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?

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 *cyA*)
  - activity of *AC* repressed by *glucose* uptake

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

**Qualitative behavior:**

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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**

**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:

ΔlacI: > 1000x;  Δcrp: > 50x

⇒ possible problems: complex links between extracellular and intracellular inducer conc.
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 txs control

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

CRP$_2 +$ cAMP $\rightleftharpoons$ CRP$_2$:cAMP

$[A] = [\text{CRP}_2]\text{tot} \cdot \frac{[\text{cAMP}]}{K_{\text{cAMP}} + [\text{cAMP}]}$

*in vitro* biochem irrelevant?
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*in vitro* biochem irrelevant?
other effects exerted by CRP-cAMP?
- 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?
IPTG dependence: cyaA- cells with [cAMP]=0

- very cooperative! (Hill coeff ≈ 4)
- Lacl 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

Quantitative characterization of mutants

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

- weakly cooperative in the presence of operator DNA (Hill coeff = 1.4 ~ 1.6)

- uninduced dimer needed for specific binding to Lac operators

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

[Matthews lab, '85]
Quantitative characterization of mutants

IPTG dependence: cyaA- cells with [cAMP]=0

- Very cooperative!
  - Lac forms tetramer (dimer of dimers)
  - Strong coupling within each dimer and weak coupling between dimers
  - LacI-IPTG binding non-cooperative
  - LacI + IPTG ≠ LacI:IPTG
  - Weakly cooperative in the presence of operator DNA (Hill coeff = 1.4 ~ 1.6)

\[ [R] \rightarrow [R]^+ \frac{L_r \cdot [lacrI]_{total}}{(1 + [IPTG]/K_{IPTG})^2} \]

- Uninduced dimer needed for specific binding to Lac operators
- Include DNA looping in model
- Local increase of [LacI] due to looping

LacI-O1 binding via DNA looping [Muller-Hill]
- Increase fold-repression from \( f = 2[LacI]/K_r \) to \( f(1+L_0) \)
- But value of \( L_0 \) not known independently

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Quantitative characterization of mutants

looping model w/ \( L_0 \approx 12, 2[LacI]/K_r = 20 \)

- Single parameter \( L_0 \) fits both fold-repression and slope
- Active repressors \( [R] = \frac{2 \cdot [LacI]_{total}}{(1 + [IPTG]/K_{IPTG})} \)
- Simple repression \( tsx \) activity \( \sim \frac{1}{1 + [R]/K_r} \)
- Include DNA looping in model

\[ [R] \rightarrow [R]^+ \frac{L_r \cdot [lacrI]_{total}}{(1 + [IPTG]/K_{IPTG})^2} \]

L_r: local increase of [LacI] due to looping
Quantitative characterization of mutants

looping model w/ $\Lambda_0 \approx 12, 2[\text{LacI}] / K_R = 20$

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

$\rightarrow$ increase fold-repression
from $f = 2[\text{LacI}] / K_R$ to $f \cdot (1 + \Lambda_0)$

but value of $\Lambda_0$ not known independently

single parameter $\Lambda_0$ fits both
fold-repression and slope

Crp-dependence of DNA looping

$\Omega = 4 \sim 12$

Fried et al, 84; Balaeff et al, 04

in vitro study found coop. factor $\Omega \approx 8$

single parameter $\Lambda_0$ fits both
fold-repression and slope
Direct probe of DNA looping in vivo

Use dimeric LacI mutant

remove auxiliary operators

\[ \text{cooperativity in IPTG response requires DNA looping (Lac tetramer + auxiliary ops)} \]

[Oehler & Muller-Hill, 06]

data well-fitted by DNA looping model

\[ \text{IPTG-Lacl-operator interaction same as in vitro} \]

back to physiology

\[ \text{lacY, cyaA, cpdA} \]

\[ \text{glucose} \]

\[ \text{lactose} \]

\[ \text{lacI} \rightarrow \text{Plac} \rightarrow \text{lacZ} \rightarrow \text{lacY} \]

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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 \( K_{crp} \)

\[ \text{repression by glucose not the intended function?} \]
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