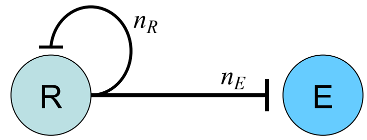
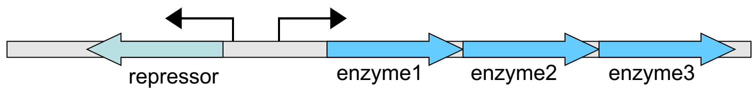
D. Metabolic Control



1. Gene regulation



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

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

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

set by DNA seq

$\approx \alpha_E/\alpha_R$ if $n_R \approx n_E \gg 1$

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

17

2. Effect of the inducer (S) dissoc const: K_S; Hill coeff: n_S

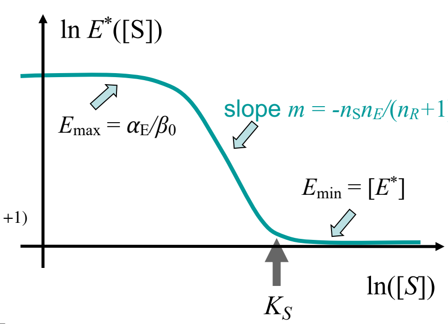
$$[RS] \equiv R_S = [R]\cdot\frac{([S]/K_S)^{n_S}}{1+([S]/K_S)^{n_S}} \approx [R]\cdot([S]/K_S)^{n_S} \quad \text{for } [S] \ll K_S$$

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

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

steady-state: $$\alpha_R\left(\frac{R_S^*}{K_R}\right)^{-n_R} \approx \beta_0[R^*]$$

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



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

➔ reduce the synthesis of E as S (product) level increases

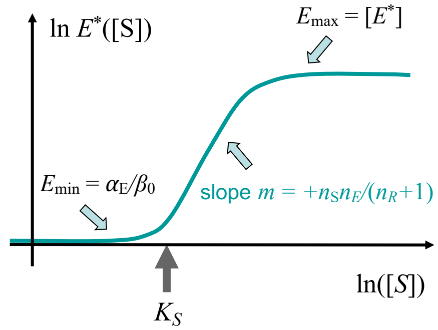
18

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

then $E^*([S]) \approx [E^*] \dfrac{1+\Omega\left([S]/K_S\right)^m}{1+\left([S]/K_S\right)^m}$,

with $m = +\dfrac{n_S \cdot n_E}{n_R+1}$,

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



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

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

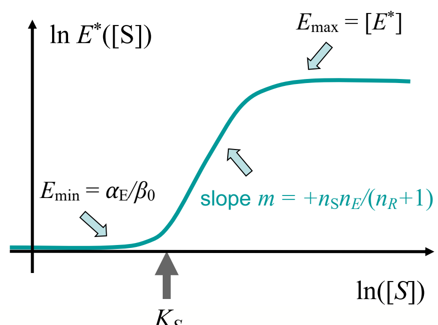
19

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

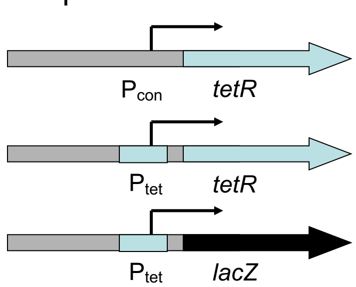
then $E^*([S]) \approx [E^*] \dfrac{1+\Omega\left([S]/K_S\right)^m}{1+\left([S]/K_S\right)^m}$,

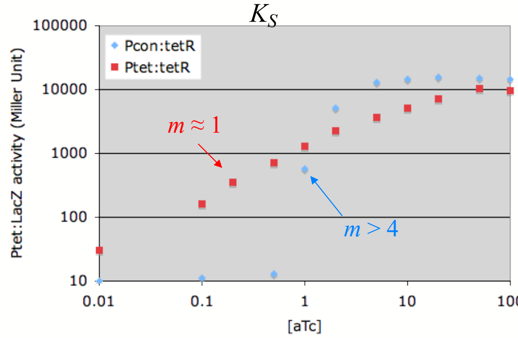
with $m = +\dfrac{n_S \cdot n_E}{n_R+1}$,

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



Expt' l test:





20

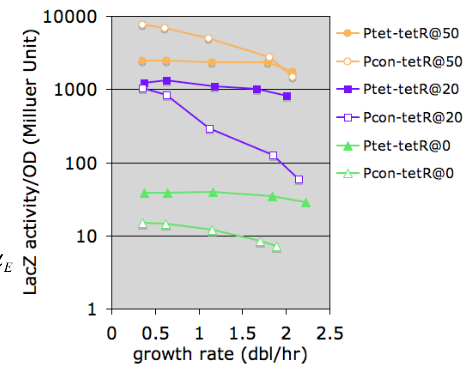
Dependence on growth conditions:

$$E^*([S]) \approx [E^*]\,\frac{1+\Omega\left([S]/K_S\right)^m}{1+\left([S]/K_S\right)^m},$$

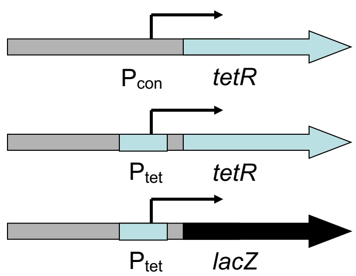
$$[E^*] = K_R\cdot\left(\frac{K_E}{K_R}\right)^{n_E}\cdot\left(\frac{\alpha_E}{\beta_0 K_R}\right)\Big/\left(\frac{\alpha_R}{\beta_0 K_R}\right)^{n_E/(n_R+1)}$$

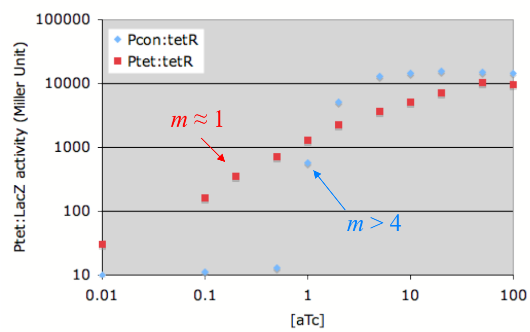
$$\approx K_R\cdot\frac{\alpha_E}{\alpha_R}\qquad \text{for } n_R = n_E \gg 1, K_R = K_E, \alpha_R \propto \alpha_E$$

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



Expt'l test:





- 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?

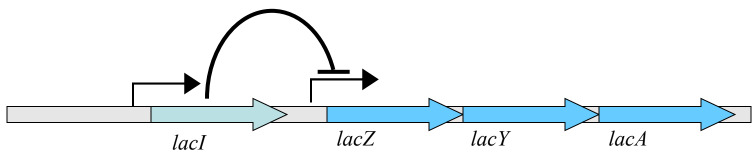
System[a]	Nature of regulator: Observed[f]	Nature of regulator: Predicted		Demand for expression: Predicted	Demand for expression: Observed[f]
Inducible catabolic pathways					
Arabinose	Activator		→	High	High
Galactose	Repressor		→	Low	Low
Glycerol	Repressor		→	Low	Low
Histidine	Repressor		→	Low	Low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

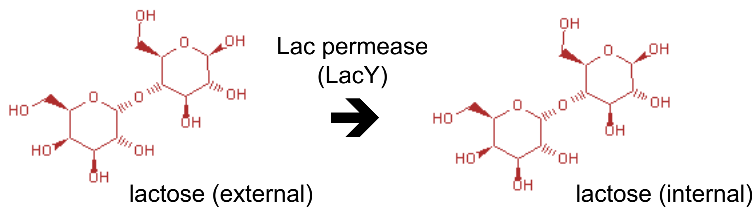
- regulation of E by S is often a form of feedback control

➔ include the synthesis of S by E

example: lactose transport and utilization

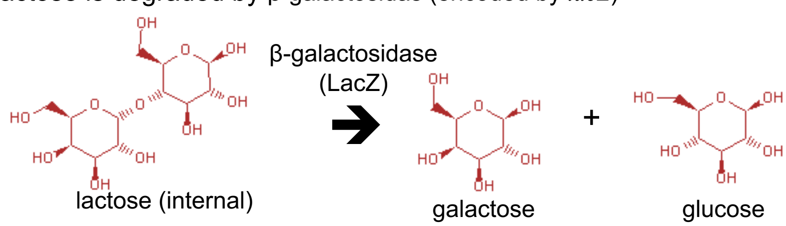


- LacR (encoded by *lacI*) weakly expressed constitutively and exerts coop strong repression of the *lacZYA* operon due to DNA looping
- want to inactivate LacR when lactose is present externally (and glucose absent)
- but entry of lactose requires the Lac permease (encoded by *lacY*)



