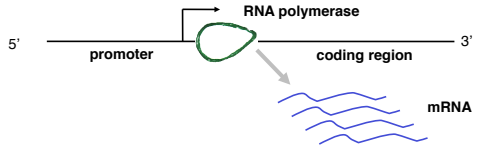
Regulation of Gene Expression



Expression = gene transcribed into RNA and translated into protein.

Not all genes are expressed all the time.

Genes can be "turned on" or "turned off".

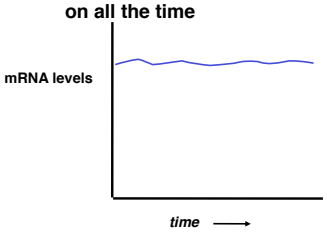
There are 3 patterns of expression:
- constitutive
- developmental
- inducible

1

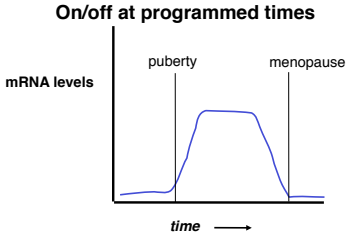
Regulation of Gene Expression

Three basic patterns of gene expression:

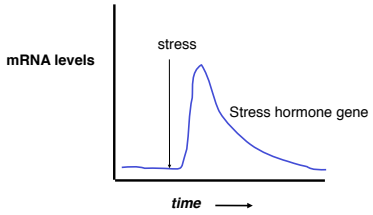
Constitutive
on all the time



Developmental
On/off at programmed times



Inducible:
Turned on/off by the environment

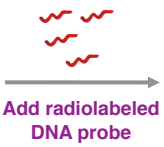


2

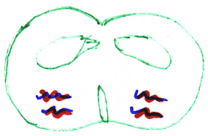
How to Detect and measure RNA

1. In situ hybridization (in the tissue itself)

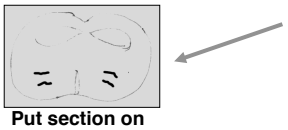
Section of tissue with mRNA in some cells



Add radiolabeled DNA probe



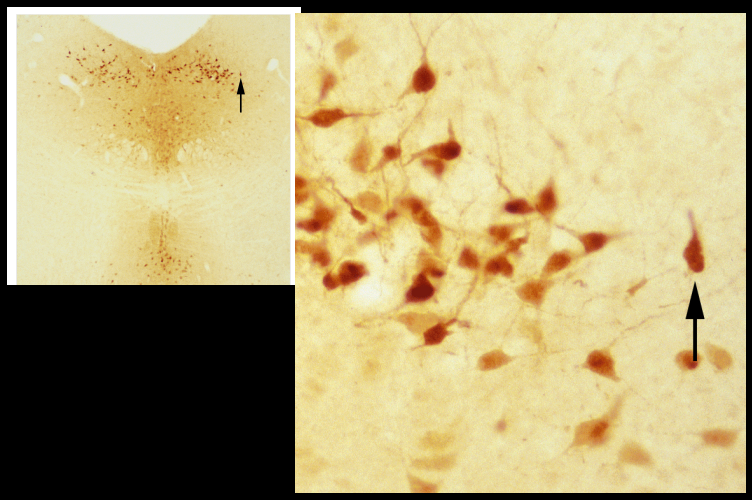
DNA probe hybridizes with specific mRNA



Put section on photographic film

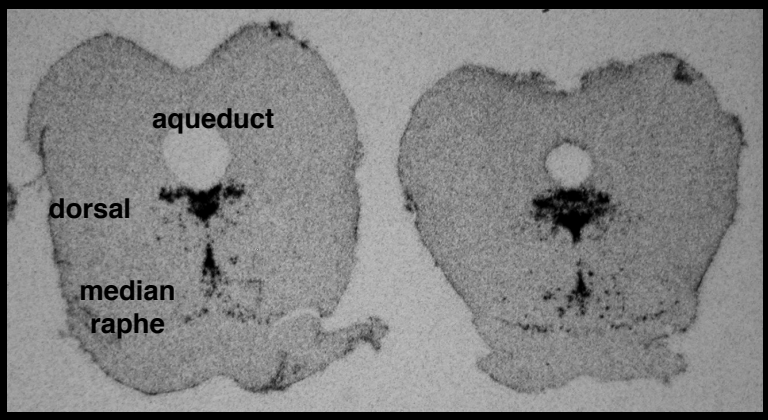
3

Cell bodies that synthesize serotonin



4

Cell bodies that express mRNA for serotonin transporter



5

Blots

Southern Blot
Separate DNA fragments by size on a gel, then transfer to a membrane (invented by Prof. Southern)

