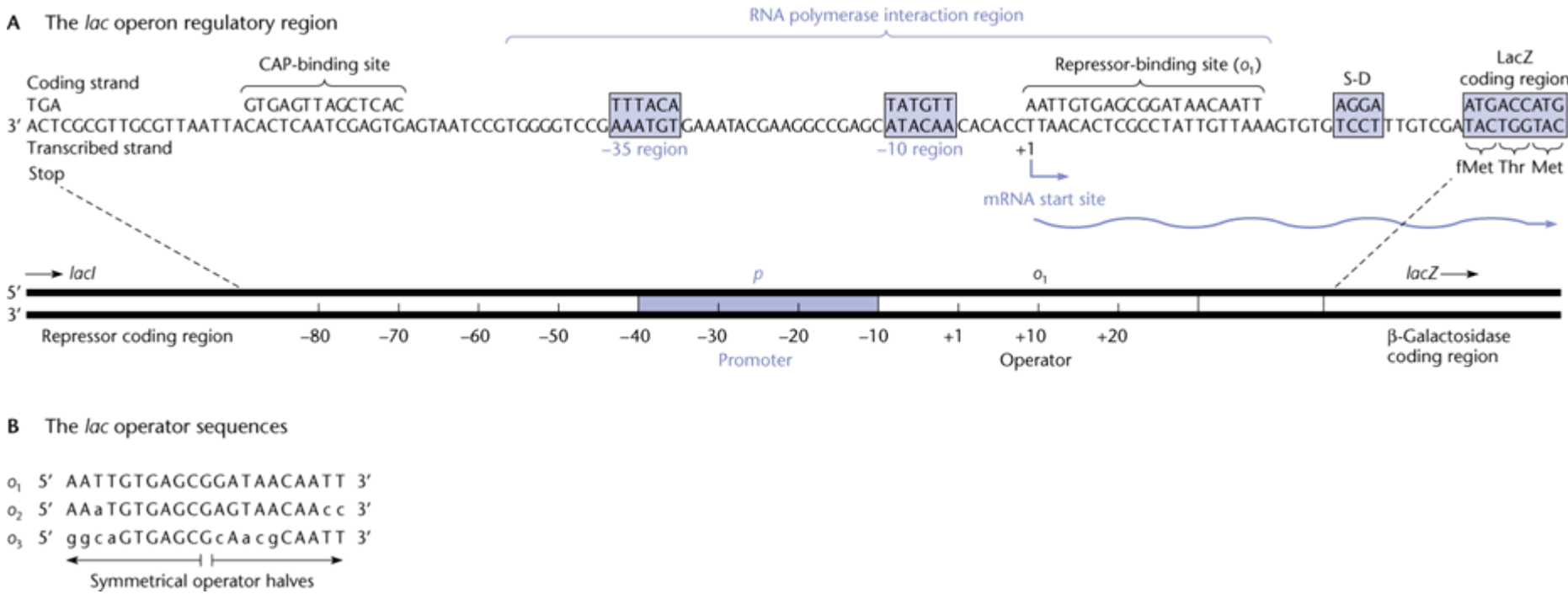
Gene regulation

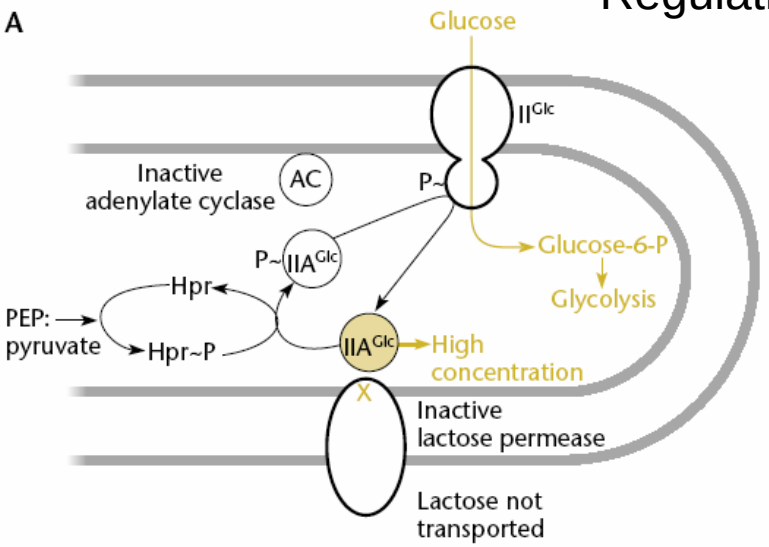
1. Promoter structure: *lac* as an example
2. Two-component regulatory systems: EnvZ/OmpR example

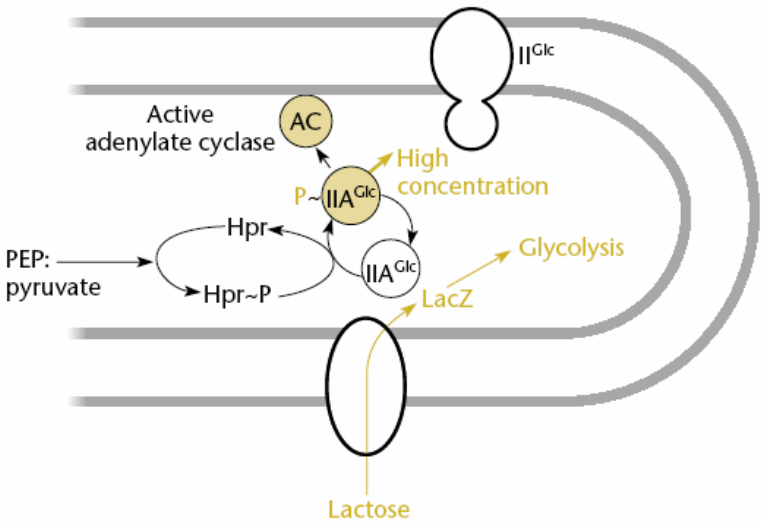
“The pencil is the wrench of the chromosome mechanic.”
John Roth

Figure 12.6


A The lac operon regulatory region
RNA polymerase interaction region
CAP-binding site
Repressor-binding site (o_1)
S-D
LacZ coding region
Coding strand
TGA
GTGAGTTAGCTCAC
TTTACA
TATGTT
AATTGTGAGCGGATAACAATT
AGGA
ATGACCATG
3′ ACTCGCGTTGCGTTAATTACACTCAATCGAGTGAGTAATCCGTGGGGTCCGAAATGTGAAATACGAAGGCCGAGCATACAACACACCTTAACACTCGCCTATTGTTAAAGTGTGTCCTTTGTCGATACTGGTAC
Transcribed strand
−35 region
−10 region
+1
fMet Thr Met
Stop
mRNA start site
lacI
p
o_1
lacZ
5′
3′
Repressor coding region
−80 −70 −60 −50 −40 −30 −20 −10 +1 +10 +20
Promoter
Operator
β-Galactosidase coding region
B The lac operator sequences
o_1 5′ AATTGTGAGCGGATAACAATT 3′
o_2 5′ AAaTGTGAGCGAGTAACAAccc 3′
o_3 5′ ggcaGTGAGCGcAacgCAATT 3′
Symmetrical operator halves

Regulation of cAMP Production





PEP (phospho enol pyruvate)-dependent sugar phosphotransferase system-transports glucose into the cells

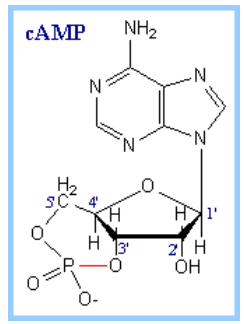
-PTS IIA^{glc} exists in two form +/- phosphate

