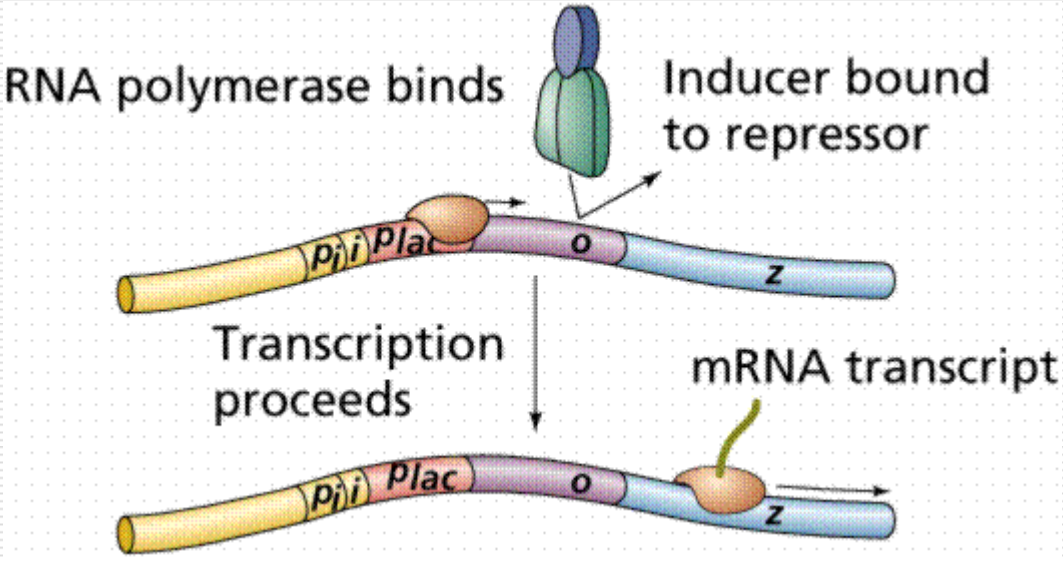
Module 3
Regulation of Gene Expression in Prokaryotes

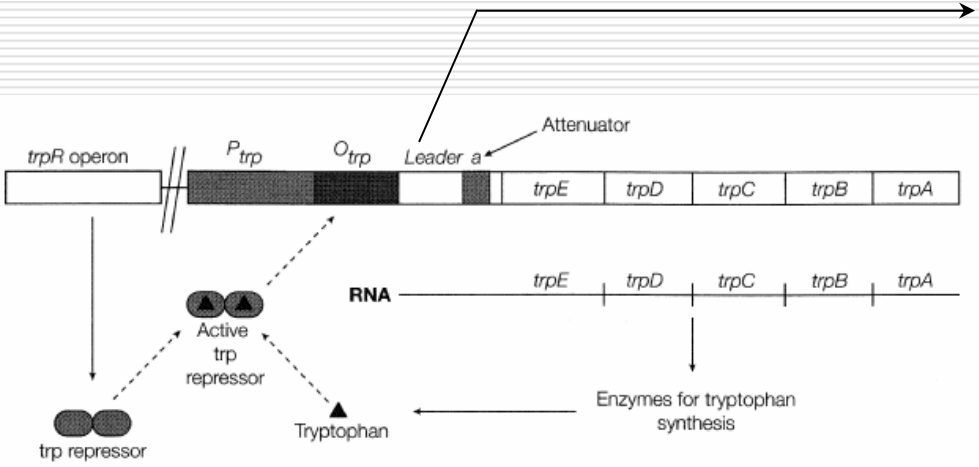
Recap- lac operon

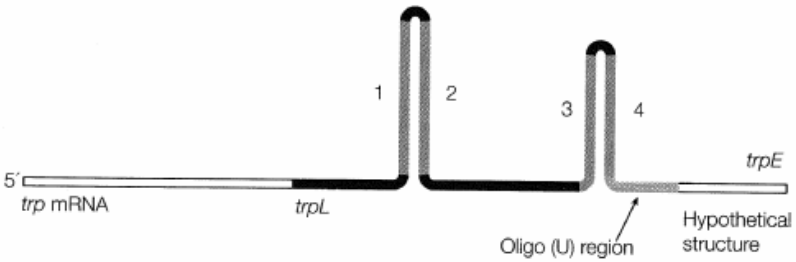
- The *lac* operon is an inducible catabolic operon. Allolactose (a lactose derivative) modifies a repressor protein, allowing transcription.

