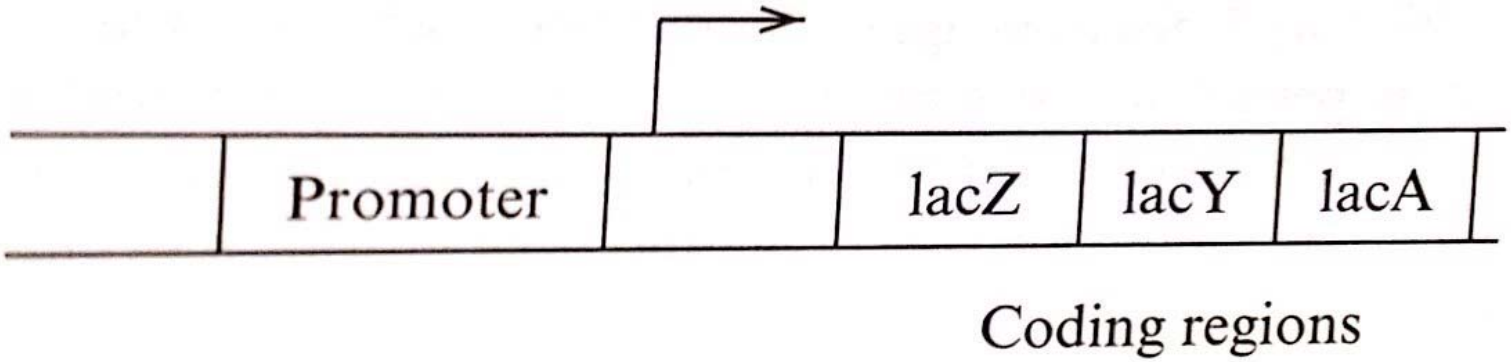
BME 5742 Biosystems Modeling and Control

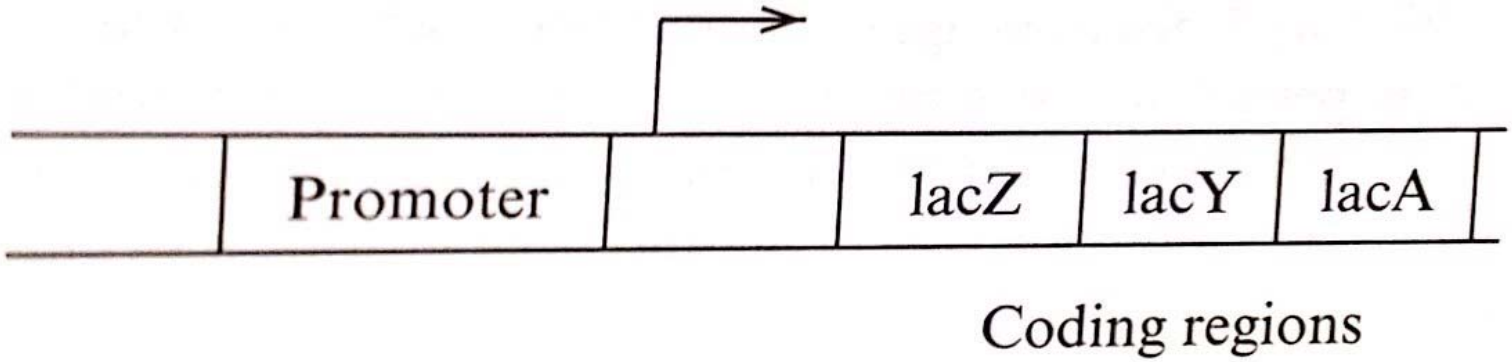
Lecture 29
Gene Regulatory Networks
Part 3
Gene Switches

Example: The *lac* Operon



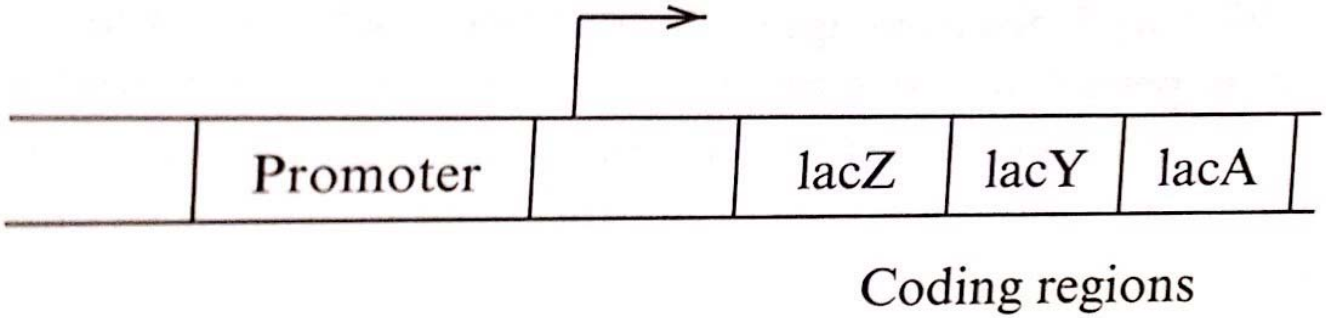
- Set of genes found in E-coli bacteria whose products allow the bacterium to metabolize lactose.
- This set of genes are contained in an operon

The *lac* Operon



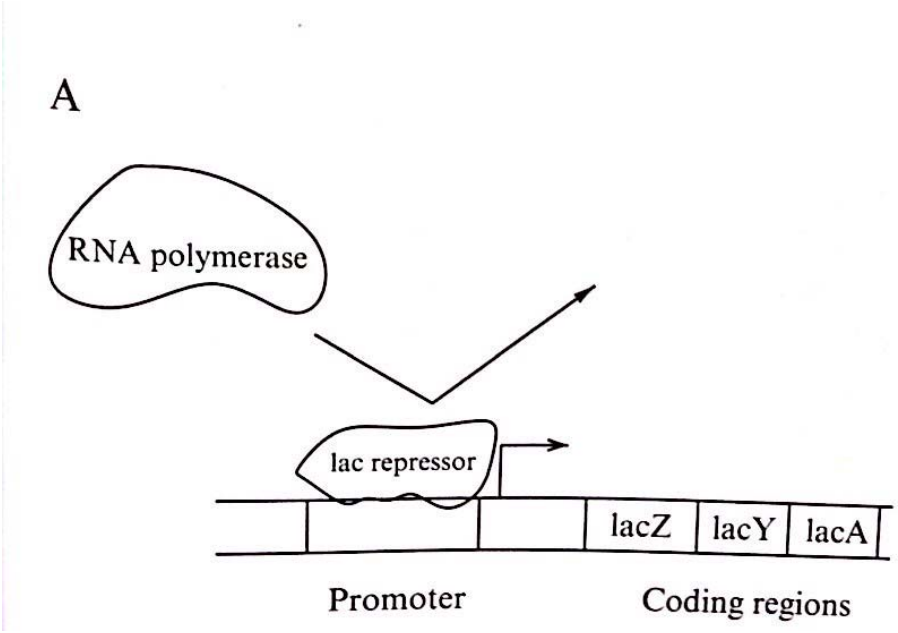
- The operon is a single promoter region followed by a set of coding regions, one for each protein product.
- The genes in an operon are expressed simultaneously and are coordinately regulated via a shared promoter.

