Gene regulation in prokaryotes

Andrew Tolonen
atolonen "at" genoscope.cns.fr
@andrew_tolonen
http://www.tolonenlab.org

Today we will discuss models of bacterial gene regulation

Lac operon (LacI repression): dual regulation involving cAMP induction and inducer exclusion

Arabinose operon (AraC activation): repression and activation by changes inDNA confirmation

Antibiotic efflux (TetR repression): repression and interaction with other regulators

Metal reduction/transport (MerR activation): DNA distortion to align -35 and -10 sites

LysR activation: (N-term HTH, C-term effector binding) bind inducer-> bend DNA to activate RNA polymerase

Tryptophan operon: repression and attenuation

95% of bacterial regulators bind DNA using a helix-turn-helix domain

Basic concepts: carbon catabolite repression to prioritize growth substrates

Jacques Monod's experiments on diauxic growth (1941)

Keys to bacterial fitness:
1 choose carbon source that supports fastest growth.

2 don't produce enzymes for carbon sources that aren't available.

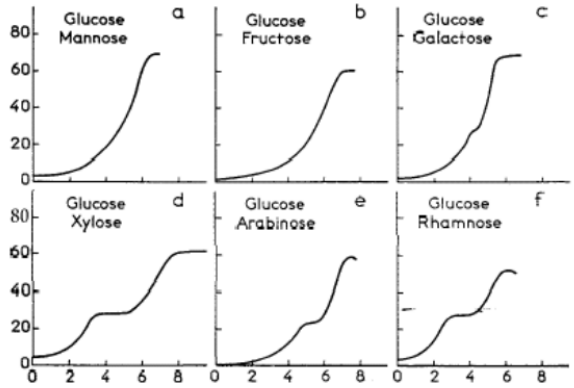
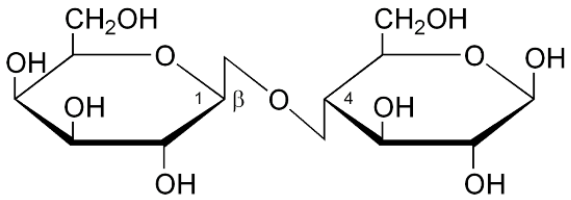


Fig.1. Growth of *Escherichia coli* in the presence of different carbohydrate pairs serving as the only source of carbon in a synthetic medium[30].

