BIOL 2416

Chapter 17: Bacterial Operons

Regulation of Gene Expression in Bacteria

- Functionally related genes clustered in **operons** (Jacob and Monod)
- Operon genes transcribed together into **polycistronic mRNA** (operon = single promoter, shared **operator**, + operon genes)
- Strong selective pressure for avoiding making unnecessary proteins (don't want to slow cellular replication, lose evolutionary foothold)
- Short prokaryotic mRNA half lives mean transcriptional control very effective.

Operons are either under POSITIVE or NEGATIVE CONTROL

NEGATIVE:	POSITIVE:
Uses a **repressor**	Uses an **activator**

Repressors and activators are PROTEINS encoded by CONSTITUTIVE (= always on) REGULATORY genes.

Operons are either REPRESSIBLE or INDUCIBLE

REPRESSIBLE:	INDUCIBLE:
Involves binding a co-repressor (metabolite/end-product) to repress/turn OFF operon transcription	Involves binding an inducer (metabolite/starting substrate) to induce/turn ON operon transcription

3 combinations found in nature:

	REPRESSIBLE	INDUCIBLE
NEGATIVE	This operon uses a repressor that binds a co-repressor to repress txn.	This operon uses a repressor that binds an inducer to induce txn.
POSITIVE	Not found.	This operon uses an activator that binds an inducer to induce txn.

Example #1: The Lac operon of E. coli makes the three enzymes needed for lactose breakdown:

- **LacZ encodes Beta-galactosidase**
  - breaks up lactose into glucose and galactose (galactose also converted to glucose for metabolism)
  - Isomerizes lactose into allolactose inducer (presence of lactose means presence of allolactose)
- **LacY encodes permease**
  - For lactose transport across cell membrane
- **LacA encodes transacetylase**
  - Poorly understood function

The Lac operon has 2 control circuits:

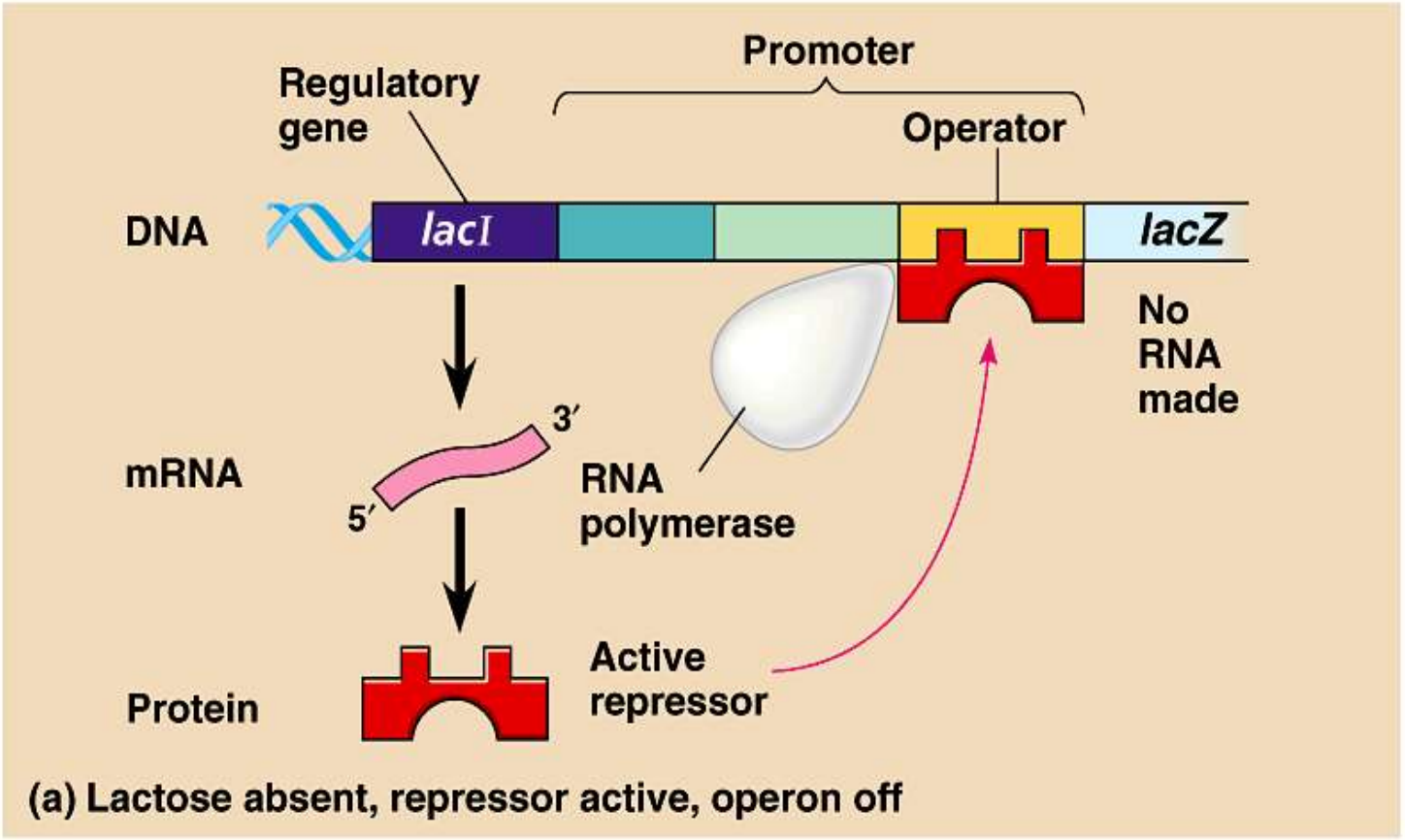
- **NEGATIVE INDUCIBLE**
  - Uses a **repressor** that binds an **inducer** (allolactose) to **induce operon txn**.
  - Car ignition
  - Requires presence of lactose
- **POSITIVE INDUCIBLE**
  - Uses an **activator** (CAP) that binds an **inducer** (cyclic AMP) to **induce operon txn**.
  - Gas pedal
  - Requires absence of (preferred) glucose

So how does it work?

