Topic 4: Genetic Circuits

A. Models and behaviors of simple genetic circuits
B. Noise in gene expression
C. Metabolic control

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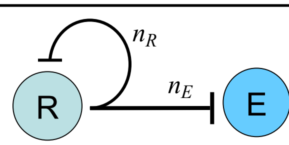
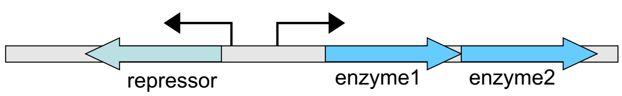
C. Metabolic Control



1. Gene regulation



$$\mathcal{G}_R = \frac{1}{1+\left(\frac{[R]}{K_R}\right)^{n_R}}$$

$$\mathcal{G}_E = \frac{1}{1+\left(\frac{[R]}{K_E}\right)^{n_E}}$$

$$\frac{d}{dt}[R] = \alpha_R \mathcal{G}_R\left(\frac{[R]}{K_R}\right) - \beta_0[R] \quad \Rightarrow \quad \frac{[R^*]}{K_R} \approx \left(\frac{\alpha_R}{\beta_0 K_R}\right)^{1/(n_R+1)} \quad \text{for } \alpha_R/\beta_0 > K_R$$

effect on enzyme: $\frac{d}{dt}[E] = \alpha_E \mathcal{G}_E\left(\frac{[R]}{K_E}\right) - \beta_0[E]$

steady-state soln: $[E^*] = K_R \cdot \left(\frac{K_E}{K_R}\right)^{n_E} \cdot \left(\frac{\alpha_E}{\beta_0 K_R}\right) \Big/ \left(\frac{\alpha_R}{\beta_0 K_R}\right)^{n_E/(n_R+1)} \equiv E_0$ (low level)

set by DNA seq; $\approx \alpha_E/\alpha_R$ if $n_R \approx n_E \gg 1$

no repressor: $[E^*] = \alpha_E/\beta_0 \equiv E_1$ (max level)

➔ $\alpha_E/\alpha_R \approx$ constant if the two promoters are in close proximity
➔ set enzyme conc independent of growth conditions

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2. Effect of the inducer (S)

dissoc const: K_S; Hill coeff: n_S

$$[RS] \equiv R_S = [R] \cdot \frac{([S]/K_S)^{n_S}}{1+([S]/K_S)^{n_S}} \approx [R]\cdot([S]/K_S)^{n_S} \quad \text{for } [S] \ll K_S$$

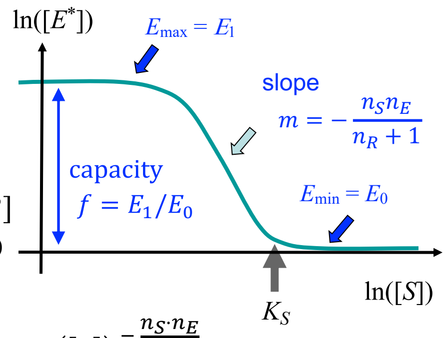
$$[R]_f \equiv R_f = [R] \cdot \frac{1}{1+([S]/K_S)^{n_S}} \approx [R]\cdot([S]/K_S)^{-n_S} \quad \text{for } [S] \gg K_S$$

- if DNA binding by R requires S
  (e.g., R=TrpR, S=Trp, E=TrpABCDE)

max repression: $[S] \gg K_S$; $[E^*] = E_0$
no repression: $[S] = 0$; $[E^*] = E_1$

intermediate: $\alpha_R \cdot (R_S/K_R)^{-n_R} \approx \beta_0 [R]$

$$\Rightarrow \frac{R_S}{K_R} \approx \left[\frac{\alpha_R}{\beta_0 K_R}\left(\frac{[S]}{K_S}\right)^{n_S}\right]^{1/(n_R+1)}$$



enzyme level: $[E^*] \approx \frac{\alpha_E}{\beta_0}\left(\frac{R_S}{K_E}\right)^{-n_E} \approx E_0 \cdot \left(\frac{[S]}{K_S}\right)^{-\frac{n_S \cdot n_E}{n_R+1}}$ for $[S] \ll K_S$

approx full expression: $[E^*] \approx E_1 \frac{1 + f^{-1}\cdot([S]/K_S)^m}{1+([S]/K_S)^m}$

