A Clarification! The Physical Target for Cro and cI DNA BINDING Is THE OPERATOR!!!
Operators Control Adjacent Promoters
As Will See For The Lac Operon

The λ Operator Controls Both the Cro & cI Promoters
Cro Binding to O Activates Cro & Represses cI
Conversely cI Binding to O Activates cI & Represses Cro By Facilitating or Preventing Access by RNA Polymerase

Why Study Bacteria?
Causal Agents of Numerous Human Diseases, Including:
- *Bacillus anthracis* Anthrax
- *Borrelia burgdorferi* Lyme disease
- *Clostridium botulinum* Botulism
- *Enterotoxigenic E.coli* Hemorrhagic diarrhea
- *Haemophilus influenzae* Bacterial pneumonia
- *Helicobacter pylori* Gastric ulcers
- *Mycobacterium leprae* Hansen’s disease
- *Mycobacterium tuberculosis* Tuberculosis
- *Staphylococcus aureus* Skin lesions, hemolysis
- *Vibrio cholerae* Cholera
- *Group A Streptococcus* Hemolysis
- *Streptococcus pneumoniae* Bacterial meningitis
- *Yersinia pestis* Plague

- Small, Haploid Genomes Amenable to Genetic Analyses
- Extremely Short Generation Times
- Easily & Inexpensively Grown in Large Numbers
- Emergence of Antibiotic-Resistant Bacterial Strains Is a Major Public Health Problem
Bacteria Must Rapidly Adapt to Changes in Nutritional Environment & Alter Metabolic Pathways

Metabolic Flexibility Is a Major Selective Pressure on Unicellular Organisms

Negative Feedback Regulation of Enzyme Activity
Enzyme 1 As Target, Shuts Down Entire Pathway

Negative Feedback Regulation of Enzyme Synthesis
Gene Encoding Enzyme 1 as Target, Shuts Down Expression of all 5 Genes
If Genes Are Organized Into a Single Operon

Converse is Also True - Can Simultaneously Activate All Genes in an Operon With a Single Inducer

Waste Not, Want Not!
Inducers Activate Synthesis of Certain Enzymes

A constitutive enzyme is always expressed At the same level +/- an inducer Can be expressed at a high level

Enzyme Absent or V. Low In Uninduced Cell
Or a low level

An Inducible Enzyme Rapidly Accumulates In Induced Cell

The E. coli lac Operon – A Paradigm for Prokaryotic Gene Regulation

The "Big Picture"

A Single Promoter Simultaneously Regulates 3 Gene Products on 1 mRNA
- Repress Operon In Absence of Lactose
- Derepress or Induce Operon in Presence of Lactose

lacI
lacZ
lacY
lacA

Lactose -> Glucose+Galactose
Can measure lacZ activity using Chromogenic lactose analogues as substrates
& use colorimetric assays both in vitro (yellow) or in vivo (blue)

lacZYA Are Expressed On a Single Polycistronic mRNA
1mRNA Encodes 3 Distinct Proteins

- Each gene is delineated by its own start & stop codons on a single mRNA
- Each AUG is preceded by its own ribosome binding site
- Ribosomes bind independently to each AUG, translate & terminate at each stop
- The majority of ribosomes are "recycled" to a "new" start codon at random
- However, a small # of ribosomes actually remain bound to the mRNA after encountering a stop codon & scan along the mRNA to the next AUG & reinitiate translation
**Lac Operon – Uninduced State**

**Lactose absent**

*LacI* binds to *Operator* sequence prevents RNA Polymerase from binding to adjacent promoter $P_{lac}$.

*lacZYA* genes not transcribed.

Same mechanism explains how *cI* represses *Cro* promoter & how *Cro* represses *cI* promoter in *λ*.

**DNA**

$Lac$ Operon – Induced State

**Lactose present**

Operon expressed as long as lactose is present. Upon depletion or deprivation, free *lacI* binds *lacO* & represses Operon.

RNA polymerase binds promoter.

Inducer (lactose) binds *lacI*. Alters conformation so that it dissociates from DNA.

Illustrates allosteric regulation.