The *lac* Operon contains coding regions for three proteins:



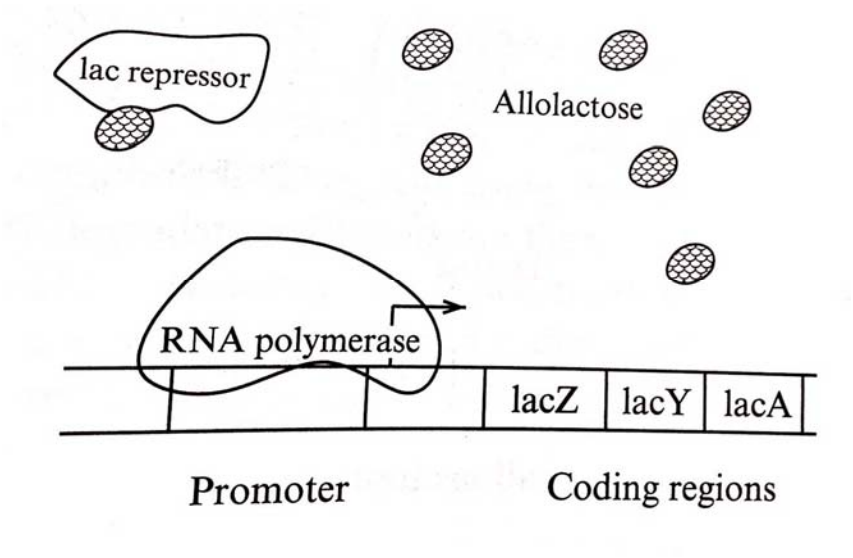
- Protein β-galactosidase – coded by the gene lacZ.
- Protein β-galactoside permease – coded by the gene lacY.
- Protein β-galactoside tranacetylase – coded by the gene lacA.

The *lac Repressor lacI*



- Whenever lactose level in the environment is low the cell represses expression from the *lac operon*
- Repression is done by a transcription factor called lacI which binds to an operator near the operon's promoter, thus blocking the transcription