-phospho IIA^{glc} activates adenylate cyclase

-ration of IIA^{glc} to IIA^{glc}-P depends on glucose availability

-Hpr Histidine protein adds phosphates

Glucose
↓
Glycolysis
↓
PEP:Pyruvate
↓
TCA Cycle



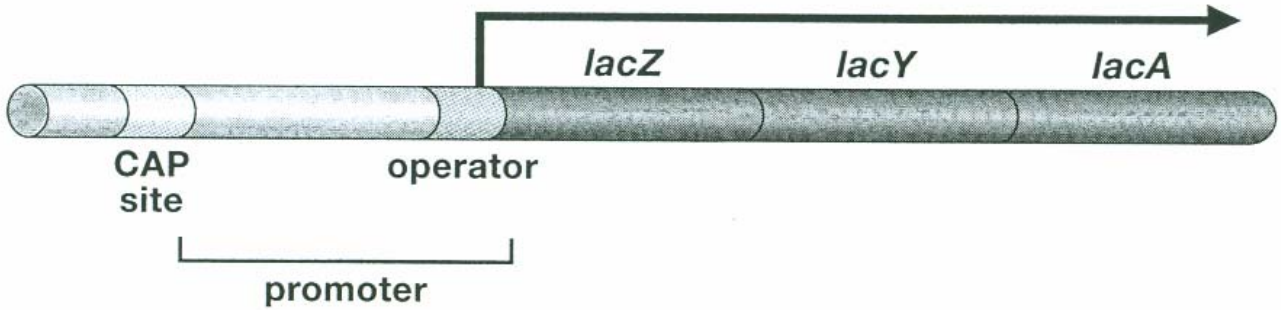


FIGURE 1.2. The *lac* operon. The *lacZ* gene is transcribed in a single mRNA along with two other genes, *lacY* and *lacA*. *lacY* encodes the permease that brings lactose into the cell, and *lacA* encodes an acetylase that is believed to detoxify thiogalactosides, which, along with lactose, are transported into cells by *lacY*. The promoter spans about 60 bp, and the CAP site and the operator (the Lac repressor-binding site) are about 20 bp each. The operator lies within the promoter, and the CAP site lies just upstream of the promoter. The picture is simplified in that there are two additional, weaker, *lac* operators located nearby (see Repression by *Lac* Repressor). It is not to scale: the *lacZ* gene, e.g., is about 3500 bp long. The entire element shown is called an "operon."

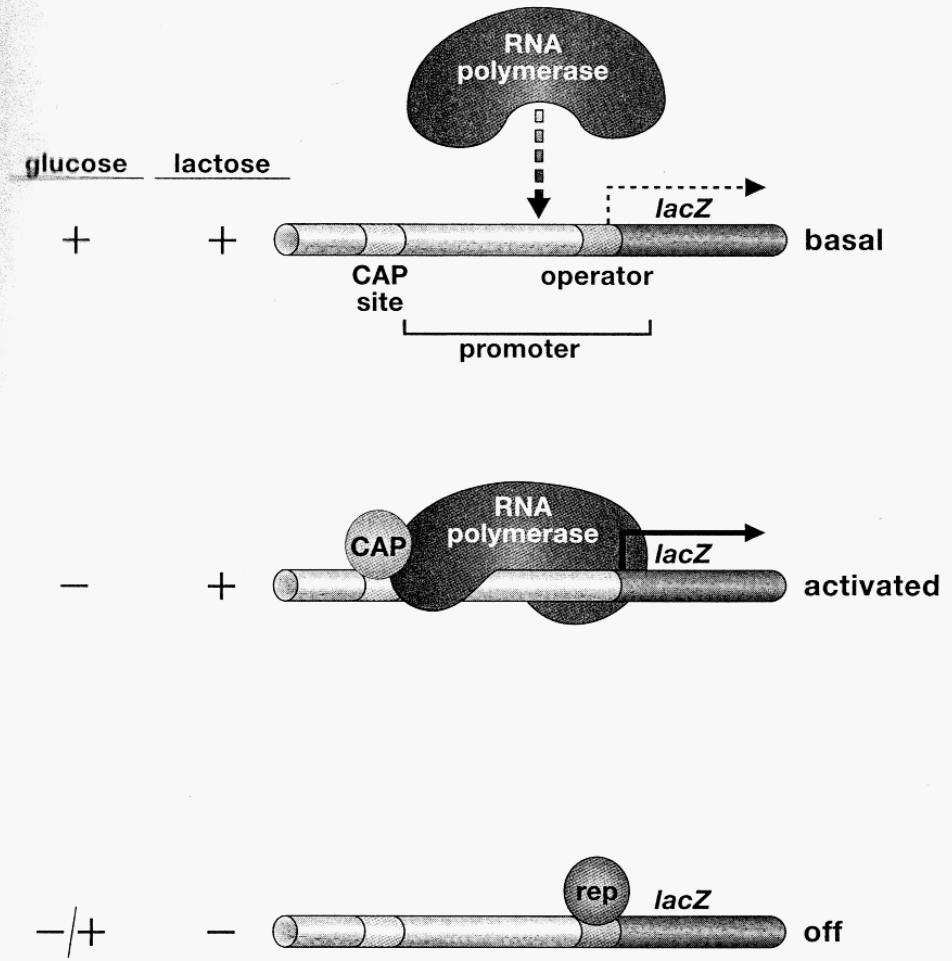


FIGURE 1.3. Three states of the *lac* genes. When bound to the operator, repressor excludes polymerase whether or not active CAP is present. Polymerase is shown in a more simplified form than in Figure 1.1: the carboxyl domains of the α subunits, which protrude from the left end of the polymerase as drawn here, are not shown. CAP actually binds DNA as a dimer, and Lac repressor as a tetramer.

1. In the presence of both sugars neither CAP or Lac repressor will bind. LacZ is produced at basal level. How much depends on RNAP level and frequency of contact

2. In the presence of lactose and no glucose, CAP is bound to CAP site, repressor is excluded, operon is on. CAP recruits RNAP to the promoter region.

3. In the absence of lactose, Lac repressor is bound, the operon transcription is off. Repressor binding is favored until lactose is present.

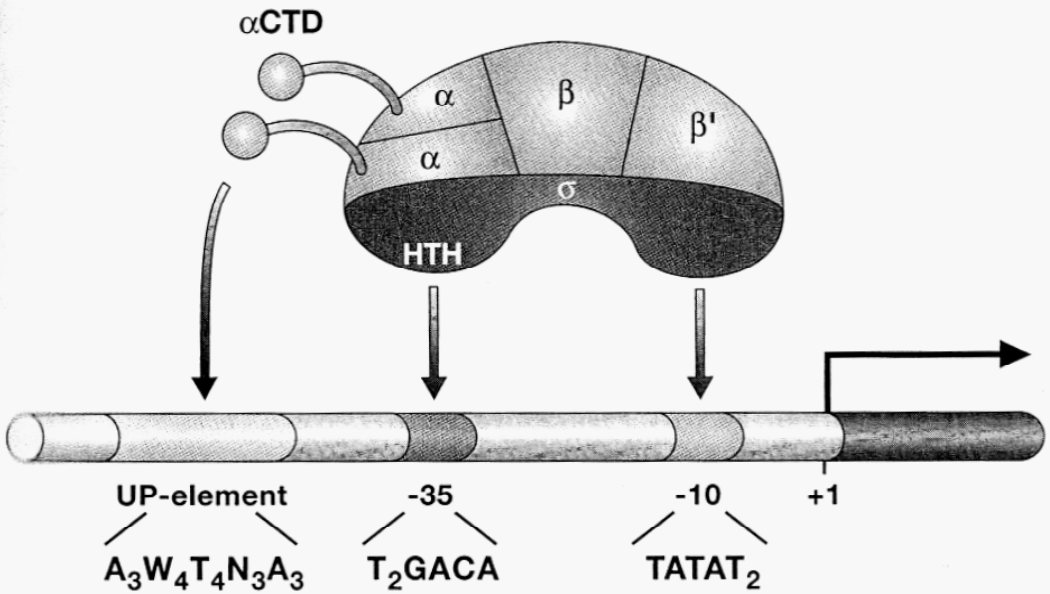


FIGURE 1.5. Sequence elements in promoters recognized by holoenzyme containing σ^{70}. The σ subunit is an elongated protein that can simultaneously contact the –10 and –35 regions of the promoter. Where present, the UP-element is recognized by the carboxy-terminal extensions of the α subunits. Although not indicated here, polymerase covers the transcription start site and an additional 20 bp downstream. Beneath each element is shown a "consensus" sequence for that element. W represents adenine or thymine; N represents any base; and a subscript indicates the number of reiterations of that base.