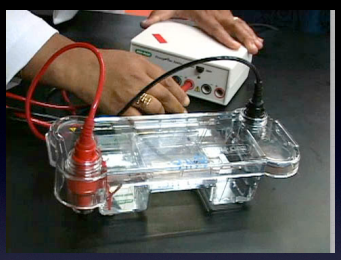
Northern Blot
Separate RNA species by size on a gel, then transfer to a membrane

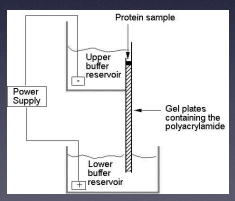
Western Blot
Separate proteins by size on a gel, then transfer to a membrane

6

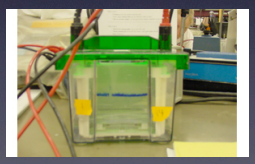
Gel Electrophoresis

Agarose Gel





Polyacrylamide Gel

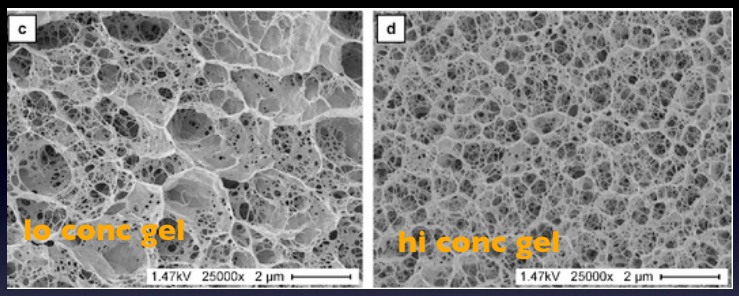


http://web.mit.edu/7.02/virtual_lab/PBC/PBC4Avirtuallab.html

http://newarkbioweb.rutgers.edu/bio301s/lab 5- mol wt sds-page.htm

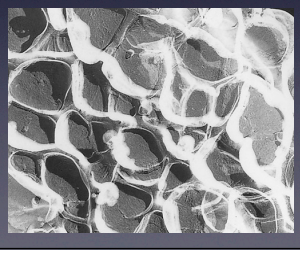
7

Agarose Gel



Tuvikene et al., J. Appl. Phycol. 20 (2008)

Polyacrylamide Gel



http://cellbiologyolm.stevegallik.org/node/78

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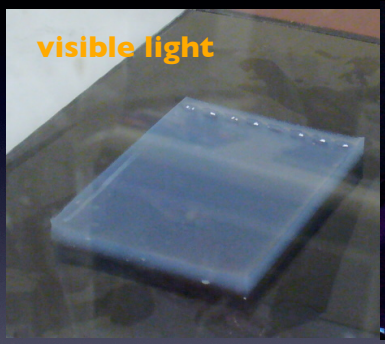
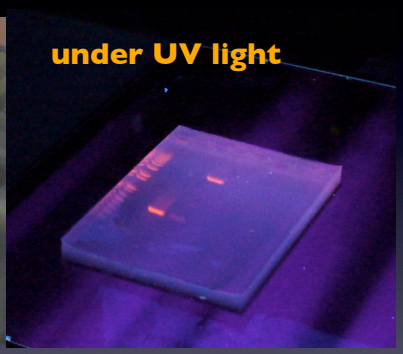
Nucleic Acid Stain of Agarose Gel