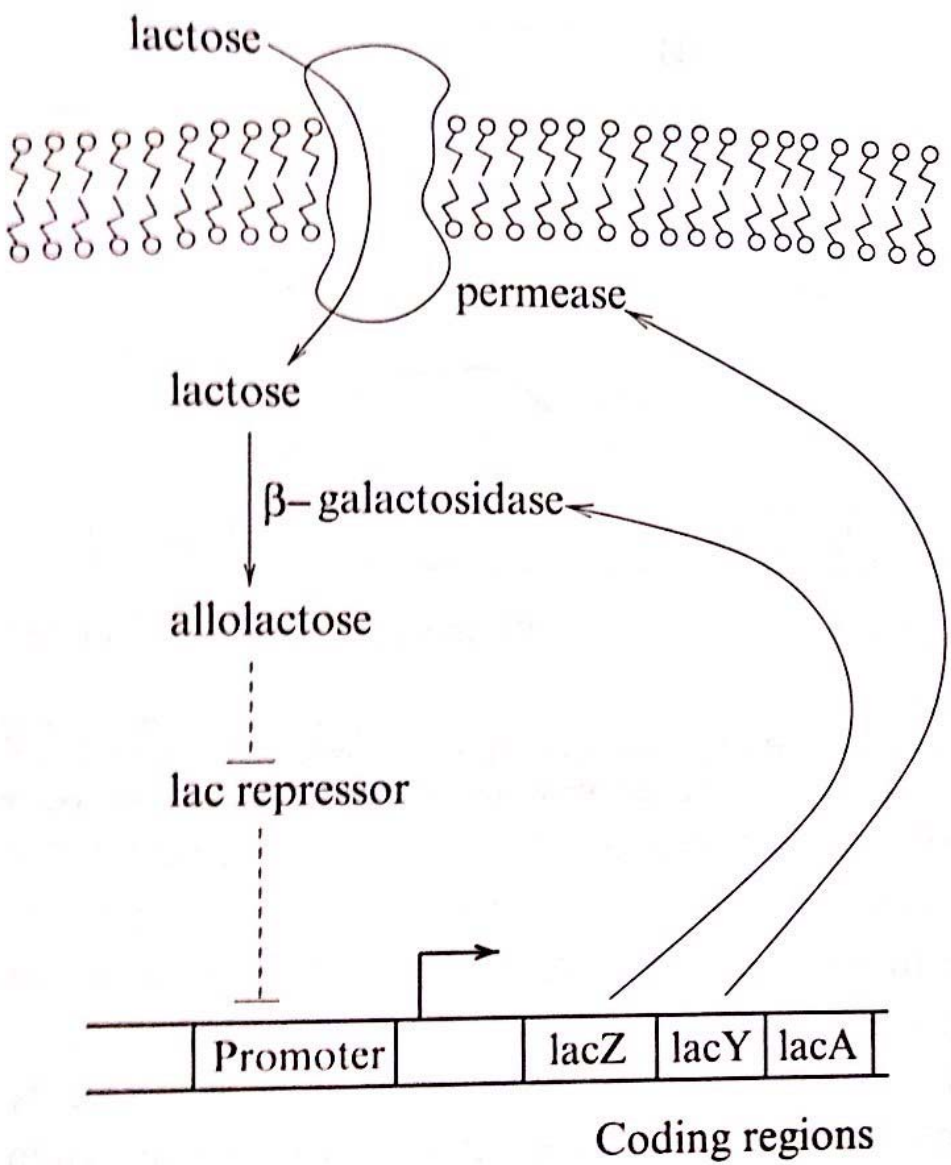
lac Repressor removal by allolactose



Allolactose acts as an inducer

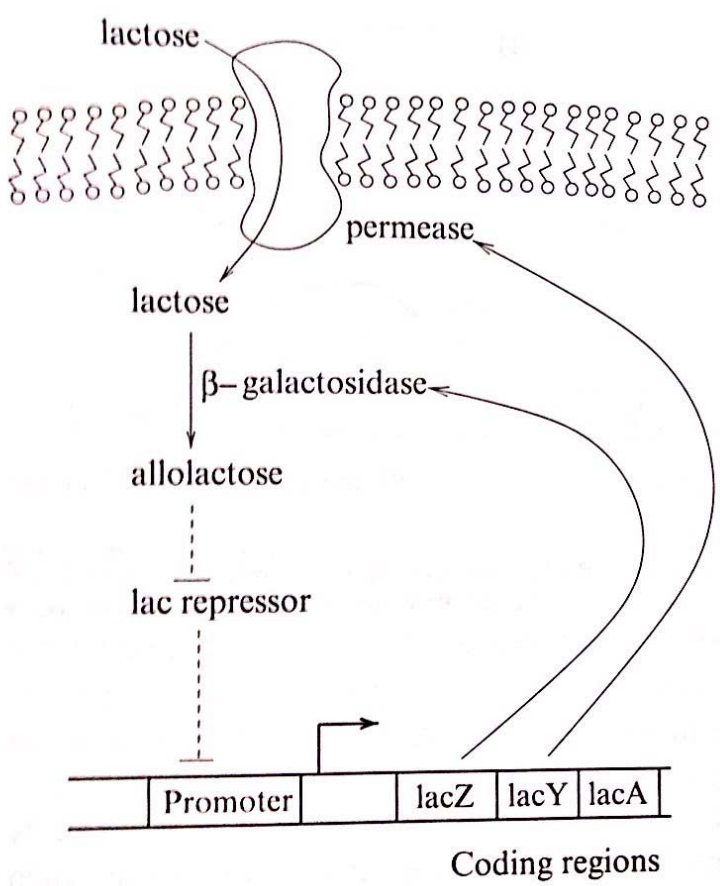
- Whenever lactose in the environment is abundant it converts in part to allolactose that binds with the lac repressor, and that lowers significantly its affinity to the operator site.
- Expression is triggered by the presence of lactose.

Feedback in the *lac* operon



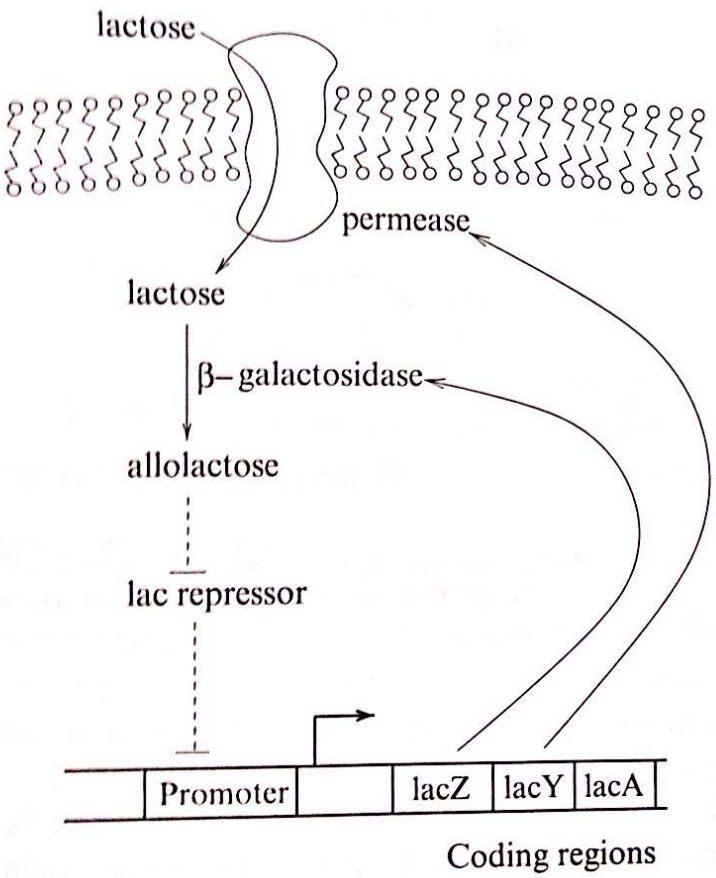
The presence of lacose sets off a positive feedback that leads to rapid expression of the *lac* genes

How does the feedback work?



- Feedback is implemented by the *lac* proteins themselves:
- β-galactoside permease is a transmembrane protein that transports lactose into the cell.

How does the feedback work?



- β-galactosidase is an enzyme that catalyzes the conversion of lactose to allolactose.
- Allolactose activates gene expression by de-repression.

One more control mechanism

- Transcription from the *lac* operonrequires the presence of an activator called catabolic gene activating protein (CAP)
- CAP is only active when glucose is absent from the environment.
- Glucose is the preferred source of energy and carbons for these cells.
- CAP ensures that when glucose is present the *lac* genes are not expressed, regardless of the availability of lactose.