First we'll need allolactose to start the car:

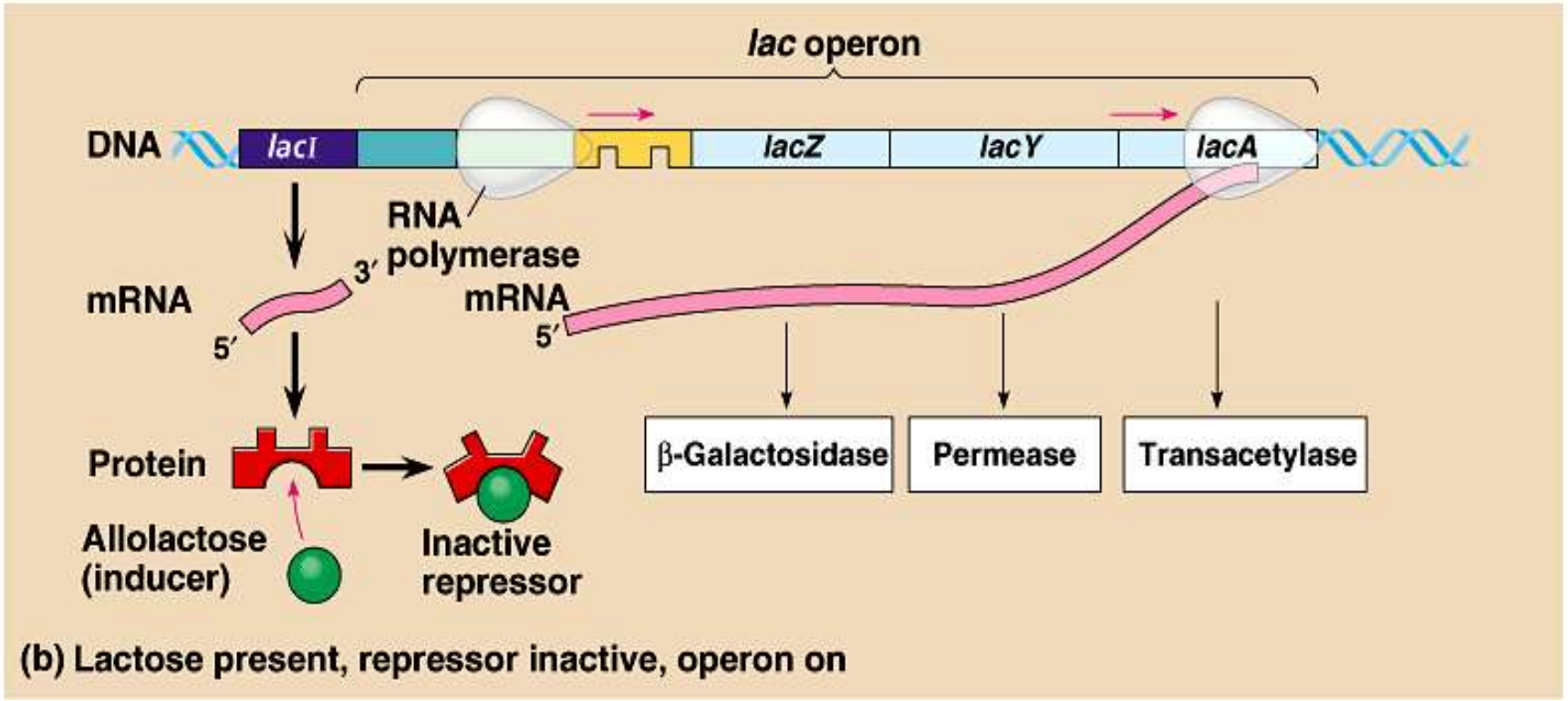
LAC OPERON NEGATIVE INDUCIBLE CONTROL CIRCUIT:

In the **absence of lactose**, an **active repressor** protein binds to the operator and **blocks transcription** by RNA Polymerase:



When **lactose is present** in the cell, **allolactose**, an isomer of lactose, binds to the repressor.

This **inactivates the repressor**, because it can no longer bind the operator. Now RNA Polymerase can **transcribe** the Lac operon:



But RNA Polymerase has low affinity for the Lac operon promoter….

so even though the Lac operon is turned on by the presence of lactose, it is transcribed at low levels (like your car merely starting to roll forward after the ignition key is turned).

So once the car is turned on, how do we step on the gas?

- **LAC OPERON POSITIVE INDUCIBLE CONTROL CIRCUIT**

  - If **glucose levels are low** (along with overall ATP energy levels), then **cAMP is high**.
  - **cAMP binds** to **CAP** (a.k.a. CRP) which **activates** Lac operon transcription.