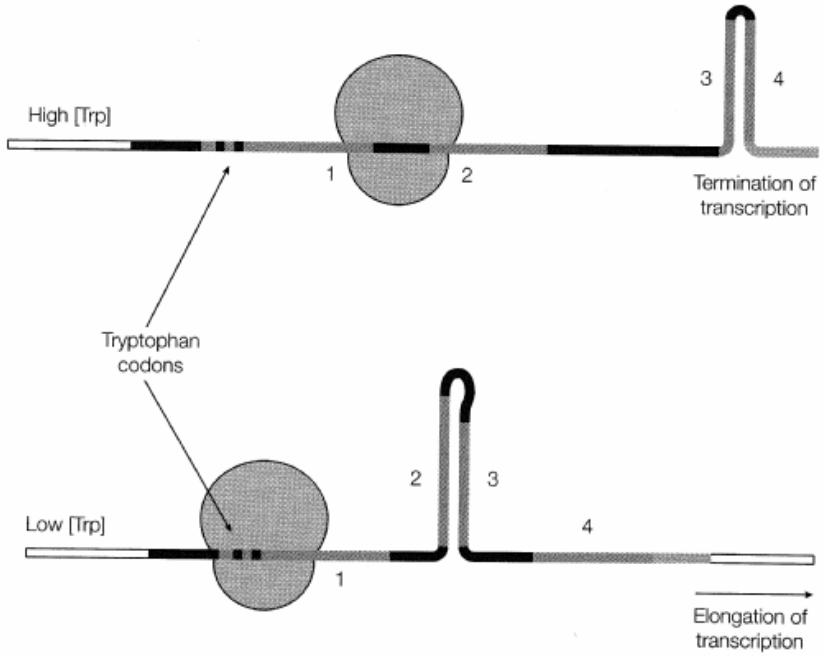


Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates

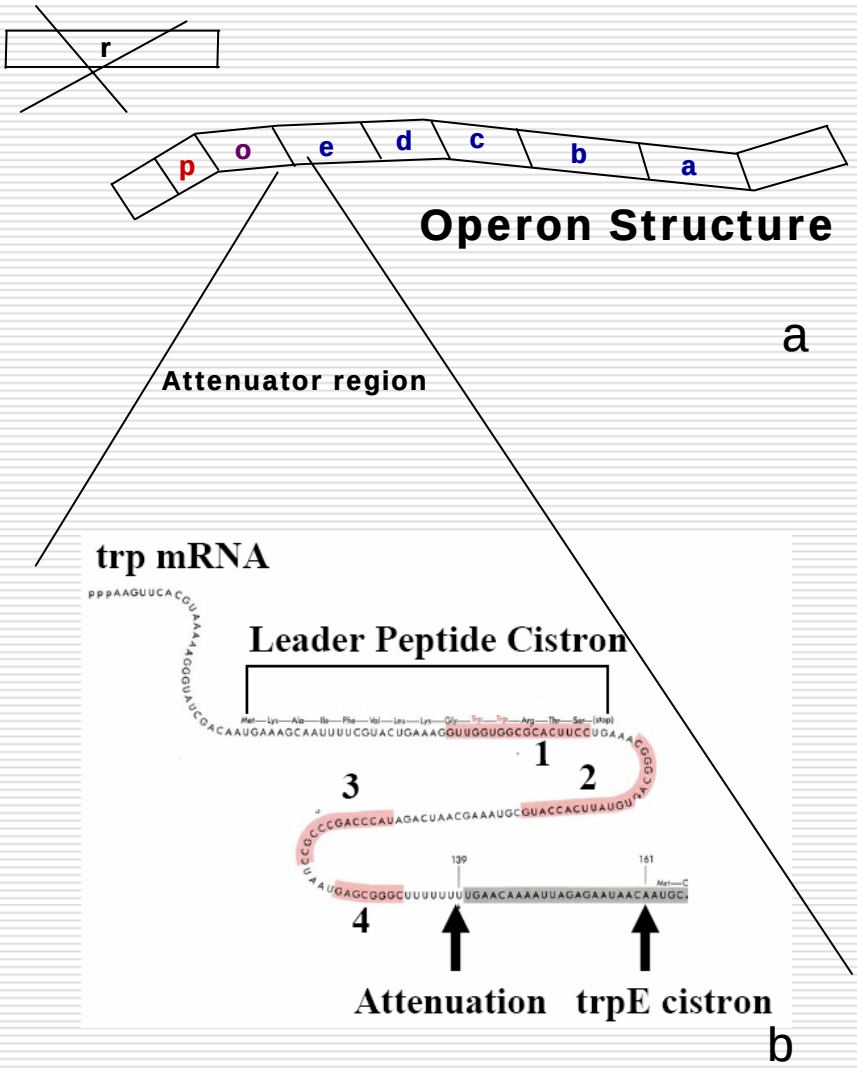
Recap- Summary of Trp gene regulation

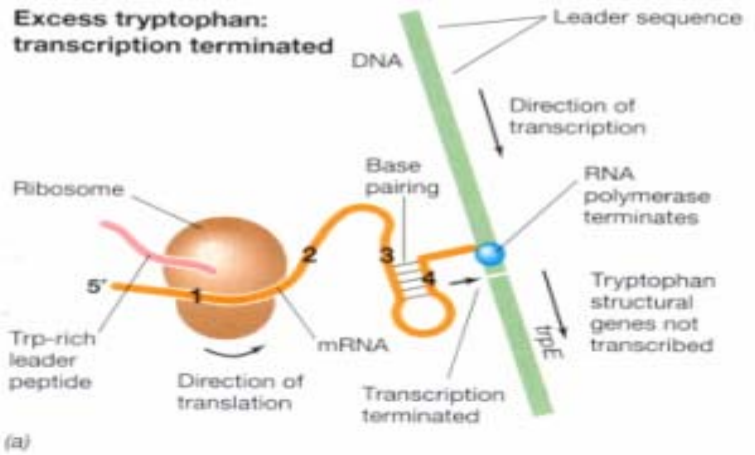


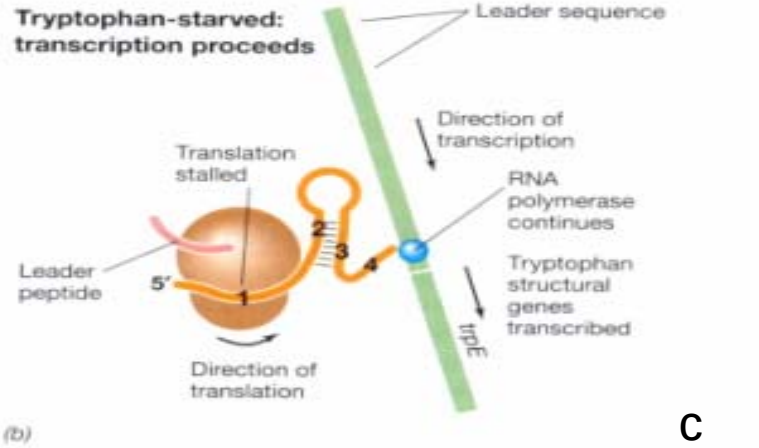




Structure of Trp operon and function of the Trp repressor



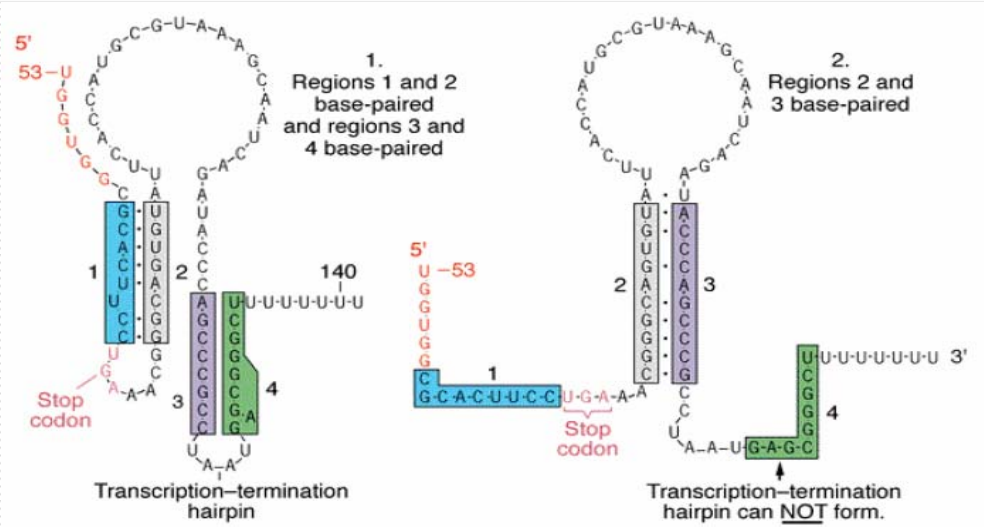




Mechanism of attenuation. Control of transcription of tryptophan operon structural genes by attenuation in *Escherichia coli*. The leader peptide is encoded by regions 1 and 2 of the mRNA. Two regions of the growing mRNA chain are able to form double-stranded loops, shown as 2:3 and 3:4. (a) Under conditions of excess tryptophan, the ribosome translates the complete leader peptide, and so region 2 cannot pair with region 3. Regions 3 and 4 then pair to form a loop that terminates RNA polymerase. (b) If translation is stalled because of tryptophan starvation, loop formation via 2:3 pairing occurs, loop 3:4 does not form, and transcription proceeds past the leader sequence.

Recap of last lecture