CTD-C-terminal domain of RNA polymerase interacts with regulatory proteins bound to regions upstream of the -35 region.
This domain is flexible.
Sigma 70 is the house-keeping sigma factor

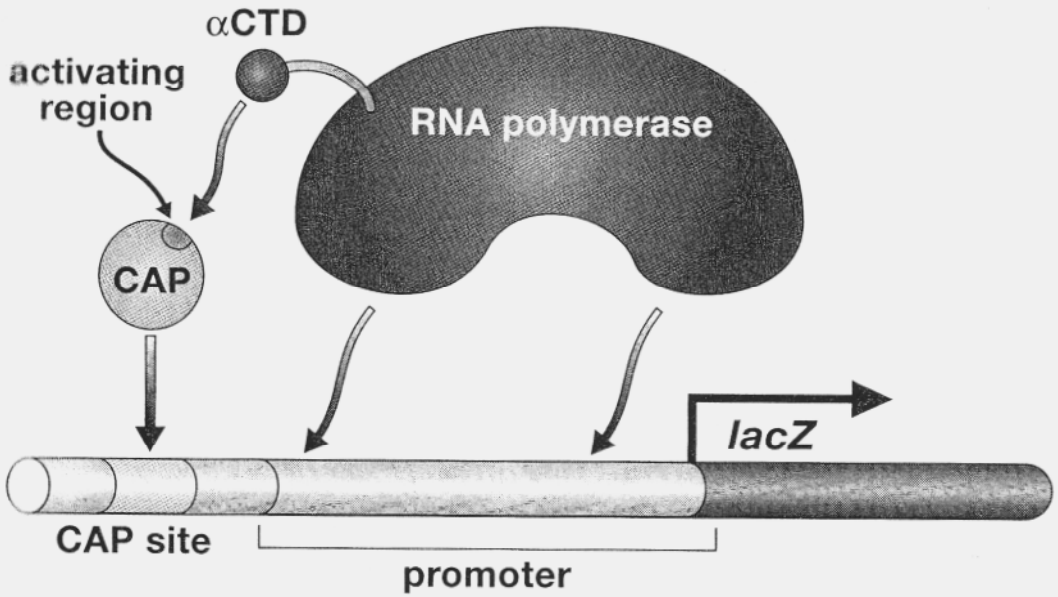


FIGURE 1.7. Cooperative binding of CAP and polymerase to DNA. The picture is simplified in that only one CAP monomer and only one αCTD are shown. It is also believed that in the absence of an UP-element (see Figure 1.5), αCTD is bound to DNA nonspecifically when contacted by CAP.

1. Binding of CAP recruits RNAP to the promoter region
2. CAP-RNAP complex causes helix to open
3. Transcription can proceed.

Interaction of CAP and RNAP requires DNA, does not require ATP.
Does result in 40+ fold induction of the lac operon over basal rate.

Biochemical and Genetic Evidence for CAP and RNAP interaction

1. Chemical cross linking and “in vivo” foot print analysis. In the absence of lactose, very few cells will have RNAP bound to the promoter region. In the presence of lactose, all cells will. These methods used chemicals to cross-link CAP and RNAP bound to DNA in living cells exposed to either glucose or lactose.

2. “Positive control mutants”. CAP has two surfaces, one that binds RNAP α-CTD and one that binds DNA. Mutants that are unable to bind RNAP but still bind DNA cannot activate transcription. This is evidence that protein-protein interaction is required for activation of operon.

3. Polymerase mutants. Deletion of the α-CTD of the alpha subunit abolishes activation by CAP.

4. DNA binding mutants. CAP mutants that are unable to bind DNA are unable to interact with RNAP to activate transcription

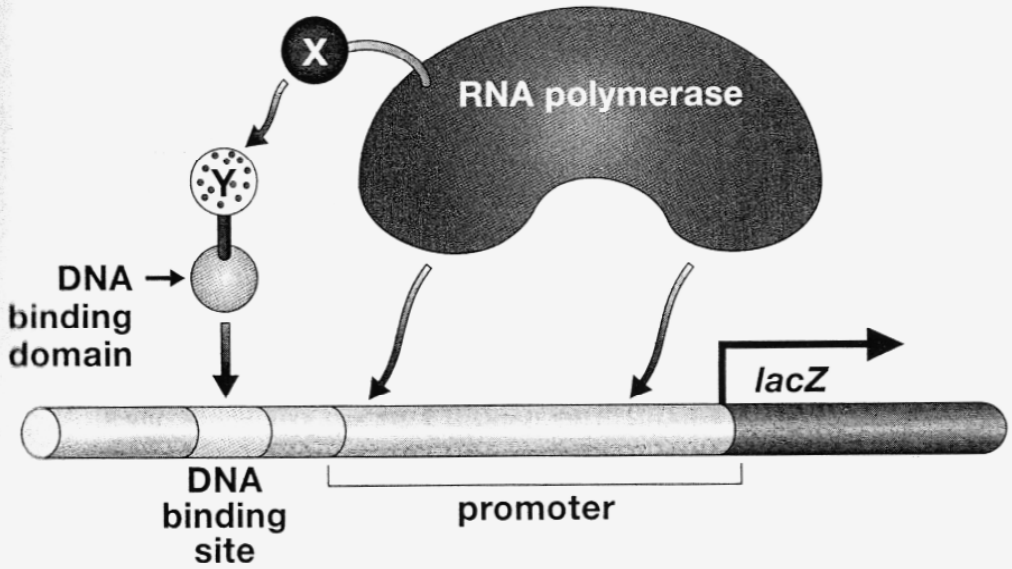


FIGURE 1.8. Activator bypass experiment 1. In this example, the αCTD has been replaced by another protein domain, labeled *X*, that is known to interact with protein *Y*. Protein *Y* has been fused to a DNA-binding domain, and the site recognized by that domain has been placed near the *lac* genes. Examples of such experiments include a case in which *X* is a domain of a yeast protein (called Gal11P), and *Y* is a domain of a yeast protein that interacts with Gal11P (the dimerization region of Gal4). These proteins are discussed in Chapter 2. In another case, both *X* and *Y* can be the interacting carboxyl domains of λ repressor, discussed later in this chapter.

By-Pass experiment 1.

Evidence for the interation of α-CTD of RNAP.

1. Replace the α-CTD with another protein
2. Replace the CAP binding region with another DNA binding sequence

LacZ expression will now be under the control of the substituted controls

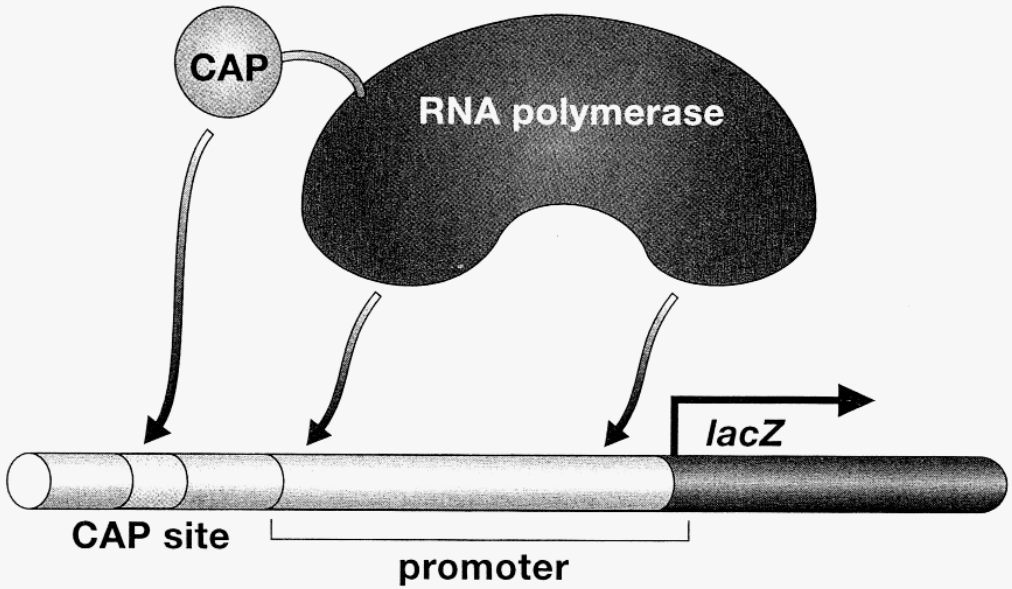


FIGURE 1.9. Activator bypass experiment 2. In this example, the αCTD has been replaced by the DNA-binding portion of CAP. Other experiments show that attaching a DNA-binding domain to ω (see Footnote 1) also works in such an experiment.

By-pass experiment 2

1. The CAP DNA binding domain is fused to the region were α-CTD would be.
2. Now, high level of lacZ expression is observed

This experiment shows that the CAP- α-CTD binding region is not needed, but simply efficient recruitment of RNAP to the promoter results in activation.