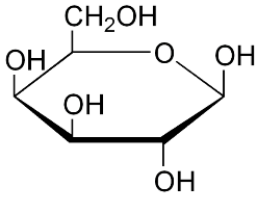
diauxic growth=growth in 2 phases

Growth rates:
glucose=mannose=fructose>galactose>>xylose,arabinose,rhamnose

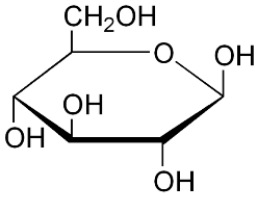
Metabolism of lactose by the lac operon



Lactose

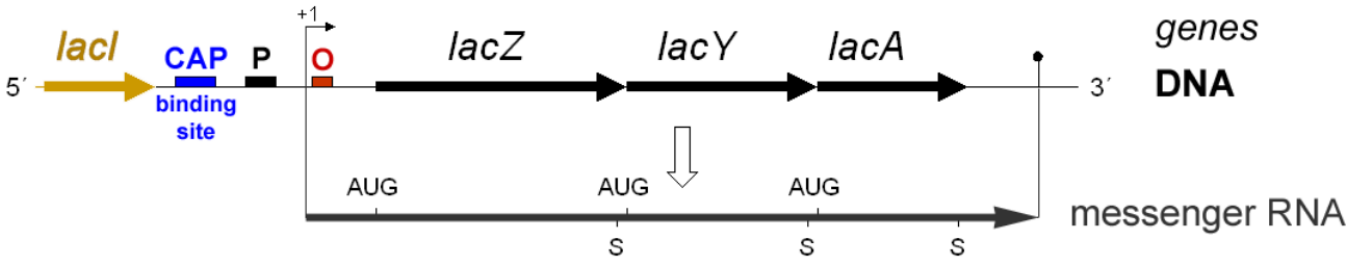


D-galactose



D-glucose

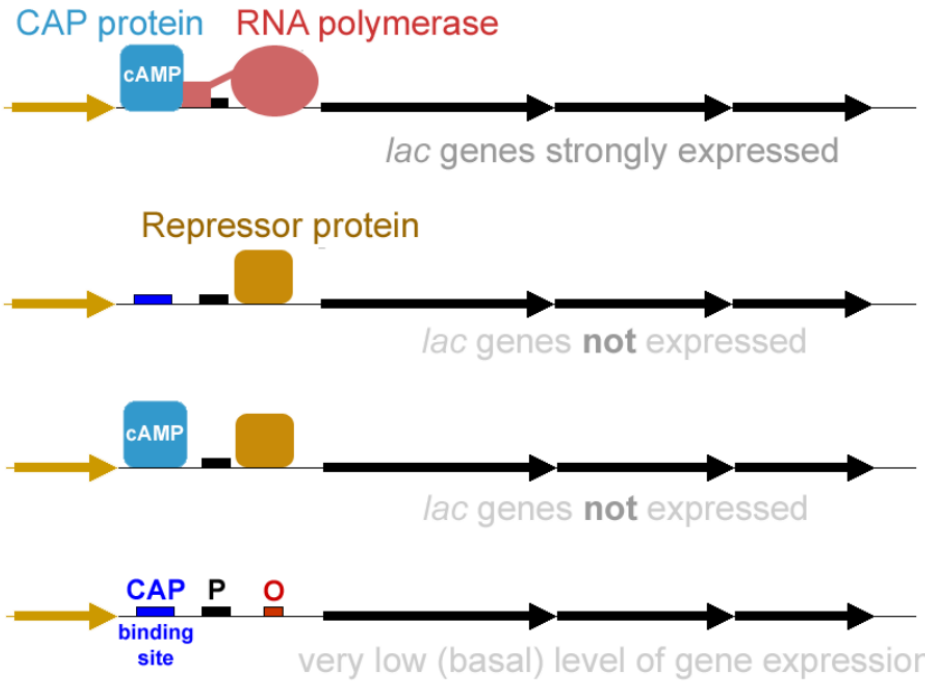
genetic elements in the lac operon



LacI represses expression of lac operon
CAP site binding site for Crp activator
P promoter
O binding site for LacI (operator)
lacZ b-galactosidase enzyme (cutting)
lacY lactose permease (transport)
lacA galactoside O-acetyltransferase (unknown)

en.wikipedia.org

Dual regulation (activation and repression) of lac operon



Low glucose
Lactose available

High glucose
Lactose unavailable

Low glucose
Lactose unavailable

High glucose
Lactose available

activation

cAMP-binds CRP

↓

cAMP-CRP binds lac promoter and recruits RNA polymerase

↓

lac operon expressed

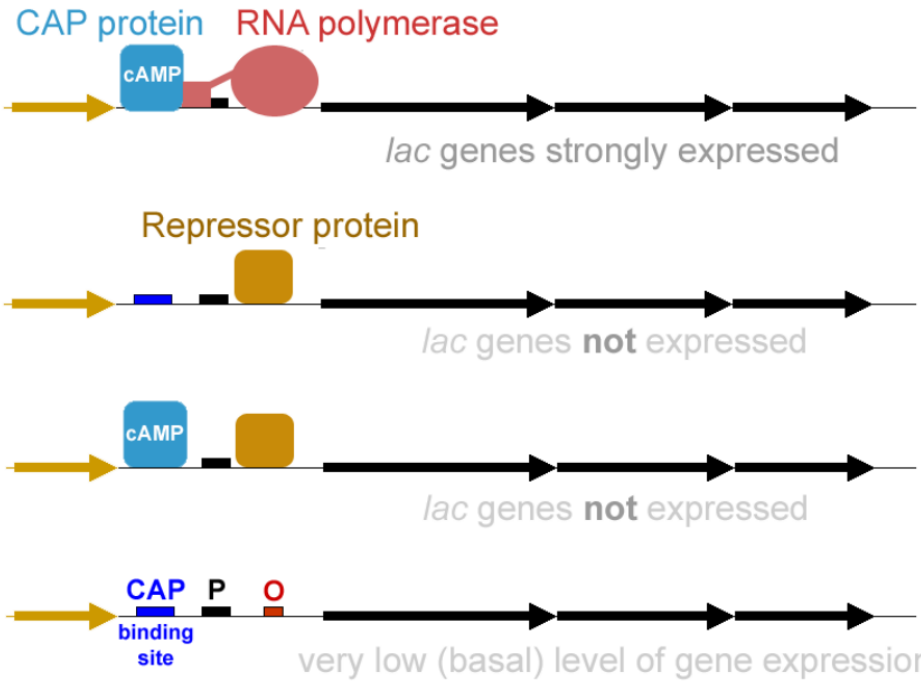
repression

LacI binds lac operator

↓

lac operon repressed

Lac expression only in presence of lactose and absence of glucose



Low glucose
Lactose available

-Glc, +Lac: Crp activates lac expression

Lac expression

High glucose
Lactose unavailable

+Glc,-Lac: no activation, lacI repression

No expression

Low glucose
Lactose unavailable

-Glc,-Lac: activation and repression

No expression

High glucose
Lactose available

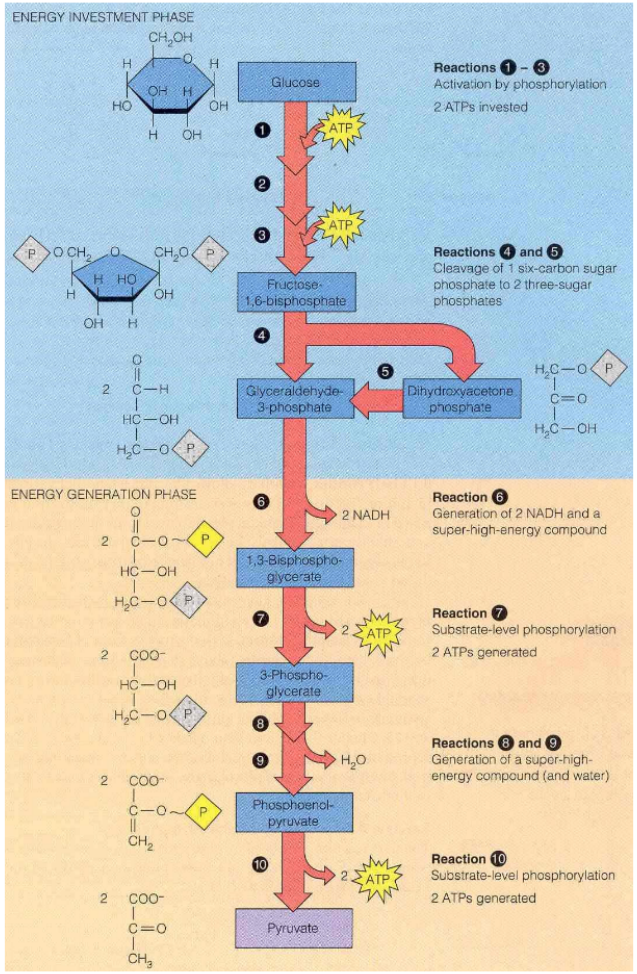
+Glc, +Lac, no activation, no repression