- The *trp* operon is a repressible anabolic operon. Tryptophan (the end product of the biosynthetic pathway, and co-repressor of this operon) binds to the repressor and prevents transcription.
- It is also regulated by an attenuation system. Tryptophan levels govern the formation of an early transcription termination stem-loop structure in a leader sequence.



New lecture – Lecture 4

Induction vs Repression as transcriptional regulation

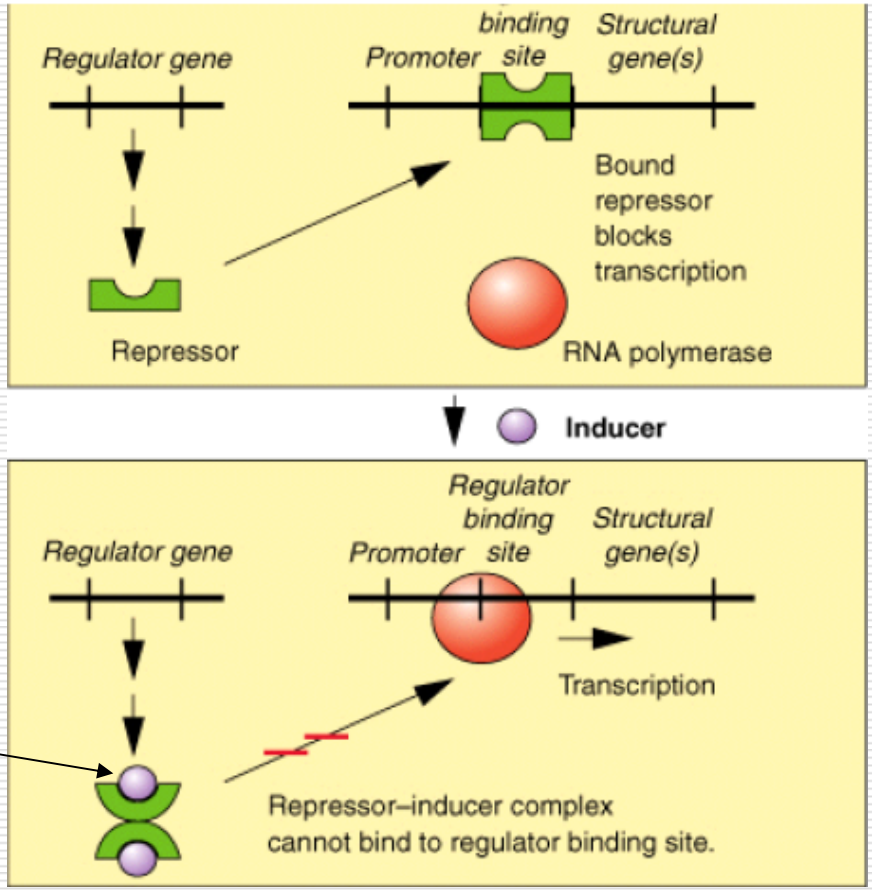
- So far, we have seen induction and repression of gene expression as methods of regulation.
- The terms relate to the impact of a particular small molecule on the gene group's level of expression.
  - Induction: The presence of the small molecule (inducer) turns transcription on (lac operon)- repressor binds to switch off the operon unless lactose (allolactose) is present
  - Repression: The presence of the small molecule (repressor, co-repressor) turns transcription off (trp operon)- repressor is activated by tryptophan & binds to switch off the operon (end-product inhibition)

