FOGA I - IS THERE SUCH A THING AS A GENE? = FORMATTING THE GENOME FOR PROTEIN SYNTHESIS

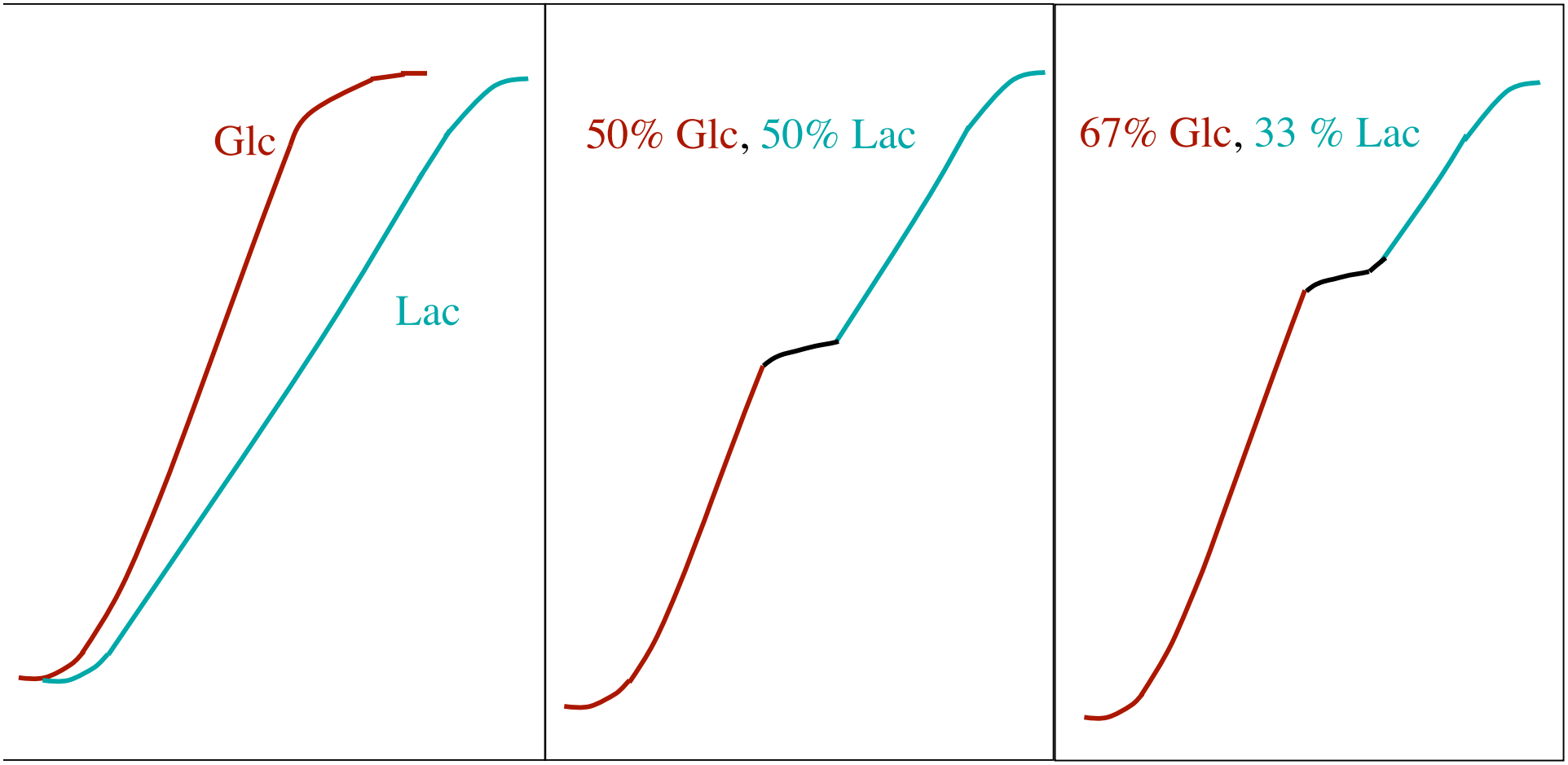
Dilemma in choosing lecture style:

• Schematic presentation of all exceptions to classical view

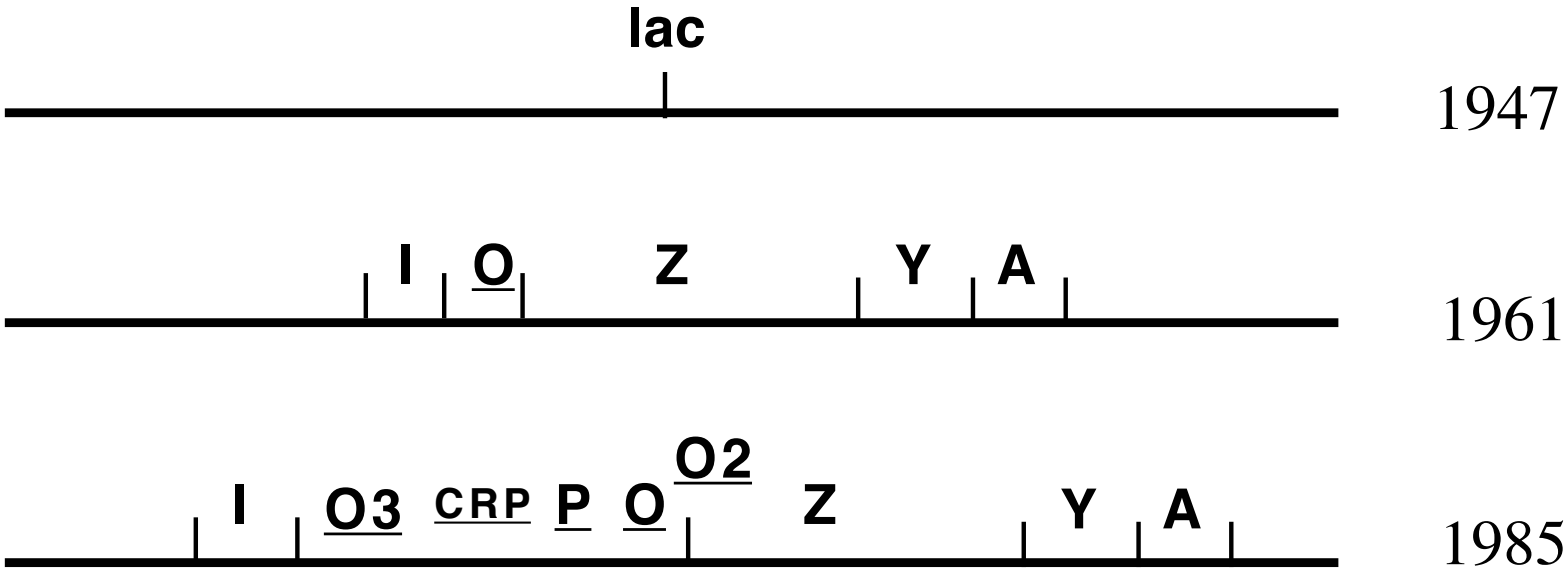
•• In-depth examination of basic model system & discussion of general principles

Lectures I & III - model system approach; lecture II - schematic approach

Diauxic Growth - a Cognitive Problem (Monod, 1942)



Historical Deconstruction of *lac*



Cis-dominance of operator mutations ==> operator does not encode protein

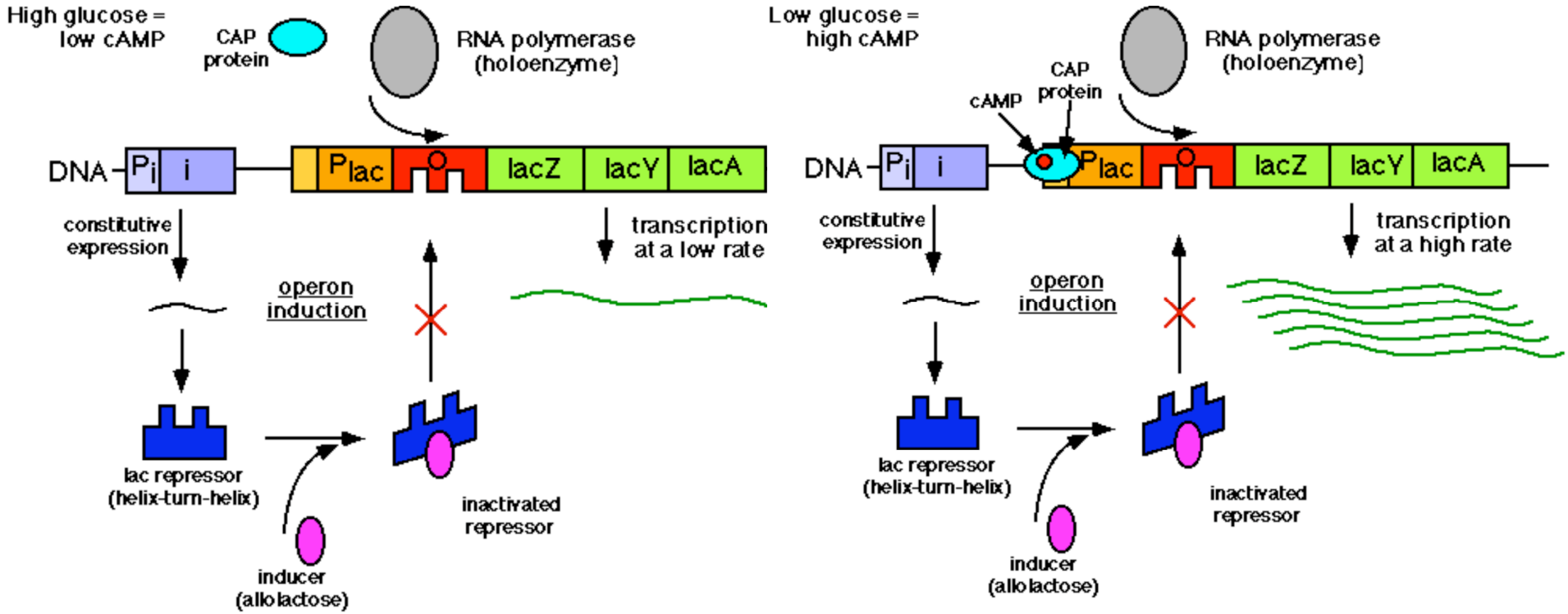
- $I^+ O^+ Z^- Y^+ / I^- O^+ Z^+ Y^-$ = inducible for Z and Y

