Bistability in ODE and Boolean models

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Algebraic Biology
Bistability

A system is bistable if it has two stable steady-states separated by an unstable state.

The threshold ODE: \( y' = -ry(1 - \frac{y}{M})(1 - \frac{y}{T}) \).

In the threshold model for population growth, there are three steady-states, \( 0 < T < M \):

- \( M \) = carrying capacity (stable),
- \( T \) = extinction threshold (unstable),
- \( 0 \) = extinct (stable).
Types of bistability

The *lac* operon exhibits bistability.

The expression level of the *lac* operon genes are either almost zero ("basal levels"), or very high (thousands of times higher). There’s no “inbetween” state.

The exact level depends on the concentration of intracellular lactose. *Let’s denote this parameter by $p$."

Now, let’s “tune” this parameter. The result might look like the graph on the left.

![Diagram](image)

This is *reversible* bistability. In other situations, it may be *irreversible* (at right).
Hysteresis

For reversible bistability, the *up-threshold* $L_2$ of $p$ is higher than the *down-threshold* $L_1$ of $p$.

This is hysteresis: a dependence of a state on its current state and past state.

**Thermostat example**

Consider a home thermostat.

- If the temperature is $T < 18$ (e.g., in winter), the heat is on.
- If the temperature is $T > 23$ (e.g., in summer), the AC is on.
- If $18 < T < 23$, then we don’t know whether it’s spring or autumn.
Hysteresis and the *lac* operon

If lactose levels are medium, then the state of the operon depends on whether or not a cell was grown in a lactose-rich environment.

**Lac operon example**

Let $[L]$ = concentration of intracellular lactose.

- If $[L] < L_1$, the operon is OFF.
- If $[L] > L_2$, the operon is ON.
- If $L_1 < [L] < L_2$, the operon might be ON or OFF.

In the region of bistability $(L_1, L_2)$, one can find both induced and un-induced cells.
An ODE model of the *lac* operon

The Boolean models we've seen are too simple to capture bistability.

We will derive two different ODE models of the *lac* operon that exhibit bistability: one with 3 variables, and another with 5 variables.

These ODE models were designed using Michaelis–Menten equations from mass-action kinetics which we learned about earlier.

They will also incorporate other features, such as:

- dilution of protein concentration due to bacterial growth
- degradation (decay) of protein concentration
- time delays

After that, we'll see how bistability can indeed be captured by a Boolean model.

In general, bistable systems tend to have positive feedback loops (in their “wiring diagrams”) or double-negative feedback loops (＝positive feedback).
Modeling dilution in protein concentration due to bacterial growth

*E. coli* grows fast! It can double in 20 minutes. Thus, ODE models involving concentration can’t assume volume is constant.

Let’s define:

- \( V \) = average volume of an *E. coli* cell.
- \( x \) = number of molecules of protein \( X \) in that cell.

Assumptions:

- cell volume increases exponentially in time: \( \frac{dV}{dt} = \mu V \).
- degradation of \( X \) is exponential: \( \frac{dx}{dt} = -\beta x \).

The concentration of \( X \) is \([x] = \frac{x}{V}\). The derivative of this is (by the quotient rule):

\[
\frac{d[x]}{dt} = (x'V - V'x) \frac{1}{V^2} = (\beta xV - \mu VX) \frac{1}{V^2} = -(\beta + \mu) \frac{x}{V} = -(\beta + \mu)[x].
\]
Modeling of lactose repressor dynamics

Assumptions

- *Lac* repressor protein is produced at a constant rate.
- Laws of mass-action kinetics.

- **Repressor binds to allolactose:**

  \[
  R + nA \xrightleftharpoons[K_1]{1} RA_n
  \]

  \[
  \frac{d[RA_n]}{dt} = K_1[R][A]^n - [RA_n]
  \]

  Assume the reaction is at equilibrium: \( \frac{d[RA_n]}{dt} = 0 \), and so \( K_1 = \frac{[RA_n]}{[R][A]^n} \).