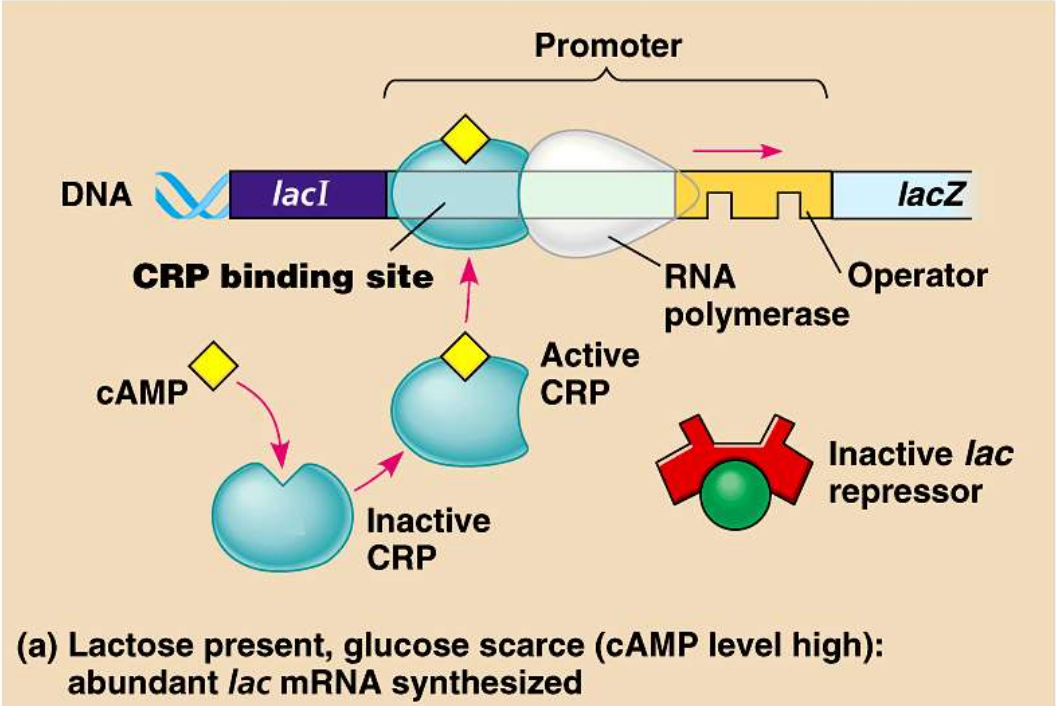
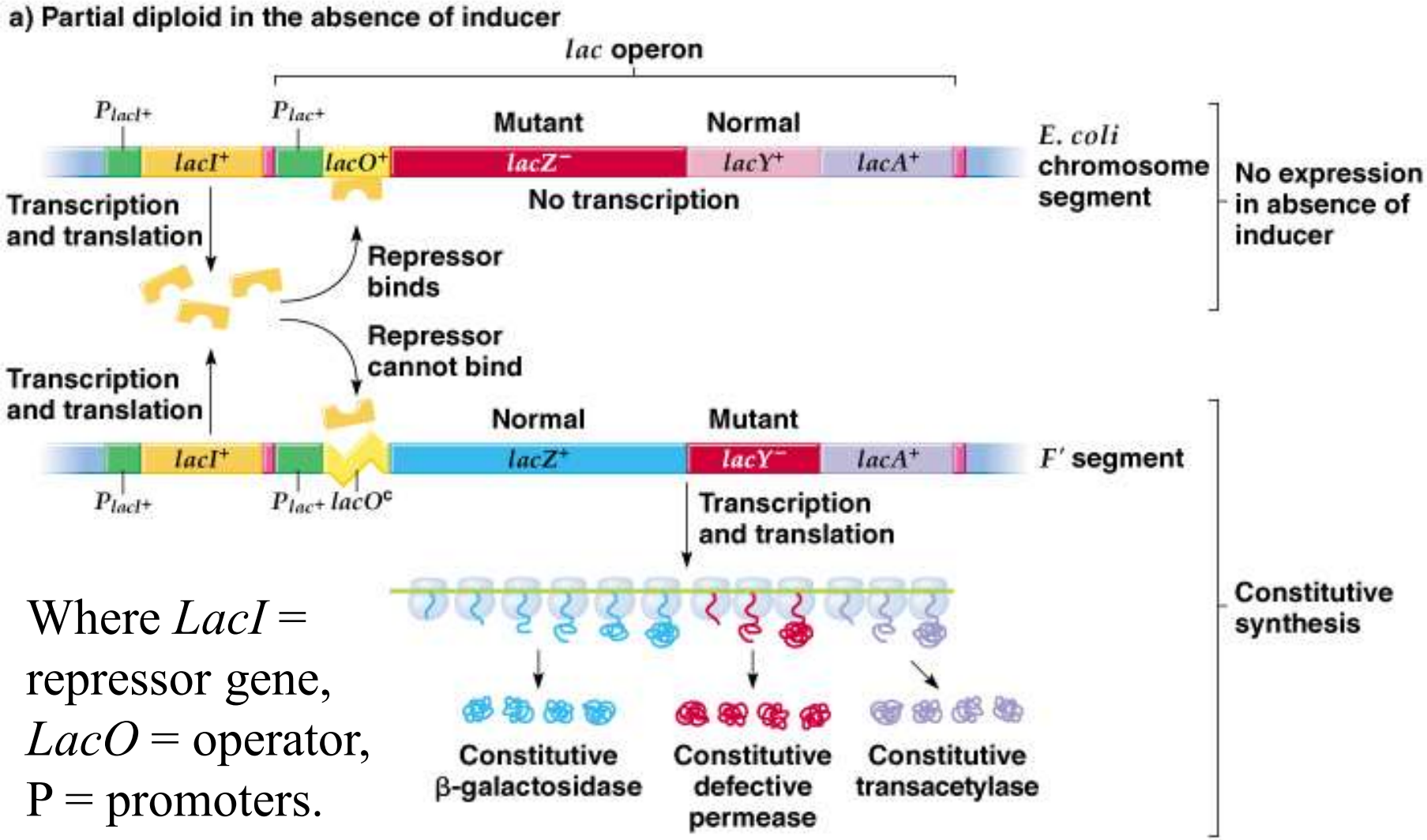