Constitutive I^- mutation is **recessive** for both the active *lacZ* sequence in *cis* and for the active *lacY* sequence in *trans*

- $I^+O^+Z^-Y^+ / I^+O^cZ^+Y^-$ = constitutive for Z but inducible for Y

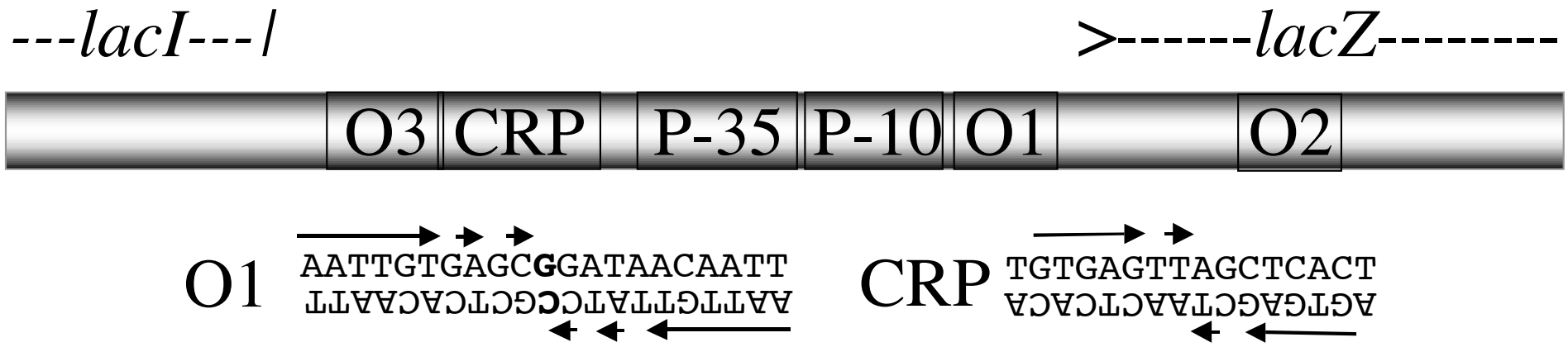
Constitutive o^c mutation is **dominant** in *cis* and conveys its constitutive phenotype to the adjacent active *lacZ* sequence, but the same mutation is **recessive** in *trans* and does not affect expression of the active *lacY* sequence on another DNA molecule