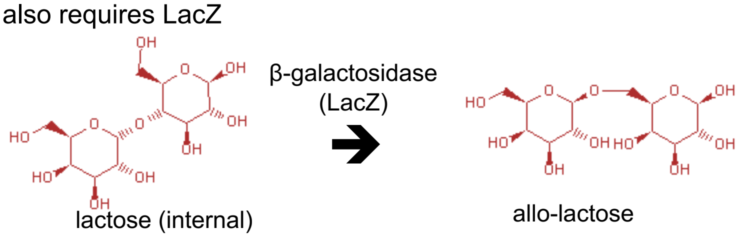
23

- 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

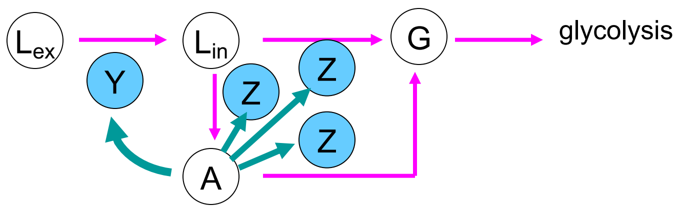


➔ induction of the lac operon (by allo-lactose) requires expression of the operon (LacY + LacZ) = positive feedback

➔ allo-lactose further degraded by LacZ

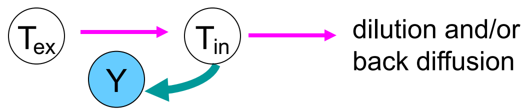
24

regulatory circuit for lactose transport/utilization:



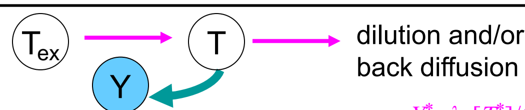
simplified system: use lactose analogue (TMG)

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



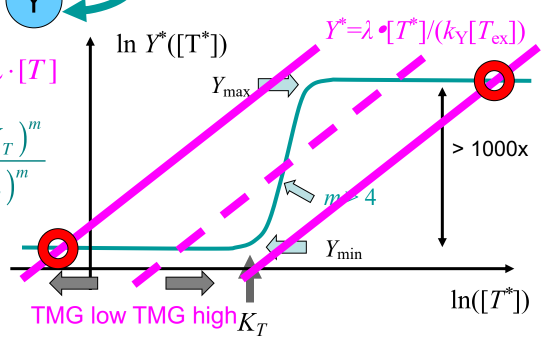
25

simplified system:



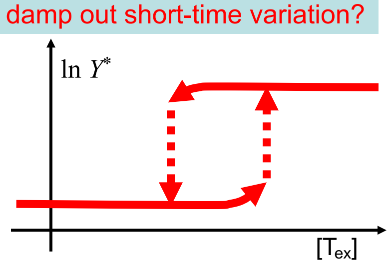
[Novick, 1957]

$$\begin{cases} \frac{d}{dt}[T] = k_Y [T_{ex}] \cdot Y^*([T]) - \lambda \cdot [T] \\ Y^*([T]) \approx Y_{\min} \frac{1 + \Omega([T]/K_T)^m}{1 + ([T]/K_T)^m} \end{cases}$$