Positive vs negative transcriptional regulation

- Negative control: a repressor is normally present to block transcription unless inactivated or activated by the presence of a small molecule.
  - Induction: The presence of the small molecule (inducer) turns transcription on (lac operon)- repressor binds to switch off the operon unless lactose (allolactose) is present
  - Repression: The presence of the small molecule (repressor, co-repressor) turns transcription off (trp operon)- repressor is activated by tryptophan & binds to switch off the operon (end-product inhibition)
- Positive control: an activator is required for transcription to occur. Ex. AraC operon

Induction by negative control

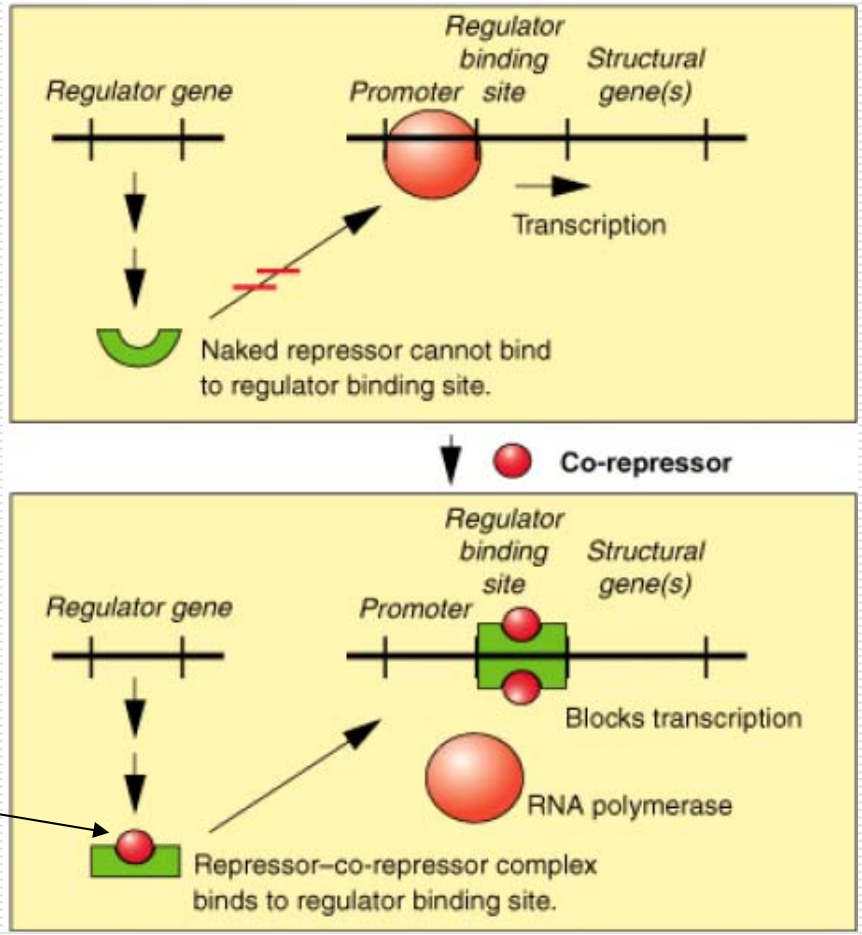
- The repressor is prevented from binding to the regulator binding site when the inducer is present.
  - Eg: the *lac* operon.



Eg Allolactose, inducer

Repression by negative control

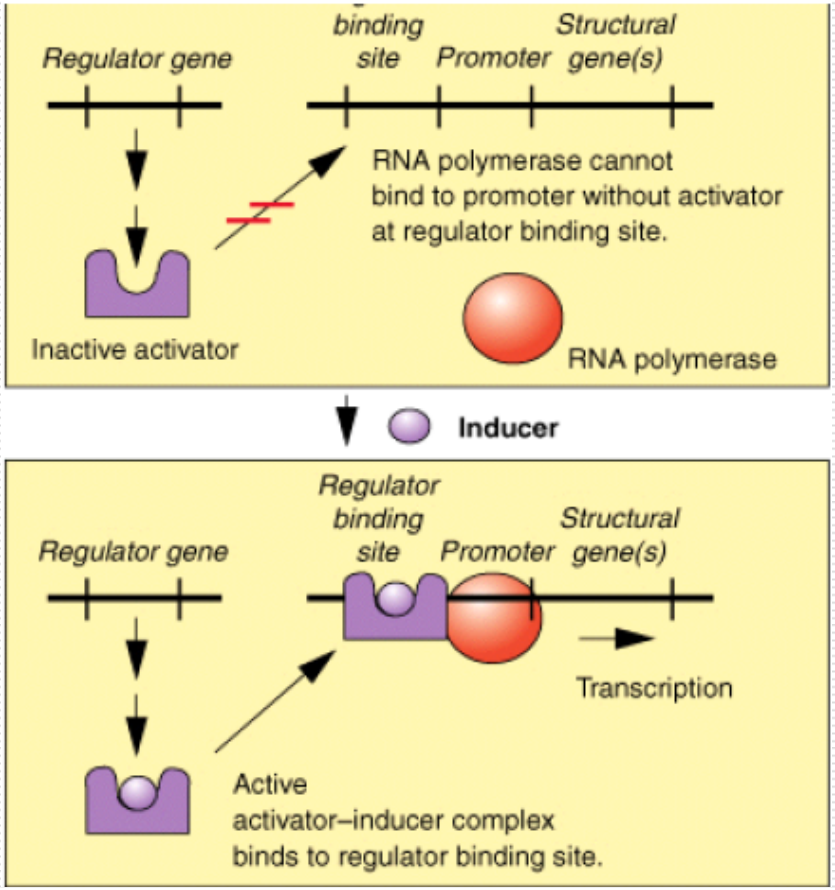
- The repressor-co-repressor complex binds to the regulator binding site and prevents transcription. Eg: the *trp* operon.