- **The repressor protein binds to the operator region if there is no allolactose:**

  \[
  O + R \xrightleftharpoons[K_2]{1} OR
  \]

  \[
  \frac{d[OR]}{dt} = K_2[O][R] - [OR].
  \]

  Assume the reaction is at equilibrium: \( \frac{d[OR]}{dt} = 0 \), and so \( K_2 = \frac{[OR]}{[O][R]} \).
Modeling of lactose repressor dynamics

Let $O_{\text{tot}} = \text{total operator concentration (a constant)}$. Then, using $K_2 = \frac{[OR]}{[O][R]}$,

$$O_{\text{tot}} = [O] + [OR] = [O] + K_2[O][R] = [O](1 + K_2[R]).$$

Therefore, $\frac{[O]}{O_{\text{tot}}} = \frac{1}{1 + K_2[R]}$. “Proportion of free (unbounded) operator sites.”

Let $R_{\text{tot}}$ be total concentration of the repressor protein (constant):

$$R_{\text{tot}} = [R] + [OR] + [RA_n]$$

Assume only a few molecules of operator sites per cell: $[OR] \ll \max \{[R], [RA_n]\}$:

$$R_{\text{tot}} \approx [R] + [RA_n] = [R] + K_1[R][A]^n$$

Eliminating $[RA_n]$, we get $[R] = \frac{R_{\text{tot}}}{1 + K_1[A]^n}$.

Now, the proportion of free operator sites is:

$$\frac{[O]}{O_{\text{tot}}} = \frac{1}{1 + K_2[R]} = \frac{1}{1 + K_2\left(\frac{R_{\text{tot}}}{1 + K_1[A]^n}\right)} \cdot \frac{1 + K_1[A]^n}{1 + K_1[A]^n} = \frac{1 + K_1[A]^n}{K + K_1[A]^n},$$

where $K = 1 + K_2 R_{\text{tot}}$. 
Modeling of lactose repressor dynamics

Summary

The proportion of free operator sites is

\[
\frac{[O]}{O_{tot}} = \frac{1 + K_1[A]^n}{K + K_1[A]^n}, \quad \text{where } K = 1 + K_2R_{tot}.
\]

Remarks

- The function \( f([A]) \) is (almost) a Hill function of coefficient \( n \).
- \( f([A] = 0) = \frac{1}{K} > 0 \) “basal level of gene expression.”
- \( f \) is increasing in \([A]\), when \([A] \geq 0\).
- \( \lim_{[A] \to \infty} f([A]) = 1 \) “with lots of allolactose, gene expression level is max’ed.”
Modeling time-delays

The production of mRNA from DNA via transcription is not instantaneous; suppose it takes time $\tau > 0$.

Thus, the production rate of mRNA is not a function of allolactose at time $t$, but rather at time $t - \tau$.

Suppose protein $P$ decays exponentially, and its concentration is $p(t)$.

$$\frac{dp}{dt} = -\mu p \quad \implies \quad \int_{t-\tau}^{t} \frac{dp}{p} = -\mu \int_{t-\tau}^{t} dt.$$  

Integrating yields

$$\ln p(t) \bigg|_{t-\tau}^{t} = -\mu t \bigg|_{t-\tau}^{t} dt = \ln \frac{p(t)}{p(t-\tau)} = -\mu [t - (t - \tau)] = -\mu \tau.$$  

Exponentiating both sides yields $\frac{p(t)}{p(t-\tau)} = e^{-\mu \tau}$, and so

$$p(t) = e^{-\mu \tau} p(t - \tau).$$
A 3-variable ODE model of the lac operon

Consider the following 3 quantities, which represent concentrations of:

- \( M(t) = \text{mRNA} \),
- \( B(t) = \beta\)-galactosidase,
- \( A(t) = \text{allolactose} \).