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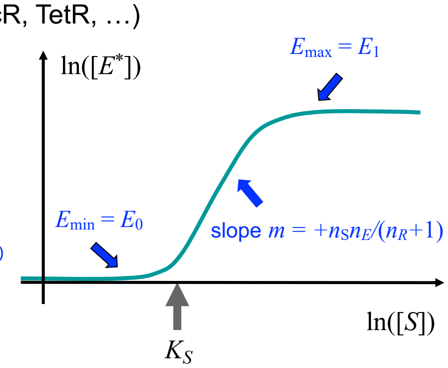
- if DNA binding requires R_f (e.g., LacR, TetR, …)

$$[E^*] \approx E_0 \frac{1 + f \cdot ([S]/K_S)^m}{1+([S]/K_S)^m}$$

$$m = +\frac{n_s \cdot n_E}{n_R + 1}$$

$$f = \frac{E_1}{E_0} = \left(\frac{K_E}{K_R}\right)^{n_E} \Big/ \left(\frac{\alpha_R}{\beta_0 K_R}\right)^{n_E/(n_R+1)}$$



note: $m = \pm \frac{n_S \cdot n_E}{n_R + 1}$ can take on large range of values

if $|m| \gg 1$, abrupt transition or strong buffer
if $|m| \ll 1$, gradual control (dimmer dial)

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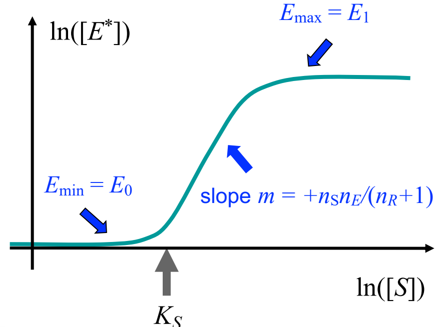
• if DNA binding requires R_f (e.g., LacR, TetR, …)

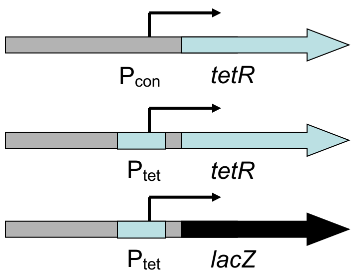
$$[E^*] \approx E_0 \frac{1 + f \cdot ([S]/K_S)^m}{1 + ([S]/K_S)^m}$$

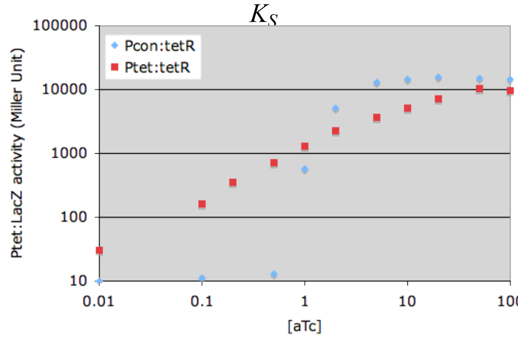
$$m = +\frac{n_s \cdot n_E}{n_R + 1}$$

$$f = \frac{E_1}{E_0} = \left(\frac{K_E}{K_R}\right)^{n_E} \Big/ \left(\frac{\alpha_R}{\beta_0 K_R}\right)^{n_E/(n_R+1)}$$



Expt' l test:





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Dependence on growth conditions:

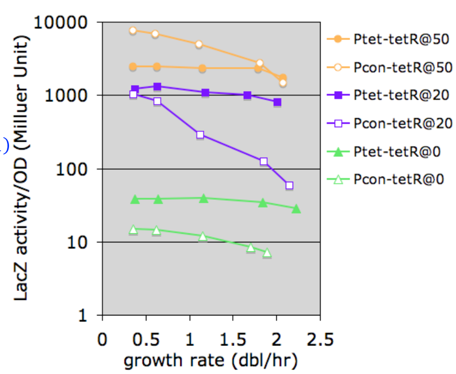
$$[E^*] \approx E_0 \frac{1 + f \cdot ([S]/K_S)^m}{1 + ([S]/K_S)^m}$$

$$E_0 = K_R \cdot \left(\frac{K_E}{K_R}\right)^{n_E} \left(\frac{\alpha_E}{\beta_0 K_E}\right) \Big/ \left(\frac{\alpha_R}{\beta_0 K_R}\right)^{n_E/(n_R+1)}$$

$$\approx K_R \cdot \frac{\alpha_E}{\alpha_R} \quad \text{for } n_R = n_E \gg 1, K_R = K_E$$

$$E_1 = \alpha_E/\beta_0$$

➔ near independence with -ve feedback
➔ still tunable by inducer



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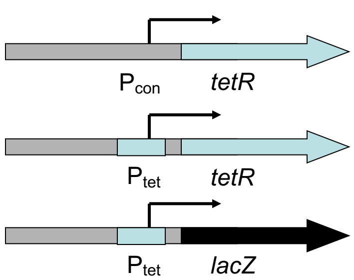