Eg Tryptophan, corepressor

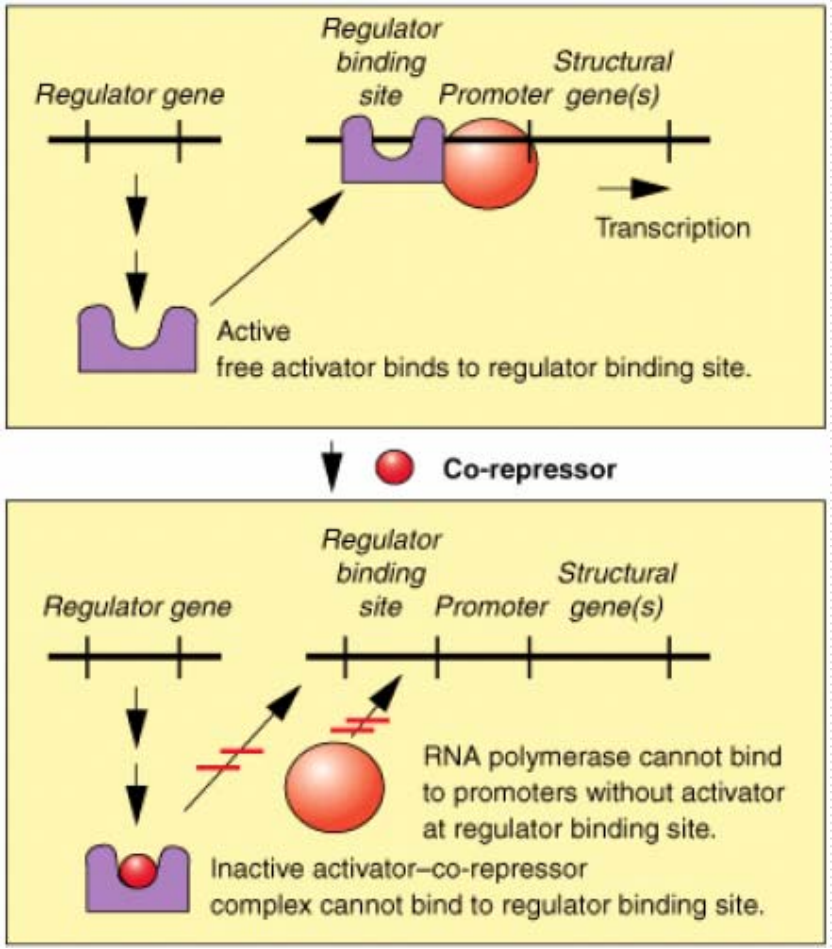
Induction by positive control

- The activator-inducer complex binds to the regulator binding site and allows transcription. Eg: the *ara* operon, catabolite repression.

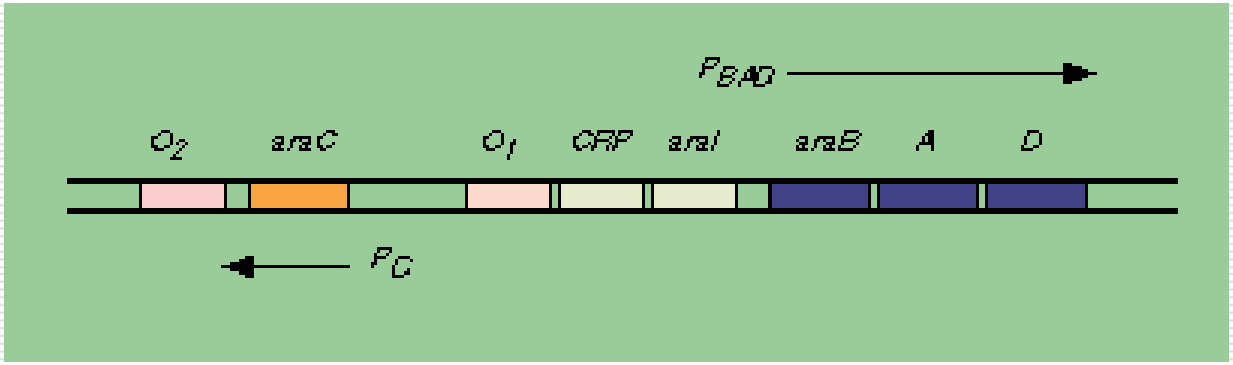


Repression by positive control

- The activator-repressor complex cannot bind to the regulator binding site, preventing transcription.