Wikimedia Commons: Agarosegel.jpg

Wikimedia Commons: AgarosegelUV.jpg

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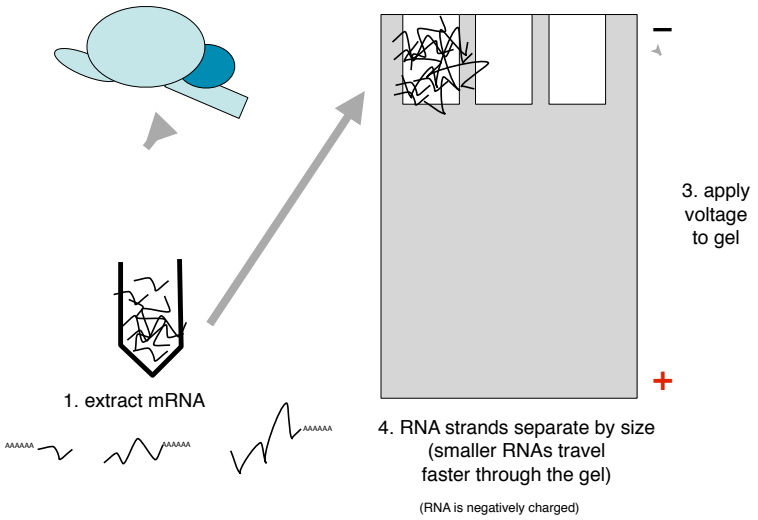
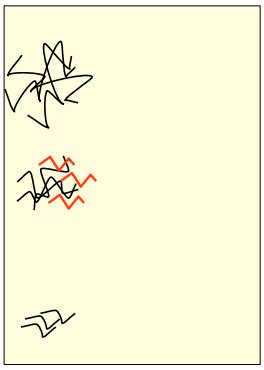
Northern Blot



10

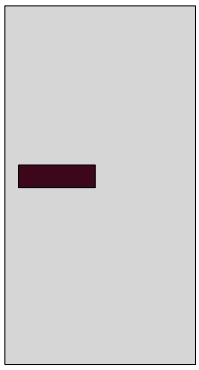
Northern Blot

5. Blot RNA onto nylon membrane



6. Incubate RNA blot with radiolabeled DNA probes

Expose Blot to Film



For example:

DNA probe: ATCCGCATTAG *(every T is radioactive)*

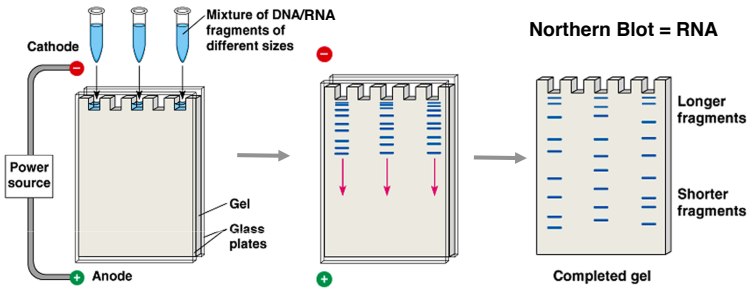
RNA in blot: TTAGCTTTAGGAGTAATCCGAATATGGC