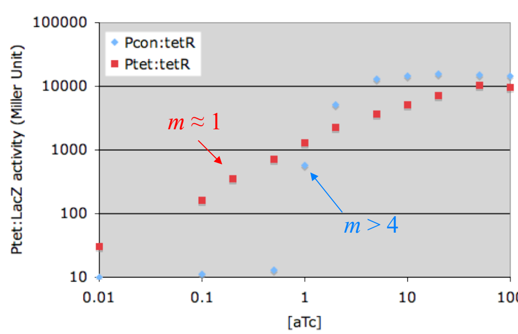

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- similar inducer-enzyme relation can be obtained for tsx activators, e.g., with inducer activating activators (AraC, MalT, …)
- "Mode of regulation" (activating activator vs inhibiting repressor)?
- empirical relation between the mode of regulation and the "demand" of gene product (e.g., lactose vs arabinose) [ref: Savageau, 1974]

➔ evolutionary use-it-or-lose-it principle?

System[a]	Nature of regulator: Observed[f]	Nature of regulator: Predicted		Demand for expression: Predicted	Demand for expression: Observed[f]
Inducible catabolic pathways					
Arabinose	Activator		→	High	High
Galactose	Repressor		→	Low	Low
Glycerol	Repressor		→	Low	Low
Histidine	Repressor		→	Low	Low
Lactose	Repressor		→	Low	Low
Maltose	Activator		→	High	High
Rhamnose	Activator		→	High	High
Mannose	?	Activator	←		High
Tryptophan	?	Activator	←		High
Xylose	?	Activator	←		High

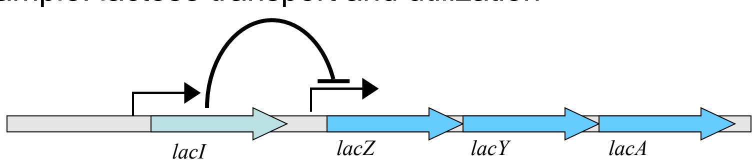
System[a]	Nature of regulator: Observed[f]	Nature of regulator: Predicted		Demand for expression: Predicted	Demand for expression: Observed[f]
Repressible biosynthetic pathways					
Arginine	Repressor		→	Low	Low
Cysteine	Activator		→	High	High
Isoleucine–valine[b]	Activator		→	High	High
Lysine	Repressor		→	Low	Low
Tryptophan	Repressor		→	Low	Low
Histidine	?	Activator	←		High
Isoleucine–valine	?	Activator	←		High
Inducible biosynthetic enzymes (within repressible biosynthetic pathways)					
Isoleucine–valine	Activator		→	High	High
Tryptophan[c]	Repressor		→	Low	?

3. Metabolic feedback

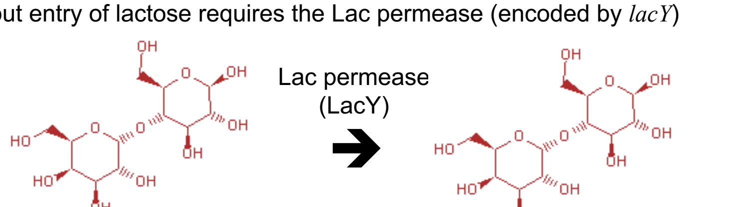
- regulation of E by S is often a form of <u>feedback control</u>

➔ include the synthesis of S by E

example: lactose transport and utilization

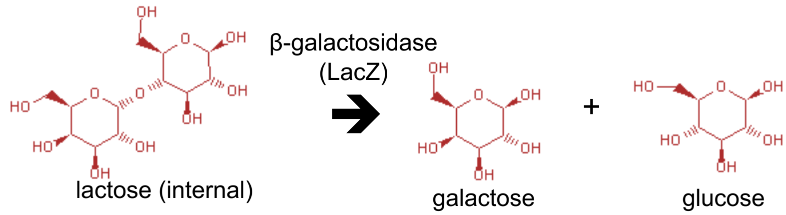


- LacR (encoded by *lacI*) weakly expressed constitutively and exerts coop strong repression of the *lacZYA* operon due to DNA looping
- want to inactivate LacR when lactose is present externally (and glucose absent)
- but entry of lactose requires the Lac permease (encoded by *lacY*)

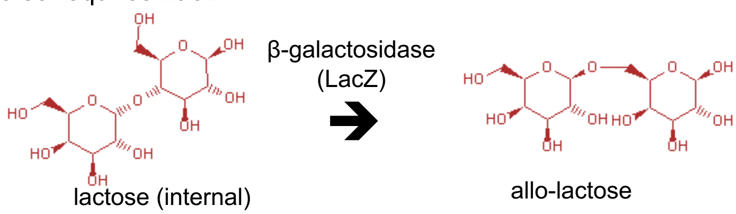


lactose (external) lactose (internal)