Cartoon of *lac* operon induction

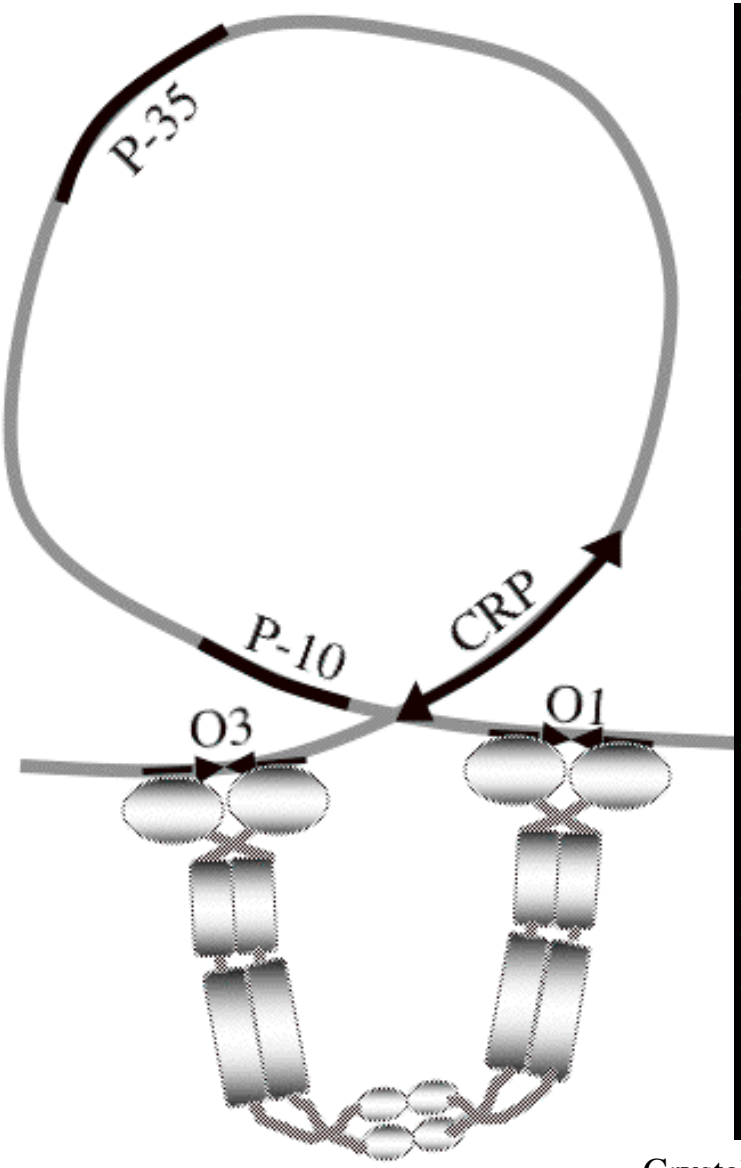


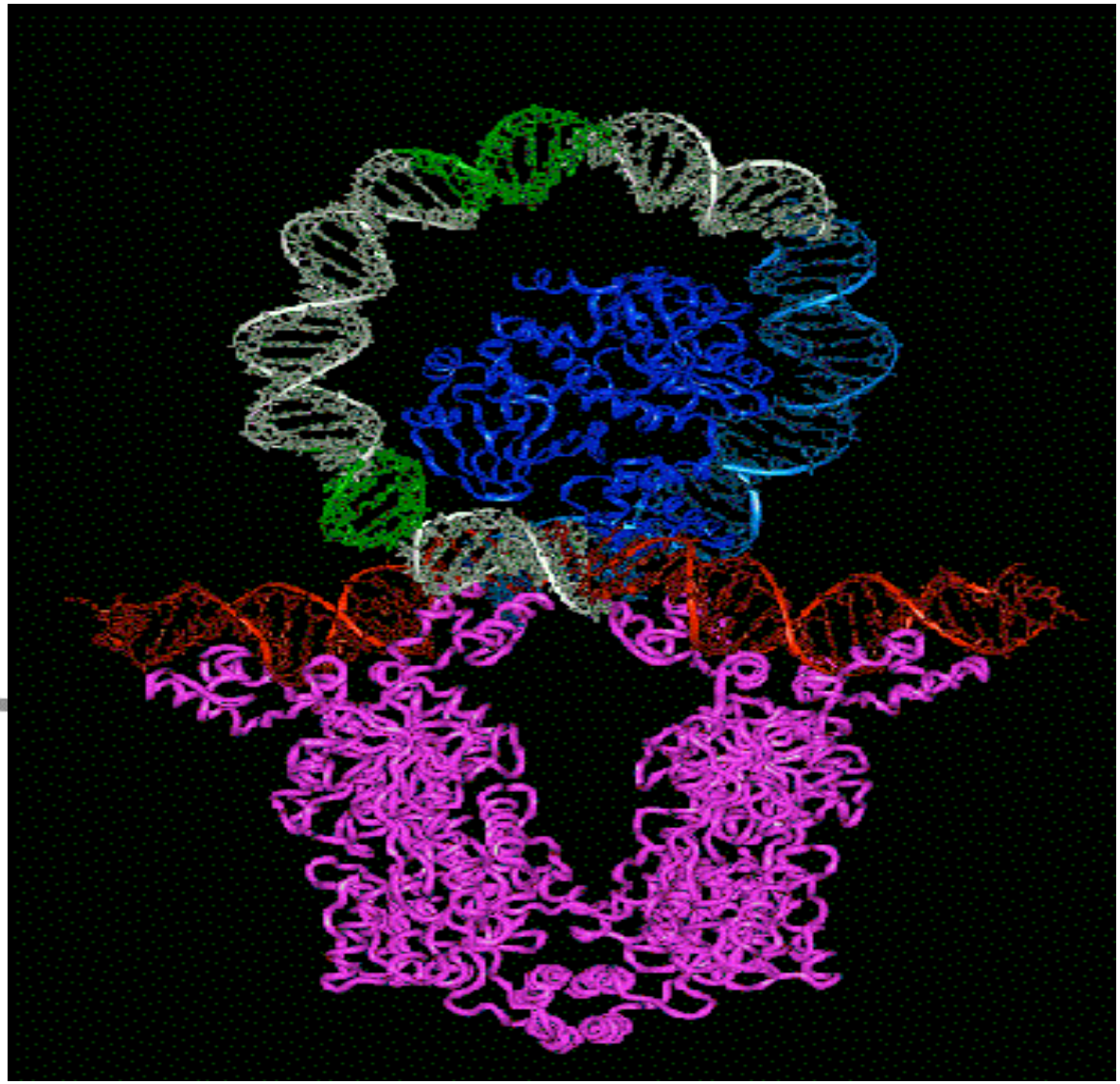
http://www.blc.arizona.edu/marty/411/Modules/catrep.html