No expression

Crp activates in presence of cAMP
LacI represses in absence of lactose

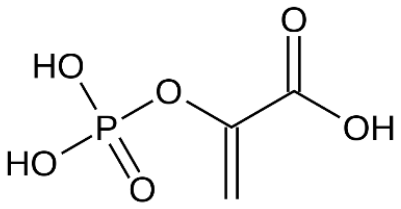
So how does cell control concentrations of cAMP (activator) and lactose (inducer)?

Glycolysis: breakdown of glucose (C6) into pyruvate (C3)

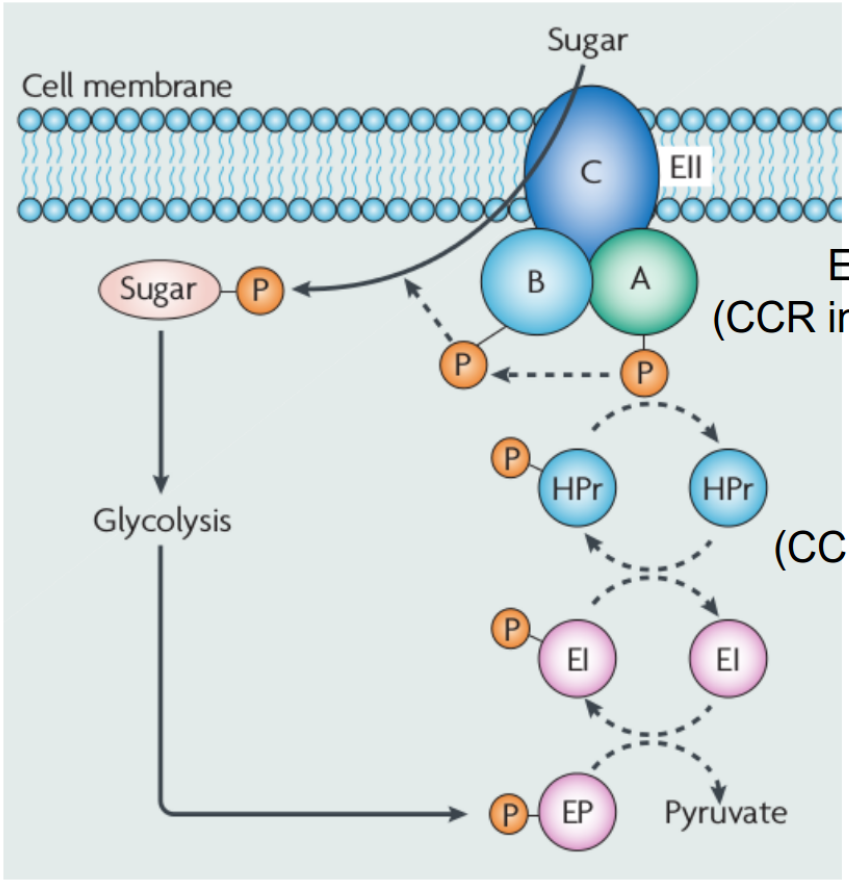


high glucose leads to high PEP/pyruvate ratio

PEP=phosphoenolpyruvate



Sugar transport by the phosphotransferase system (PTS)



EII
(CCR in gram-)

HPr
(CCR in gram+)

Glucose → PEP → sugar transport/ phosphorylation

high glucose leads to high PEP (glycolysis intermediate)

↓

PEP transfers Pi to EI

↓

EI transfers Pi to HPr

↓

HPr transfer Pi to EII (A,B,C subunits)

↓

EII transfers Pi to sugar

↓

transport and phosphorylation of sugar (glucose)

PMID 18628769

Carbon catabolite repression (*E.coli*)

glucose
(repression by inducer exclusion)

EII phosphorylates glucose

↓

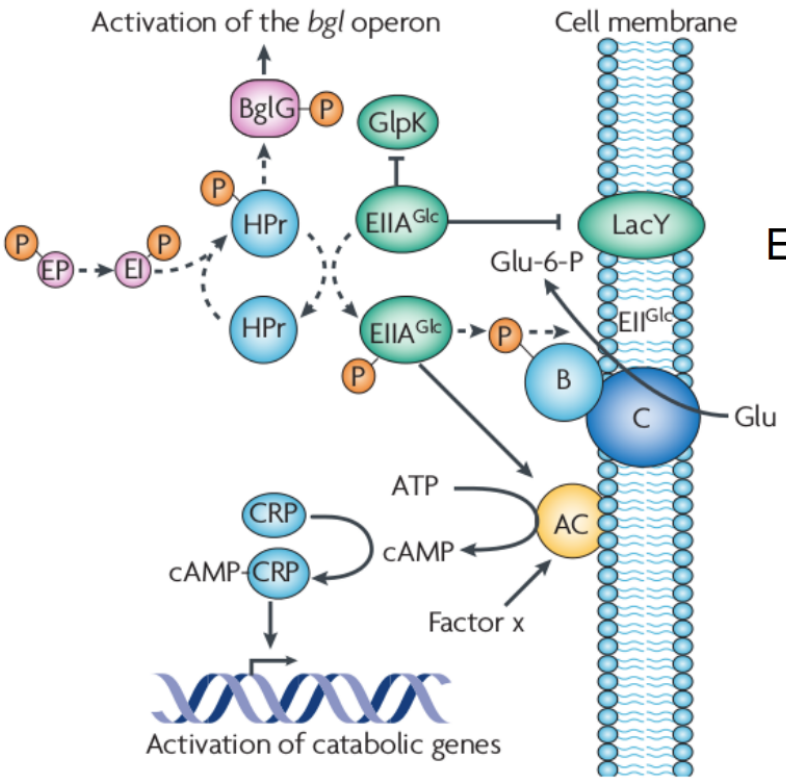
EII binds and inactivates
lactose transport (LacY)

