E. Quantitative characterization of the *lac* promoter

***lac* promoter of *E. coli*:**

- best-studied system of molecular biology
  - all molecular components characterized
  - many mutants studied *in vivo*
  - most parameters measured *in vitro*
- exemplary model system of combinatorial gene regulation
  - involves activation, repression, and DNA looping

Quantitative confrontation of model and experiment

➔ applicability of the thermodynamic description of tsx control?

➔ can the *in vivo* behavior of a system be understood in terms of its molecular parts?

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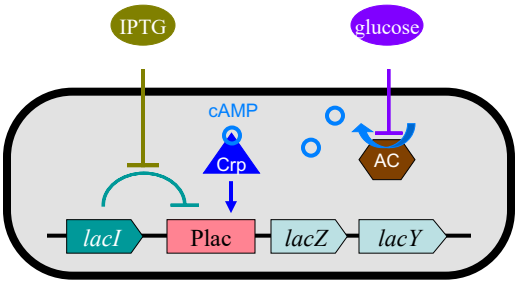
Review: regulation of the *lac*-operon of *E. coli*

Physiology:

- lac-operon: utilization of lactose
- repressed by the **Lac Repressor** (encoded by ***lacI***)
- repression alleviated by allo-lactose (by-product of lactose metabolism) or the synthetic inducer **IPTG**
- activated by the global regulator **Crp**; requires the inducer **cAMP**
- cAMP synthesized endogenously by **Adenylate Cyclase** (encoded by *cyaA*)
- activity of **AC** repressed by **glucose** uptake

Function: expression ONLY in the presence of lactose **AND** absence of glucose



qualitative behavior:

IPTG	glucose	expression
low	high	OFF
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high	high	OFF
high	low	ON

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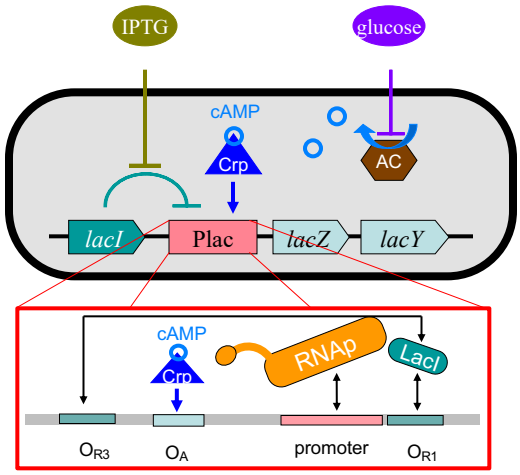

qualitative behavior:

IPTG	glucose	expression
low	high	**OFF**
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high	high	**OFF**
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molecular ingredients:

- specific protein-DNA binding
- protein-protein interaction
- protein-mediated DNA looping

➔ theory: quantitative prediction of gene regulation by LacI, cAMP-Crp

➔ expt: characterize LacZ activity for different levels of regulatory proteins
-- control protein levels by varying the inducers (IPTG and cAMP)

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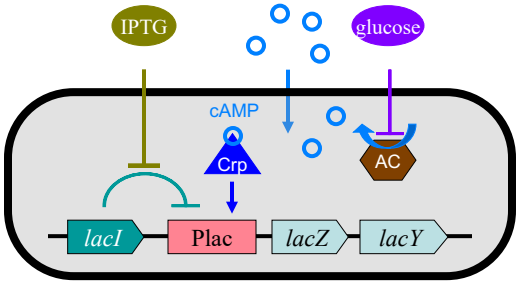
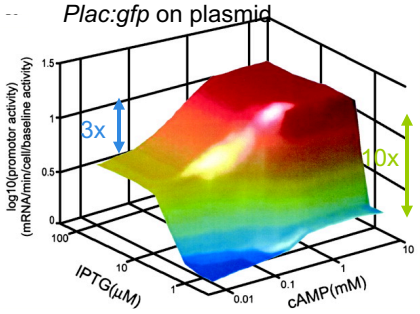
Quantitative characterization



Plac:gfp on plasmid



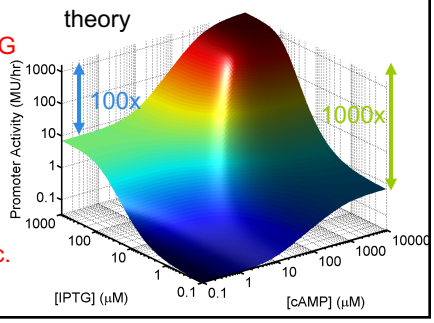
Previous expt: [Setty et al, PNAS, 2003]