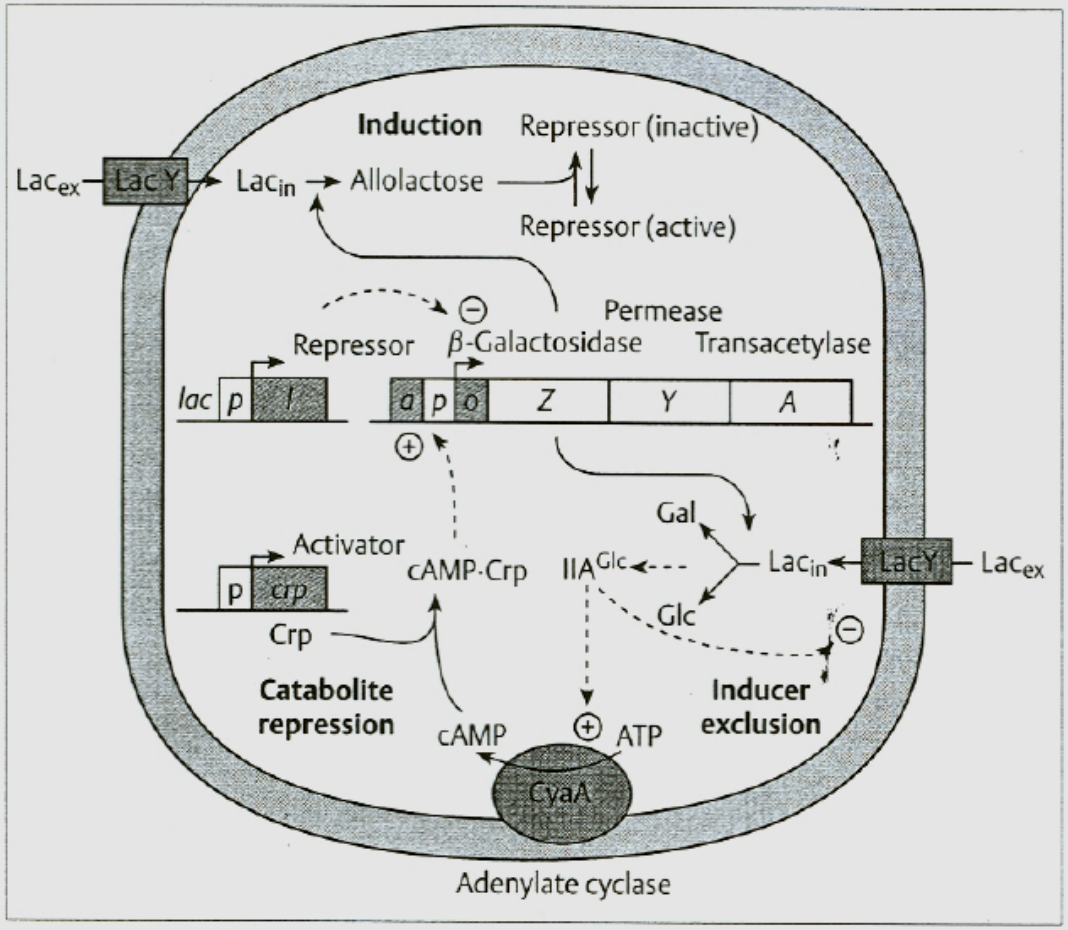
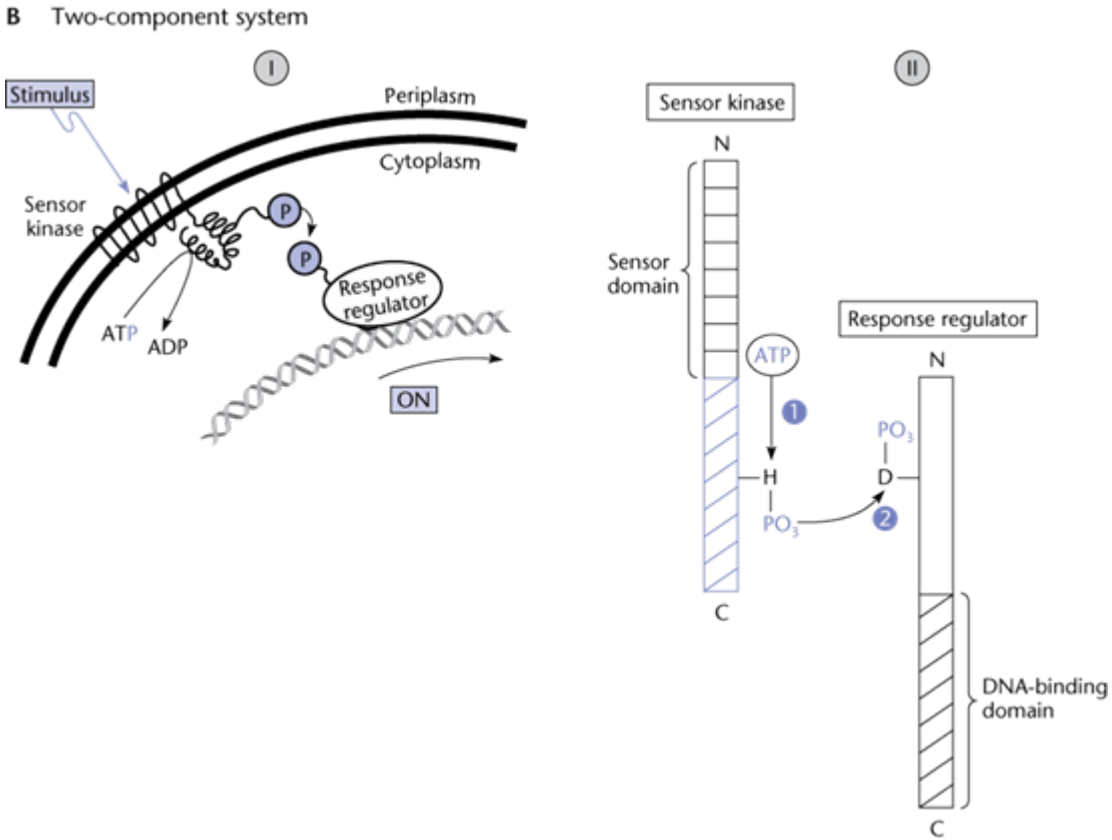


Fig. 20.4 **Schematic view of the *lac* operon and of lactose (Lac) metabolism as elements of a primitive sensory system.** In this model, induction corresponds to sensory input, hydrolysis of lactose corresponds to the response (output), and catabolite repression together with inducer exclusion corresponds to adaptation

The lac operon and CAP system constitute an primitive sensory system

Box 13.4B



Two-component regulatory systesms

1. All bacteria have these systems: 1% of genome ~61 systems
2. All have a sensor domain to sense the extracytoplasmic environment
3. All have a transmitter-receiver that “transmits” the signal to the response regulator
4. The response regulator is usually a DNA binding protein.