↓

no lactose inducer
in presence of glucose

↓

LacI represses
lac operon



No glucose
(cAMP activates lac expression)

EII retains Pi in absence of glucose

↓

EII-P activates adenylate cyclase (AC)

↓

cAMP formed

↓

cAMP binds CRP

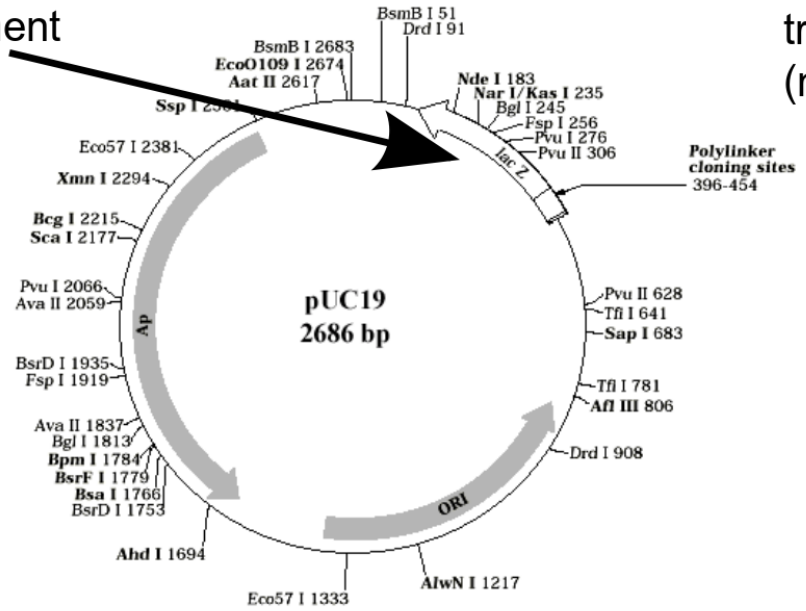
↓

cAMP-CRP activates
lac operon

PMID 18628769

Alpha complementation with pUC19

lacZ alpha fragment



bio.classes.ucsc.edu

transform pUC19 into *E.coli* with lacZalpha deletion (missing residues 11-41)

↓

add IPTG (inducer) and X-gal (turns blue when cut by B-gal)