Grow cells in medium with glucose, cAMP, IPTG
-- use glucose to suppress cAMP synthesis
-- control cAMP-level extracellularly

inconsistent with behavior of mutants:
Δ*lacI*: > 1000x; **Δ*crp* > 50x**

➔ possible problems: complex links between extracellular and intracellular inducer conc.

theory



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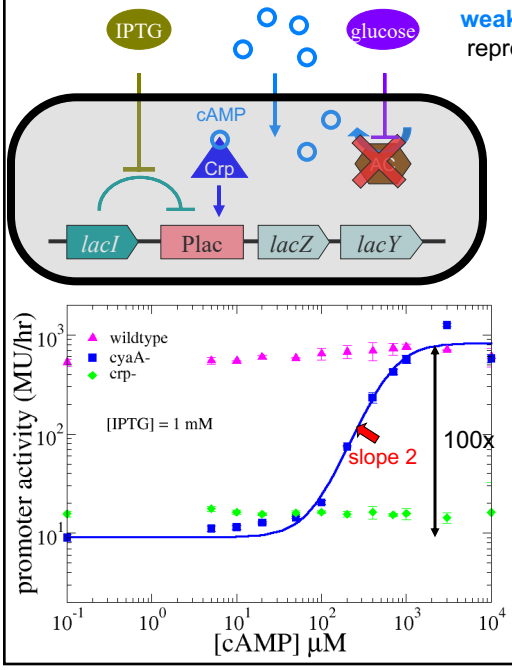
Quantitative characterization of mutants



weak cAMP dependence: glucose-mediated repression of AC activity may be incomplete

- ➔ delete ***cyaA*** gene (encoding **AC**)
- ➔ find ~100x change in LacZ activity
- ➔ Hill coeff ≈ 2

incompatible w/ biochem and thermodynamic model of tsx control

$$\text{Plac activity} \propto \frac{1+\omega_{A-p}[A]/K_A}{1+[A]/K_A}$$

CRP_2 + cAMP ⇌ CRP_2:cAMP

$$[A] = [CRP_2]_{total} \cdot \frac{[cAMP]}{K_{cAMP}+[cAMP]}$$

in vitro biochem irrelevant?
other effects exerted by CRP-cAMP?

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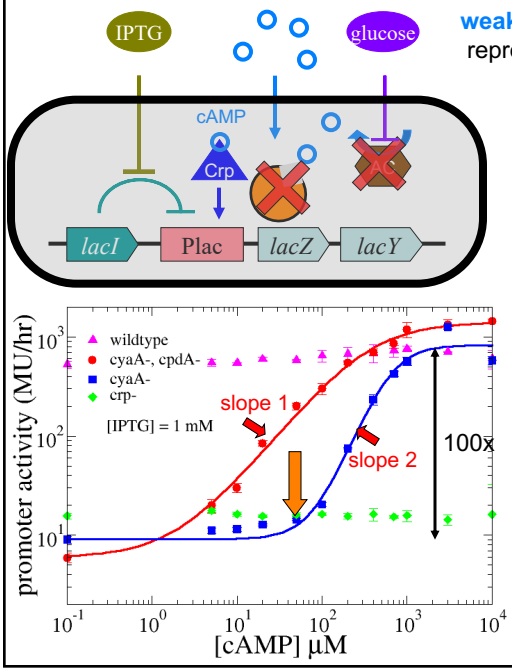
Quantitative characterization of mutants



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- ➔ cAMP degraded by **PDE** (*cpdA*)
- ➔ effect of *cpdA* deletion?
- ➔ Hill coeff ≈ 1, agrees with model
- ➔ role of PDE: no known phenotype [insulation of ext cAMP?]
- ➔ mechanism of cooperativity?

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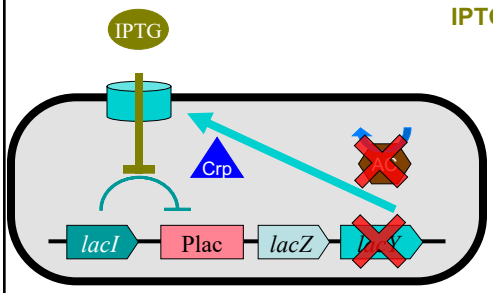
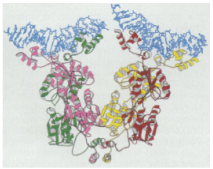
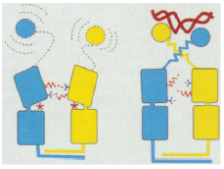
Quantitative characterization of mutants

