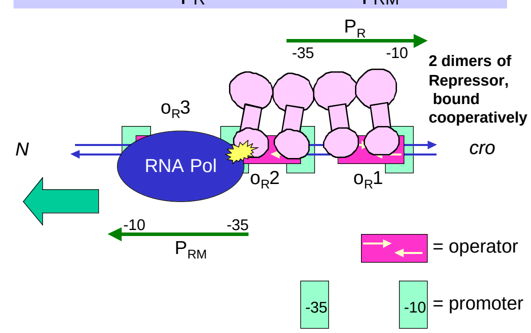
The CTD of the alpha subunit of RNA Pol can interact with activators



Class I promoters: CAP binding sites upstream of -35, E.g. centered at -62, -83, -93.

Class II promoters: CAP binding sites centered at -42, Overlaps -35 box.

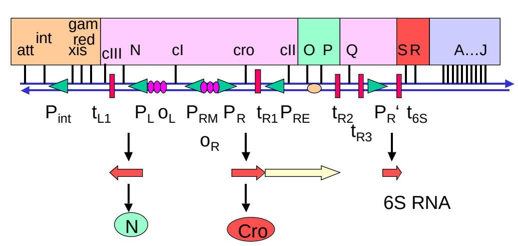
Binding of repressor blocks transcription from p_R but activates p_{RM}



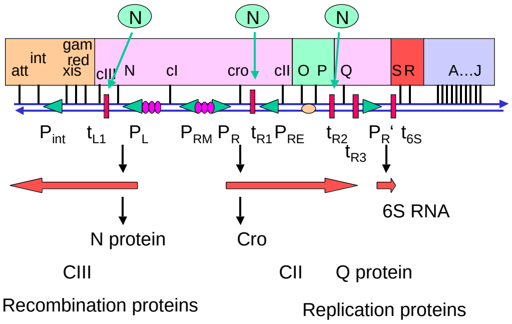
Antitermination occurs at two stages in the λ life cycle

Immediate early transcription

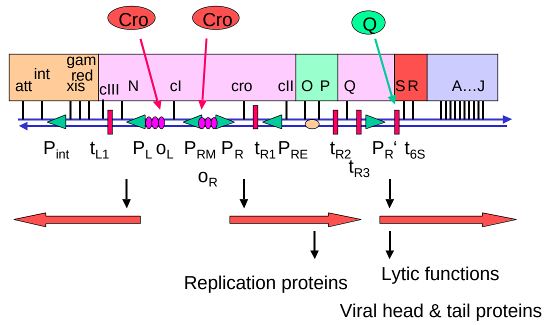
Transcription by E. coli RNA polymerase initiates at strong promoters P_R , P_R', and P_L , and terminates at t's.



Antitermination by N protein leads to early gene expression



Lytic cascade: Cro turns off *cI*, Q protein action leads to late gene expression



Review of ρ-dependent termination of transcription

Termination of transcription in *E. coli*: Rho-**dependent** site

5' ...AUCGCUACCUCAUAUCCGCACCUCCUCAAACGCUACCUCGACCAGAAAGGCGUCUCUU

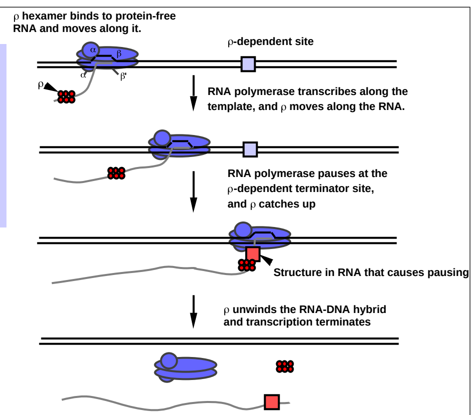
Termination occurs at one of these 3 nucleotides.

- Little sequence specificity: rich in C, poor in G.
- Requires action of rho (ρ) *in vitro* and *in vivo*.
- Many (most?) genes in *E. coli* have rho-dependent terminators.