↓

blue colonies

clone gene into MCS between Plac and lacZalpha

↓

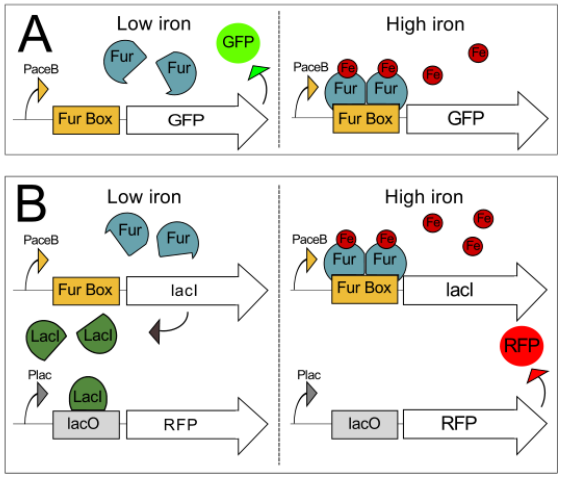
disrupt lacZ alpha expression

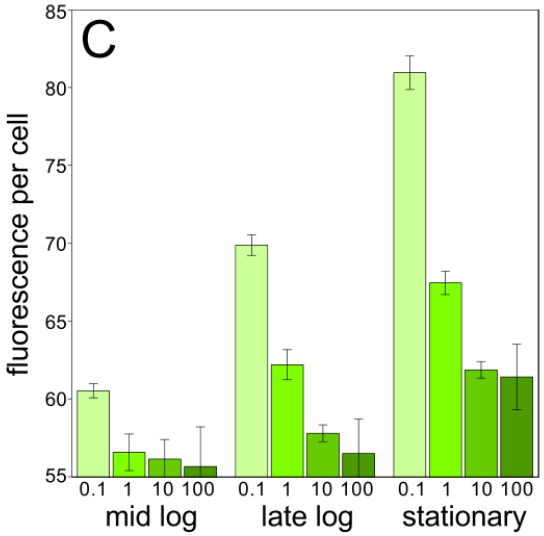
↓

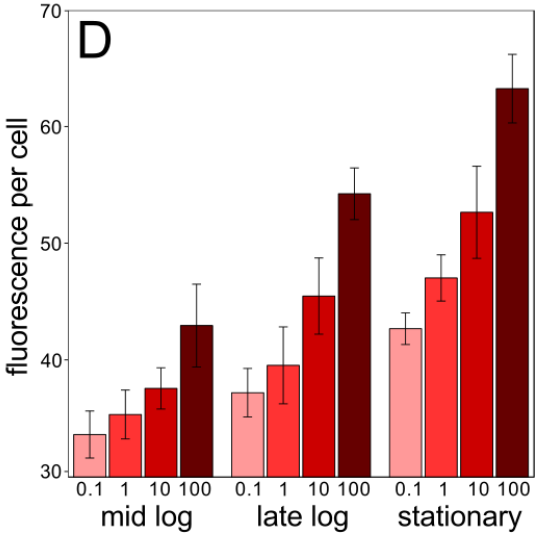
white colonies in +IPTG, +X-gal

A LacI-based Fur inverter to activate gene expression in response to iron

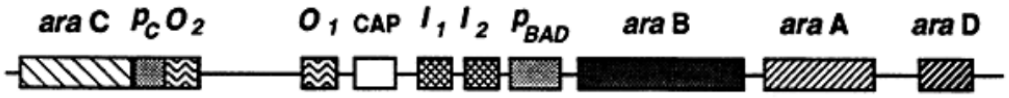
iGEM Evry 2013







Regulation of the L-arabinose operon



L-arabinose $\xrightarrow{\text{AraA}}$ L-ribulose

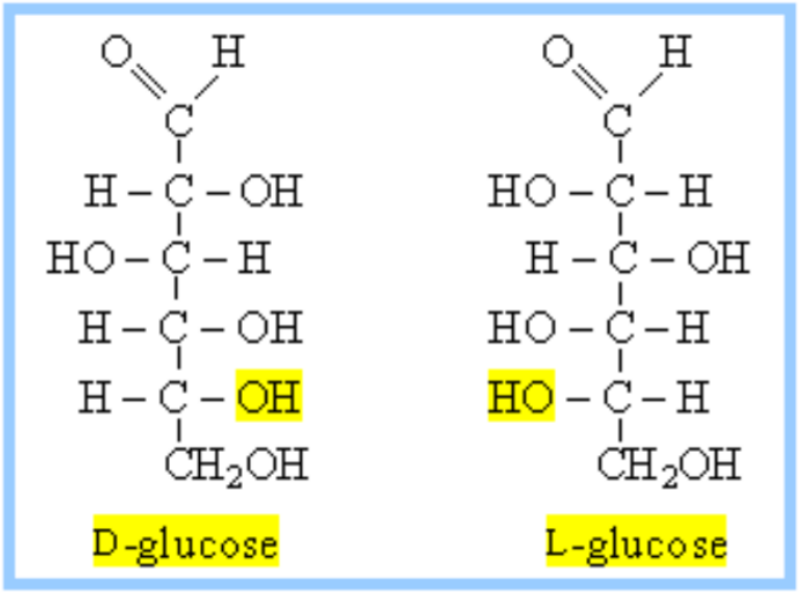
L/D-ribulose $\xrightarrow{\text{AraB}}$ L/D-ribulose-5P

L-ribulose-5P $\xrightarrow{\text{AraD}}$ D-xylulose-5P

AraEFGH=arabinose transporters

AraC=regulator

Review: D and L sugars are stereoisomers



most biological sugars
are D, but arabinose is L

https://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/sugar.htm

Review: D-xylulose-5P is assimilated by the pentose phosphate pathway

generate NADPH (reducing)

Ribose-5-P for nucleotide synthesis

Xylulose-5P converted into fructose-6P and glyceraldehyde-3P by transketolase and transaldolase

http://en.wikipedia.org

Repression of arabinose operon by 'no arabinose'

No arabinose

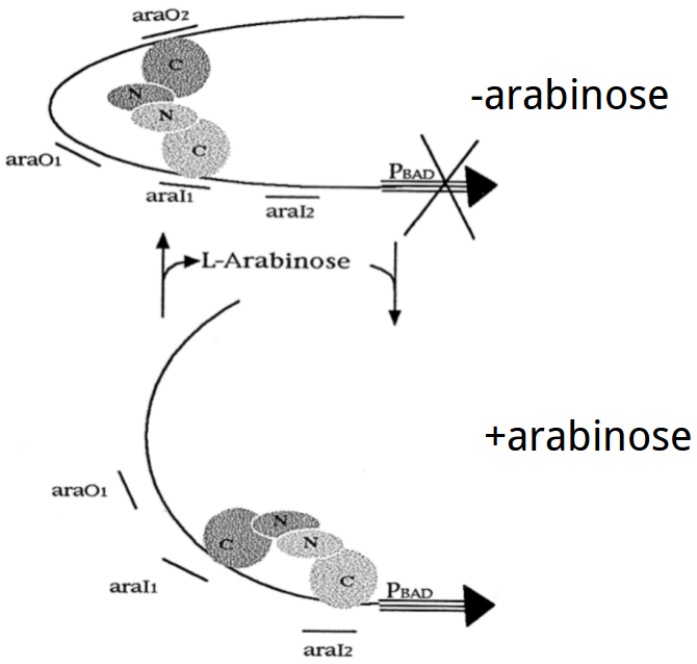
AraC dimer binds I1, **O2**

↓

AraC on O2 and I1 multimerize

↓

DNA bends to block transcription of arabinose operon



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AraC domain structure: residues 1-170 bind arabinose and dimerize protein; 170-8 flexible linker; 178-286 site-specific DNA binding and RNA polymerase recognition.