- lactose is not an inducer of LacR
- lactose is degraded by β-galactosidas (encoded by *lacZ*)



- actual inducer is allo-lactose (minor by-product of lactose degradation)
  ➔ also requires LacZ



➔ induction of the lac operon (by allo-lactose) requires expression of the operon (LacY + LacZ) = positive feedback
➔ allo-lactose further degraded by LacZ

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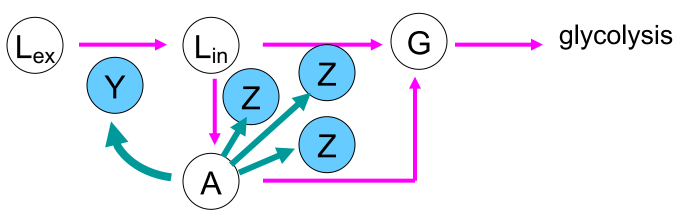
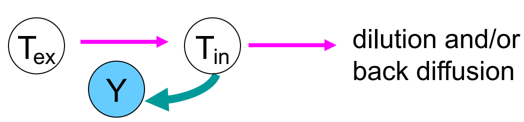
regulatory circuit for lactose transport/utilization:



simplified system: use lactose analogue (TMG)

- inducer of LacR
- non-hydrolyzable
- still requires LacY for entry



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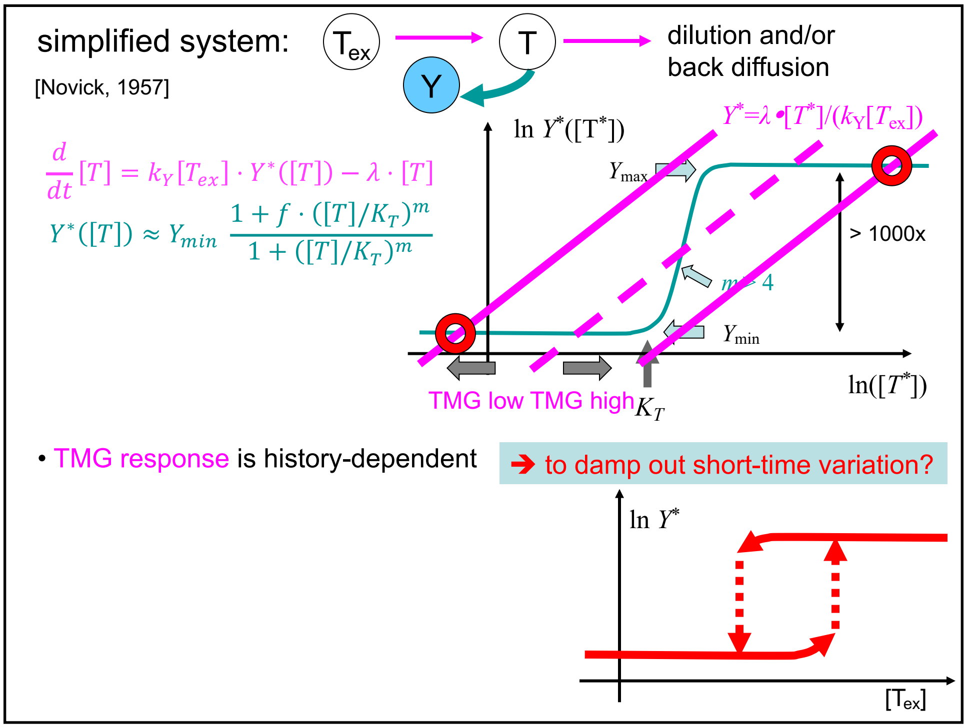
simplified system:
[Novick, 1957]
Tex
T
Y
dilution and/or
back diffusion
$\frac{d}{dt}[T] = k_Y[T_{ex}] \cdot Y^*([T]) - \lambda \cdot [T]$
$Y^*([T]) \approx Y_{min} \frac{1 + f \cdot ([T]/K_T)^m}{1 + ([T]/K_T)^m}$
ln Y*([T*])
Y*=λ•[T*]/(kY[Tex])
Ymax
> 1000x
m ≈ 4
Ymin
ln([T*])
TMG low TMG high
KT
• TMG response is history-dependent
➔ to damp out short-time variation?
ln Y*
[Tex]