**Assumption:** Internal lactose (\( L \)) is available and is a parameter.

**The model (Yildirim and Mackey, 2004)**

\[
\frac{dM}{dt} = \alpha_M \frac{1 + K_1(e^{-\mu \tau_M} A_{\tau_M})^n}{K + K_1(e^{-\mu \tau_M} A_{\tau_M})^n} - \gamma_M M
\]
\[
\frac{dB}{dt} = \alpha_B e^{-\mu \tau_B} M_{\tau_B} - \gamma_B B
\]
\[
\frac{dA}{dt} = \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \gamma_A A
\]

These are *delay differential equations*, with discrete time delays due to the transcription and translation processes.

There should (?) be a self-loop \( \xrightarrow{\text{X}} \) at \( M \), \( B \), and \( A \), but we’re omitting them for clarity.
A 3-variable ODE model of the \textit{lac} operon

### ODE for $\beta$-galactosidase ($B$)

\[
\frac{dB}{dt} = \alpha_B e^{-\mu \tau_B} M_{\tau_B} - \tilde{\gamma}_B B,
\]

**Justification:**

- $\tilde{\gamma}_B B = \gamma_B B + \mu B$ represents loss due to $\beta$-galactosidase degradation and dilution from bacterial growth.

- Production rate of $\beta$-galactosidase, is proportional to mRNA concentration.

- $\tau_B = \text{time required for translation of } \beta\text{-galactosidase from mRNA, and}$
  
  $M_{\tau_B} := M(t - \tau_B)$.

- $e^{-\mu \tau_B} M_{\tau_B}$ accounts for the time-delay due to translation.
A 3-variable ODE model of the \textit{lac} operon

ODE for mRNA ($M$)

\[
\frac{dM}{dt} = \alpha_M \frac{1 + K_1 (e^{-\mu \tau_M} A_{\tau_M})^n}{K + K_1 (e^{-\mu \tau_M} A_{\tau_M})^n} - \tilde{\gamma}_M M
\]

\textit{Justification:}

- $\tilde{\gamma}_M M = \gamma_M M + \mu M$ represents loss due to mRNA degradation and dilution from bacterial growth.

- Production rate of mRNA [=expression level!] is proportional to the fraction of free operator sites,

\[
\frac{[O]}{O_{tot}} = \frac{1 + K_1 A^n}{K + K_1 A^n} = f(A).
\]

- $\tau_M = \text{time required for transcription of mRNA from DNA, and } A_{\tau_M} := A(t - \tau_M)$.

- The term $e^{-\mu \tau_M} A_{\tau_M}$ accounts for the time-delay due to transcription.
A 3-variable ODE model of the \textit{lac} operon

**ODE for allolactose (A)**

$$\frac{dA}{dt} = \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \tilde{\gamma}_A A$$

**Justification:**

- $\tilde{\gamma}_A A = \gamma_A A + \mu A$ represents loss due to allolactose degradation and dilution from bacterial growth.

- The first two terms models the chemical reaction catalyzed by the enzyme $\beta$-galactosidase:

  $$L \xrightarrow{\alpha_A} A \xrightarrow{\beta_A} glucose + galactose.$$
A 3-variable ODE model of the *lac* operon

### Steady-state analysis

To find the steady states, we must solve the nonlinear system of equations:

\[
0 = \alpha_M \frac{1 + K_1(e^{-\mu M} A_{t M})^n}{K + K_1(e^{-\mu M} A_{t M})^n} - \tilde{\gamma}_M M
\]

\[
0 = \alpha_B e^{-\mu B} M_{t B} - \tilde{\gamma}_B B
\]

\[
0 = \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \tilde{\gamma}_A A
\]

This was done by Yildirim et al. (2004). They set \( L = 50 \times 10^{-3} \) mM, which was in the “bistable range.”

They estimated the parameters through an extensive literature search.

Finally, they estimated \( \mu = 3.03 \times 10^{-2} \) min\(^{-1}\) by fitting ODE models to experimental data.