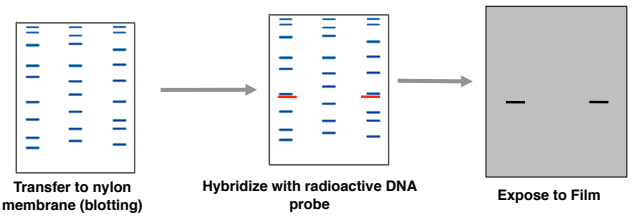
11

2. Gel electrophoresis and Blotting

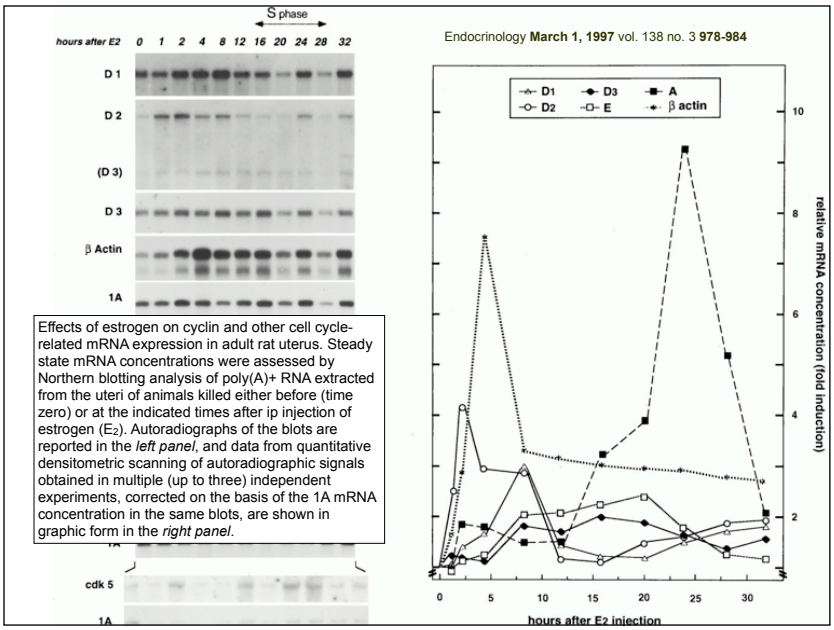
Southern Blot = DNA

Northern Blot = RNA





12



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Controls of Gene Expression

1. DNA packing in nucleosomes

In chromosomes, DNA is wrapped around protein complexes called histones to form nucleosomes.

In order to transcribe the DNA of a gene to RNA, DNA has to be unpacked from histone to expose the gene.

2. Binding of transcription factors to the promoter

Prokaryotic vs. Eukaryotic gene regulation

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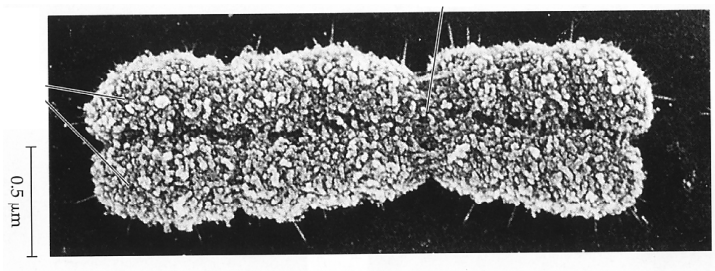
Unpacking a chromosome to transcribe RNA



15

Regulation of Gene Expression

Expression is controlled by:

DNA-binding proteins = transcription factors

that bind to

specific sequences in genes = control elements

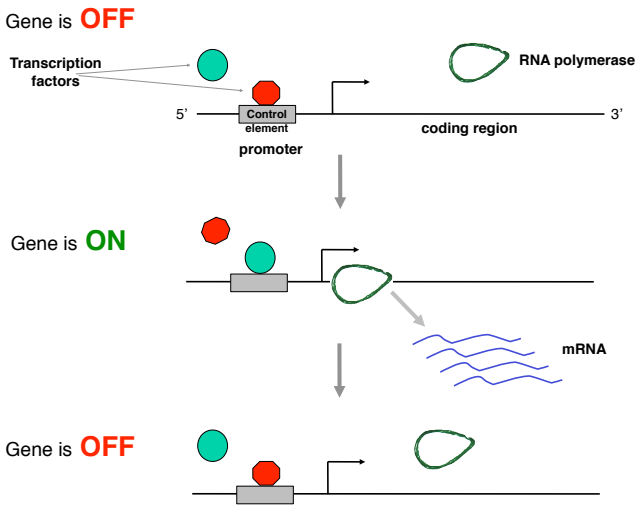
that alter the rate of transcription.

Because transcription factors are proteins, there are

genes for transcription factors = regulatory genes

Transcription factors can be either positive or negative (enhance or repress transcription).