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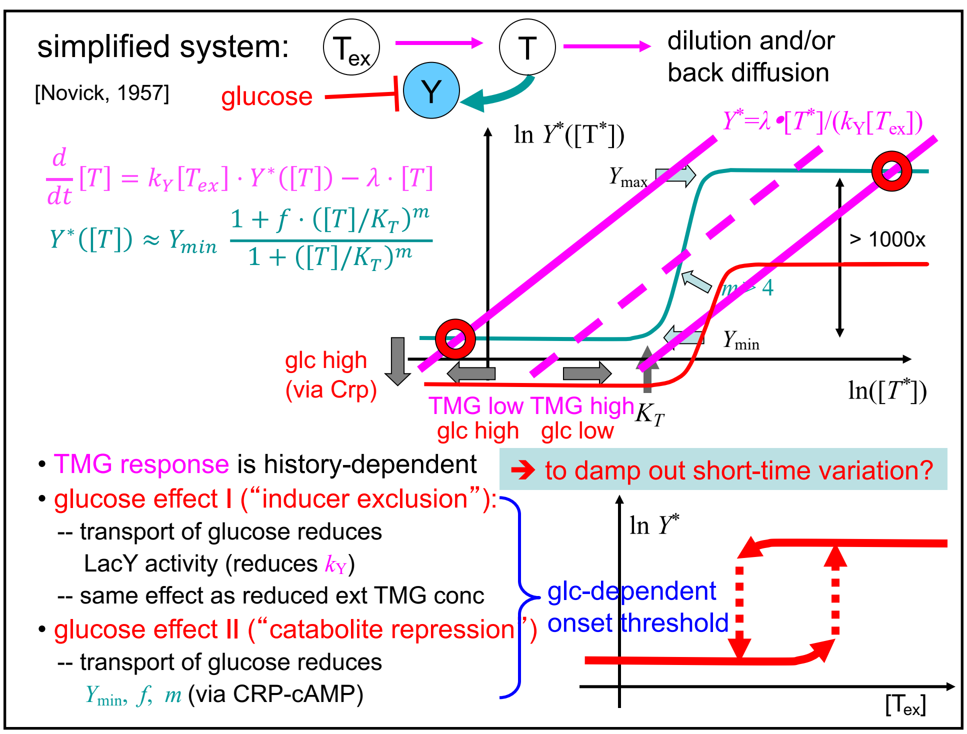
simplified system:
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ln Y*([T*])
Y*=λ•[T*]/(kY[Tex])
Ymax
> 1000x
m ≈ 4
Ymin
glc high
(via Crp)
ln([T*])
TMG low TMG high
glc high glc low
KT
• TMG response is history-dependent
➔ to damp out short-time variation?
• glucose effect I ("inducer exclusion"):
-- transport of glucose reduces
LacY activity (reduces kY)
-- same effect as reduced ext TMG conc
• glucose effect II ("catabolite repression")
-- transport of glucose reduces
Ymin, f, m (via CRP-cAMP)
glc-dependent
onset threshold
ln Y*
[Tex]

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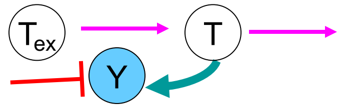
simplified system:

[Novick, 1957]



Multistability in the lactose utilization network of *Escherichia coli*

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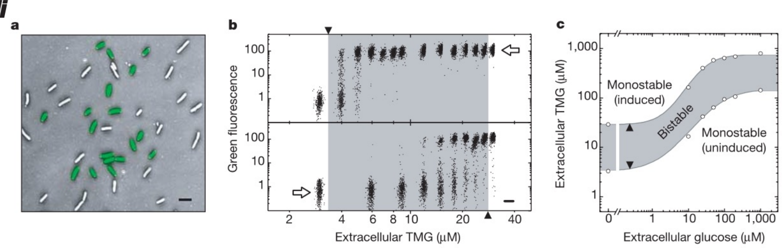
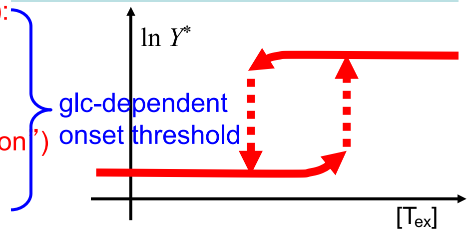


- TMG response is history-dependent
- glucose effect I ("inducer exclusion"):
  - -- transport of glucose reduces LacY activity (reduces k_Y)
  - -- same effect as reduced ext TMG conc
- glucose effect II ("catabolite repression")
  - -- transport of glucose reduces Y_{min}, f, m (via CRP-cAMP)

➔ to damp out short-time variation?