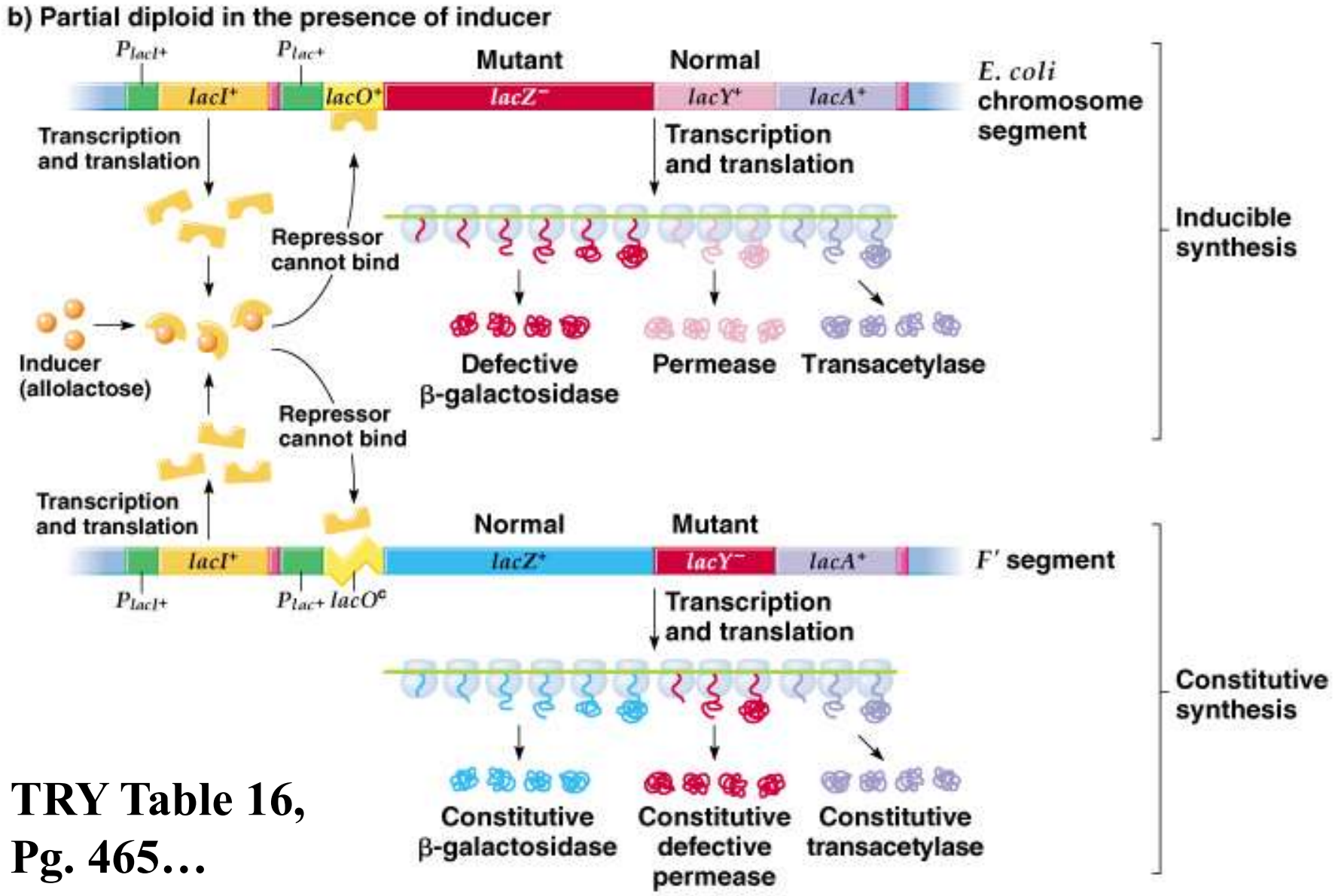