Rho factor, or ρ

- Rho is a hexamer, subunit size is 46 kDa
- Is an RNA-dependent ATPase
- Is an essential gene in *E. coli*
- Rho binds to **protein-free** RNA and moves along it (tracks)
- Upon reaching a paused RNA polymerase, it causes the polymerase to dissociate and unwinds the RNA-DNA duplex, using ATP hydrolysis. This terminates transcription.

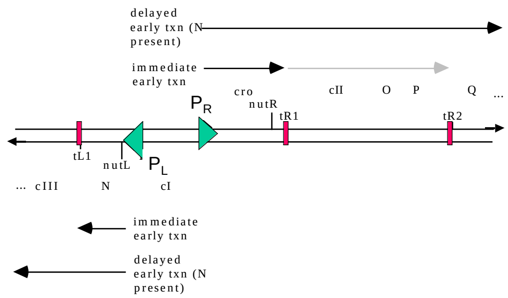
Model for action of rho factor



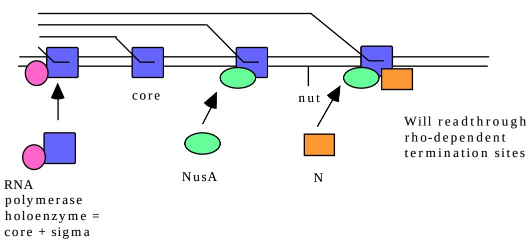
Components needed for antitermination

- Sites on DNA
  - *nut* sites (N utilization sites) for N protein, *qut* sites for Q protein
  - Are found **within** the transcription unit
  - *nut* sites are 17 bp sequences with dyad symmetry
- Proteins
  - Antiterminators: N protein and Q protein encoded by λ
  - Host proteins (encoded by *E. coli*)
    - Nus A (encoded by *nusA*, N-utilization substqance)
    - Rho protein

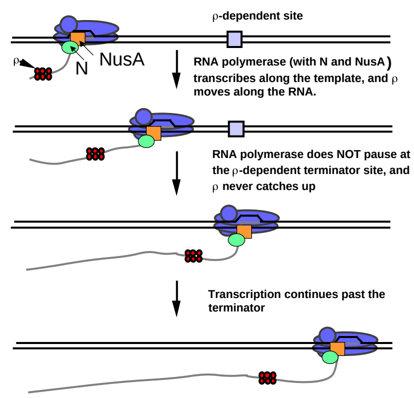
Arrangement of *nut* sites in transcription units



Model for antitermination by N protein



N plus Nus factors block rho action

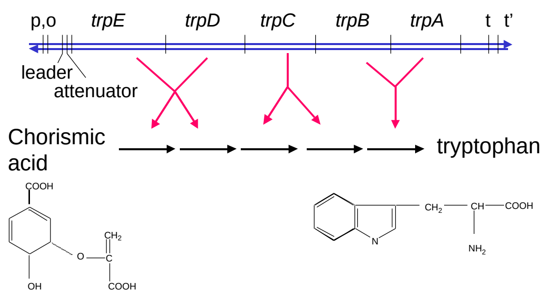


NusG and elongation

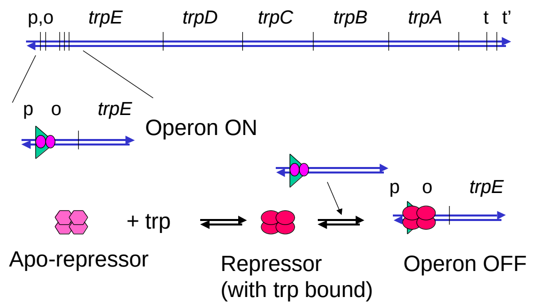
- NusG is another *E. coli* protein needed for lambda N to prevent termination
- Homolog of a family of proteins involved in elongation in prokaryotes and eukaryotes
- Eukaryotic DSIF
  - DRB-sensitivity inducing factor (Flies and mammals)
    - DRB is a drug that blocks transcriptional elongation
  - Two subunits
    - 160 kDa, homolog to yeast Spt5
    - 14 kDa, homolog to yeast Spt4
  - Implicated in positive and negative control of elongation