Mathematical Model of the *lac* Operon Transcription

$$O + R \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} OR$$

$$\frac{dm(t)}{dt} = a_1 \frac{1}{1 + \frac{r(t)}{K_1}} - \delta_M m(t)$$

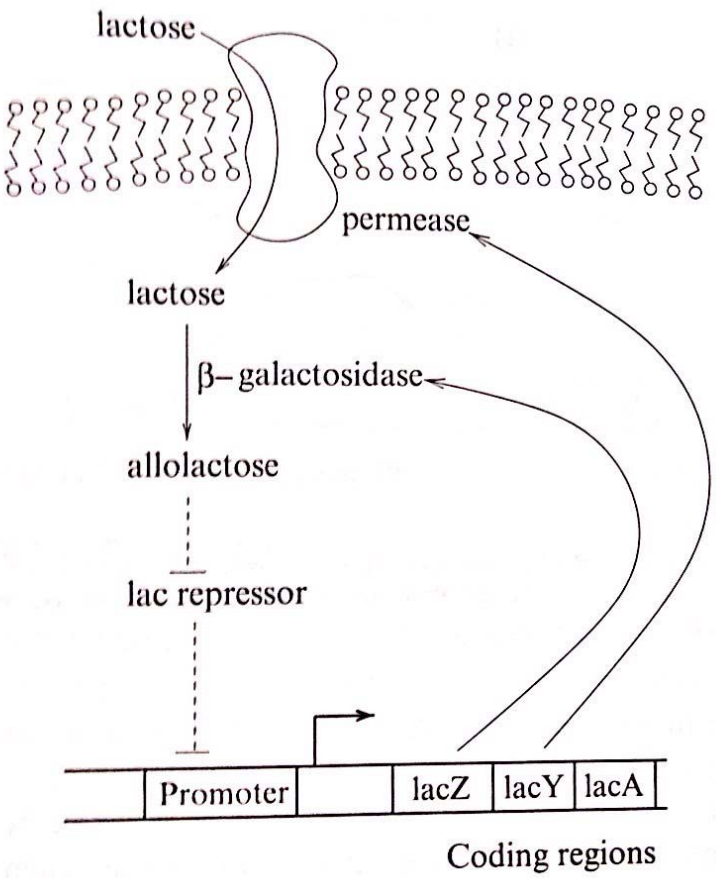
- O is the unbound operator
- R is the *lac* repressor
- OR is the repressor-bound operator
- Repressor concentration is r
- The dissociation constant is $K_1 = k_{-1}/k_1$
- Concentration of operon mRNA = m
- Maximal rate of transcription = a_1
- mRNA degradation/dilution rate = δ_M

Mathematical Model of the *lac* Operon Translation

$$\frac{dy(t)}{dt} = c_1 m(t) - \delta_Y y(t)$$

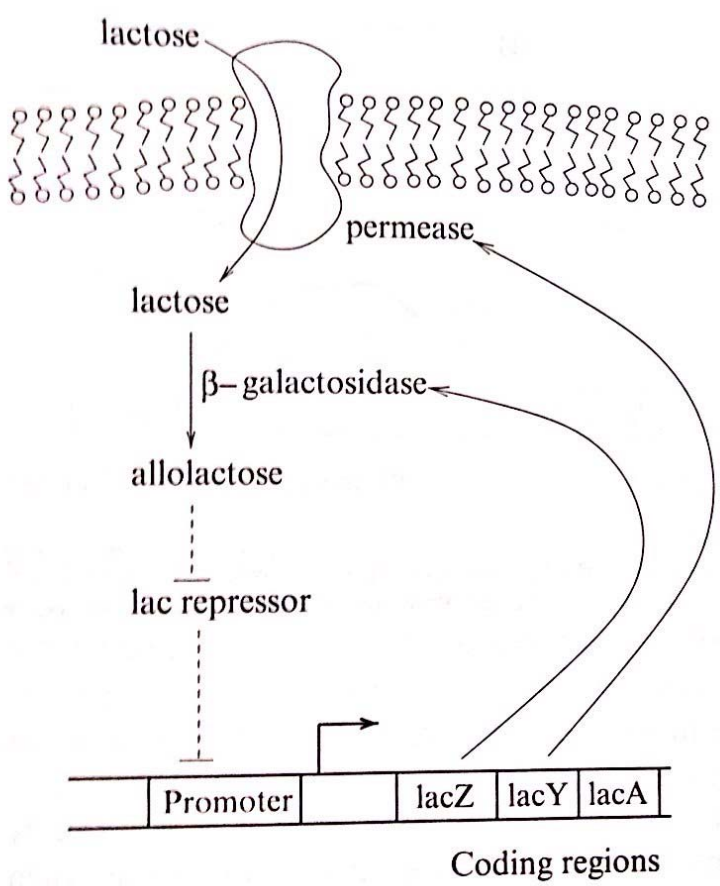
- Permease concentration = y(t)
- Rate of translation = c_1
- Permease degradation/dilution rate = δ_Y

Modeling of the *lac* operon feedback - Notation



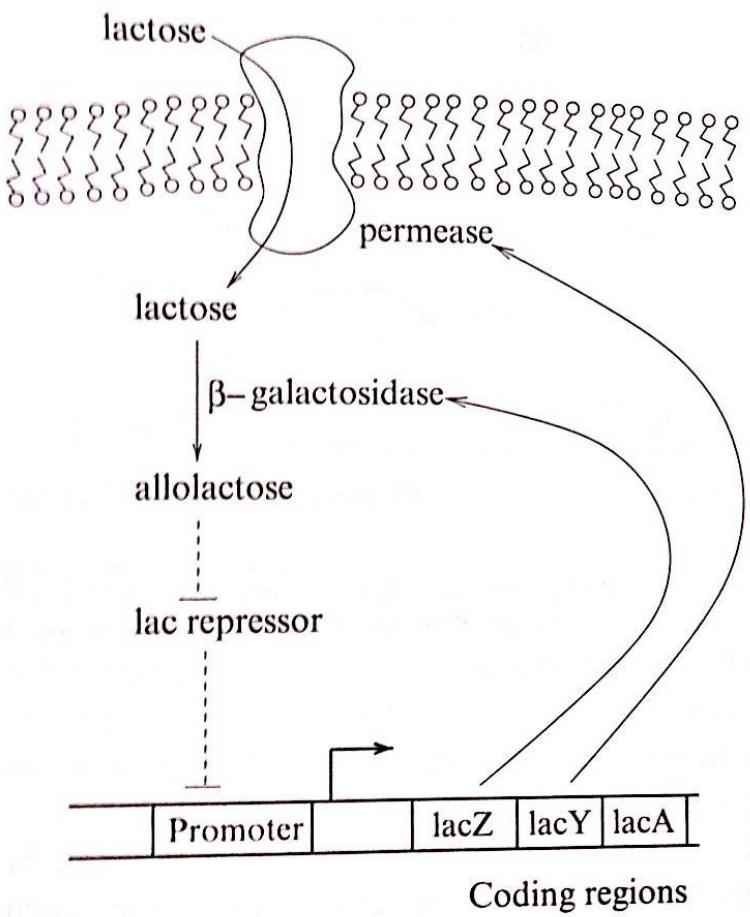
- Permease concentration = y
- Concentration of β-galactosidase = b
- Both are translated from the same mRNA transcript → same translation rate and same degradation rate