Fig. 16.8a Cis-dominant effect of *lacO*^{c} mutation in a partial-diploid



Where *LacI* = repressor gene, *LacO* = operator, P = promoters.

Fig. 16.8b Cis-dominant effect of *lacO*^{c} mutation in a partial-diploid



TRY Table 16, Pg. 465…

Example #2: The Trp operon of E. coli encodes five enzymes needed to catalyze the synthesis of Tryptophan:

- TrpE gene product
- TrpD gene product
- TrpC gene product
- TrpB gene product
- TrpA gene product

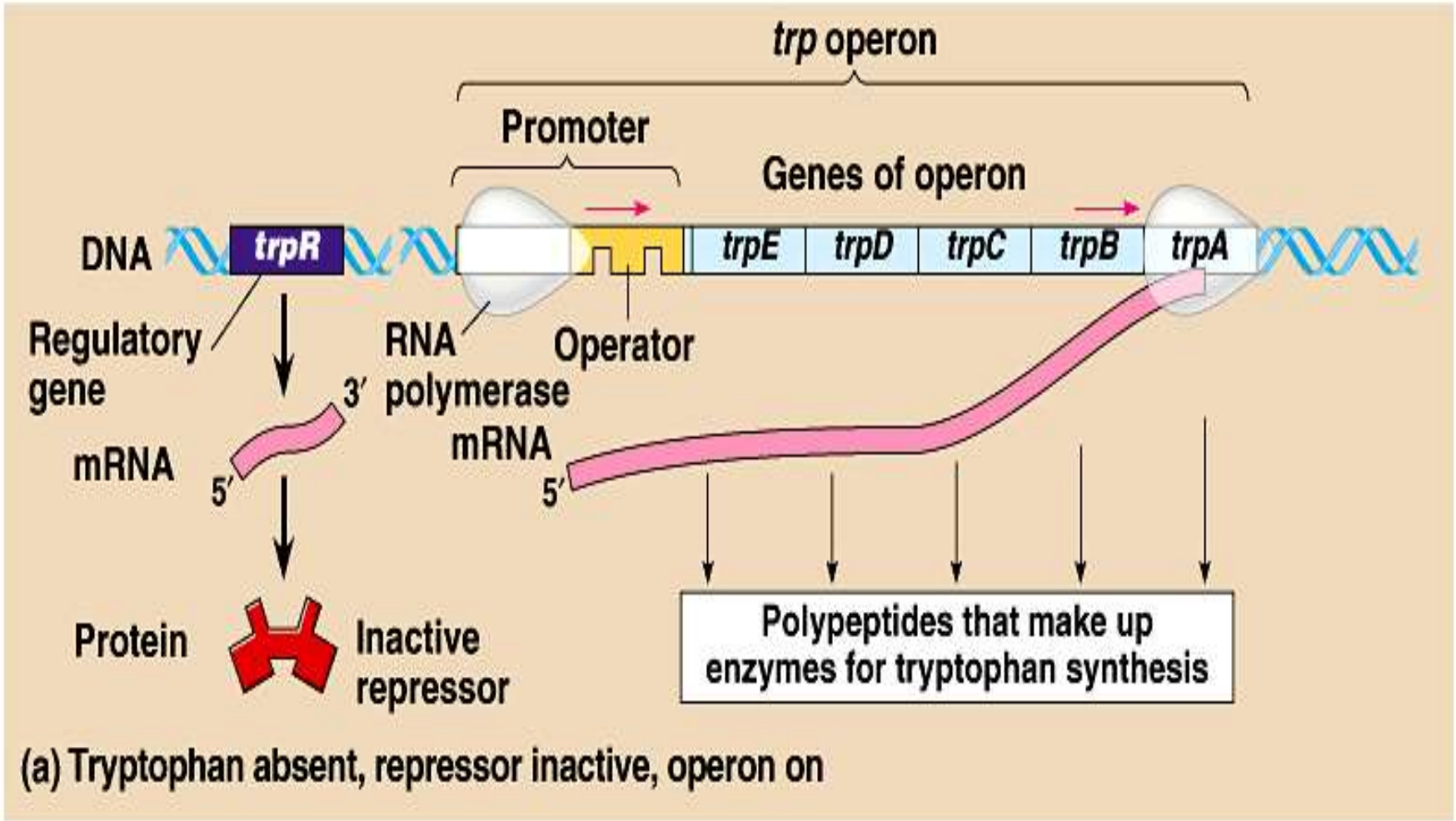
The Trp operon has 2 control mechanisms

- **NEGATIVE REPRESSIBLE OPERON**
  - Uses a repressor that binds a co-repressor (end product Trp) to repress operon transcription by 70-fold
  - Requires presence of Trp