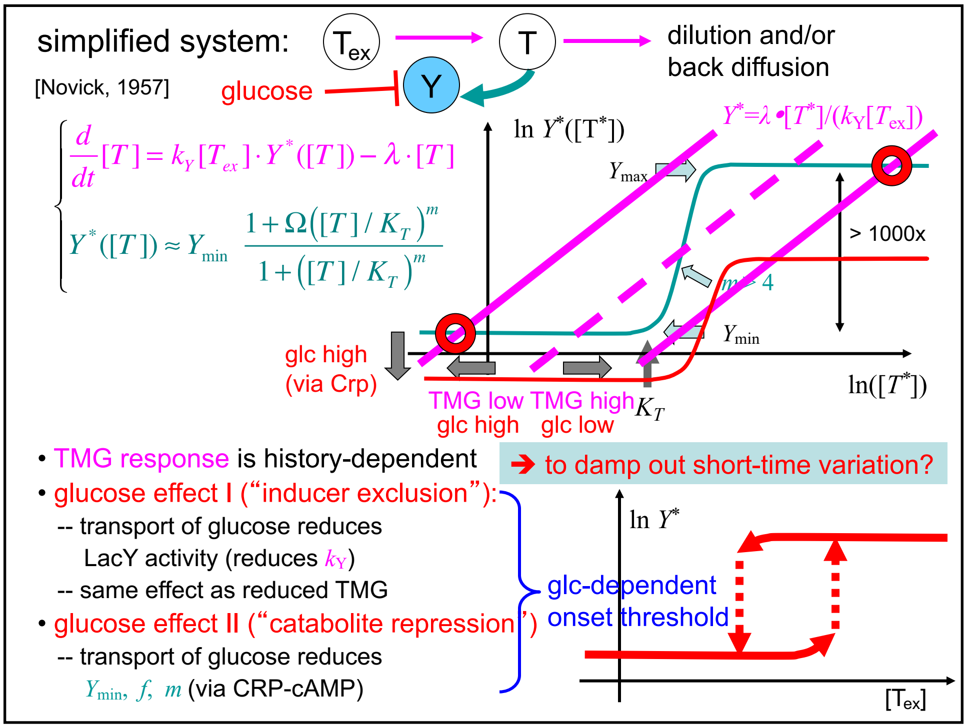


- TMG response is history-dependent

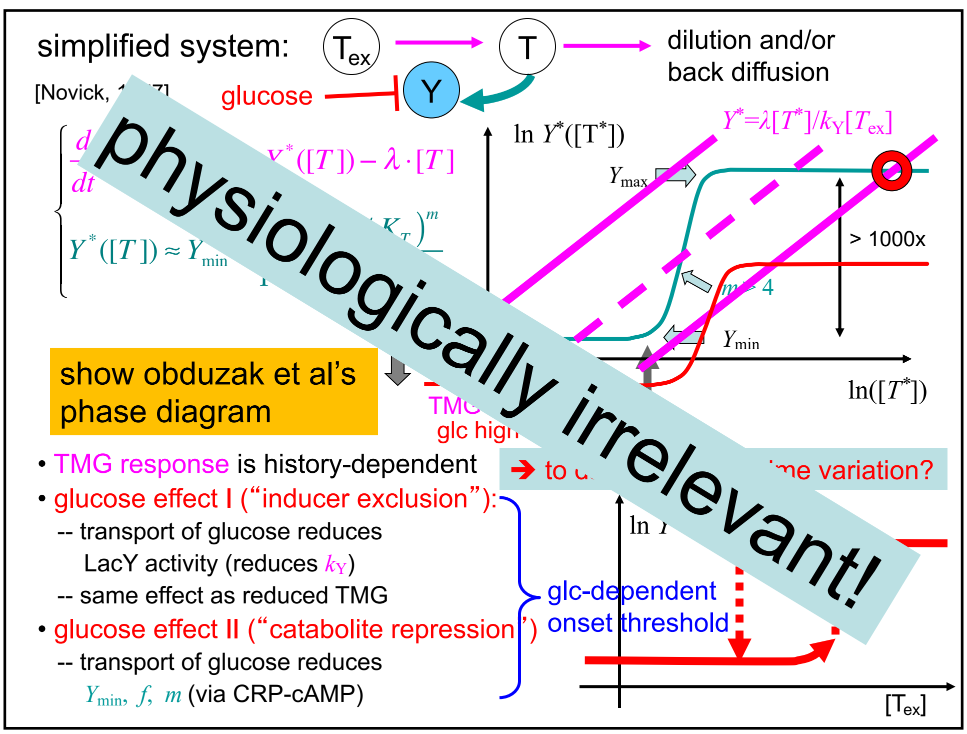
➔ to damp out short-time variation?