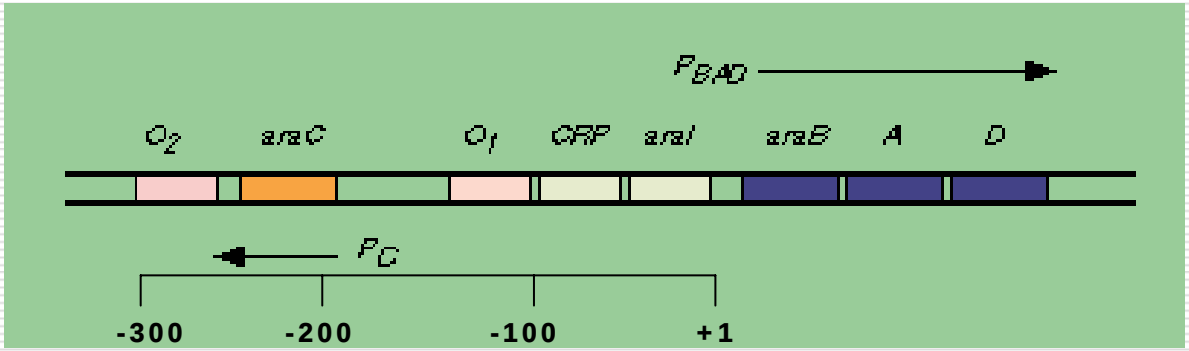


The arabinose operon- structure



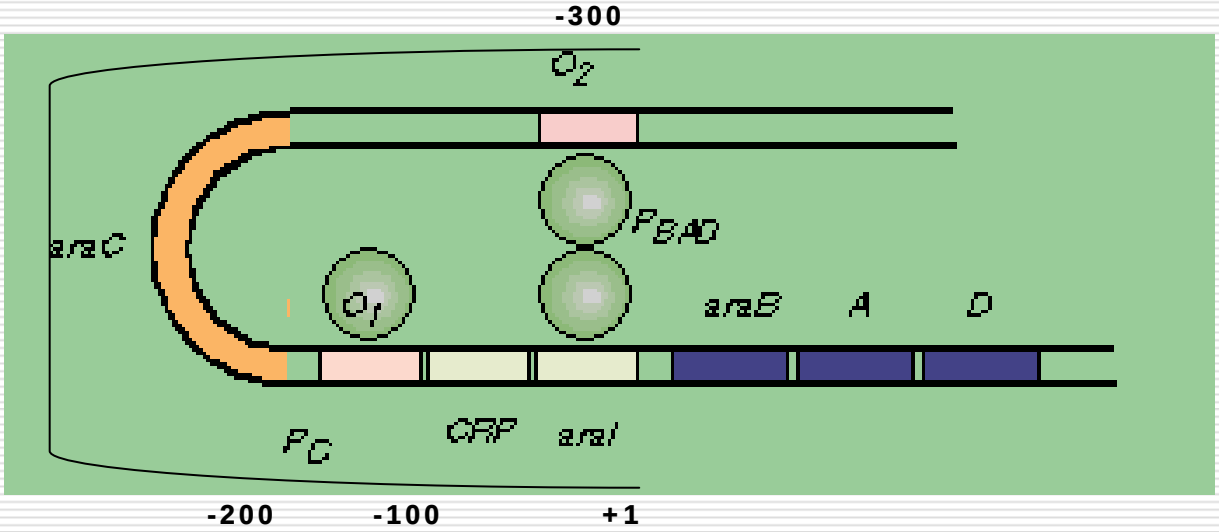
- The ***ara*** operon (known as pBAD) codes for three enzymes that are required to catalyze the metabolism of arabinose.
  - Arabinose isomerase - encoded by ***araA*** - coverts arabinose to ribulose
  - Ribulokinase - encoded by ***araB -***- phosphorylates ribulose
  - Ribulose-5-phosphate epimerase - encoded by ***araD -***- converts ribulose-5-phosphate to xylulose-5-phosphate which can then be metabolized via the pentose phosphate pathway.

The arabinose operon- structure (cont'd)



- The three pBAD structural genes are arranged in an operon that is regulated by the ***araC*** gene product (a regulator protein). There are four important regulatory sites:
  - ***$araO_1$*** is an operator site. **AraC** binds to this site and represses its own transcription from the P_C promoter. In the presence of arabinose, however, **AraC** bound at this site helps to activate expression of the P_{BAD} promoter.
  - ***$araO_2$*** is also an operator site. **AraC** bound at this site can simultaneously bind to the ***araI*** site to repress transcription from the P_{BAD} promoter
  - ***araI*** is also the inducer site. **AraC** bound at this site can simultaneously bind to the ***$araO_2$*** site to repress transcription from the P_{BAD} promoter. In the presence of arabinose, however, **AraC** bound at this site helps to activate expression of the P_{BAD} promoter.
  - **CRP** binds to the **CRP** binding site. It does not directly assist RNA polymerase to bind to the promoter in this case. Instead, in the presence of arabinose, it promotes the rearrangement of **AraC** when arabinose is present from a state in which it represses transcription of the P_{BAD} promoter to one in which it activates transcription of the P_{BAD} promote