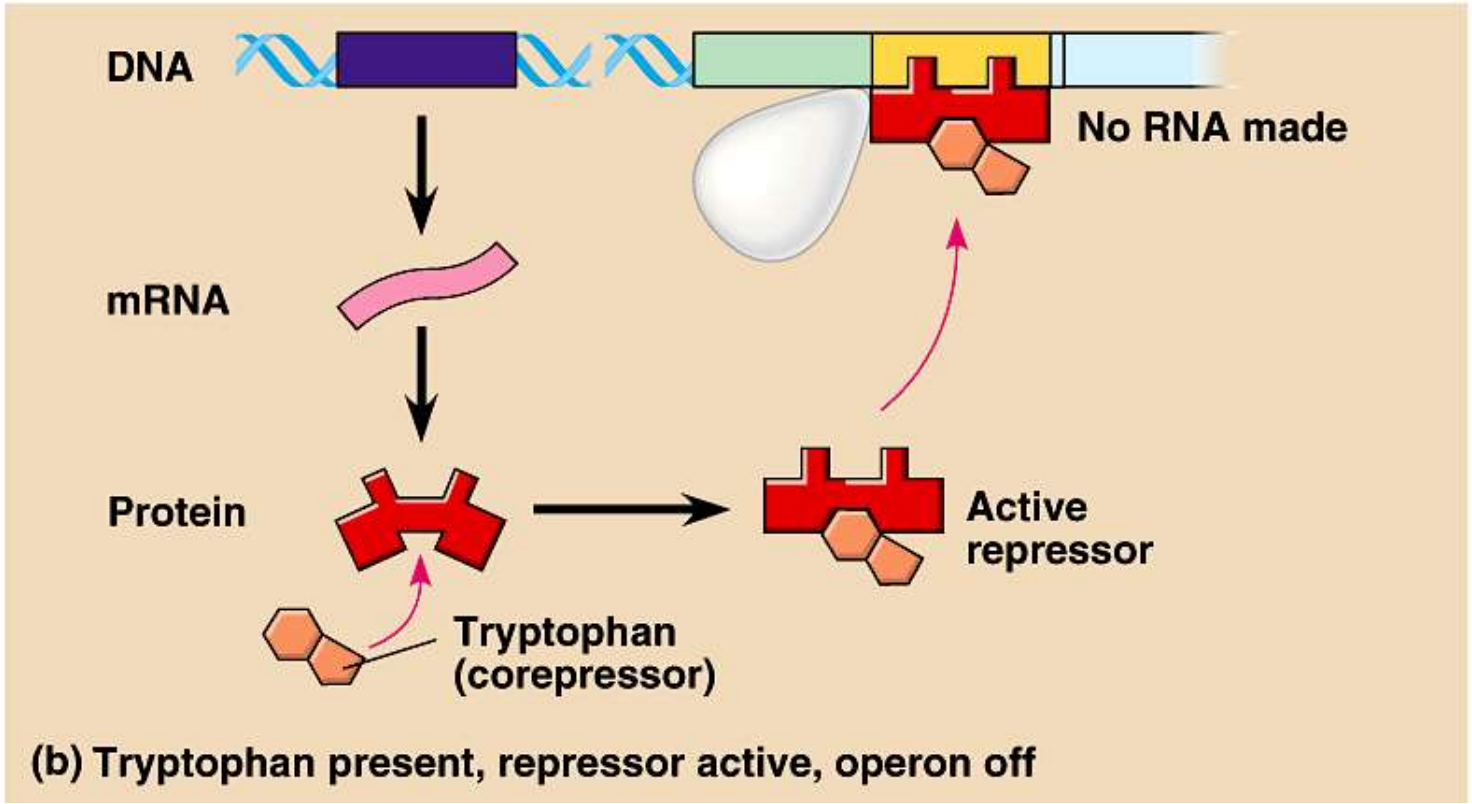
- **ATTENUATION**
  - Acts / represses on top of above mechanism by another 8 to 10-fold
  - Involves premature txn termination
  - Requires high Trp levels

TRP OPERON NEGATIVE REPRESSIBLE CONTROL

CIRCUIT: By itself, the operon is on. RNA polymerase can bind to the promotor and moves freely through the operator to transcribe the genes:

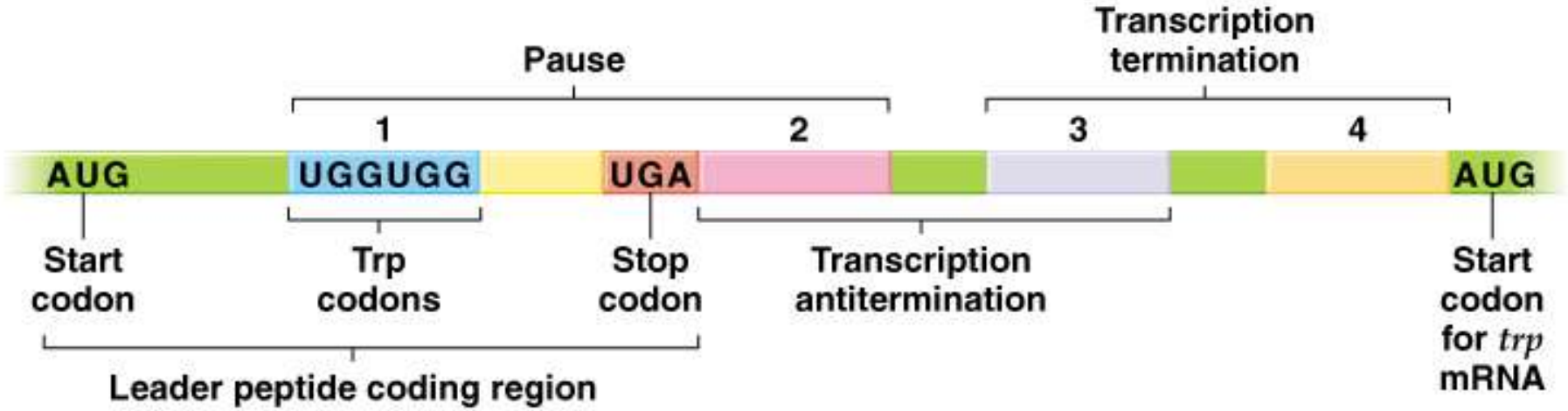


When **co-repressor (end-product) Trp is present**, it **binds to the repressor**. This activates the repressor, causing it to bind the operator to **block** Trp operon transcription:

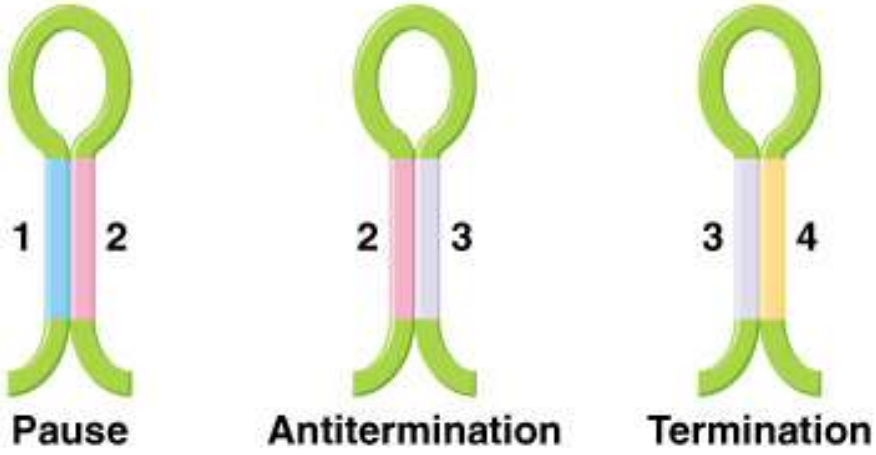


TRP OPERON ATTENUATION MECHANISM: involves 3 alternate ways to fold mRNA:

Organization of region:



Alternative RNA structures:



Pairing of 1 and 2 causes ribosomes to load onto mRNA right after RNA Pol. (COUPLING txn + tln)

Fig. 16.17a Models for attenuation in the *trp* operon of *E.coli*

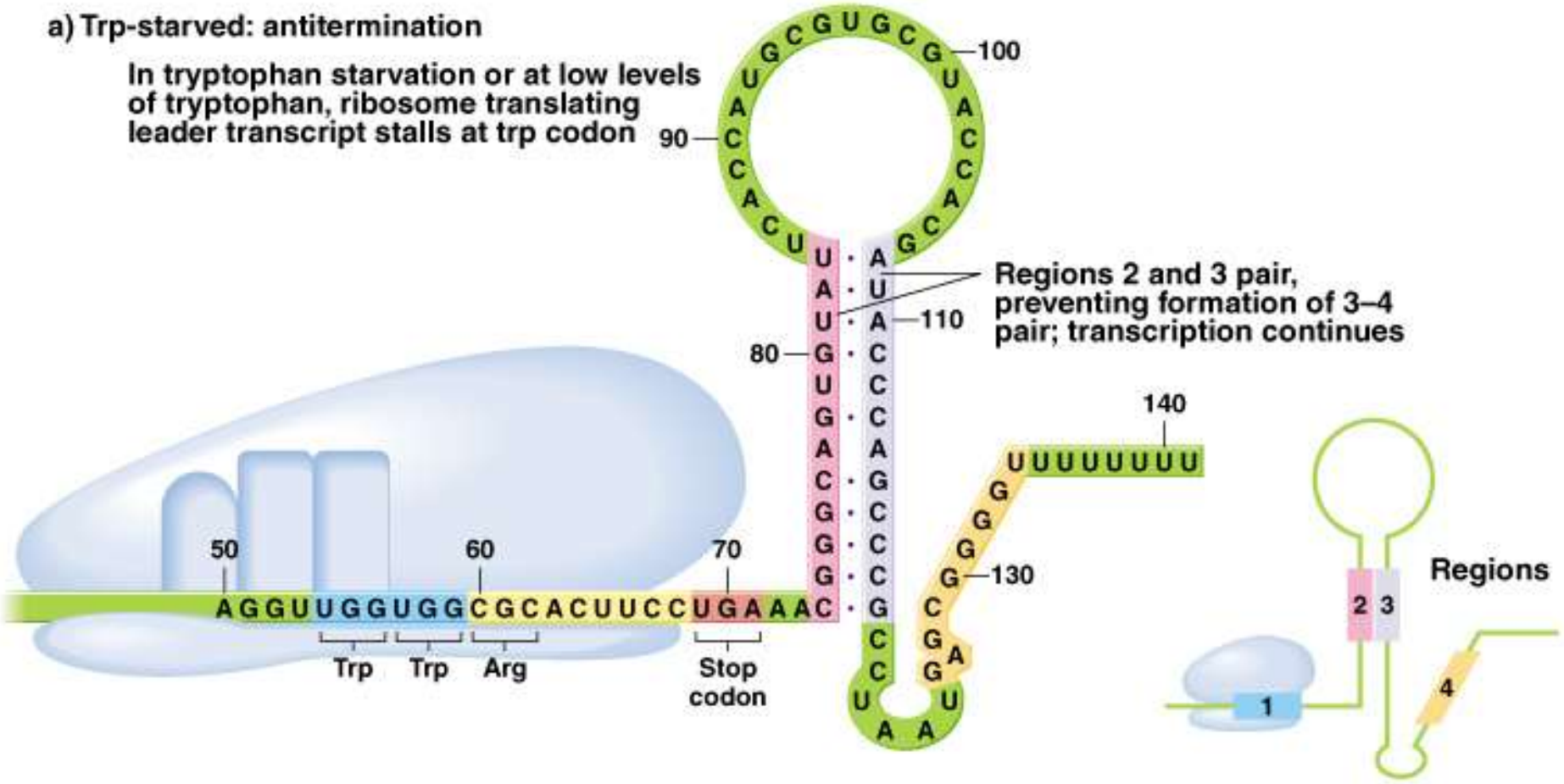
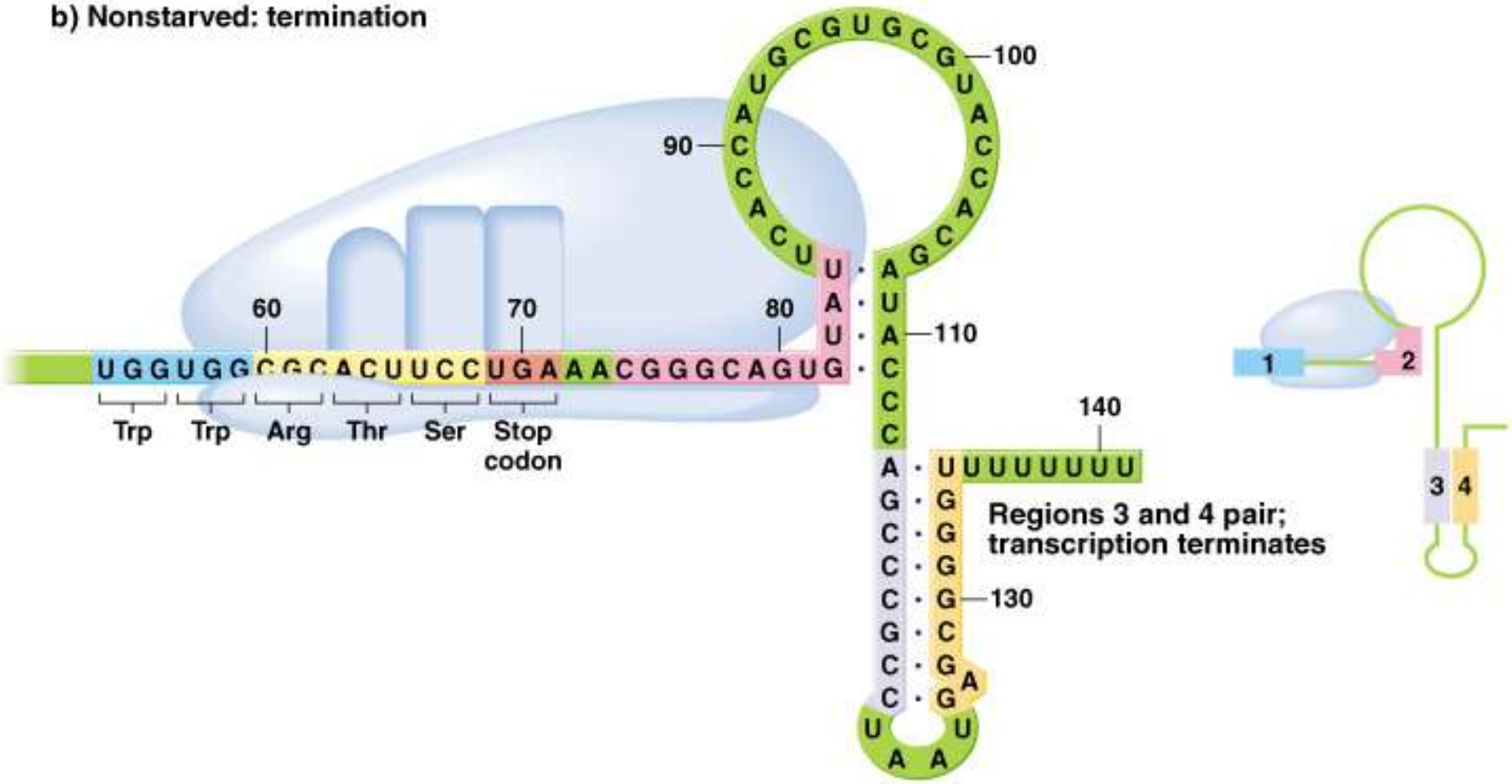