<table>
<thead>
<tr>
<th>Steady states</th>
<th>( M^* ) (mM)</th>
<th>( B^* ) (mM)</th>
<th>( A^* ) (mM)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>( 4.57 \times 10^{-7} )</td>
<td>( 2.29 \times 10^{-7} )</td>
<td>( 4.27 \times 10^{-3} )</td>
<td>low (stable)</td>
</tr>
<tr>
<td>II.</td>
<td>( 1.38 \times 10^{-6} )</td>
<td>( 6.94 \times 10^{-7} )</td>
<td>( 1.16 \times 10^{-2} )</td>
<td>medium (unstable)</td>
</tr>
<tr>
<td>III.</td>
<td>( 3.28 \times 10^{-5} )</td>
<td>( 1.65 \times 10^{-5} )</td>
<td>( 6.47 \times 10^{-2} )</td>
<td>high (stable)</td>
</tr>
</tbody>
</table>
3-variable ODE model

Figure: The fixed points of the allolactose concentration $A^*$ in ODE model as a function of the parameter $L$ (lactose). For a range of $L$ concentrations there are 3 coexisting steady states, which is the phenomenon of bistability.
3-variable ODE model

Figure: Numerical solutions of $M(t)$ (mRNA), $B(t)$ ($\beta$-galactosidase), and $A(t)$ (allolactose), using $L = 50 \times 10^{-3}$.
5-variable ODE model

Consider the following 5 variables, which represent concentrations of:

- \( M(t) = \) mRNA,
- \( B(t) = \) \( \beta \)-galactosidase,
- \( A(t) = \) allolactose.
- \( P(t) = \) lac permease.
- \( L(t) = \) intracellular lactose.

The model (Yildirim and Mackey, 2004)

\[
\begin{align*}
\frac{dM}{dt} &= \alpha_M \frac{1 + K_1 (e^{-\mu T_M} A)^n}{K + K_1 (e^{-\mu T_M} A)^n} + \Gamma_0 - \tilde{\gamma}_M M \\
\frac{dB}{dt} &= \alpha_B e^{-\mu T_B} M - \tilde{\gamma}_B B \\
\frac{dA}{dt} &= \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \tilde{\gamma}_A A \\
\frac{dP}{dt} &= \alpha_P e^{-\mu (T_B + T_P)} M - \tilde{\gamma}_P P \\
\frac{dL}{dt} &= \alpha_L P \frac{L}{K_{L_e} + L} - \beta_{L_e} P \frac{L}{K_{L_e} + L} - \alpha_A B \frac{L}{K_L + L} - \tilde{\gamma}_L L
\end{align*}
\]
Remarks

- The only difference in the ODE for $M$ is the extra term $\Gamma_0$ which describes the basal transcription rate ($L_e = 0$).

- The ODEs for $B$ and $A$ are the same as in the 3-variable model.

- The ODE for $P$ is very similar to the one for $B$:
  - production rate of lac permease $\propto$ mRNA concentration, with a time-delay.
  - the 2nd term accounts for loss due to degradation and dilution.

- The ODE for lactose,

$$\frac{dL}{dt} = \alpha_L P \frac{L_e}{K_{L_e} + L_e} - \beta_{L_e} P \frac{L}{K_{L_1} + L} - \alpha_A B \frac{L}{K_L + L} - \tilde{\gamma}_L L,$$

is justified by:

- The first two terms model the transport lactose by lac permease:

$$L_e \xleftarrow{\alpha_L} L \xrightarrow{\beta_{L_e}} L$$

- The 3rd term describes the reaction catalyzed by $\beta$-galactosidase: $L \xrightarrow{\alpha_A} A$.

- the 4th term accounts for loss due to degradation and dilution.
A 5-variable ODE model

To find the steady states, we set $M' = A' = B' = L' = P' = 0$ and solve the resulting nonlinear system of equations.

This was done by Yildirim et al. (2004). They set $L_e = 50 \times 10^{-3}$ mM, in the “bistable range.”

They also estimated the parameters through an extensive literature search.

Finally, they estimated $\mu = 2.26 \times 10^{-2}$ min$^{-1}$ by fitting the ODE models to experimental data.