Modeling of the *lac* operon feedback – Relationship between y and b



- Permease has one active site
- β-galactosidase has 4 active sites
- Simplifying assumption:
- Relationship of concentrations: $b(t)=y(t)/4$

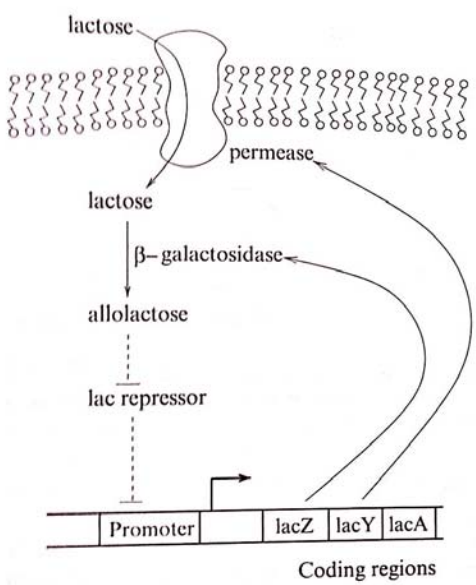
Modeling of the *lac* operon feedback – Relationship between L and L_e



- Lactose uptake is mediated by the permease.
- Assume simple Michaelis-Menten kinetics for the transport:

$$Lactose_uptake = \frac{k_L y(t) L_e}{K_{ML} + L_e}$$

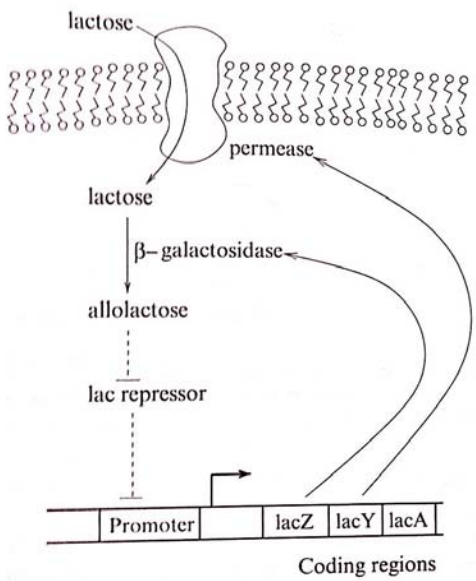
Modeling of the *lac* operon feedback – b(t) influence on L(t)



- Once lactose is transported across the membrane, it is either converted to allolactose or is metabolized (into simpler sugars)
- Denote ICF concentration of lactose by L(t)

$$\frac{dL(t)}{dt} = \frac{k_L y(t) L_e}{K_{ML} + L_e} - \frac{k_g b(t) L(t)}{K_{Mg} + L(t)} - \frac{k_a b(t) L(t)}{K_{Ma} + L(t)} - \delta_L L(t)$$

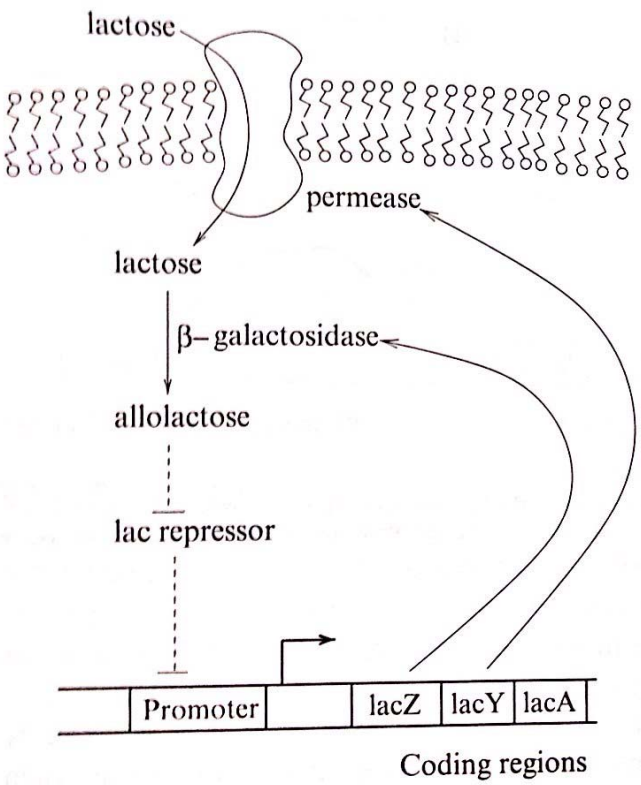
Modeling of the *lac* operon feedback – Simplified influence of b(t) on L(t)



- Dilution rate of lactose = δ_L
- It is assumed that both conversion to allolactose and metabolism each follows a simple enzyme kinetics, with the appropriate constants
- Assumption: $k_g=k_a$, $K_{Mg}=K_{Ma}$

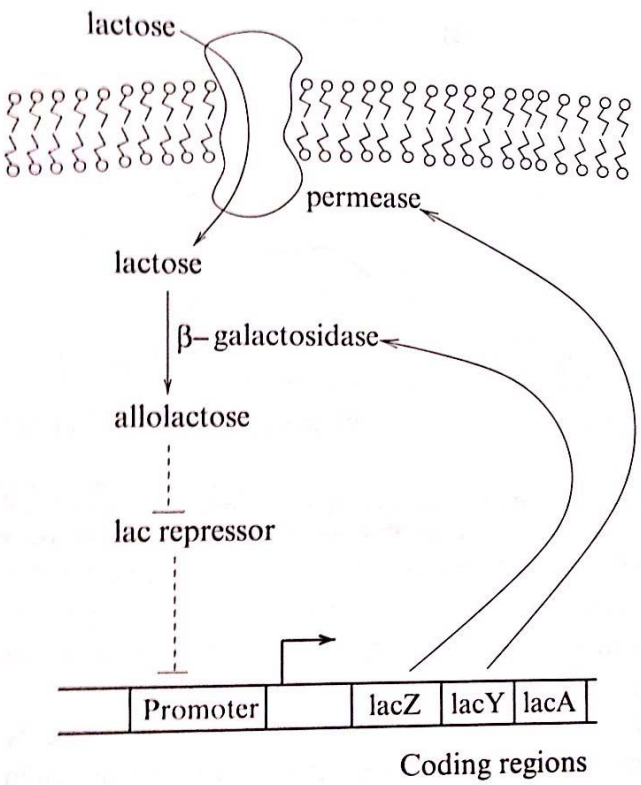
$$\frac{dL(t)}{dt} = \frac{k_L y(t) L_e}{K_{ML} + L_e} - 2\frac{k_g b(t) L(t)}{K_{Mg} + L(t)} - \delta_L L(t)$$