16



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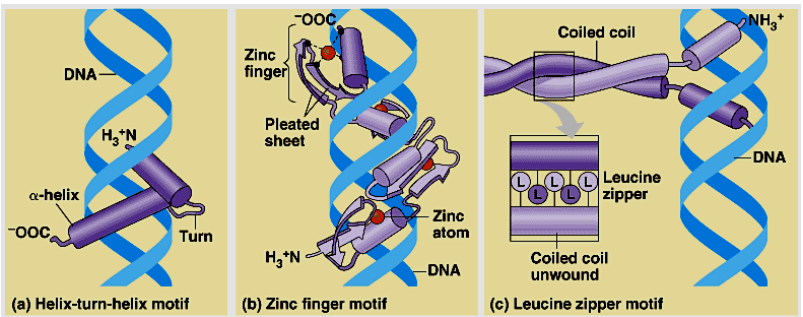
Transcription Factors: proteins that bind DNA



18

Prokaryotic Gene Regulation

Bacterial genes come in clusters called **operons.**
1 operon codes for several related proteins
Example: ***Tryptophan operon***
5 genes for 5 enzymes that synthesize tryptophan, all in one cluster

Operator = DNA sequence in the gene's promoter DNA.

Repressor = a protein that sticks to the DNA sequence in the operator.

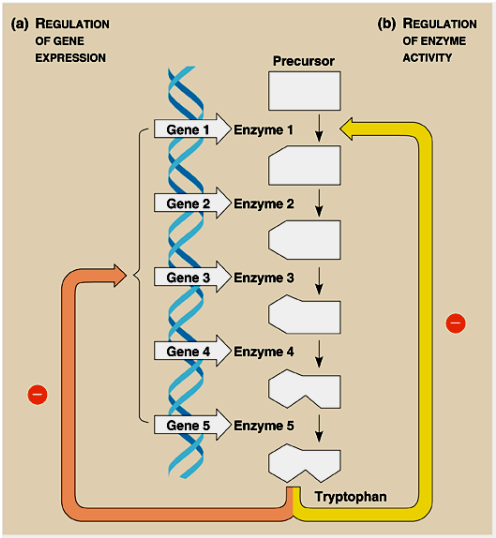
The 4° structure of a repressor is **changed** by a chemical to put it in the active or inactive state.

inactive repressor -> gene expression

active repression -> no gene expression.

19

Prokaryotic Gene Regulation

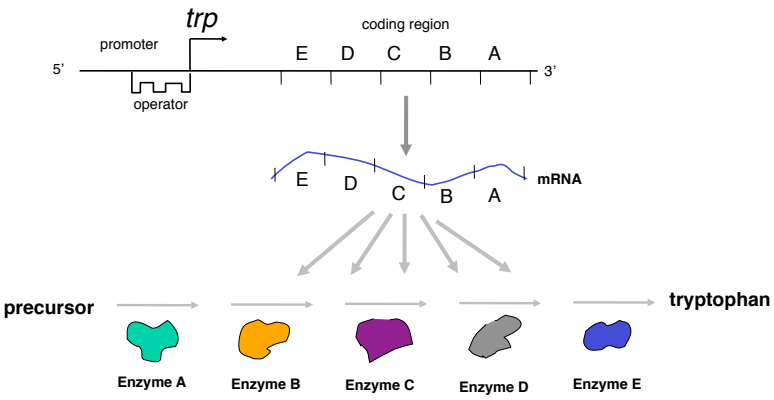


20

Prokaryotic Gene Regulation

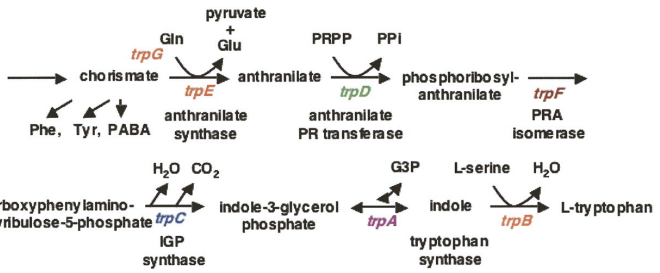
Bacterial genes come in clusters called **operons.**

Example: **Tryptophan operon**



So 1 operon codes for several related proteins

21



The genes, enzymes, and reactions of the tryptophan biosynthetic pathway. The seven genes, or genetic segments, seven enzymes, or enzyme domains, and seven reactions, involved in tryptophan formation are shown (Yanofsky and Crawford 1987). Only one of the reactions is reversible. The products of four other pathways contribute carbon and/or nitrogen during tryptophan formation. Two of the tryptophan pathway enzymes often function as polypeptide complexes: anthranilate synthase, consisting of the TrpG and TrpE polypeptides, and tryptophan synthase, consisting of the TrpB and TrpA polypeptides.