Fig. 16.17b Models for attenuation in the *trp* operon of *E.coli*



Regulation of gene expression in Lambda Phage - overview

- Recall lysogeny (integrated prophage) *vs*. lytic cycle:

- The lambda phage which infects *E. coli* demonstrates the cycles of a temperate phage.

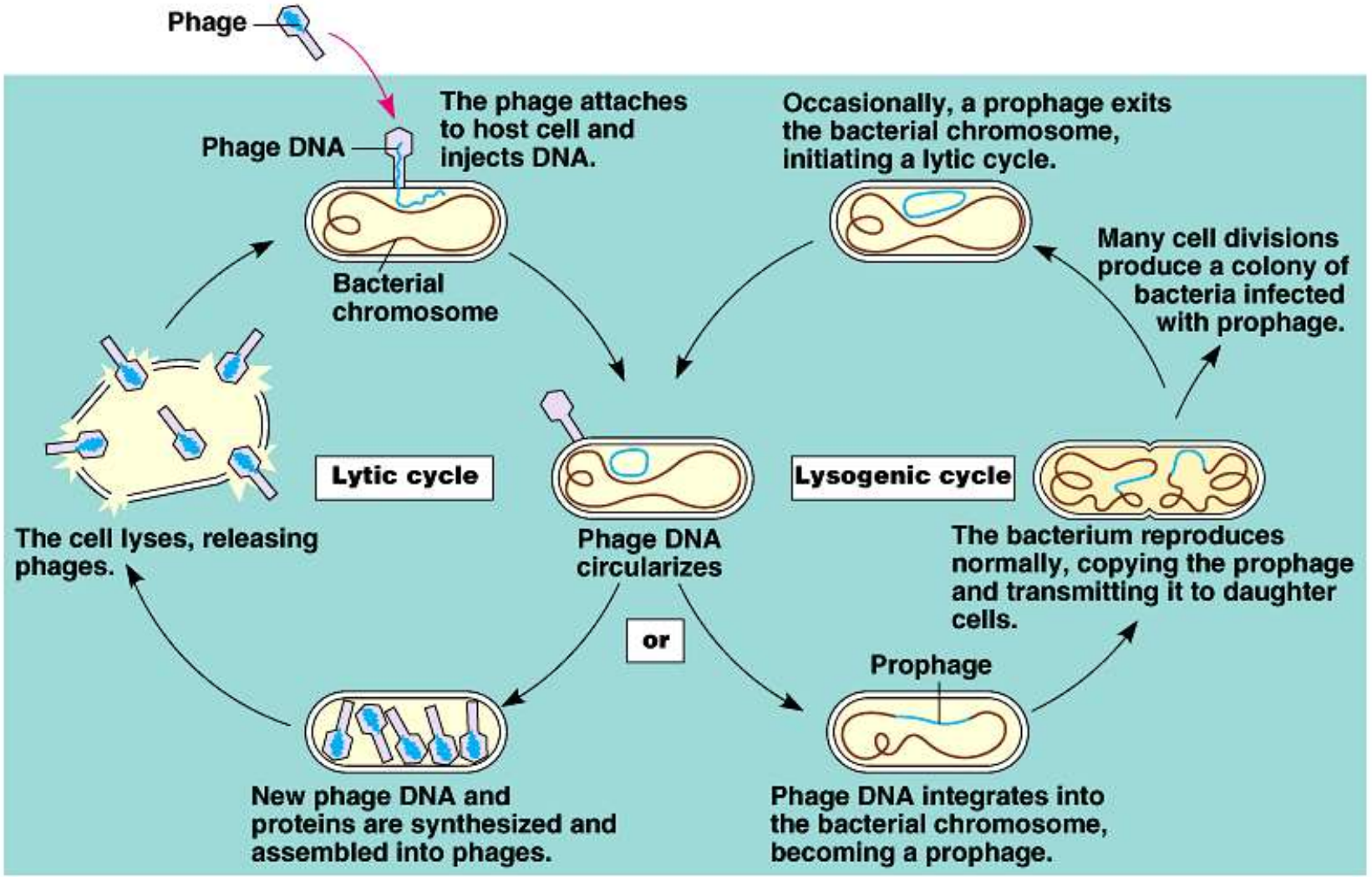
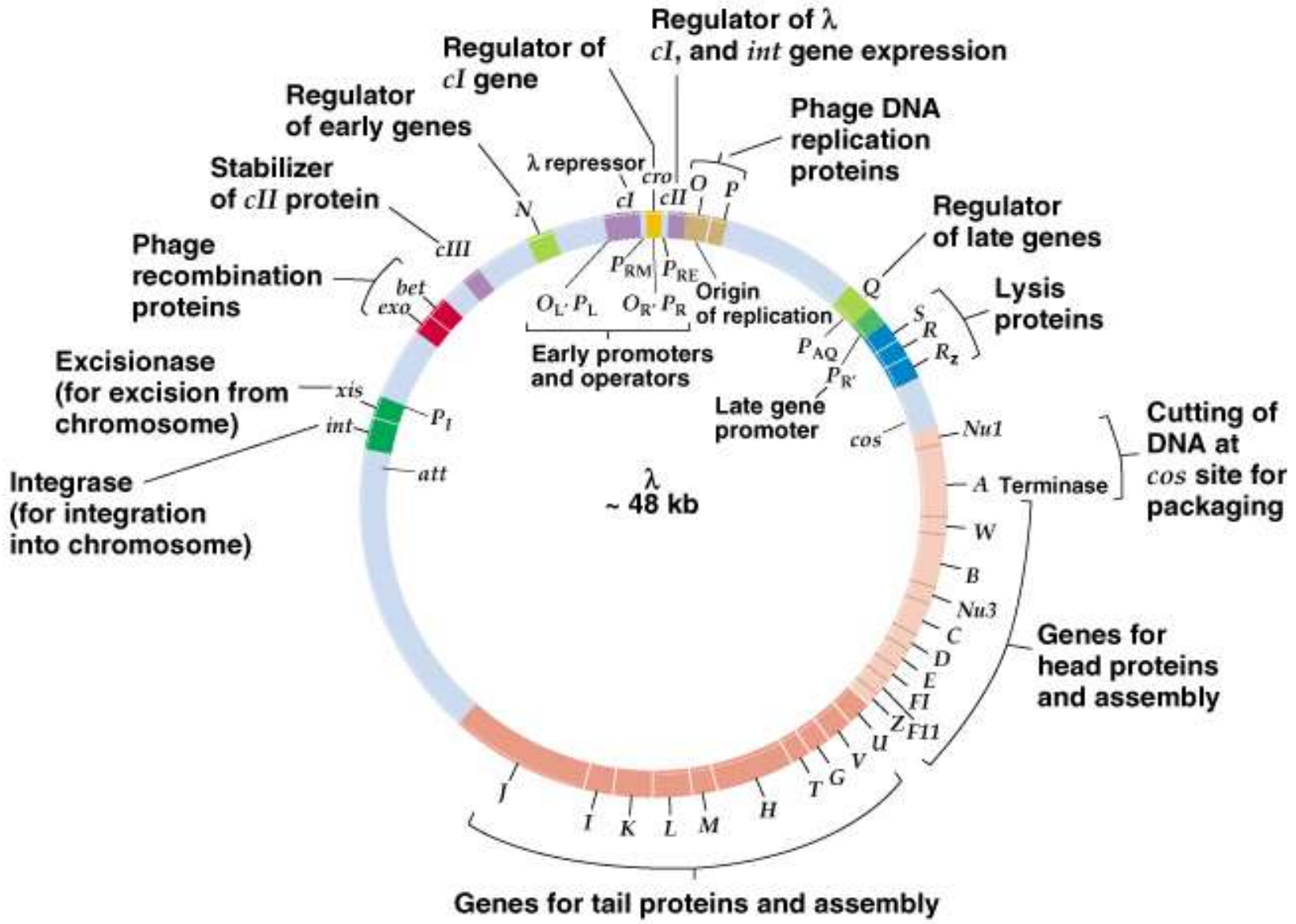


Fig. 18.5

How does Lambda choose between lysogeny and lytic cycle?

Fig. 16.20 A map of phage λ, showing the major genes



Establishing lysogeny:

- Usually when nutrients are low, virus will lay dormant until things improve.
- Starving E. coli cells make lots of cAMP
- Upon infection, a very unstable CII protein is made.
- High cAMP inhibits the host enzymes that would normally rapidly degrade CII
- CII stimulates production of CI repressor and integrase
- CI stimulates its own production and inhibits txn of lytic genes.

Entering the lytic cycle:

- Usually due to stress (e.g. UV light) resulting in DNA damage.
- Host cell DNA damages initiates host cell SOS response, including making host cell RecA protein.
- Host cell RecA protein destroys CI repressor and stimulates CRO production.
- CRO represses CI and CII genes and stimulates lytic genes.