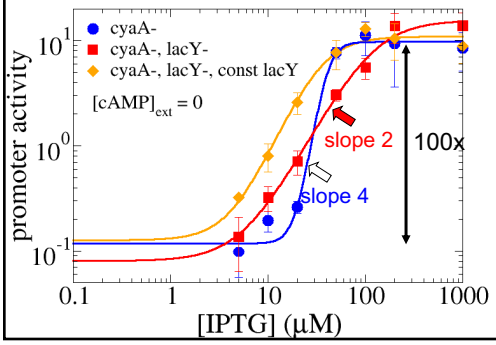


IPTG dependence: *cyaA-* cells with [cAMP]=0
➔ very cooperative! (Hill coeff ≈ 4)

- LacI forms tetramer (dimer of dimers)
- strong coupling within each dimer and weak coupling between dimers







➔ suggest Hill coeff = 2
(widely cited in literature)

- other effect: despite its permeability, IPTG uptake increased by LacY; large coop. from +ve feedback?

➔ delete *lacY* Hill coeff ≈ 2
➔ constitutive expression of LacY only shifted IPTG dependence

great, but… Hill coeff = 2 is one of the many **pseudo-facts** regarding Lac

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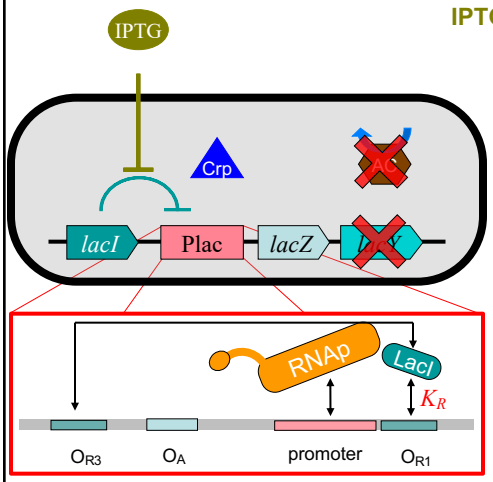
Quantitative characterization of mutants



auxiliary Lac operators stabilize LacI-O1 binding via **DNA looping** [Muller-Hill]

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- $LacI_4$-IPTG binding non-cooperative
  $LacI_4$ + IPTG ⇌ $LacI_4$:IPTG
- weakly cooperative in the presence of operator DNA (Hill coeff = 1.4 ~ 1.6)

[Matthews lab, '85]

➔ uninduced dimer needed for specific binding to Lac operators

active repressors $[R] = \frac{2 \cdot [\mathrm{LacI}_4]_{total}}{\left(1 + [\mathrm{IPTG}] / \mathrm{K}_{\mathrm{IPTG}}\right)^2}$

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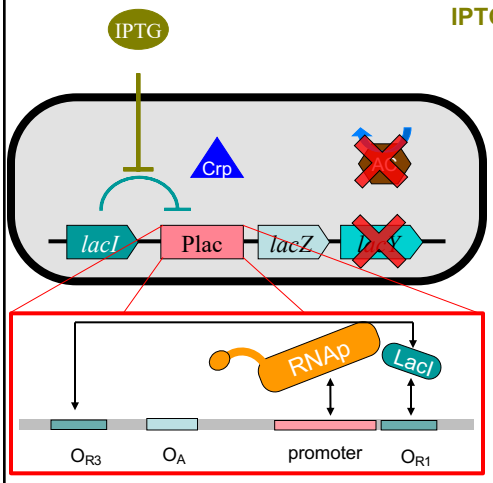

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$\mathcal{L}_o$: local increase of [LacI] due to looping

30

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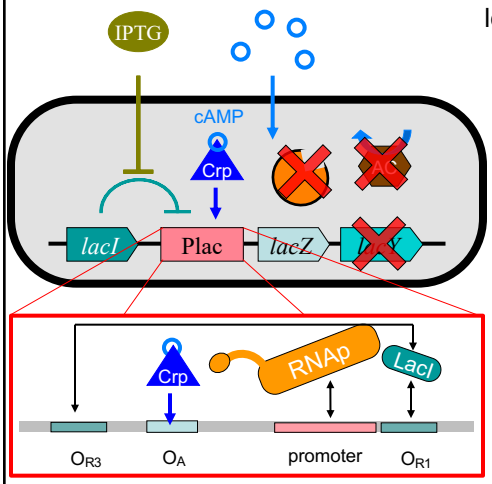