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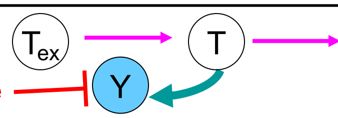

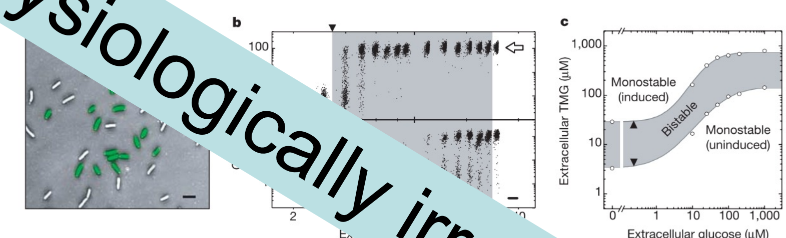

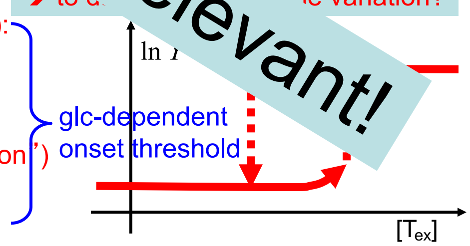

physiologically irrelevant!

simplified system:

- TMG response is history-dependent
- glucose effect I ("inducer exclusion"):
  - -- transport of glucose reduces LacY activity (reduces k_Y)
  - -- same effect as reduced ext TMG conc
- glucose effect II ("catabolite repression")
  - -- transport of glucose reduces Y_{min}, f, m (via CRP-cAMP)

glc-dependent onset threshold

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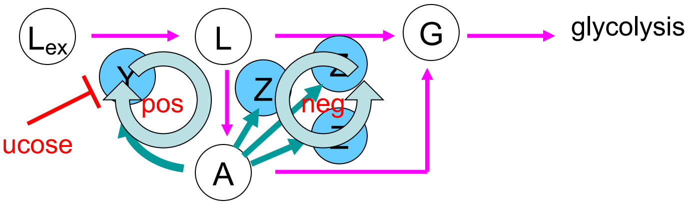
full lac system:



include hydrolysis of substrate

$$\frac{d}{dt}[L] = k_Y[L_{ex}]\cdot Y^*([A]) - k_Z[L]\cdot Z^*([A]) - \cancel{\lambda\cdot[L]}$$

$$\frac{d}{dt}[A] = k_{Z1}[L]\cdot Z^*([A]) - k_{Z2}[A]\cdot Z^*([A]) - \cancel{\lambda\cdot[A]}$$

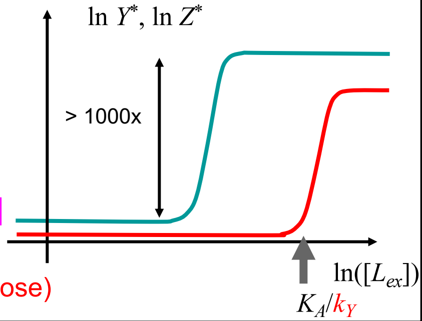
(note: for $L_{ex} < K_M$ of LacY)

$$Y^*([A]) \approx Y_{min}\frac{1+f\cdot([A]/K_A)^m}{1+([A]/K_A)^m}$$

- pos & neg feedback
- dilution negligible
- at steady-state: $[A]=(k_{z1}/k_{z2})[L]$
  $\sim(k_{Z1}/k_{Z2})(k_Y/k_Z)[L_{ex}]$

➔ no bistability; no history-dependence

➔ onset depends on k_Y (controlled by glucose)



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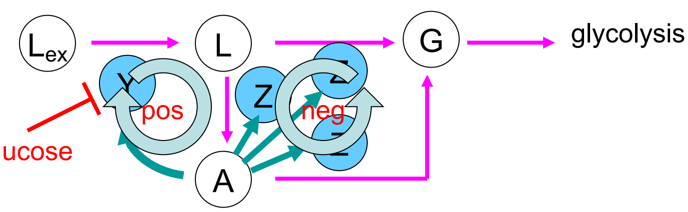
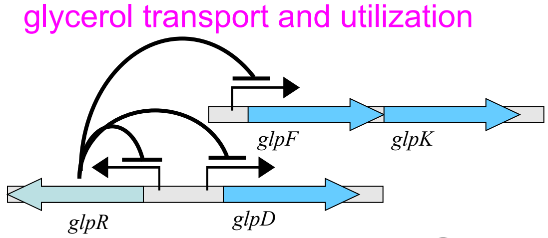
full lac system:



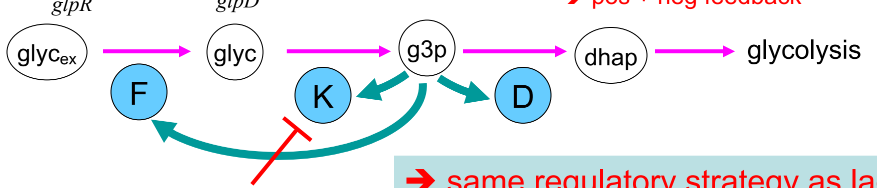
Is the lac system an exception?