RNA 2007. 13: 1141–1154

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Repressible Operon: tryptophan operon

Tryptophan synthesis is controlled by a **tryptophan repressor**

= protein that binds to operator DNA sequence and blocks RNA polymerase from transcribing mRNA for tryptophan synthetic enzymes.

Repressor is **usually inactive**, so bacteria **transcribes** RNA for tryptophan enzymes

Repressor is **activated** whenever there is too much tryptophan, so *trp* operon is **repressed** by tryptophan.

The 4° structure of a repressor is **changed** by a chemical to put it in the active or inactive state.

inactive repressor -> gene expression

active repression -> no gene expression.

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Repressible Operon: tryptophan operon

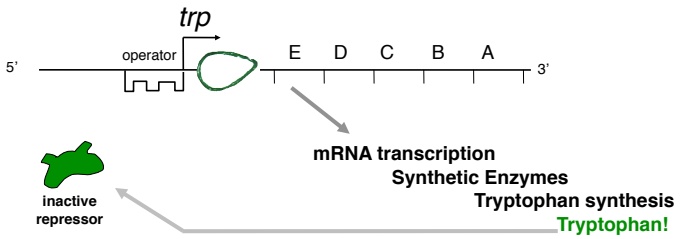
no tryptophan:

inactive
repressor

24

Repressible Operon: tryptophan operon

no tryptophan:

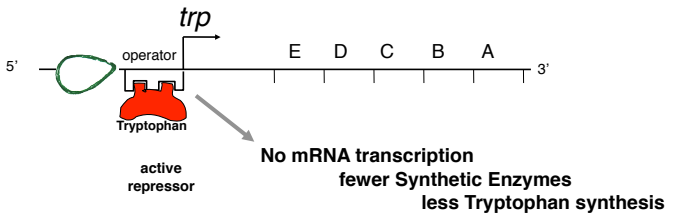


mRNA transcription
Synthetic Enzymes
Tryptophan synthesis
Tryptophan!

25

Repressible Operon: tryptophan operon

Lots o' tryptophan:



No mRNA transcription
fewer Synthetic Enzymes
less Tryptophan synthesis
lessTryptophan...

26

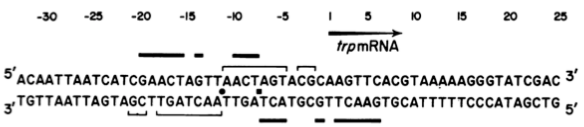


FIG. 4. Deduced DNA sequence of the *trp* mRNA initiation region. The arrow indicates the initiation site for *trp* mRNA synthesis *in vitro*, numbered position 1. The centers of the hyphenated symmetries are indicated by a dot and a square between the two DNA strands. The nucleotide pairs involved in each symmetry are denoted by the presence of similar bars above and below the sequence.

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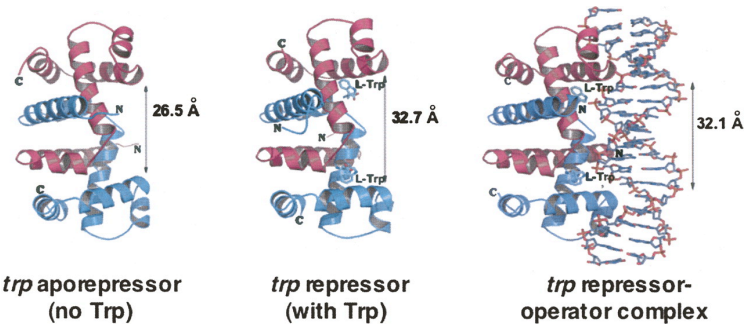

The basic structures of the trp aporepressor, trp repressor, and trp repressor–operator complex. The aporepressor is a dimer of identical polypeptide chains; it has two tryptophan binding sites. When tryptophan is bound, the repressor's two helix–turn–helix domains assume conformations that prepare them for binding at specific operator sequences. Binding of the trp repressor at a trp operator site—located within the trp promoter—interferes with RNA polymerase binding, hence binding prevents transcription initiation

RNA 2007. 13: 1141–1154

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Inducible Operon: lac operon

Usually (when there is no lactose around), the repressor is **usually active** and **blocks** gene expression all the time.