Modeling of the *lac* operon feedback – Simplified relationship between lactose and allolactose



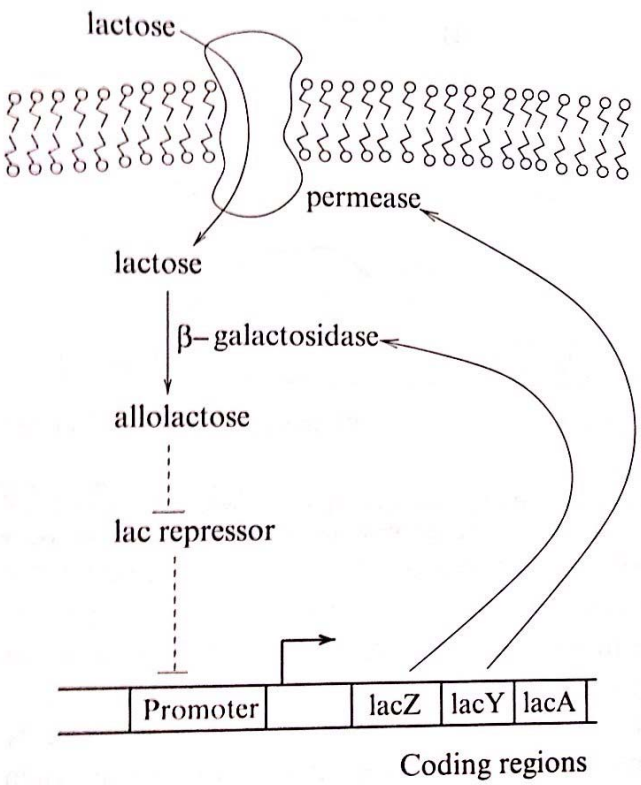
- One can also show that the reaction by which L(t) is converted to allolactose A(t) reaches equilibrium very fast
- L(t) and A(t) equilibrium condition is approximately:
- A(t) = L(t)

Modeling of the *lac* operon feedback – Inactivation of the *lac* repressor



- The repressor consists of 4 identical parts, each one has one active site.
- If allolactose binds to any one of these active site the repressor becomes inactivated

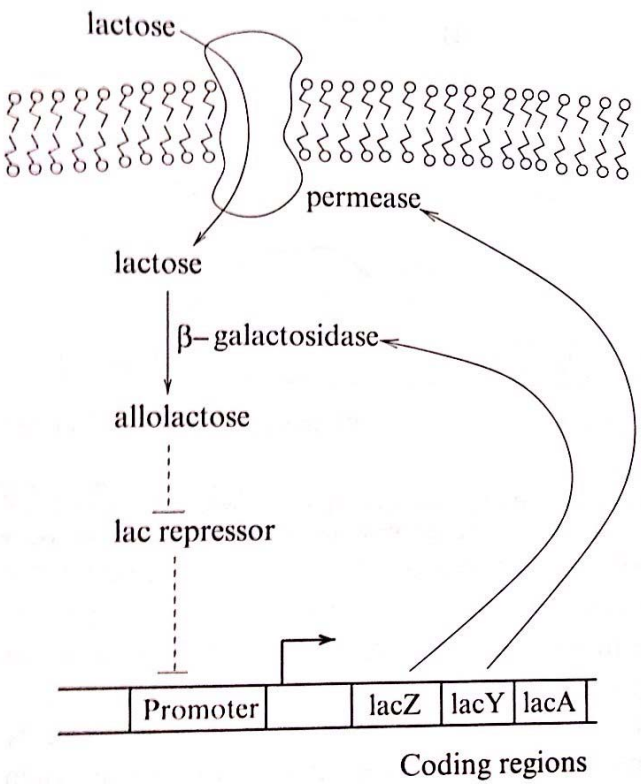
Modeling of the *lac* operon feedback – Inactivation of the *lac* repressor



- These binding events are independent
- K_2 is the dissociation constant

$$Fraction_of_unbound_repressor_parts = \frac{K_2}{K_2 + A(t)}$$

Modeling of the *lac* operon feedback – Inactivation of the *lac* repressor



- Repressor is active if all its active site are unbound by A

$$r(t) = R_T \left(\frac{K_2}{K_2 + A(t)}\right)^4$$

The full model:

$$\frac{dm(t)}{dt} = a_1 \frac{1}{1+\frac{r(t)}{K_1}} - \delta_M m(t)$$

$$b(t) \approx \frac{y(t)}{4}$$

$$A(t) \approx L(t)$$

$$\frac{dy(t)}{dt} = c_1 m(t) - \delta_Y y(t)$$

$$r(t) = R_T \left(\frac{K_2}{K_2 + A(t)}\right)^4$$

$$\frac{dL(t)}{dt} = \frac{k_L y(t) L_e}{K_{ML} + L_e} - 2\frac{k_g b(t) L(t)}{K_{Mg} + L(t)} - \delta_L L(t)$$

Typical Parameter Values

$\delta_M = 0.48 _[\min^{-1}]$ $\delta_Y = 0.03 _[\min^{-1}]$ $\delta_L = 0.02 _[\min^{-1}]$