Regulation of *E. coli trp* operon by attenuation of transcription

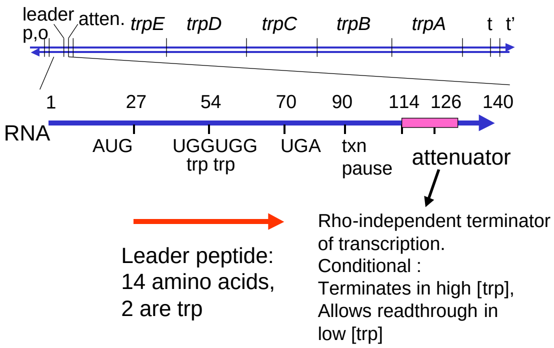
Organization of the *E. coli trp* operon



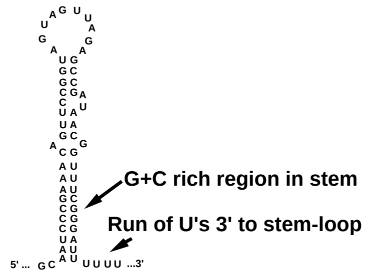
The *trp* operon is regulated in part by an apo-repressor



The *trp* operon is also regulated by attenuation



Termination of transcription in *E. coli*: Rho-**independent** site



How attenuation works

- The [trp] determines the [trp-tRNA].
- The [trp-tRNA] determines whether a translating ribosome will add trp to the leader peptide.
- If trp is added:
  - The ribosome moves on to the translation stop codon.
  - This places the attenuator in a secondary structure that causes termination of transcription (OFF).
- If trp is not added:
  - A different secondary structure forms in the leader RNA
  - Allows readthrough transcription into the structural genes (ON).

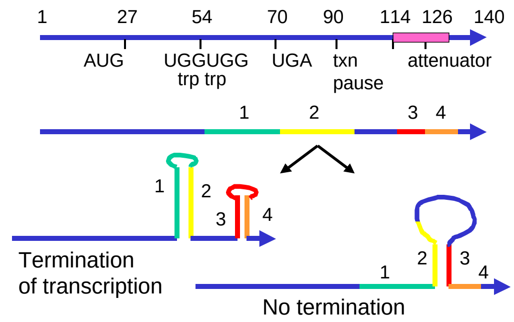
Basic components for attenuation in *trp*

[trp-tRNA]	translation of trpL	secondary structures formed in RNA	Attenuator	Operon
High	complete	3-4 stem	terminate txn	OFF
Low	stalls at trp codons	2-3 stem	allow read-through txn	ON

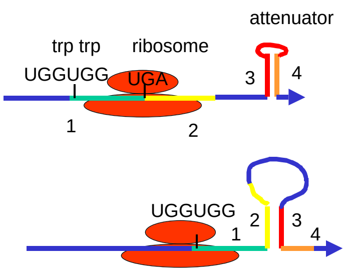
Requirements for attenuation in *trp* operon

- Simultaneous transcription and translation.
- A segment of RNA that can serve as a terminator because of its base-paired (secondary) structure.
- An alternative secondary structure in the RNA that does not allow termination of transcription.
- Does **NOT** need an additional protein, such as a repressor.

Alternative base-paired structures in leader RNA



Progress of ribosome determines secondary structure of *trp* leader RNA



High [trp], termination of transcription

Low [trp], No termination

Examples of mutational analysis of *trp*

- Translation of *trp* leader is needed for regulation
  - Mutation of AUG prevents transcription past the attenuator
  - Without translation, the 1:2 and 3:4 stem-loops form, and thus causing termination
- Specific secondary structures are needed
  - Mutations that decrease the number of base pairs in the 3:4 stem-loop increase expression (less termination) in high [trp].
  - Compensatory mutations that restore the wild-type number of base pairs allow termination in high [trp].

Many biosynthetic operons are regulated by attenuation

- Amino acid biosynthetic operons
- E.g., *his, phe, leu, thr, ilv*
- In each case, a short leader RNA and polypeptide precede the structural genes. This leader polypeptide is rich in the amino acid that is the product of the pathway.