glycerol transport and utilization



- glp operons repressed by GlpR and activated by Crp-cAMP
- GlpF: allows glycerol influx
- GlpK: converts glycerol to g3p
- GlpD: "consumes" g3p
- g3p: inhibits GlpR

➔ pos + neg feedback



➔ same regulatory strategy as lac

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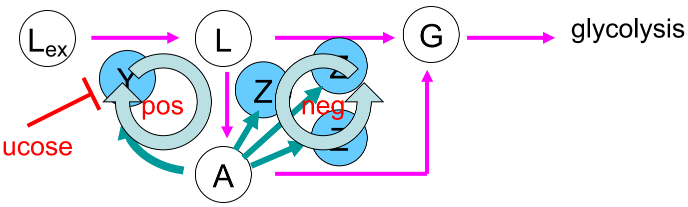
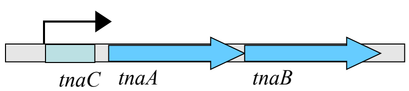
full lac system:



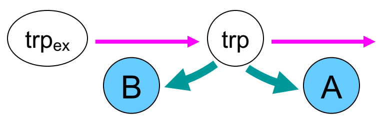
Is the lac system an exception?

Tryptophan transport & degradation



- promoter activated by CRP-cAMP and tsx attenuation (TnaC) relieved by trp
- TnaB: high capacity trp transporter
- TnaA: degrades trp into pyr, NH4, indole

➔ pos + neg feedback



central metabolites

Note: high capacity (low affinity) transporter is typically dominant when the ext substrate conc is high; used for general catabolic purpose

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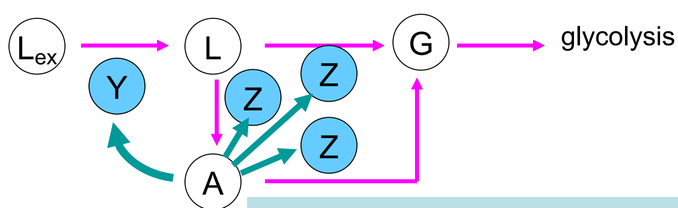
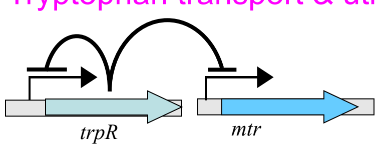
full lac system:



Is the lac system an exception?

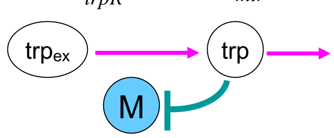
lac typical of catabolic systems; different from anabolic systems

Tryptophan transport & utilization



- Mtr: high affinity Trp transporter (specific for trp usage)
- repressed by TrpR
- repression of TrpR requires trp

➔ negative feedback

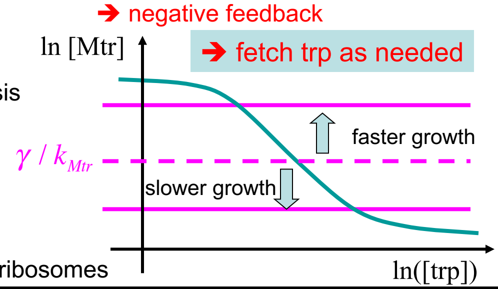


protein synthesis

$$\frac{d}{dt}[T] = k_{Mtr} \cdot \mathrm{Mtr}^*([T]) - \gamma$$

rate of trp "consumption" by ribosomes

➔ fetch trp as needed



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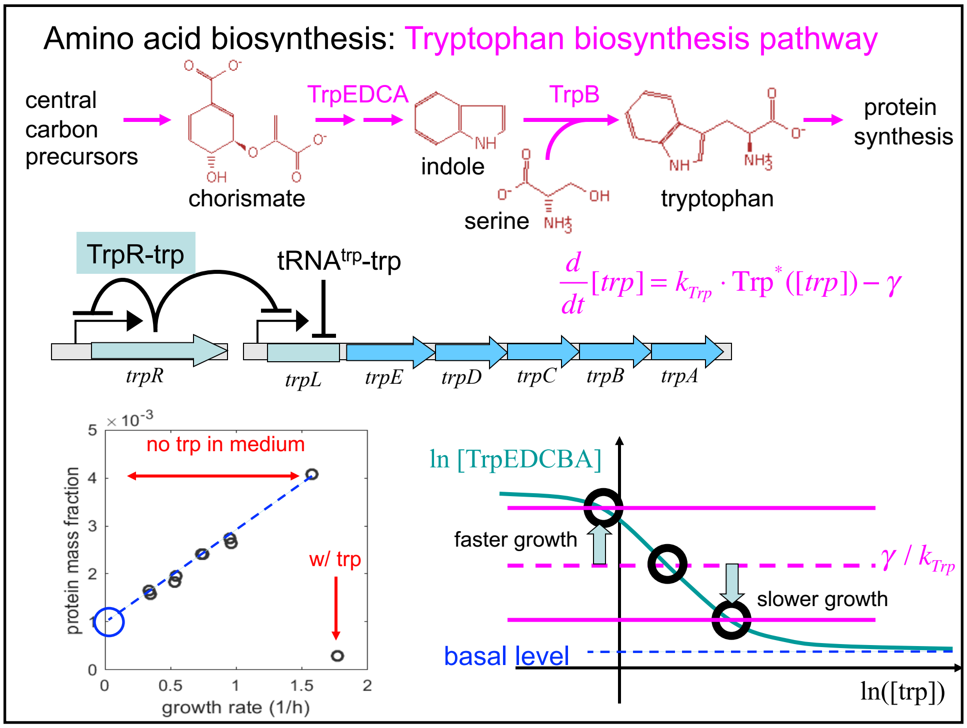