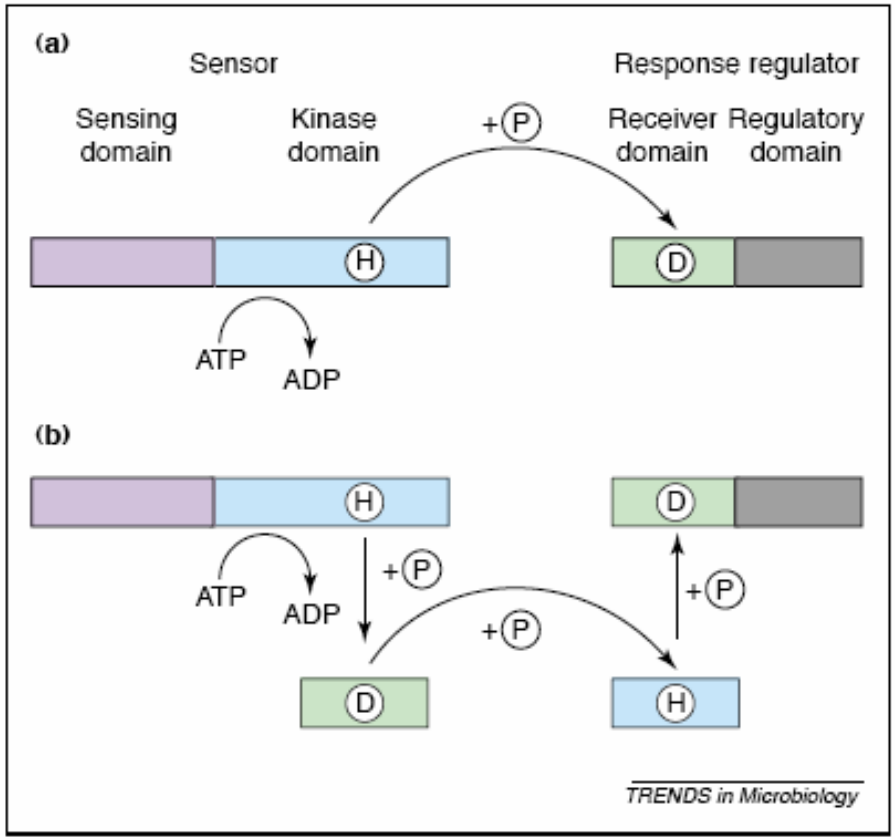


Fig. 1. Two-component systems consist of a sensor protein (histidine kinase) that transfers a high-energy phosphoryl group to the response regulator, which is often a transcription factor. (a) The sensor protein autophosphorylates on a conserved histidine residue (H) in the kinases domain (blue), often in response to signal sensing in the sensing domain (purple). The high-energy phosphoryl group (P) is then transferred to a conserved aspartate (D) residue in the receiver domain (green) of the response regulator, often changing the ability of its regulatory domain (grey) to bind DNA. (b) The His-Asp-His-Asp three-step phosphorelay consists of the transfer of the phosphoryl group from the conserved histidine in the sensor to additional phosphorylatable aspartate and histidine residues in auxiliary domains before transfer to the aspartate in the receiver domain of the response regulator. Many different arrangements of these extra phosphorylatable domains exist within proteins and pathways. They are either present in separate proteins, a representative example of which is depicted here, or part of multidomain (hybrid) sensors.