AraC consensus binding site: AGC-N7-TCCATA

Activation of *araC* by arabinose

Arabinose

AraC dimer binds arabinose

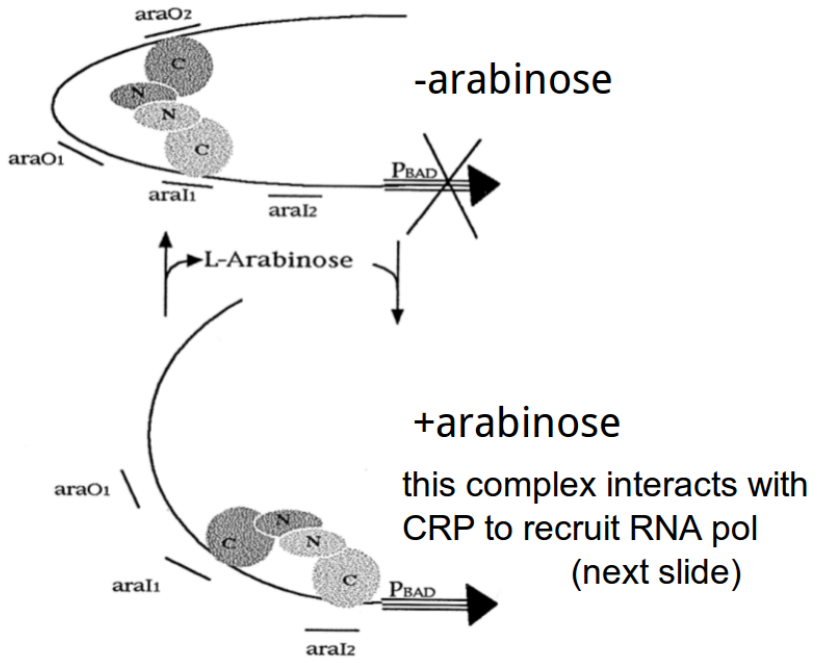
↓

AraC dimer binds I1, **I2**

↓

up-regulate AraC

*Arabinose binds AraC->change affinity of AraC for spatial orientation of binding sites.



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Activation of arabinose operon by 'no glucose'

no glucose

Low glucose stimulates cAMP (see slide 9)