26

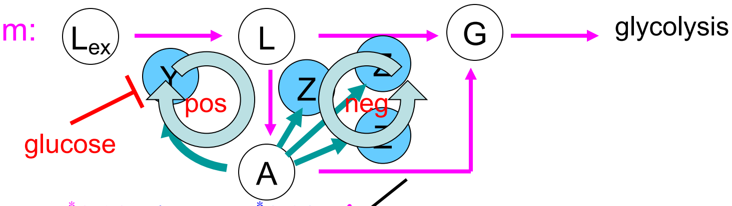

simplified system:
Tex
T
Y
dilution and/or
back diffusion
[Novick, 1957]
glucose
d/dt [T] = k_Y [T_ex] · Y*([T]) − λ · [T]
Y*([T]) ≈ Y_min (1 + Ω([T]/K_T)^m) / (1 + ([T]/K_T)^m)
ln Y*([T*])
Y*=λ•[T*]/(k_Y[T_ex])
Y_max
> 1000x
m ≈ 4
Y_min
glc high
(via Crp)
TMG low
glc high
TMG high
glc low
K_T
ln([T*])
• TMG response is history-dependent
➔ to damp out short-time variation?
• glucose effect I ("inducer exclusion"):
-- transport of glucose reduces
LacY activity (reduces k_Y)
-- same effect as reduced TMG
• glucose effect II ("catabolite repression")
-- transport of glucose reduces
Y_min, f, m (via CRP-cAMP)
glc-dependent
onset threshold
ln Y*
[Tex]

27


simplified system:
Tex
T
Y
dilution and/or
back diffusion
glucose
ln Y*([T*])
Y*=λ[T*]/k_Y[T_ex]
Y_max
> 1000x
m ≈ 4
Y_min
ln([T*])
show obduzak et al's
phase diagram
physiologically irrelevant!
• TMG response is history-dependent
• glucose effect I ("inducer exclusion"):
-- transport of glucose reduces
LacY activity (reduces k_Y)
-- same effect as reduced TMG
• glucose effect II ("catabolite repression")
-- transport of glucose reduces
Y_min, f, m (via CRP-cAMP)
glc-dependent
onset threshold
[Tex]

28

full lac system:



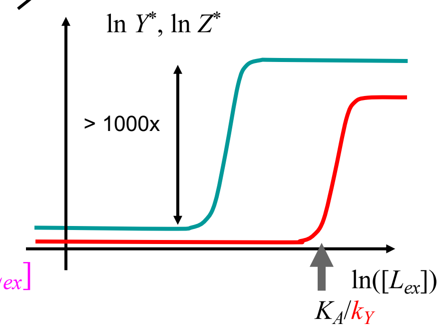
$$\begin{cases} \frac{d}{dt}[L] = k_Y \cdot [L_{ex}] \cdot Y^*([A]) - k_Z \cdot [L] \cdot Z^*([A]) - \lambda \cdot [L] \\ \frac{d}{dt}[A] = k_{Z1} \cdot [L] \cdot Z^*([A]) - k_{Z2} \cdot [A] \cdot Z^*([A]) - \lambda \cdot [A] \end{cases}$$

$$Y^*([A]) \propto Z^*([A]) \propto \frac{1+\Omega([A]/K_A)^m}{1+([A]/K_A)^m}$$

- include hydrolysis of substrate
- 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

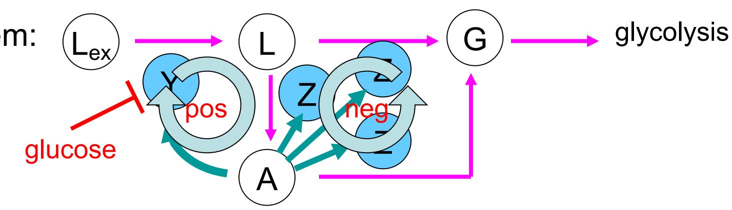
➔ onset depends on k_Y (controlled by glucose)



(note: max of L_{ex} limited by by K_M of LacY)

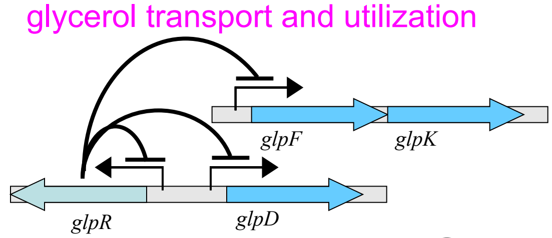
29

full lac system:



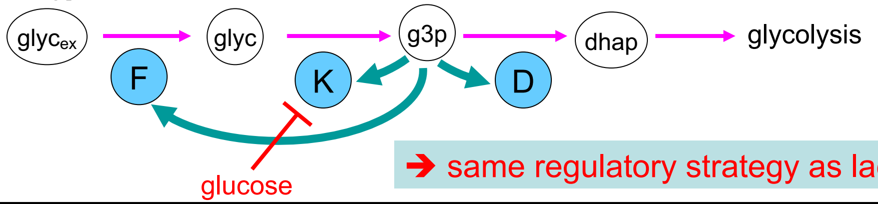
Is the lac system an exception?