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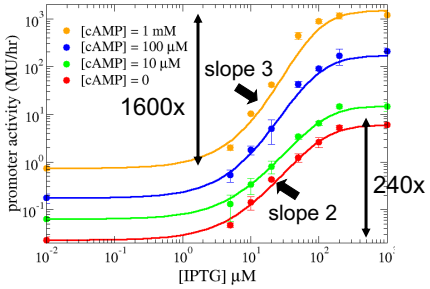
looping model w/ $\mathcal{L}_0 \approx 12$, $2[\mathrm{LacI_4}]/K_R = 20$



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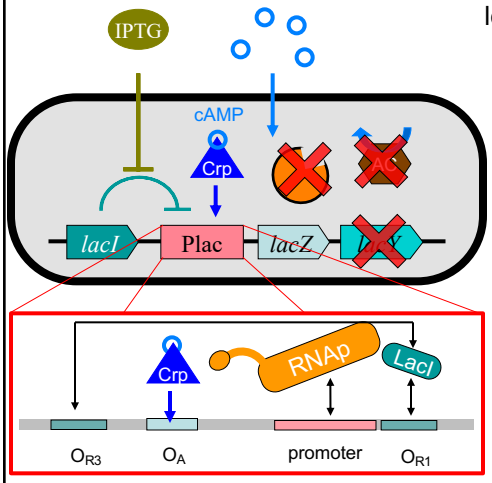

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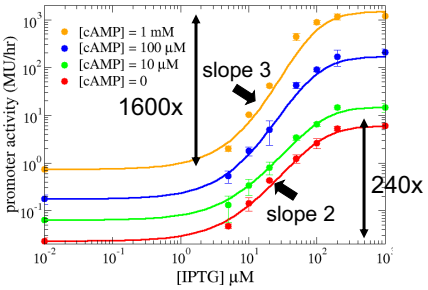

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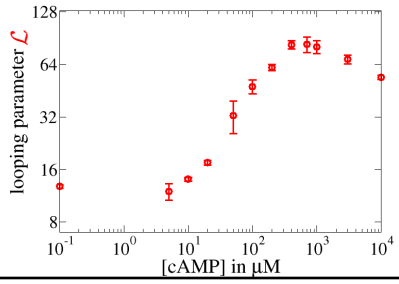

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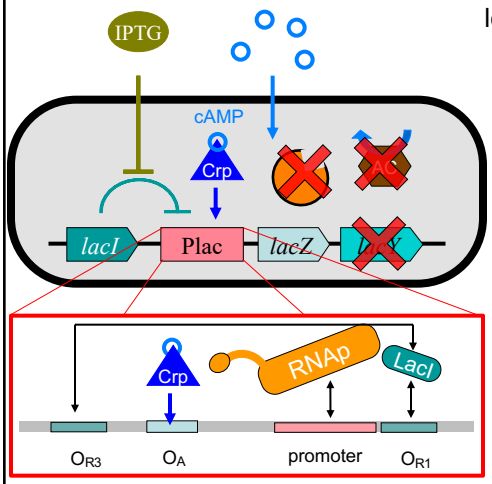

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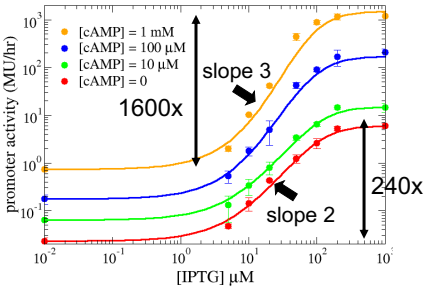

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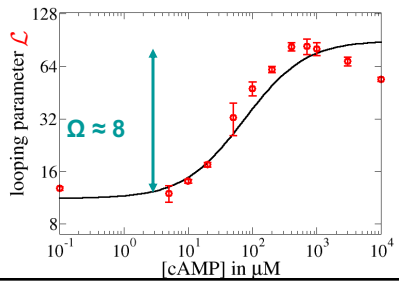
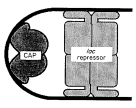


Crp-dependence of DNA looping



Fried et al, 84;
Balaeff et al, 04

in vitro study found coop. factor **Ω = 4 ~12**

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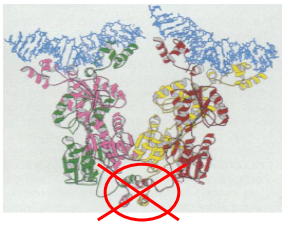
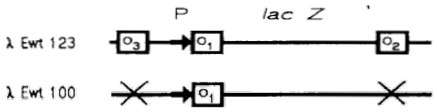
Direct probe of DNA looping *in vivo*