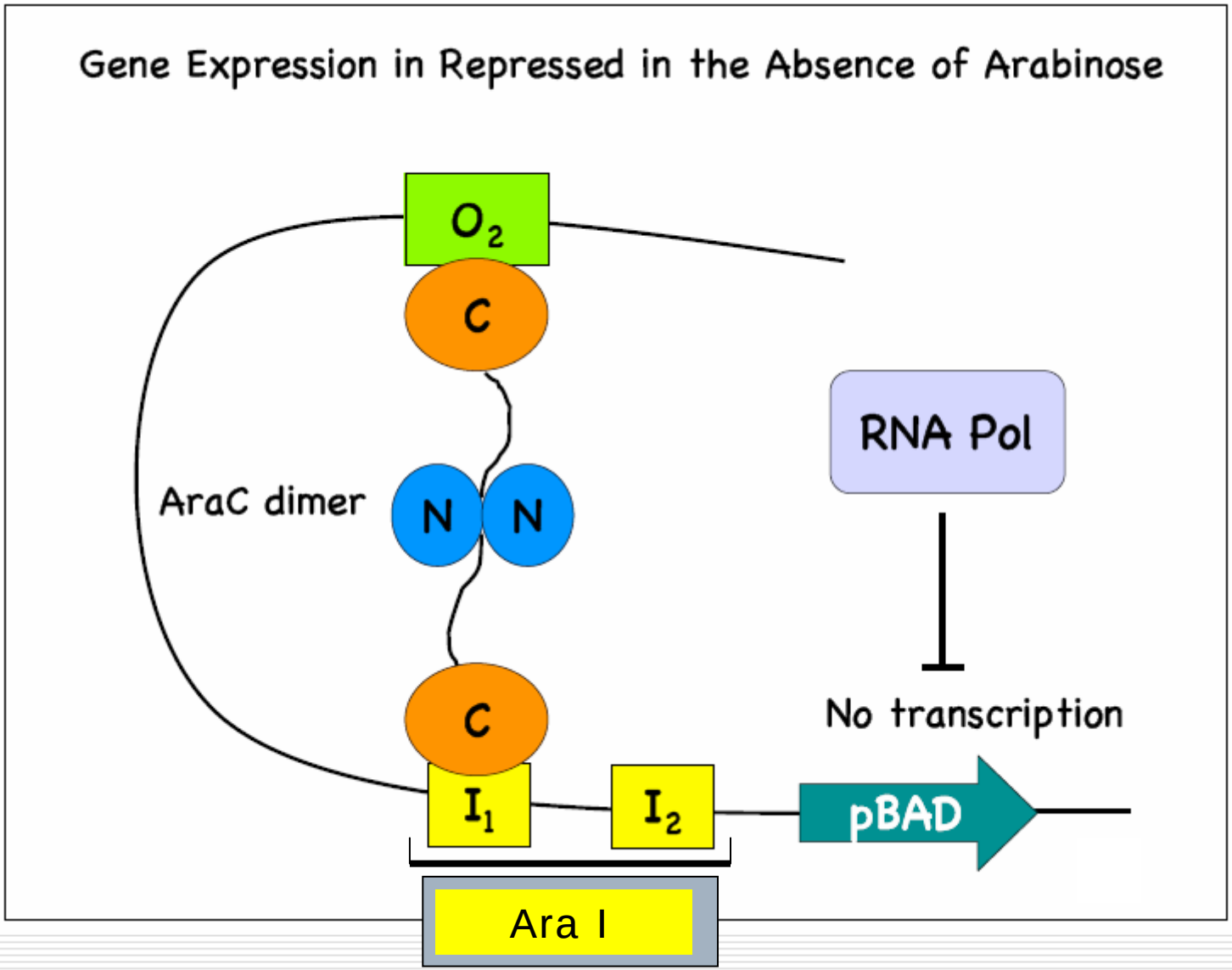
The arabinose operon- arabinose absent



Regulation of the arabinose operon is, clearly, much more complex than the lactose operon:

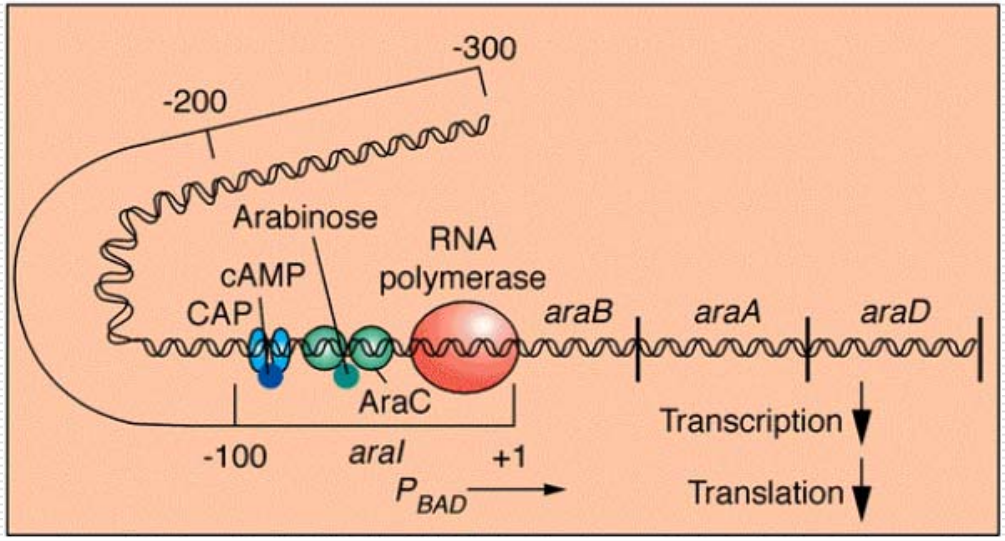
When arabinose is absent, there is no need to express the structural genes (negative regulation)