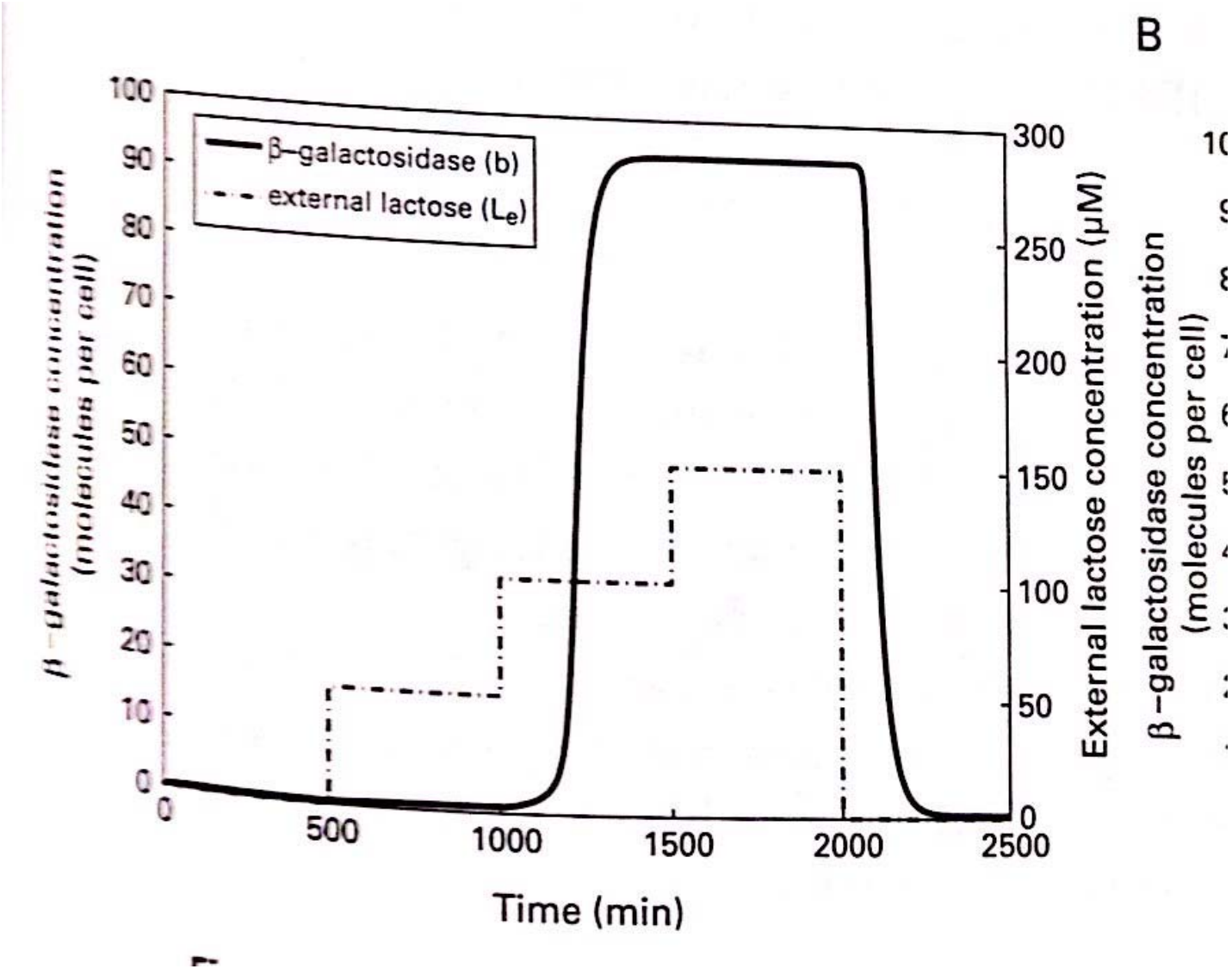
$a_1 = 0.29 _[molecules \cdot \min^{-1}]$ $K_2 = 2.92 \cdot 10^6 _[molecules]$

$K_1 R_T = 213.2$ $c_1 = 18.8 _[\min^{-1}]$ $k_L = 6.0 \cdot 10^4 _[\min^{-1}]$

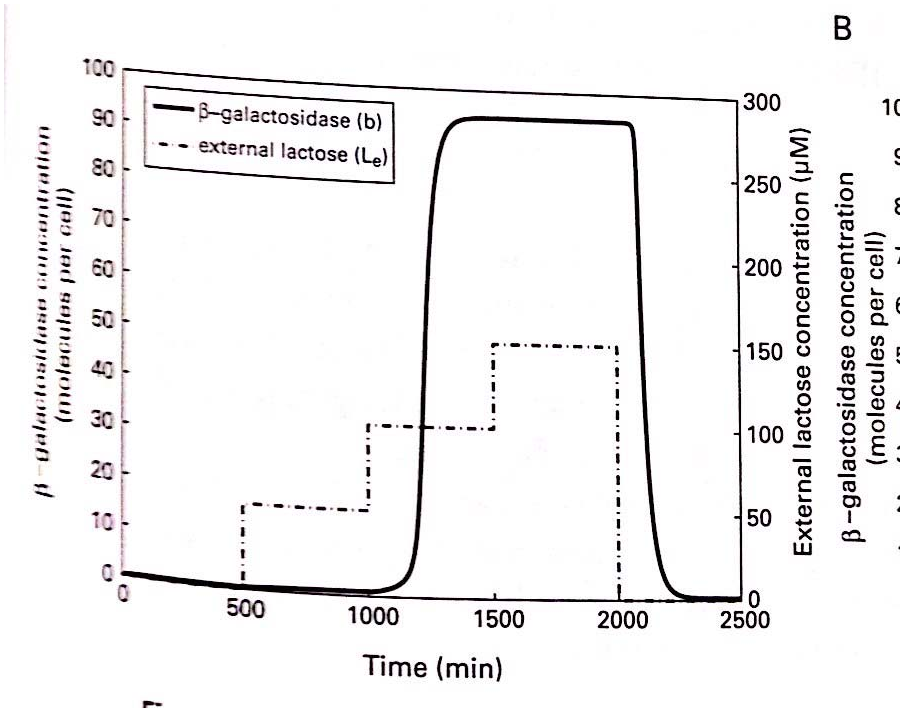
$k_{ML} = 680 _[\mu M]$ $k_g = 3.6 \cdot 10^3 _[\min^{-1}]$