Use dimeric LacI mutant



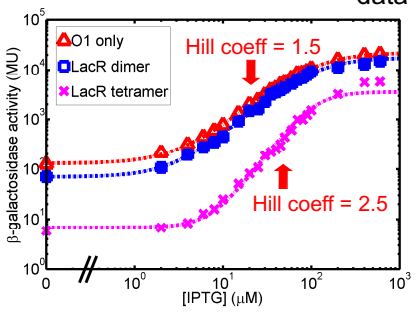
remove auxiliary operators

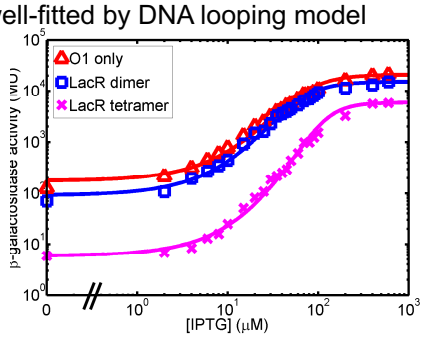


➔ cooperativity in IPTG response requires DNA looping (Lac tetramer + auxiliary ops)

[Oehler & Muller-Hill, 06]

data well-fitted by DNA looping model





➔ IPTG-LacI-operator interaction same as *in vitro*

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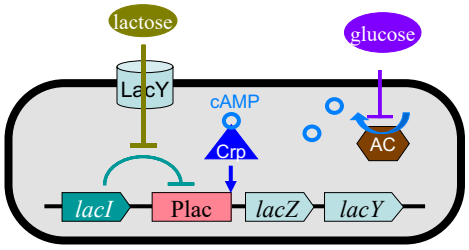
back to physiology



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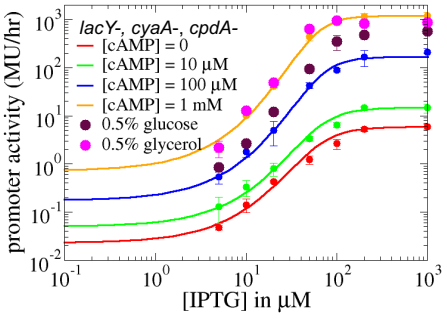

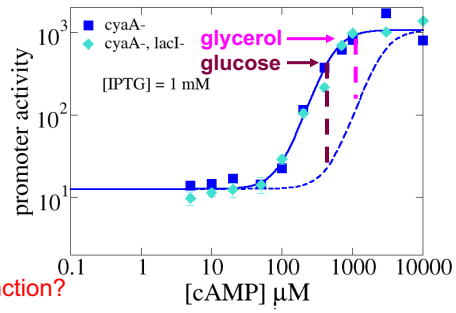

- only ~3x decrease from glucose to glycerol
- small fraction of dynamic range; (operating in saturation of cAMP-CRP)
- 10x change possible by reducing K_{crp}

➔ repression by glucose not the intended function?

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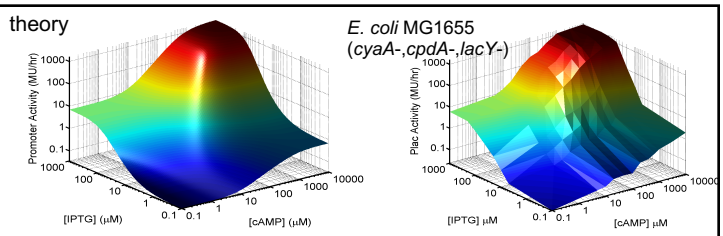
Summary



- **main findings for the *lac* promoter:**
  - Crp enhances DNA looping
  - abrupt IPTG response despite non-cooperative LacI-IPTG interaction;

  ➔ **suggests physiological role of Crp-cAMP as enhancer of repression**
  - mechanism of Crp-LacI interaction?
  - coop cAMP response due to PDE; physiological function? mechanism?
- **general lessons for quantitative systems biology:**
  - hidden interaction and pseudo-facts abound even for the "best studied" system
  - quantitative description of *in vivo* biology is possible
  - need **solid, qualitative** knowledge of the components (e.g., Hill coeff)
    (*in vitro* results surprisingly robust in this regard)
  - **(semi) quantitative** characterization generates spectrum of phenotypes

  ➔ provides clues for identifying unknown components and mechanisms

  ➔ provides **phenomenological description** of Plac for high-level studies

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