Composite Nature of Operator, Promoter and Crp Sites



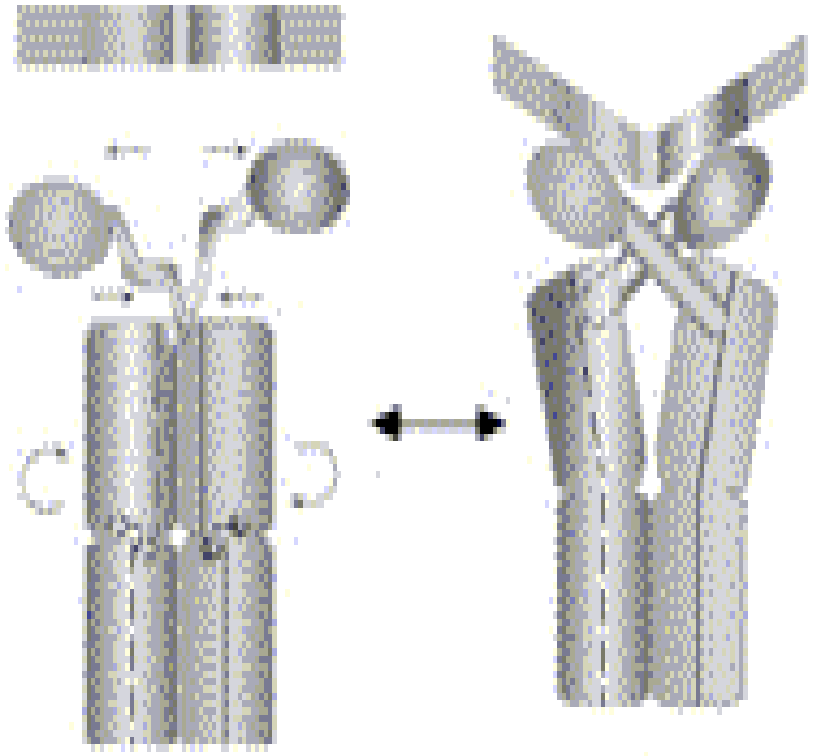
LacI Repressor Binding to *lacO* Sites and DNA Looping - Cooperativity Plus



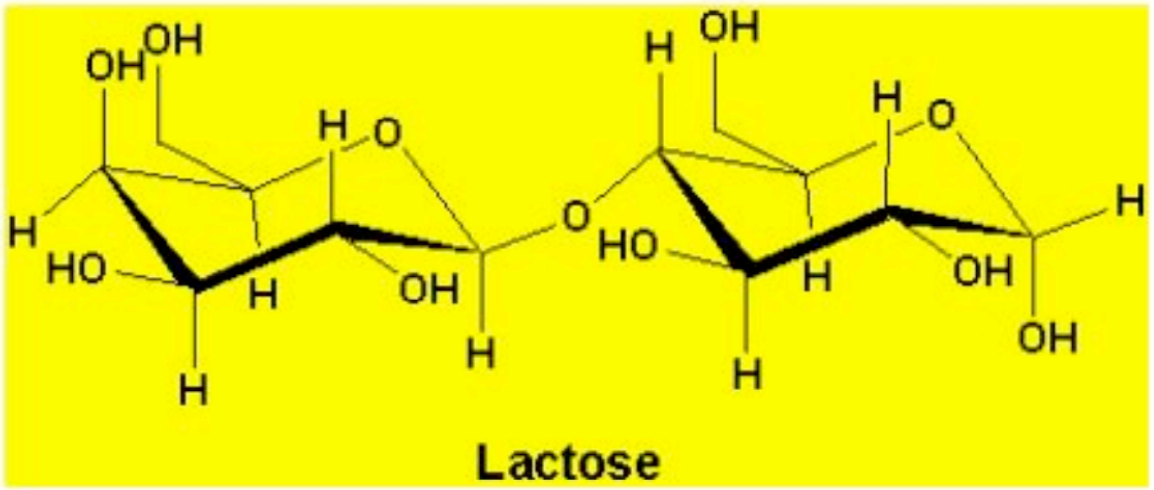


Crystal Structure of the Lactose Operon Repressor and Its Complexes with DNA and Inducer. Mitchell Lewis, et al. Science, Vol. 271(Mar. 1, 1996), pp. 1247-1254.