Simple sensor-response

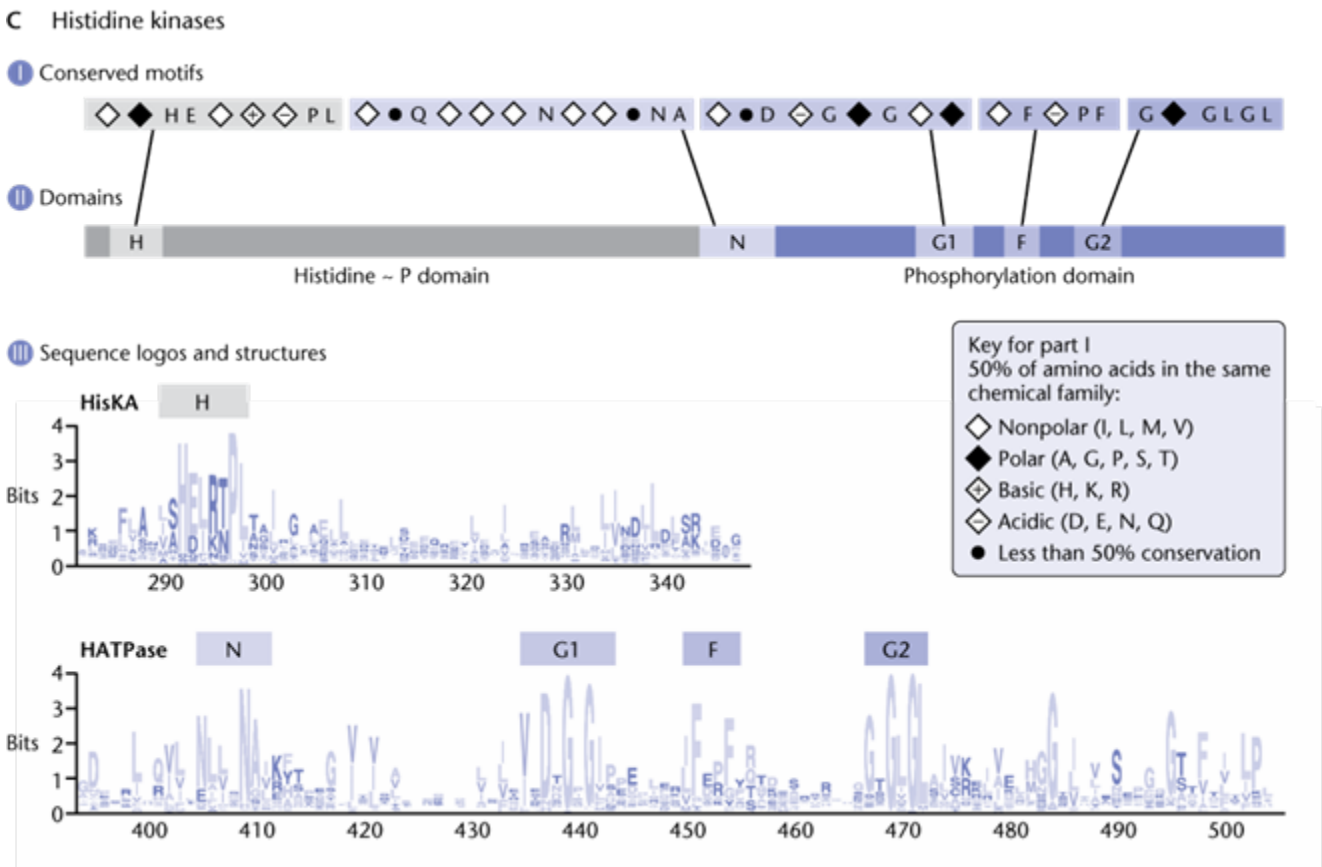
Phospho-relay system

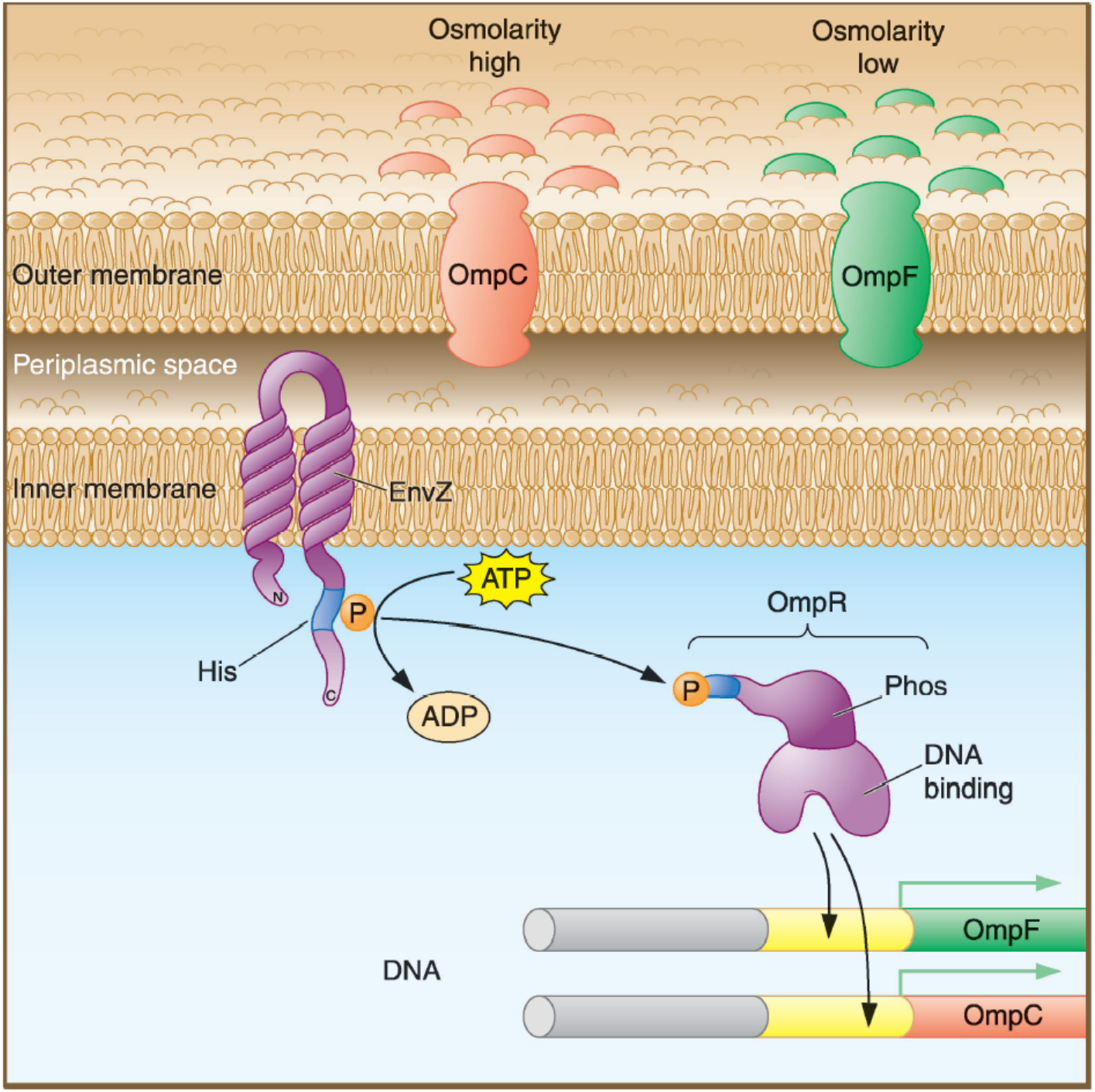
Eduardo Groisman

TRENDS in Microbiology Vol.11 No.8 August 2003

Box 13.4C

Sensor Kinases are very conserved. The histidine in the sensor-kinase is very conserved.

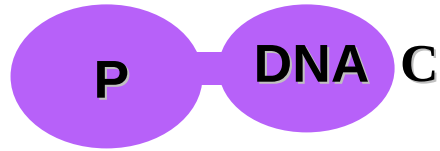




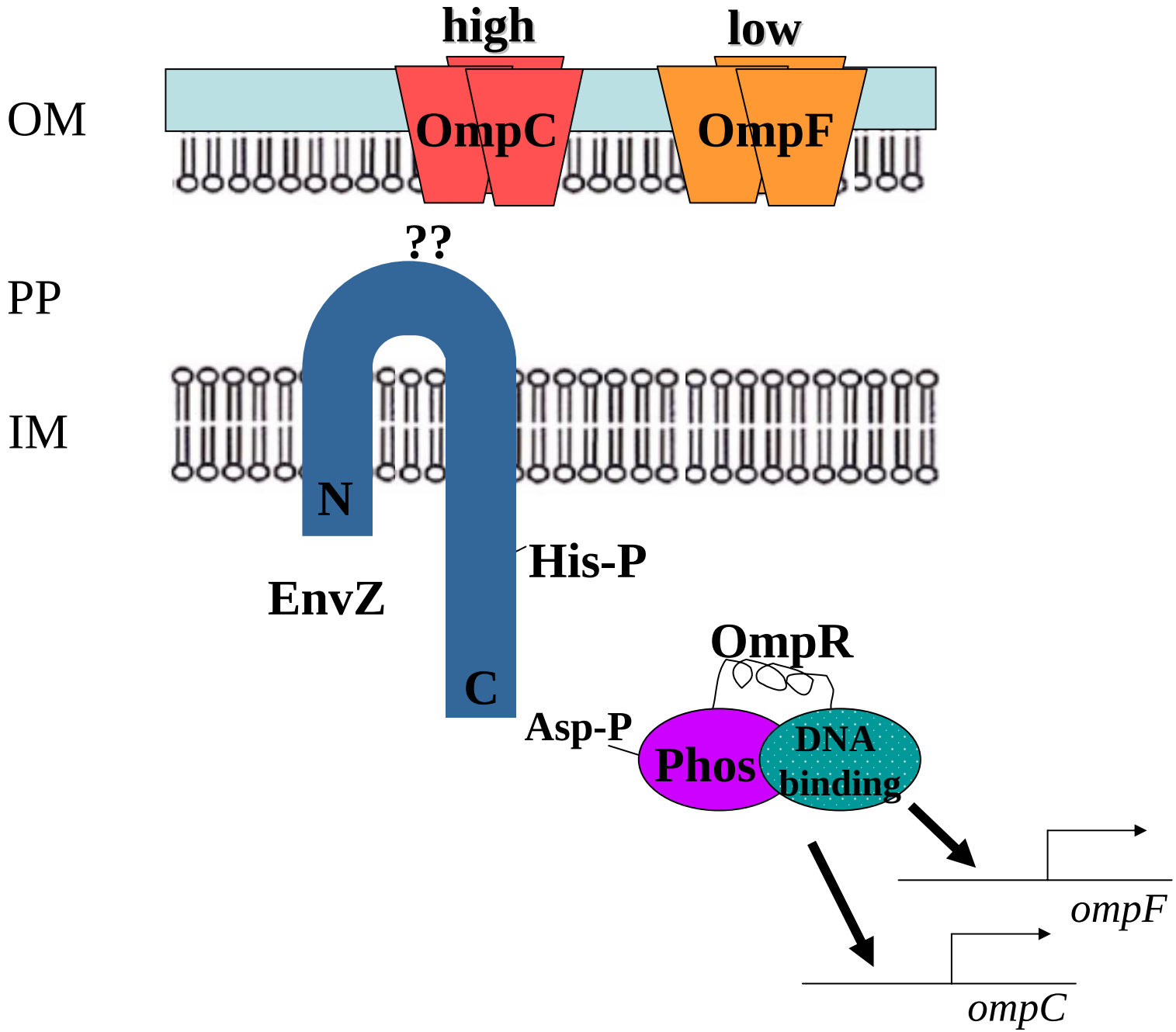
Linda Kenney
Univ. of Ill.

OmpR

- 239 amino acids (27 kDa)



- Two domains that are separated by a flexible linker region
- N-terminal domain contains the site of phosphorylation, Asp55
- C-terminal domain binds to DNA via a winged HTH-motif
- Regulates curli, fimbriae
- Regulates resistance to microcin, an antimicrobial peptide
- In *Salmonella*, regulates ability to replicate in macrophages
- Regulates flagellar operon
- Regulates porins


high
low
OM
OmpC
OmpF
??
PP
IM
N
His-P
EnvZ
C
OmpR
Asp-P
Phos
DNA
binding
ompF
ompC

Osmoregulation in E.coli/Salmonella: EnvZ/OmpR

1. The function of this system is to regulate porin proteins OmpC and OmpF
2. Porins function to regulate the concentration of ions across the cell membrane and hence the salt balance in the cytoplasm.
3. Cells grown in high salt will have more OmpC than OmpF, and more OmpF when in low salt.
4. Ratio of OmpC/F also depends on temp, oxidative stress, organic solvents, bile salts

Genetics: how to find genes that regulate OmpF and OmpC

1. Porin mutants were resistant to a particular phage and were not killed by bacteriocins (like colicins)
2. Two other loci were identified that affected OmpF and OmpC expression: EnvZ and OmpR
3. OmpR looked like a transcriptional regulatory protein, similar to the systems that regulate ammonia utilization

Experiment: make *ompF* and *ompC lacZ* fusions and isolate mutants in OmpR and EnvZ that affect expression.