$K_{Mg} = 7.0 \cdot 10^5 _[molecules]$

Simulation Results



Simulation Results

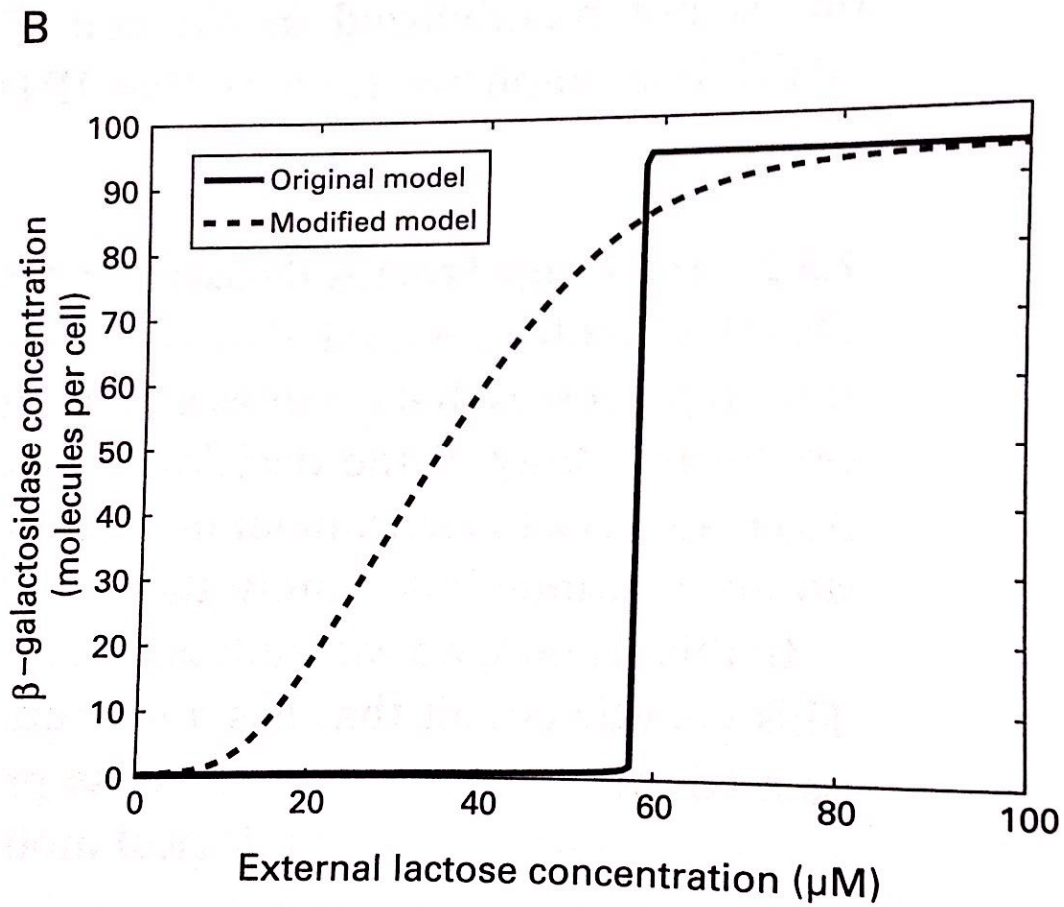


Switching action at a specific level of the external lactose concentration:

Below threshold low activity of the *lac* operon

Dramatic increase past the threshold

Simulation Results – Positive Feedback Removed



The full model: (with removed feedback)

$$\frac{dm(t)}{dt} = a_1 \frac{1}{1+\frac{r(t)}{K_1}} - \delta_M m(t)$$

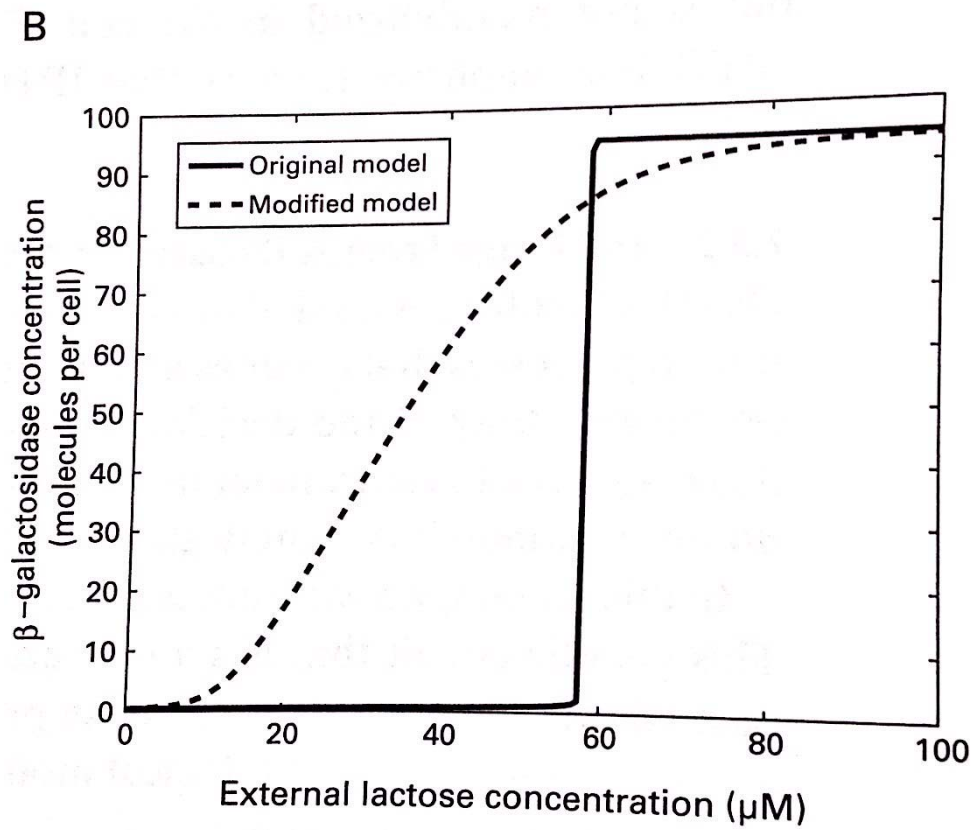
$$b(t) \approx \frac{y(t)}{4}$$

$$A(t) \approx L(t)$$

$$\frac{dy(t)}{dt} = c_1 m(t) - \delta_Y y(t)$$

$$r(t) = R_T \left(\frac{K_2}{K_2 + A(t)}\right)^4$$

$$\frac{dL(t)}{dt} = \frac{4k_L E L_e}{K_{ML} + L_e} - \frac{k_g b(t) L(t)}{K_{Mg} + L(t)} - \frac{k_g E L(t)}{K_{Mg} + L(t)} - \delta_L L(t)$$

E is constant

Simulation Results: Abrupt change in the original model vs. moderate change if there is no feedback