Amino acid biosynthesis: Tryptophan biosynthesis pathway
central carbon precursors
chorismate
TrpEDCA
indole
TrpB
serine
tryptophan
protein synthesis
TrpR-trp
tRNAtrp-trp
$\frac{d}{dt}[trp] = k_{Trp} \cdot \mathrm{Trp}^*([trp]) - \gamma$
trpR
trpL
trpE
trpD
trpC
trpB
trpA
no trp in medium
w/ trp
protein mass fraction
growth rate (1/h)
ln [TrpEDCBA]
faster growth
slower growth
γ / k_{Trp}
basal level
ln([trp])

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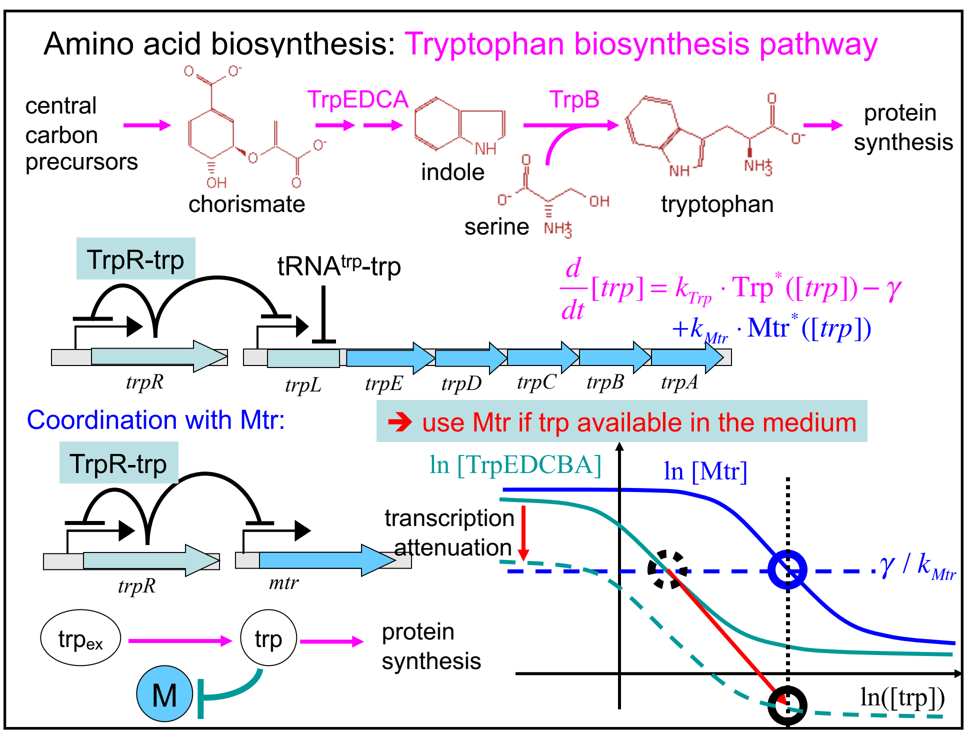

Amino acid biosynthesis: Tryptophan biosynthesis pathway
central carbon precursors
chorismate
TrpEDCA
indole
TrpB
serine
tryptophan
protein synthesis
TrpR-trp
tRNAtrp-trp
$\frac{d}{dt}[trp] = k_{Trp} \cdot \mathrm{Trp}^*([trp]) - \gamma$
$+k_{Mtr} \cdot \mathrm{Mtr}^*([trp])$
trpR
trpL
trpE
trpD
trpC
trpB
trpA
Coordination with Mtr:
➔ use Mtr if trp available in the medium
TrpR-trp
trpR
mtr
ln [TrpEDCBA]
ln [Mtr]
transcription attenuation
γ / k_{Mtr}
trpex
trp
protein synthesis
M
ln([trp])

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