Affinity model of porin gene regulation

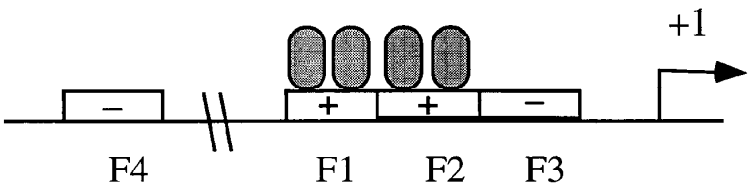
♉ Low Osmolarity

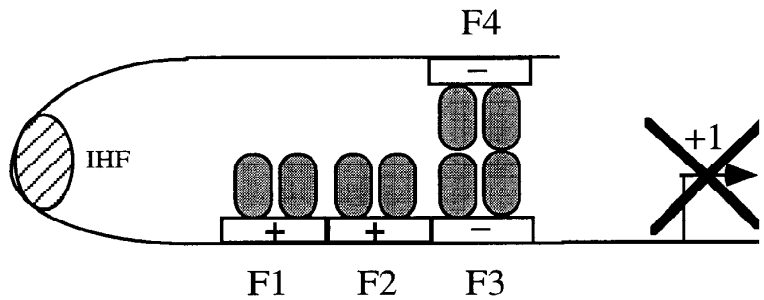
♉ ↓ OmpR-P

♉ High Osmolarity

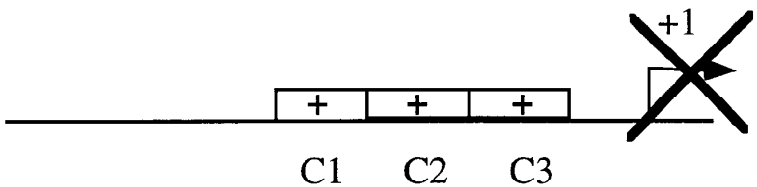
♉ ↑ OmpR-P

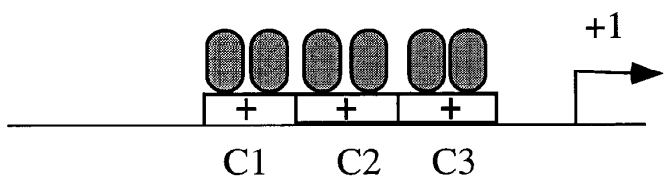
ompF





ompC





Phenotype of EnvZ and OmpR mutants

Genotype	Predicted Phenotype		Actual Phenotype	
	OmpC	OmpF	OmpC	OmpF
envZ+ ompR+	N/A	N/A	$+^{high}$	$+^{low}$
envZ+ ompR1	-	-	-	-
envZ(null) ompR+	-	+	-	+/-

The unexpected result!

Summary of Table 13.3 p578

Phenotype of OmpR- constitutive mutants

Genotype	Phenotype OmpC	OmpF
envZ+ ompR+	+ high	+ low
envZ(null) ompR+	**-**	**+/-**
envZ+ ompR2(con)	**-**	**+** ★
envZ(null) ompR2(con)	**-**	**+** ★
envZ+ ompR3(con)	**+**	**-**

★ Under high and low osmolarity

OmpR(con): a form of OmpR that mimics the phosphorylate state without being phosphorylated

Diploid analysis using the *ompR3(con)* mutant

Genotype	Predicted Phenotype		Actual Phenotype	
	OmpC	OmpF	OmpC	OmpF
envZ+ ompR+			$+^{high}$	$+^{low}$
envZ+ ompR3(con)	+	-	+	★-
envZ+ ompR3(con) /envZ+/ompR+ **Partial diploid**	+	$+^{low}$	+	★-