glycerol transport and utilization



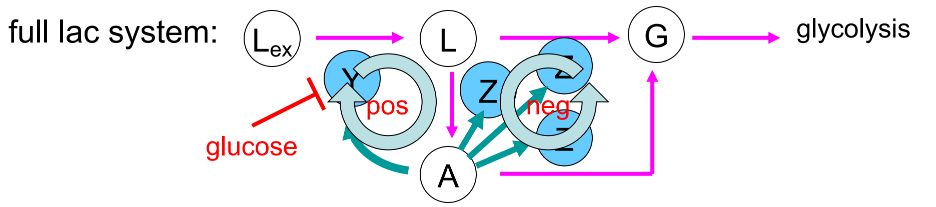
- glp operons repressed by GlpR and activated by Crp-cAMP
- GlpF: allows glycerol influx
- GlpK: converts glycerol to g3p
- GlpD: “consumes” g3p
- g3p: inhibits GlpR

➔ pos + neg feedback



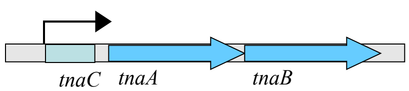
➔ same regulatory strategy as lac

30



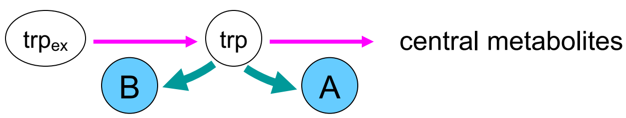
Is the lac system an exception?

Tryptophan transport & degradation



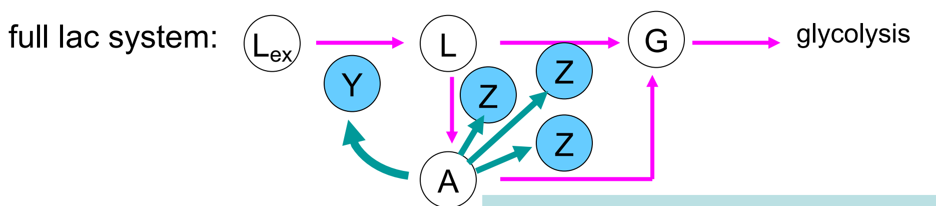
- promoter activated by CRP-cAMP and tsx attenuation (TnaC) relieved by trp
- TnaB: low affinity trp transporter
- TnaA: degrades trp into pyr, NH4, indole

➔ pos + neg feedback



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

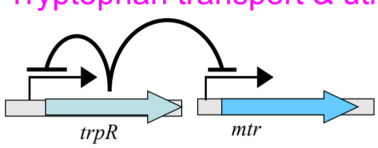
31



Is the lac system an exception?

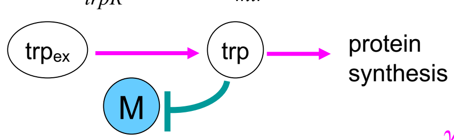
lac typical of catabolic systems; different from anabolic systems