↓

cAMP-CRP binds CAP site

↓

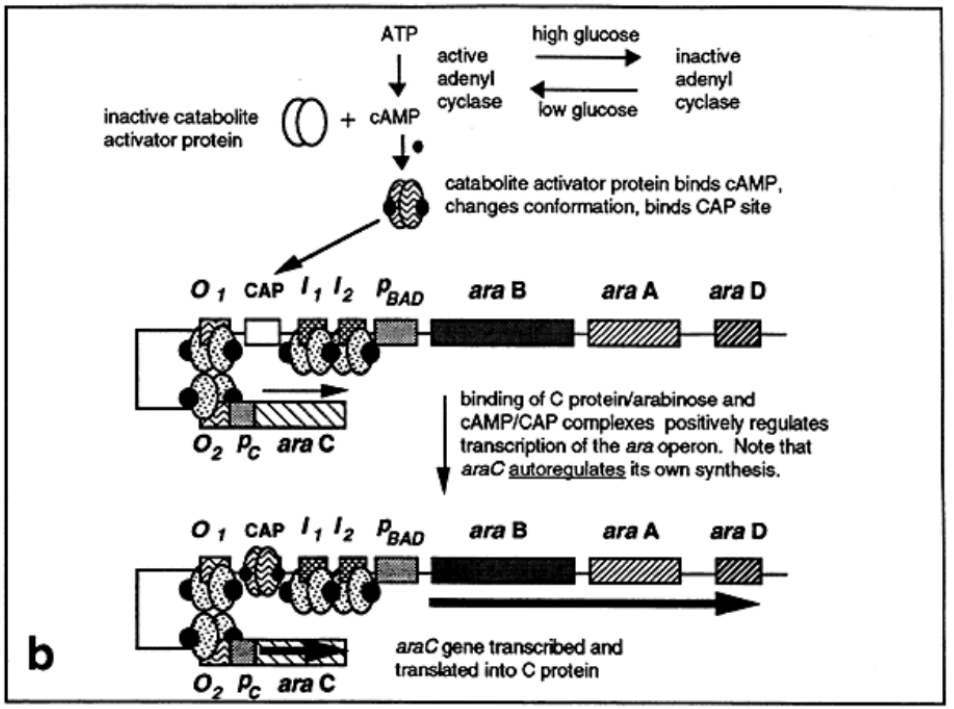
AraC (I1,I2) interacts with cAMP-CRP

↓

RNA polymerase recruited to araB promoter

↓

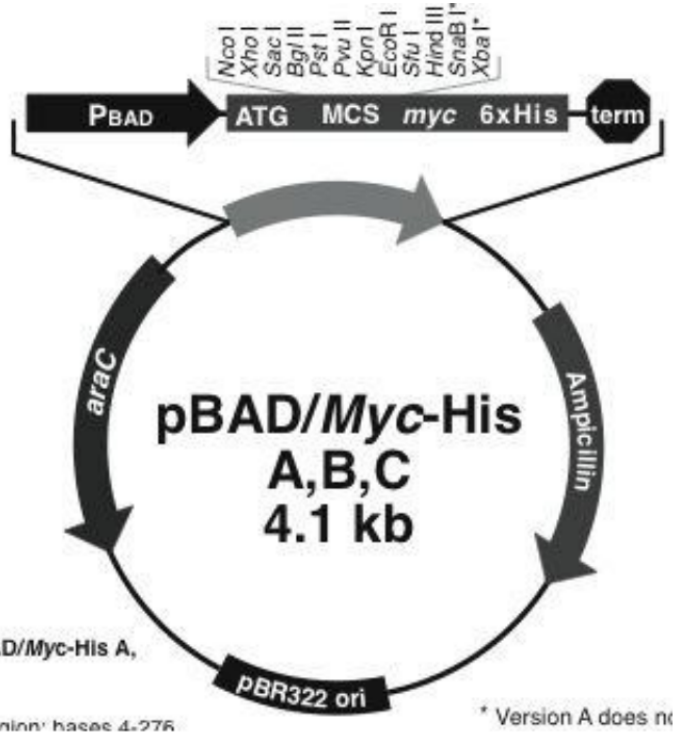
arabinose operon expressed



http://www.gravitywaves.com

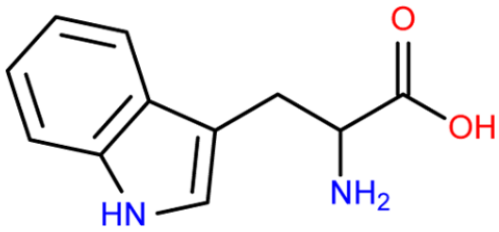
Pbad promoter

activate gene expression in +arabinose, -glucose

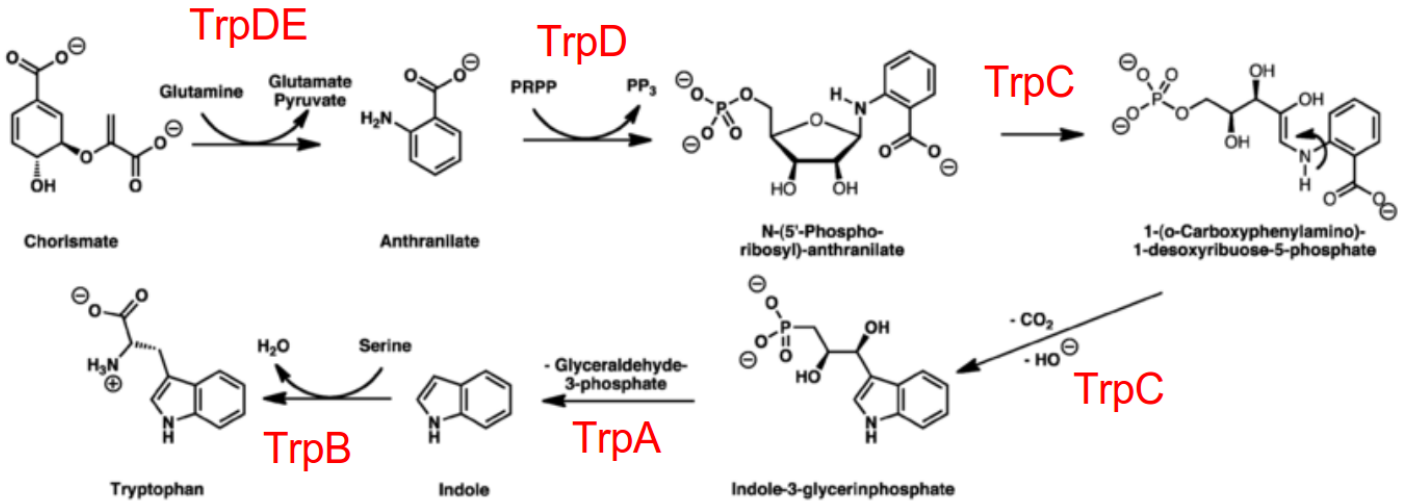


http://ecoliwiki.net

Trp operon: 5 genes for tryptophan biosynthesis

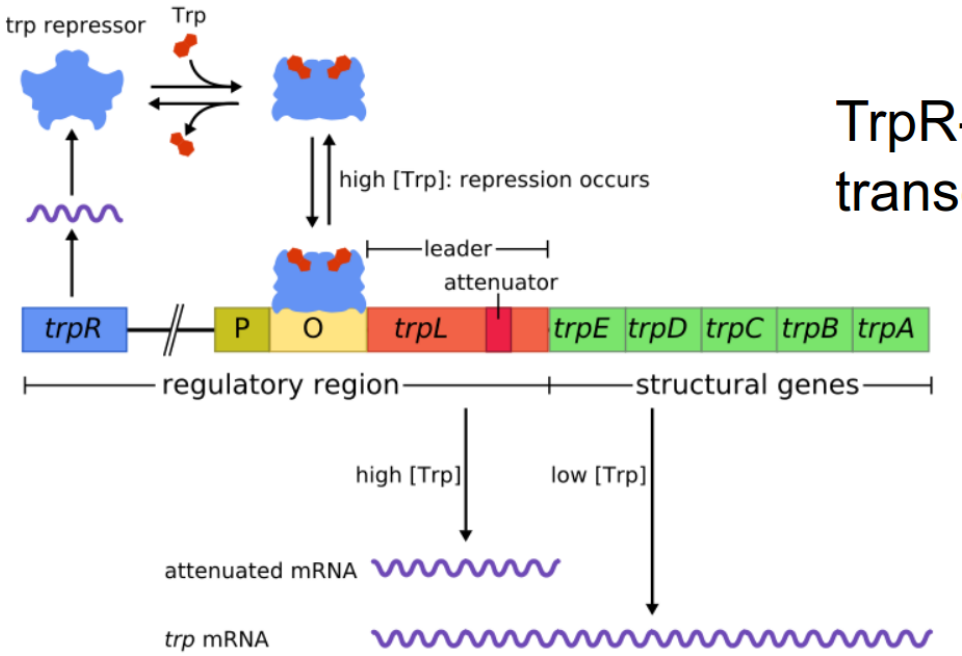


synthesis of Trp from chorismate



TrpR blocks trp operon transcription in +Trp conditions

TrpR constitutively expressed



TrpR-Trp binds operator to block transcription of Trp operon

http://en.wikipedia.org

Trp regulation by attenuation

Important points

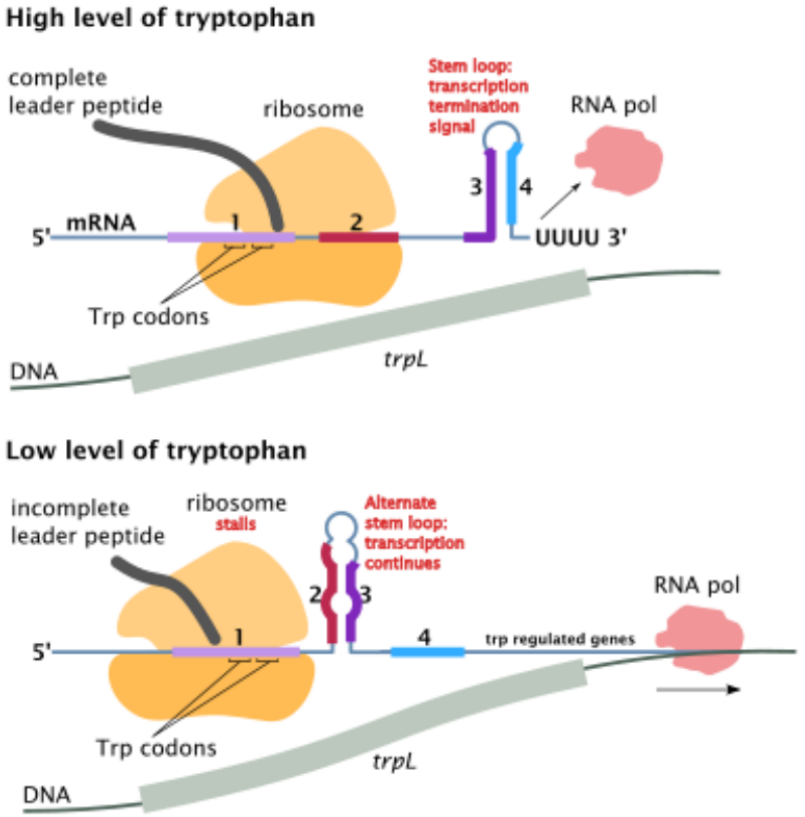