★ Under high and low osmolarity

Affinity model of porin gene regulation

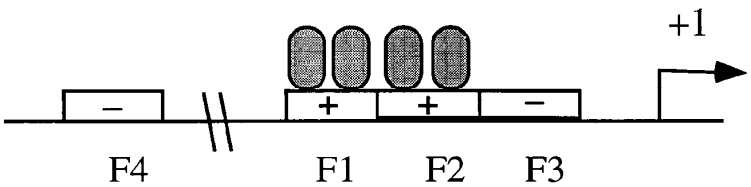
♉ Low Osmolarity

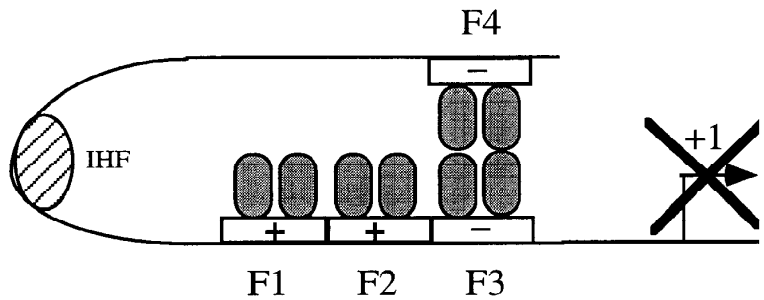
♉ ↓ OmpR-P

♉ High Osmolarity

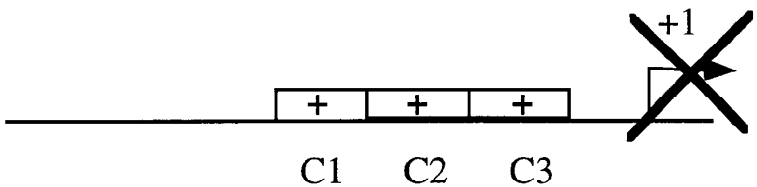
♉ ↑ OmpR-P

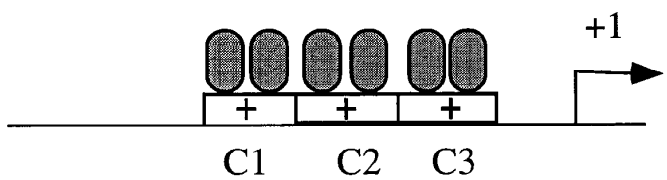
ompF



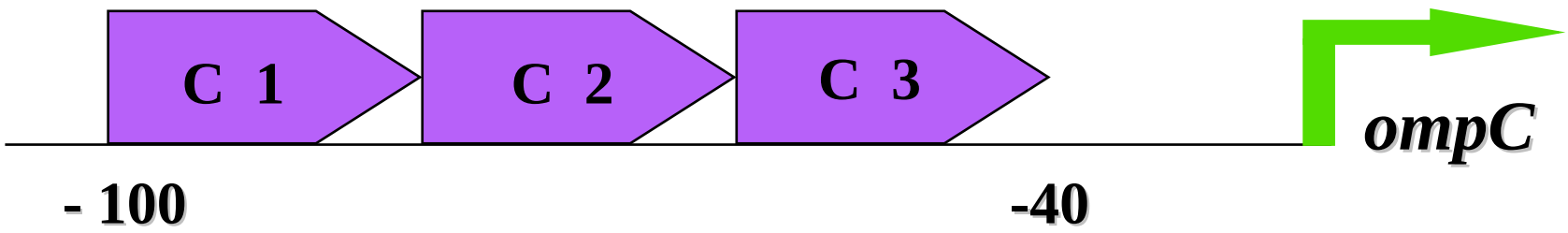


ompC

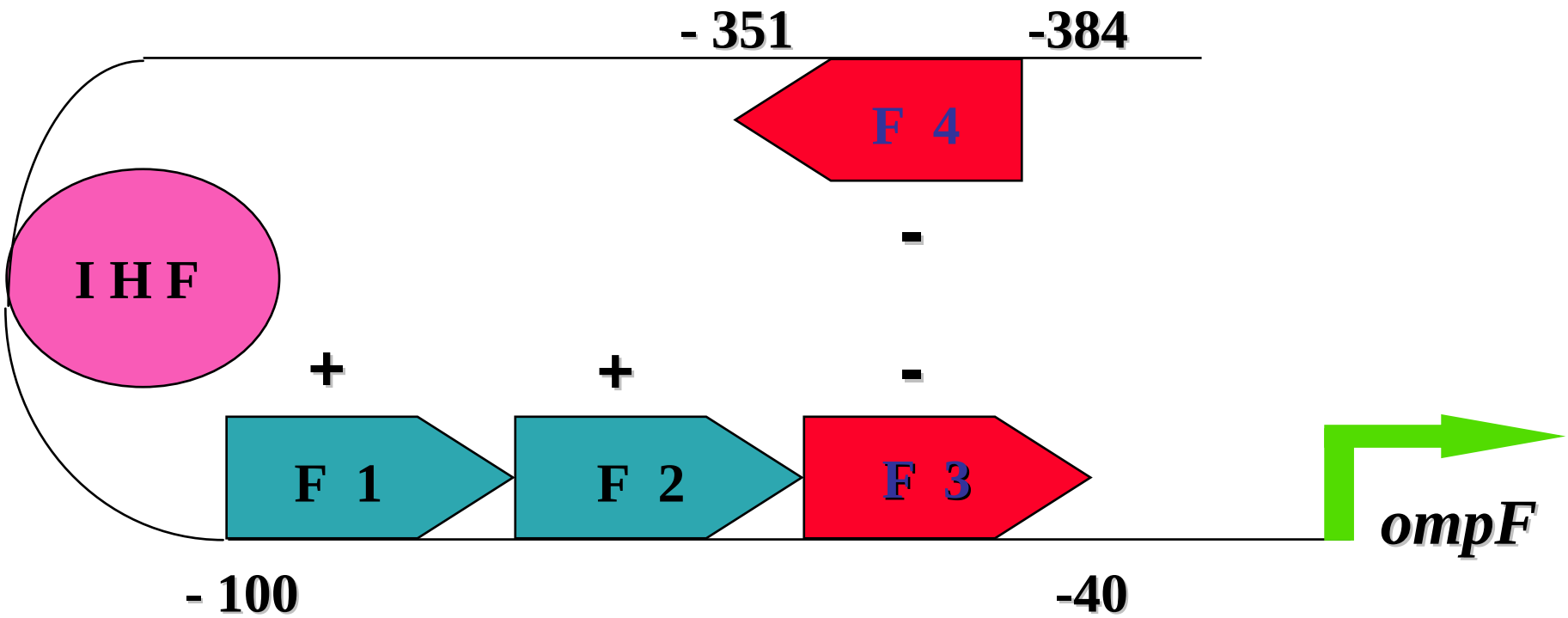




the *ompC* regulatory region

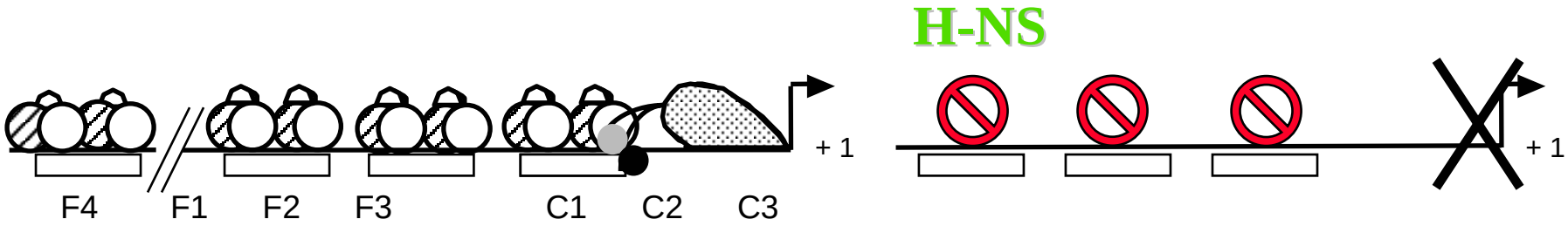


the *ompF* regulatory region

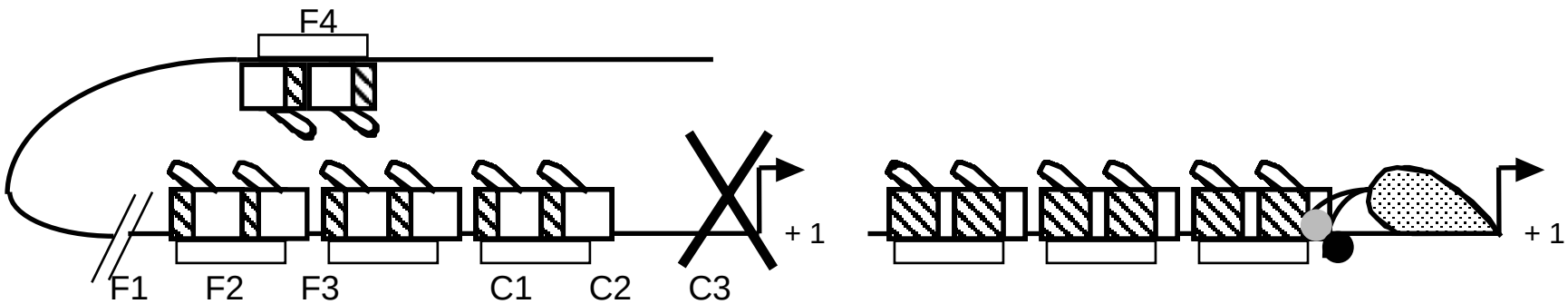


Conformational changes in OmpR-P control porin gene expression

(a) Low osmolarity



(b) High osmolarity



ompF ***ompC***

Question for the Day:
Are bacteria digital or analog?

E.coli with pBAD:gfp green fluorescent protein
-add arabinose, look under the microscope

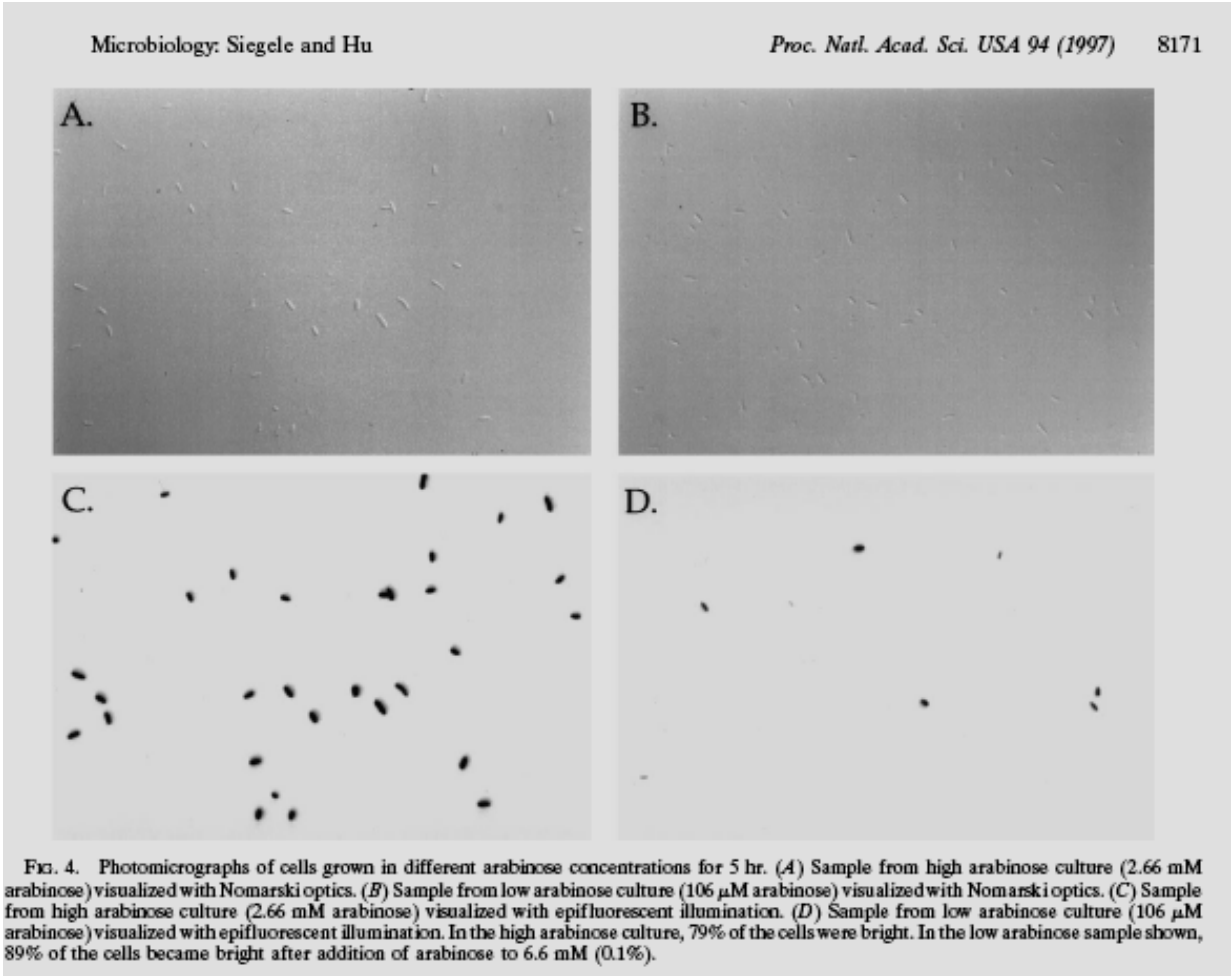


FIG. 4. Photomicrographs of cells grown in different arabinose concentrations for 5 hr. (*A*) Sample from high arabinose culture (2.66 mM arabinose) visualized with Nomarski optics. (*B*) Sample from low arabinose culture (106 μM arabinose) visualized with Nomarski optics. (*C*) Sample from high arabinose culture (2.66 mM arabinose) visualized with epifluorescent illumination. (*D*) Sample from low arabinose culture (106 μM arabinose) visualized with epifluorescent illumination. In the high arabinose culture, 79% of the cells were bright. In the low arabinose sample shown, 89% of the cells became bright after addition of arabinose to 6.6 mM (0.1%).

Light micrograph

Fluorescent micrograph