Tryptophan transport & utilization



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

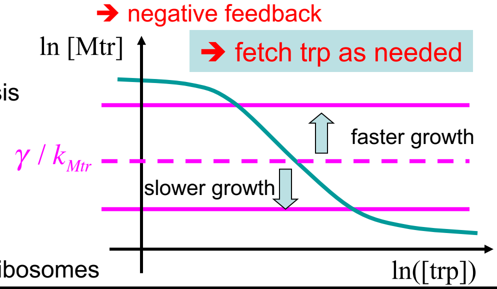
➔ negative feedback



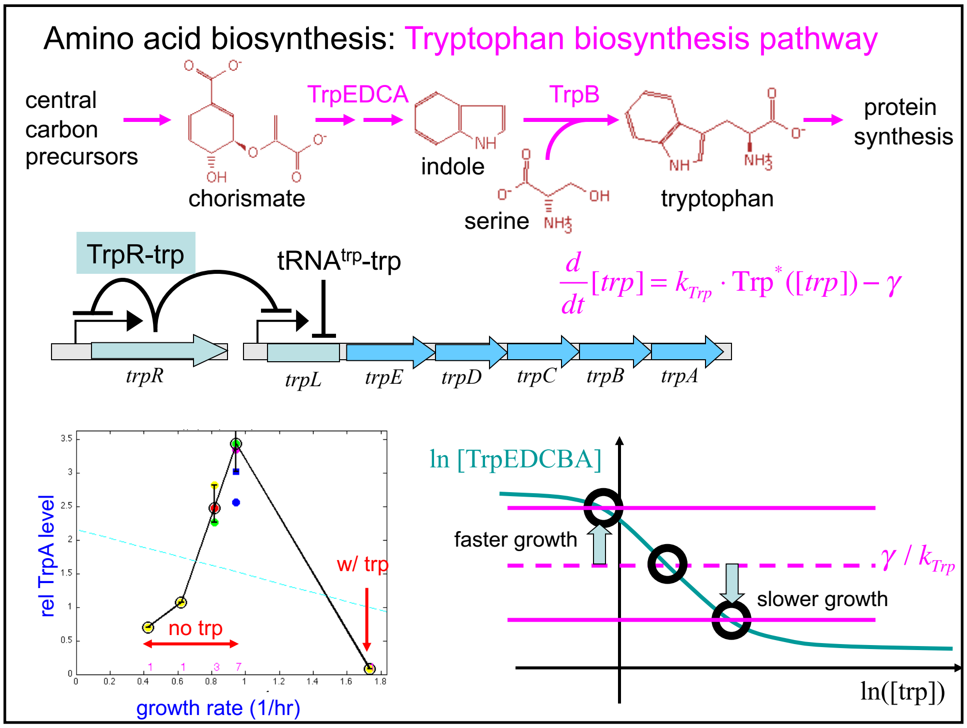
$$\frac{d}{dt}[T] = k_{Mtr} \cdot \mathrm{Mtr}^*([T]) - \gamma$$

rate of trp consumption by ribosomes

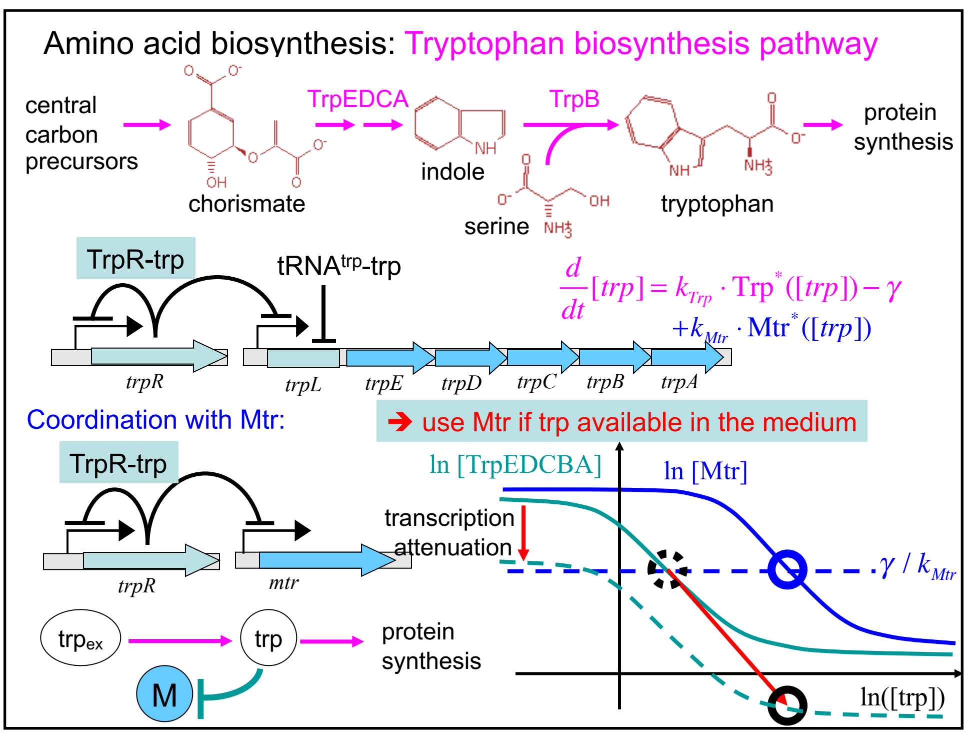
➔ fetch trp as needed



32


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
rel TrpA level
no trp
w/ trp
growth rate (1/hr)
ln [TrpEDCBA]
faster growth
slower growth
γ / k_{Trp}
ln([trp])

33


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
trpex
trp
protein synthesis
M
ln [TrpEDCBA]
ln [Mtr]
transcription attenuation
γ / k_{Mtr}
ln([trp])

34