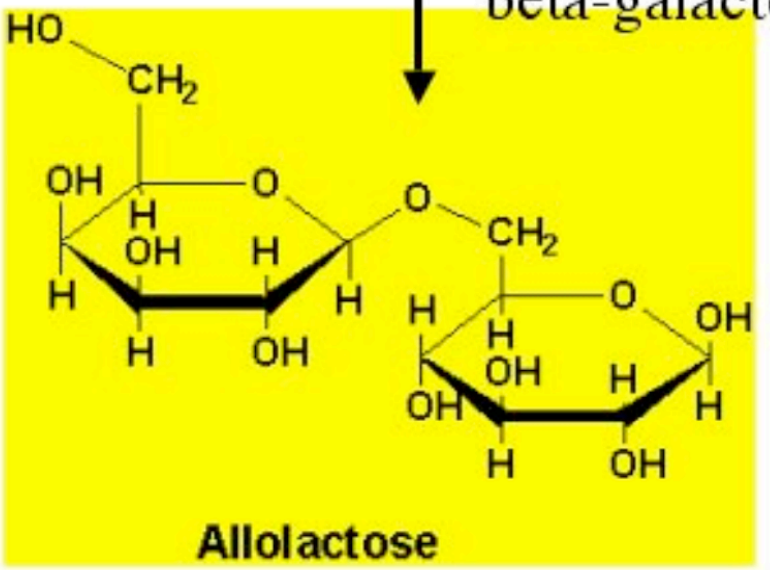
Derepression by Allosteric Effect of Inducer Binding to Repressor



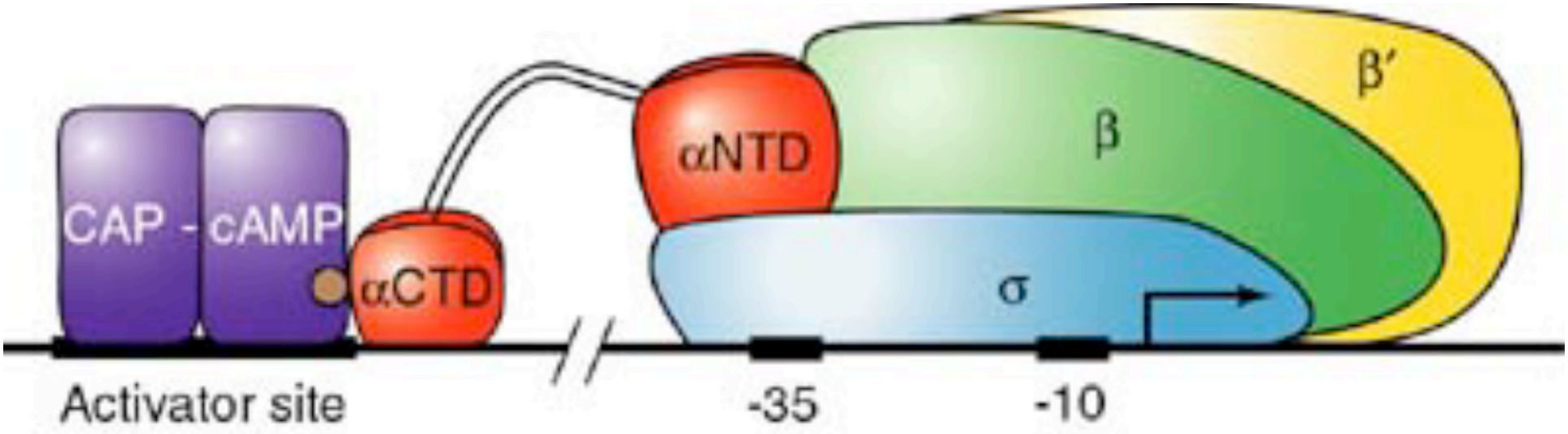
LacZ Converts Lactose to Allolactose Inducer



beta-galactosidase (LacZ)