**DNA**

Transcription proceeds.

RNA Polymerase can now bind promoter & *lacZYA* are expressed.
Why Study Eukaryotes, esp. Humans?

If presume not to God to scan; The proper study of Mankind is Man. Plac’d on this isthmus of a middle state, a being darkly wise, and rudely great.

Alexander Pope

What a piece of work is a man! How noble in reason, how infinite in faculty, in form and moving how express and admirable, in action how like an angel, in apprehension how like a god -- the beauty of the world, the paragon of animals!

William Shakespeare

Man is an ape with possibilities.

Roy Chapman Andrews

Our humanity is a poor thing, except for the divinity that stirs within us.

Francis Bacon

Human beings are the only animals of which I am thoroughly and cravenly afraid.

George Bernard Shaw

I sometimes think that God in creating man somewhat overestimated his ability.

Oscar Wilde

We’re all of us guinea pigs in the laboratory of God. Humanity is just a work in progress.

Tennessee Williams

Prokaryotic & Eukaryotic Organisms Differ Markedly in Genome Size, Organization & Mechanisms of Gene Expression

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>PROKARYOTES</th>
<th>EUKARYOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genome size (base pairs)</td>
<td>$10^6$-$10^7$</td>
<td>$10^9$-$10^{11}$</td>
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<tr>
<td>Reported sequences</td>
<td>Few</td>
<td>Many</td>
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<tr>
<td>Noncoding DNA within coding sequences</td>
<td>Rare</td>
<td>Common</td>
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<tr>
<td>Transcription and translation</td>
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<td>Yes</td>
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<tr>
<td>separated in cell DNA segregated within a nucleus</td>
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<tr>
<td>DNA bound to proteins</td>
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<td>Promoter</td>
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<td>Yes</td>
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<tr>
<td>Enhancer/silencer</td>
<td>Rare</td>
<td>Common</td>
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<tr>
<td>Capping and tailing of mRNA</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>RNA splicing required</td>
<td>Rare</td>
<td>Common</td>
</tr>
<tr>
<td>Number of chromosomes in genome</td>
<td>One</td>
<td>Many</td>
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</tbody>
</table>

So How Many More Genes Does It Take
To Go From *E.coli* to *H. sapiens*?
What Does It Take To Progress From a Unicellular Prokaryote To A Unicellular Eukaryote?

Eukaryotes Have Significantly Greater Subcellular Complexity

How Much Genetic Complexity is Required to Become Multicellular?
Nematode, *C. elegans*, Simplest Animal With Only ~1000 Cells
97,000,000 base pairs (8X > Yeast)
19,099 Proteins (4X > Yeast)

<table>
<thead>
<tr>
<th>Function</th>
<th>Protein/Domain</th>
<th>Genes</th>
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</thead>
<tbody>
<tr>
<td>Transcription control</td>
<td>Zinc finger; homeobox</td>
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<tr>
<td>RNA processing</td>
<td>RNA binding domains</td>
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<tr>
<td>Nerve impulse transmission</td>
<td>Gated ion channels</td>
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<tr>
<td>Tissue formation</td>
<td>Collagens</td>
<td>170</td>
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<tr>
<td>Cell interactions</td>
<td>Extracellular domains; glycoltransferases</td>
<td>330</td>
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<tr>
<td>Cell–cell signaling</td>
<td>G protein-linked receptors; protein kinases; protein phosphatases</td>
<td>1,290</td>
</tr>
</tbody>
</table>

*Drosophila* 10,000 cells
(10X > *C. elegans*)
~13,600 Genes
~30,000–50,000 Genes
~50% Conserved in Flies
The C Value Paradox: The Total Amount of Haploid DNA/Cell (C value) Fails To Correspond to Increasing Phylogenetic Complexity Why?

Frog 10 billion bp per cell

14.1 A Comparison of Prokaryotic and Eukaryotic Genes and Genomes

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Comparison of Steps Required To Execute The "Central Dogma" In Prokaryotes vs Eukaryotes

Prokaryotic Gene Regulation Occurs Primarily at the Transcriptional & Translational Levels