<table>
<thead>
<tr>
<th>SS’s</th>
<th>$A^*$ (nM)</th>
<th>$M^*$ (mM)</th>
<th>$B^*$ (mM)</th>
<th>$L^*$ (mM)</th>
<th>$P^*$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>$7.85 \times 10^{-3}$</td>
<td>$2.48 \times 10^{-6}$</td>
<td>$1.68 \times 10^{-6}$</td>
<td>$1.69 \times 10^{-1}$</td>
<td>$3.46 \times 10^{-5}$</td>
</tr>
<tr>
<td>II.</td>
<td>$2.64 \times 10^{-2}$</td>
<td>$7.58 \times 10^{-6}$</td>
<td>$5.13 \times 10^{-6}$</td>
<td>$2.06 \times 10^{-1}$</td>
<td>$1.05 \times 10^{-4}$</td>
</tr>
<tr>
<td>III.</td>
<td>$3.10 \times 10^{-1}$</td>
<td>$5.80 \times 10^{-4}$</td>
<td>$3.92 \times 10^{-4}$</td>
<td>$2.30 \times 10^{-1}$</td>
<td>$8.09 \times 10^{-3}$</td>
</tr>
</tbody>
</table>
5-variable ODE model

Figure: The fixed points of the allolactose concentration $A^*$ in ODE model as a function of the parameter $L_e$ (external lactose). For a range of $L_e$ concentrations there are 3 coexisting steady states, which is the phenomenon of bistability.
Figure: Numerical solutions of mRNA, β-galactosidase, allolactose, *lac* permease, and lactose concentrations, using $L_e = 50 \times 10^{-3}$. 
For bistability to exist, we need to be able to describe three levels of lactose: high, medium, and low.

In a Boolean network framework, one way to do this is to add variable(s):

**Medium levels of lactose**

Introduce a new variable $L_m$ meaning “at least medium levels” of lactose. Clearly, $L = 1$ implies $L_m = 1$.

- High lactose: $L = 1, L_m = 1$.
- Medium lactose: $L = 0, L_m = 1$.
- Low lactose levels: $L = 0, L_m = 0$.

We can ignore any state for which $L = 1, L_m = 0$.

Since $\beta$-galactosidase converts lactose into allolactose, it makes sense to add a variable $A_m$ to differentiate between high, medium, and low levels of allolactose.

It’s not necessary, but we will also introduce $R_m$ so we can speak of medium levels of the repressor protein.
A Boolean network model of the lac operon

Consider the following Boolean network model, which was published in Veliz-Cuba / Stigler (2011).

\[
\begin{align*}
M &= \text{mRNA} \\
P &= \text{lac permease} \\
B &= \beta\text{-galactosidase} \\
C &= \text{cAMP-CAP complex} \\
R &= \text{repressor protein} \\
L &= \text{lactose} \\
A &= \text{allolactose} \\
G &= \text{glucose}
\end{align*}
\]

\[
\begin{align*}
\begin{cases}
  f_M &= \overline{R} \land \overline{R_m} \land C \\
  f_P &= M \\
  f_B &= M \\
  f_C &= \overline{G_e} \\
  f_R &= \overline{A} \land \overline{A_m} \\
  f_{R_m} &= (\overline{R} \land \overline{A_m}) \lor R \\
  f_A &= L \land B \\
  f_{A_m} &= L \lor L_m \\
  f_L &= \overline{G_e} \land P \land L_e \\
  f_{L_m} &= \overline{G_e} \land ((L_{em} \land P) \lor L_e)
\end{cases}
\end{align*}
\]

Comments

- Circles denote variables, and squares denote parameters.
- The subscript \( e \) denotes extracellular concentrations.
- The subscript \( m \) denotes medium concentration.
A Boolean network model of the \textit{lac} operon