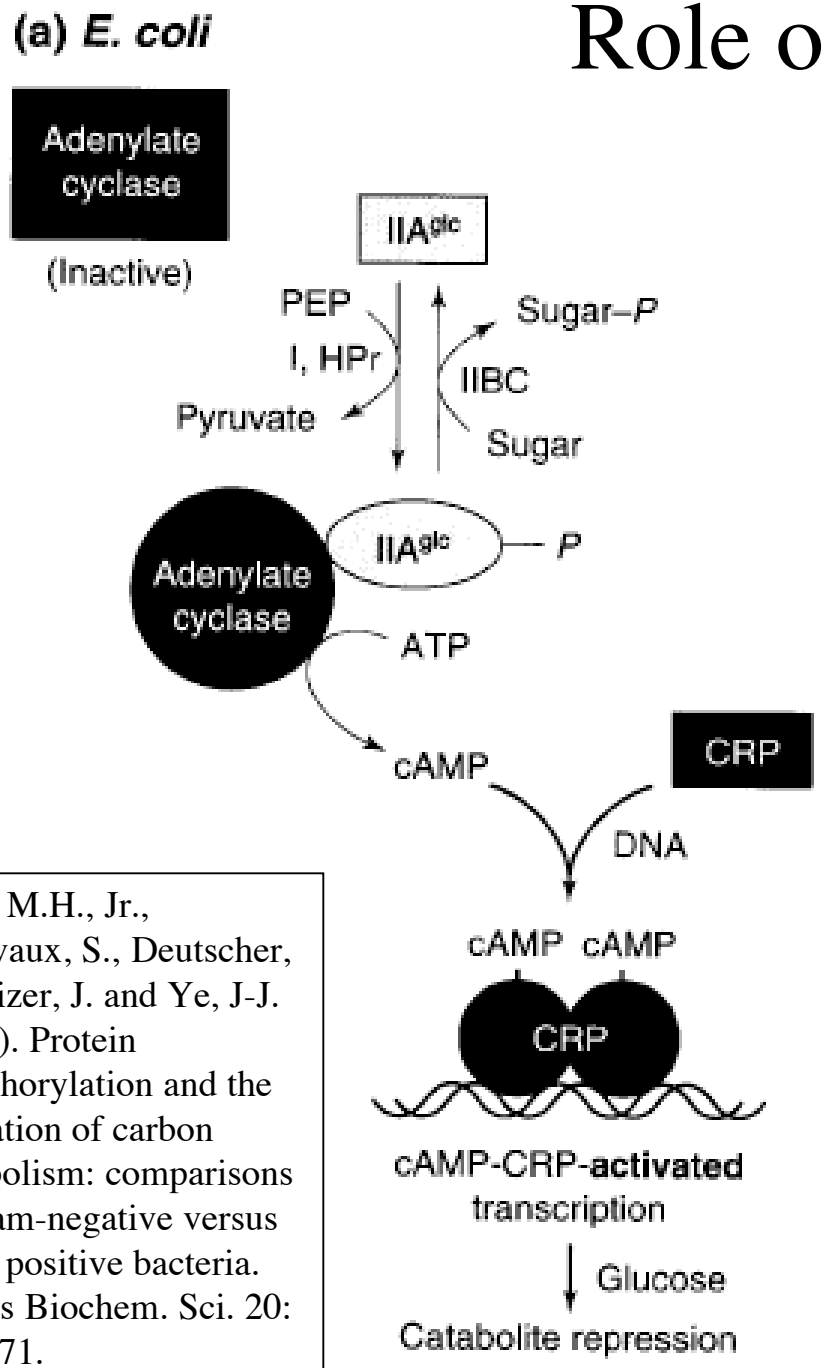


Cooperative Interactions in Crp Stimulation of Transcription from *lacP*

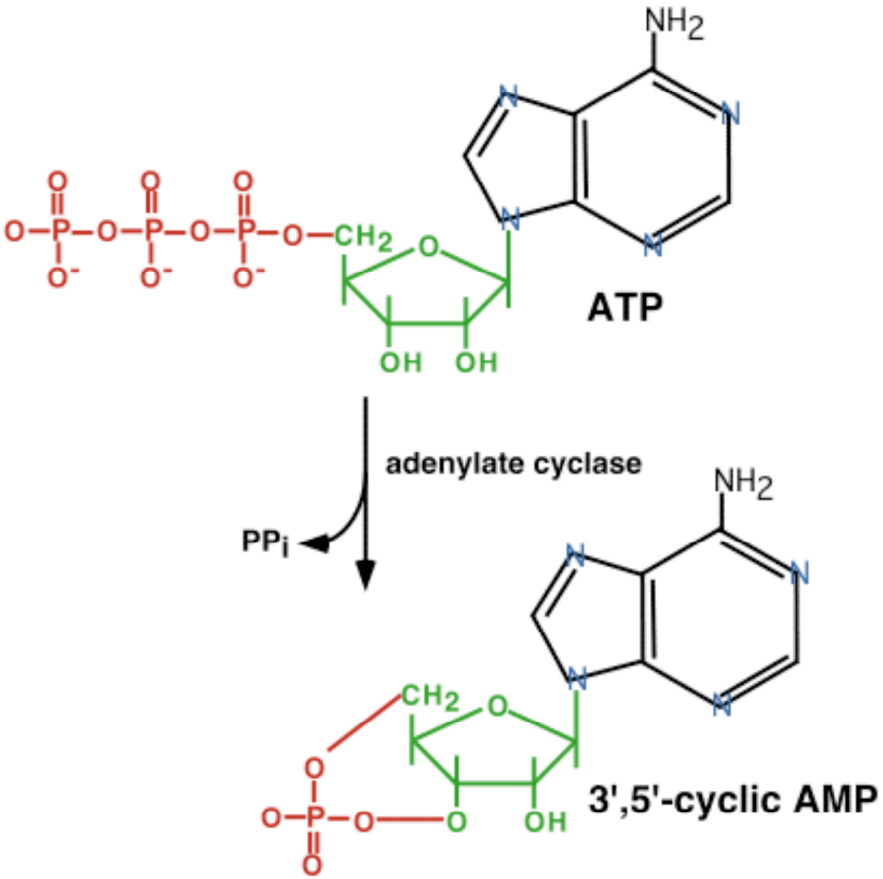


http://www.blc.arizona.edu/marty/411/Modules/catrep.html

Role of Glucose in Regulating cAMP Synthesis



Saier, M.H., Jr., Chauvaux, S., Deutscher, J., Reizer, J. and Ye, J-J. (1995). Protein phosphorylation and the regulation of carbon metabolism: comparisons in Gram-negative versus Gram positive bacteria. Trends Biochem. Sci. 20: 267-271.



Boolean interactions in *lac* operon regulation

Operations involving *lac* operon products

LacY + lactose(ext) ==> lactose)(int)	**(1)**
LacZ + lactose ==> allolactose (minor product)	**(2)**
LacI + *lacO* ==> lacI-*lacO* (repressor bound, *lacP* inaccessible)	**(3)**
LacI + allolactose ==> LacI-allolactose (repressor unbound, *lacP accessible*)	**(4)**

Operations involving glucose transport components and adenylate cyclase

IIA^{Glc}-P + glucose(ext) ==> IIA^{Glc} + glucose-6-P(int)	**(5)**
IIA^{Glc}-P + adenylate cyclase(inactive) ==> adenylate cylase(active)	**(6)**
Adenylate cyclase(active) + ATP ==> cAMP + P~P	**(7)**

Operations involving transcription factors

Crp + cAMP ==> Crp-cAMP	**(8)**
Crp-cAMP + *CAP* ==> Crp-cAMP-*CRP*	**(9)**
RNA Pol + *lacP* ==> unstable complex	**(10)**
RNA Pol + *lacP* + Crp-cAMP-*CRP* ==> stable complex, initiate *lacZYA* mRNA	**(11)**

Partial computations

No lactose ==> *lacP* inaccessible	**(3)**
Lactose + LacZ(basal) + LacY(basal) ==> *lacP* accessible	**(1, 2, 4)**
Glucose ==> low IIA^{Glc}-P ==> low cAMP ==> unstable transcription complex	**(5, 6, 7, 10)**
No glucose ==> high IIA^{Glc}-P ==> high cAMP ==> stable transcription complex	**(5, 6, 7, 8, 9, 11)**