-simultaneous transcription and translation in bacteria

-upstream is tryptophan-rich 130 bp leader peptide

-two stem loops possible:
3-4 is a transcription terminator (RNA pol falls off DNA)

2-3 prevents formation of 3-4 but does not block transcription

-attenuation senses the amount of tRNA-trp



http://en.wikipedia.org/wiki/Trp_operon

Trp regulation by attenuation

attenuation also controls His, Phe, Thr synthesis

+Tryptophan

ribosome rapidly translates leader peptide

↓

stem loop 2-3 blocked

↓

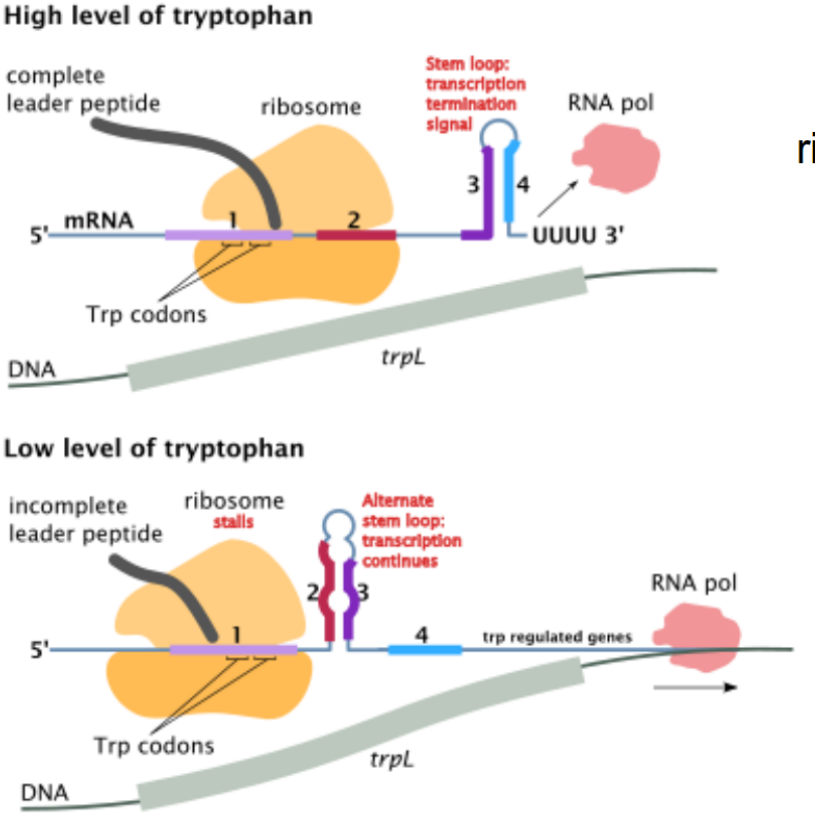
stem loop 3-4 (terminator) forms

↓

RNA pol falls off DNA

↓

trp operon repressed



http://en.wikipedia.org/wiki/Trp_operon

-Tryptophan

ribosome stalls on leader peptide (lacks tRNA-trp)

↓

stem loop 2-3 forms

↓

stem loop 3-4 (terminator) cannot form

↓

trp operon expressed