Here is that model as a polynomial dynamical system:

\[
\begin{align*}
  x_1 &= \text{lac mRNA (M)} & f_1 &= x_4(x_5 + 1)(x_6 + 1) \\
  x_2 &= \text{lac permease (P)} & f_2 &= x_1 \\
  x_3 &= \beta\text{-galactosidase (B)} & f_3 &= x_1 \\
  x_4 &= \text{cAMP-CAP complex (C)} & f_4 &= G_e + 1 \\
  x_5 &= \text{high repressor protein (R)} & f_5 &= (x_7 + 1)(x_8 + 1) \\
  x_6 &= \text{med. repressor protein (R}_m) & f_6 &= (x_7 + 1)(x_8 + 1) + x_5 + (x_7 + 1)(x_8 + 1)x_5 \\
  x_7 &= \text{high allolactose (A)} & f_7 &= x_3x_9 \\
  x_8 &= \text{med. allolactose (A}_m) & f_8 &= x_9 + x_{10} + x_9x_{10} \\
  x_9 &= \text{high intracellular lactose (L)} & f_9 &= x_2(G_e + 1)L_e \\
  x_{10} &= \text{med. intracellular lactose (L}_m) & f_{10} &= (x_2L_{em} + L_e + x_2L_{em}L_e)(G_e + 1)
\end{align*}
\]

To find the fixed points, we need to solve the following system of nonlinear equations over \( \mathbb{F}_2 \), for six choices of initial conditions, \((L_e, L_{em}, G_e)\):

\[
\{ f_i + x_i = 0, \quad i = 1, 2, \ldots, 10 \}.
\]

This is an easy task in Sage.
The bistable case

Let's compute the fixed points with medium lactose ($L_e = 0$, $L_{em} = 1$) and no glucose ($G_e = 0$), which is the case where we hope to observe bistability.

We see immediately that $x_7 = x_9 = 0$ and $x_4 = 1$.

Recall that $x_{10}^k = x_{10}$ for all $k \in \mathbb{N}$. Thus, the last equation, $x_{10}^3 + x_{10} = 0$ doesn’t give any information about $x_{10}$.

The variables $x_1$, $x_2$, $x_3$, and $x_8$ must equal $x_{10}$.

The variables $x_5$ and $x_6$ must be the opposite of $x_{10}$. We get two fixed points:

$$(M, P, B, C, R, R_m, A, A_m, L, L_m) = (0, 0, 0, 1, 1, 1, 0, 0, 0, 0) \quad \text{and} \quad (1, 1, 1, 1, 0, 0, 1, 0, 1, 0).$$
Fixed point analysis and bistability

Computing the fixed point(s) for the other 5 initial conditions is an easy task in Sage:

<table>
<thead>
<tr>
<th>$(L_e, L_{em}, G_e)$</th>
<th>$M$</th>
<th>$P$</th>
<th>$B$</th>
<th>$C$</th>
<th>$R$</th>
<th>$R_m$</th>
<th>$A$</th>
<th>$A_m$</th>
<th>$L$</th>
<th>$L_m$</th>
<th>operon</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0, 0, 1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>OFF</td>
</tr>
<tr>
<td>(0, 1, 1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>OFF</td>
</tr>
<tr>
<td>(1, 1, 1)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>ON</td>
</tr>
<tr>
<td>(0, 0, 0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>OFF</td>
</tr>
<tr>
<td>(1, 1, 0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>OFF</td>
</tr>
<tr>
<td>(0, 1, 0)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>ON</td>
</tr>
</tbody>
</table>

Suppose glucose or lactose are both absent ($L_e = L_{em} = G_e = 0$), so the operon is OFF:

$$(M, P, B, C, R, R_m, A, A_m, L, L_m) = (0, 0, 0, 1, 1, 0, 0, 0, 0, 0).$$

Now, let’s change $L_{em}$ from 0 to 1, increasing lactose to medium. We are now in the next-to-last fixed point above, so the operon remains OFF.

Conversely, suppose lactose concentration is high ($L_e = L_{em} = 1$), and so the operon is ON:

$$(M, P, B, C, R, R_m, A, A_m, L, L_m) = (1, 1, 1, 1, 0, 0, 0, 1, 0, 1).$$

Now, let’s change $L_e$ from 1 to 0, reducing lactose levels to medium. This takes us to the last fixed point above, so the operon remains ON.