When lactose is **present**, the repressor is **inactive**, and lac operon **expresses** enzymes to digest lactose.

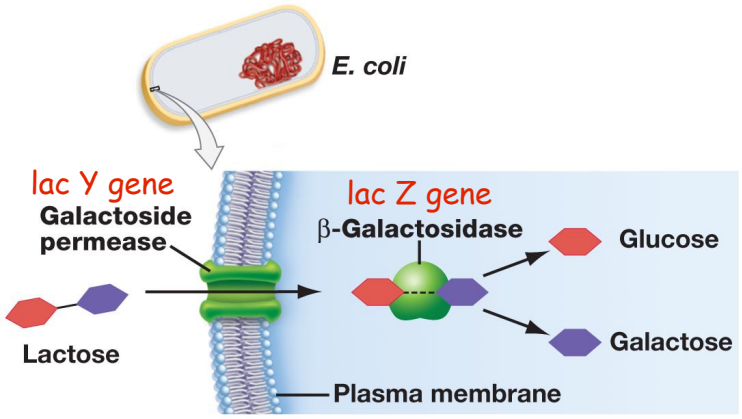
So presence of lactose causes **induction** of the lac operon.

The 4° structure of a repressor is **changed** by a chemical to put it in the active or inactive state.

inactive repressor -> gene expression

active repression -> no gene expression.

29

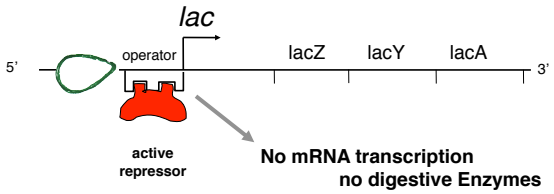


http://www.uic.edu/classes/bios/bios100/lectures/17_04_using_lactose-L.jpg

30

Inducible Operon: lac operon

no lactose:



No mRNA transcription
no digestive Enzymes
no lactose digestion

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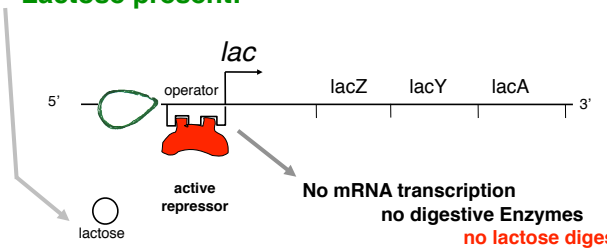
Inducible Operon: lac operon

Lactose present:

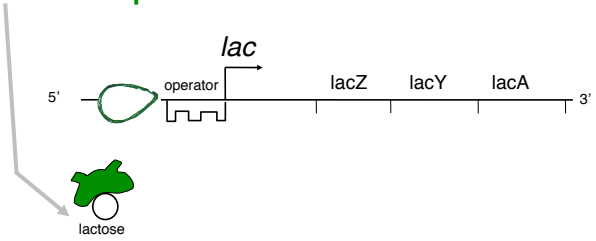


No mRNA transcription
no digestive Enzymes
no lactose digestion

32

Inducible Operon: lac operon

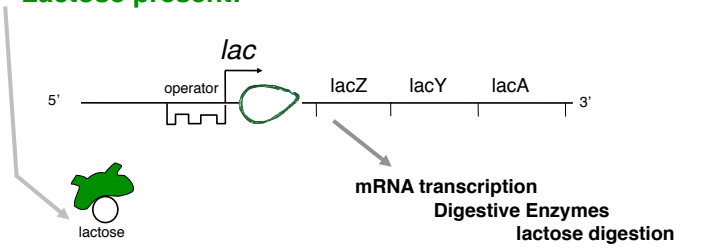
Lactose present:



33

Inducible Operon: lac operon

Lactose present:



mRNA transcription
Digestive Enzymes
lactose digestion
less lactose

34

Prokaryotic Gene Regulation

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35