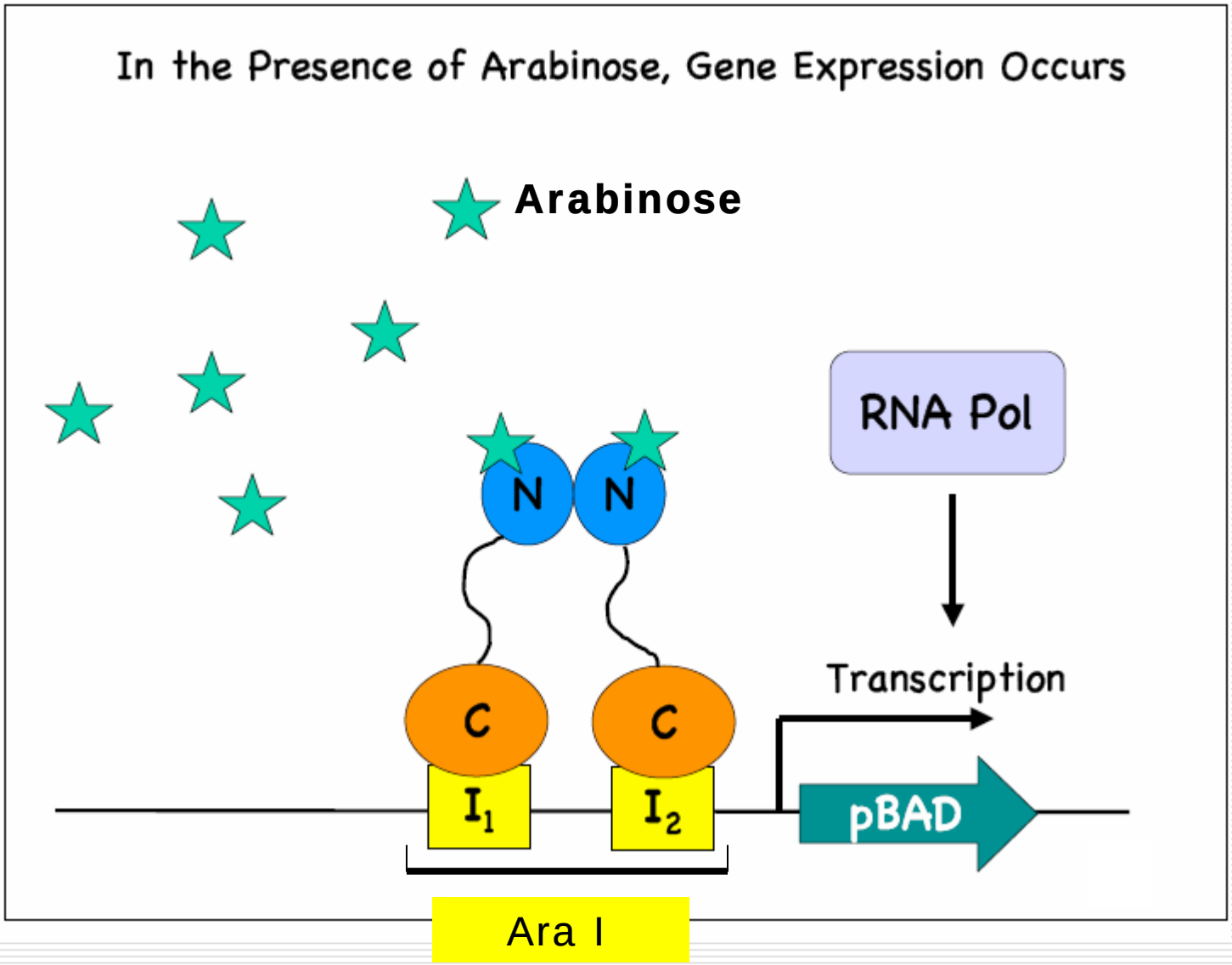
- **AraC** (dimer) does this by binding simultaneously to both ***araI*** and ***araO2***. As a result the intervening DNA is **looped**. These two events block access to the **PBAD** promoter which is, in any case, a very weak promoter (unlike the ***lac*** promoter).
- **AraC** also prevents its own expression. Thus, it is an autoregulator of its own expression. This makes sense; there is no need to over-express **AraC**. If the concentration falls too low then transcription of ***araC*** resumes until the amount of **AraC** is sufficient to prevent more transcription again.


Gene Expression in Repressed in the Absence of Arabinose
O2
C
AraC dimer
N
N
C
I1
I2
RNA Pol
No transcription
pBAD
Ara I

The arabinose operon- arabinose present

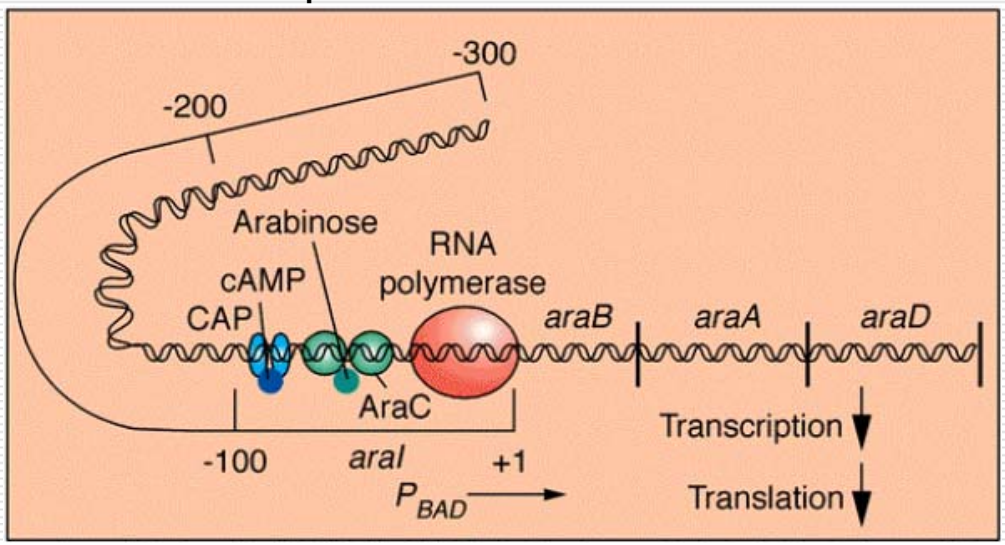
- **<u>In the presence of arabinose</u>**, AraC specificity is changed by an allosteric transition induced by binding of arabinose. The AraC duplex-arabinose complex binds preferentially to *araI*, not *araO*$_2$, activating transcription. Structural genes are expressed.
- This is <u>positive</u> regulation – induction.




In the Presence of Arabinose, Gene Expression Occurs
Arabinose
RNA Pol
N
N
C
C
I_1
I_2
Transcription
pBAD
Ara I

Catabolite repression and the *ara* operon.

- *Ara* is also regulated by catabolite repression via the Catabolite Activator Protein (CAP). In the presence of glucose, *ara* is not transcribed. High glucose ⇒ low cAMP ⇒ free CAP ⇒no activation by CAP-cAMP complex.
- Low glucose ⇒ high cAMP ⇒ CAP-cAMP complex. The complex binds at a binding site adjacent and upstream of *araI*, helping to open up the loop and improving the efficiency of arabinose-AraC binding to *araI*. Transcription is more efficient.



The arabinose operon- summary

- The AraC regulatory protein changes binding affinity depending upon the concentration of arabinose. Low arabinose leads to AraC duplexes binding to both *araO*$_2$ and *araI*, and the duplexes interact to form a DNA loop.
- In the presence of arabinose, binding affinity of the duplex changes, and it binds to *araI* and not *araO*$_2$. The DNA loop doesn't form, and transcription may occur.
- Formation of the DNA loop is less efficient in the presence of CAP-cAMP complex (formed when glucose is low) bound to the binding site adjacent to *araI*.