Overall computation = <u>IF</u> lactose present <u>AND</u> glucose not present
<u>AND</u> cell can synthesize active LacZ and LacY,
<u>THEN</u> transcribe *lacZYA* from *lacP*

General Principles Deducible from *lac* Operon Case

* Functional requirement to interpret cognitive inputs
* Systemic (modular-interactive) nature of each protein molecule and DNA element
* Weak interactions, specific binding & cooperativity essential aspects of molecular computations in cells
* Layering of weak and "fuzzy" interactions provides overall precision to integrated cellular responses
* Allostery(the fact that binding of one ligand affects binding a distinct ligand) confers communication and processing capabilities on individual molecules
* Repetition in DNA and proteins ==> specific logical operations arise through combinations of basic circuit elements (complex regulatory regions in DNA, domain accretion & swapping in proteins)
* Cells use chemical symbols to represent physiological information (allolactose, phosphorylation of IIA^{Glc}, cAMP levels)
* No fundamental separation between control molecules and execution molecules ==> no "Cartesian" dualism in *E. coli* cell
* Participation of DNA directly in formation of repression and transcription nucleoprotein complexes ==> no Turing separation of "machine" and "tape" (also seen in computations that do not involve DNA)

Further Topics Pertinent to Continued Relevance of Classical Genetic Concepts

- Modular nature of proteins, protein machines and protein networks
- Complexity of transcriptional regulatory determinants
- Multiple proteins from a single DNA segment (overlapping coding regions, alternative splicing)
- Variation and *de novo* assembly of coding sequences
- Networked determination of phenotypic characters