Bistability in self-activating genes regulated by non-coding RNAs

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Abstract. Non-coding RNA molecules are able to regulate gene expression and play an essential role in cells. On the other hand, bistability is an important behaviour of genetic networks. Here, we propose and study an ODE model in order to show how non-coding RNA can produce bistability in a simple way. The model comprises a single gene with positive feedback that is repressed by non-coding RNA molecules. We show how the values of all the reaction rates involved in the model are able to control the transitions between the high and low states. This new model can be interesting to clarify the role of non-coding RNA molecules in genetic networks. As well, these results can be interesting in synthetic biology for developing new genetic memories and biomolecular devices based on non-coding RNAs.

1. Introduction
Non-coding RNA (ncRNA) molecules are strands of RNA that are not codified in proteins and are involved in important cell functions [1]. These ncRNA molecules are able to regulate gene expression by repressing the activity of messenger RNA (mRNA) molecules. The regulation of a genetic network by ncRNAs can produce bistability and oscillations [2, 3, 4, 5]. Bistability is important in cellular processes such as cell differentiation and signalling. A way to produce bistability in genetic networks is a self-activating gene, i.e., a single gene that activates its own transcription creating a positive feedback loop [6]. Cooperative reactions or multimers are necessary in the positive feedback in order to obtain bistability. An alternative way to produce bistability, without cooperative reactions or multimers, is a gene with positive feedback in which the protein is sequestered by a repressor molecule [7]. In this case, the bistable dynamics has been poorly studied if the sequestered molecule is the mRNA instead of the protein. Here, we present and study an ODE model that shows how to obtain bistability in a simple way in a self-activating gene repressed by ncRNA molecules. We also show how all the parameters involved in the model are able to control the transitions between the low and high states.

2. Results and Discussion
The model comprises a gene with positive feedback that is repressed by non-coding RNA molecules (Fig. 1A). The gene is transcribed into mRNA molecules. And the mRNAs are translated into proteins. These proteins are able to bind to the promoter of its own gene increasing the transcription rate. Therefore, a positive feedback loop is created in the system. On the other hand, ncRNA molecules are synthesized at constant rate in the system. These
ncRNA molecules recognize and bind to mRNA molecules forming a complex mRNA-ncRNA. In this complex, the mRNA can not be translated, and therefore, the gene expression is silenced. The positive feedback does not produce bistability by itself because it does not include the formation of multimers or cooperative binding reactions. The repression of the gene expression by ncRNA molecules introduces in the system the necessary non-linearity to produce bistability. The biochemical reactions that described the gene with positive feedback and the repression carried out by the ncRNA molecules are as follows:

\[
\begin{align*}
\text{Positive feedback} & \quad \text{Repression} \\
G + P & \xrightarrow{k_1} G_a \quad (\text{Activation}) \\
G_a & \xrightarrow{k_{-1}} G + P \quad (\text{Deactivation}) \\
G & \xrightarrow{k_2} G + M \quad (\text{Slow transcription}) \\
G_a & \xrightarrow{k_3} G_a + M \quad (\text{Fast transcription}) \\
M & \xrightarrow{k_4} \phi \quad (\text{mRNA degradation}) \\
M & \xrightarrow{k_5} \phi \quad (P \text{ degradation}) \\
\end{align*}
\]

where \( G \) denotes the gene without \( P \) bound to its promoter, \( M \) denotes mRNA transcribed from \( G \), \( P \) denotes the activator protein translated from \( M \), \( G_a \) denotes the gene with \( P \) bound to its promoter, \( S \) denotes the ncRNA molecules and \( C \) denotes \( S \) bound to \( M \). The description of the rates is as follows: \( k_1 \) is the binding rate of \( P \) to the promoter of \( G \), \( k_{-1} \) is the unbinding rate of \( P \) from the promoter of \( G \), \( k_2 \) is the basal transcription rate, \( k_3 \) is the activated transcription rate, \( k_4 \) is the degradation rate of \( M \), \( k_5 \) is the translation rate, \( k_6 \) is the degradation rate of \( P \), \( k_7 \) is the binding rate of \( S \) to \( M \), \( k_{-7} \) is the decay rate of \( C \) into \( S \) and \( P \), \( k_8 \) is the degradation rate of the complex \( C \), \( k_9 \) is the synthesis rate of \( S \) and \( k_{10} \) is the degradation rate of \( S \).

The dynamics of the biochemical reactions (1) follows the law of mass action and can be
Figure 2. Bifurcation diagrams for all the reaction rates of the model. Solid and dashed lines represent stable and unstable points, respectively.

described by the following ordinary differential equations:

\[
\begin{align*}
\frac{dG}{dt} &= -k_1GP + k_{-1}G_a \\
\frac{dG_a}{dt} &= k_1GP - k_{-1}G_a \\
\frac{dM}{dt} &= k_2G + k_3G_a - k_4M - k_7SM + k_{-7}C \\
\frac{dP}{dt} &= -k_1GP + k_{-1}G_a + k_5M - k_6P \\
\frac{dS}{dt} &= -k_7MS + k_{-7}C + k_9 - k_{10}S \\
\frac{dC}{dt} &= k_7MS - (k_{-7} + k_8)C,
\end{align*}
\]

(2)

where all the biochemical species are measured in molecules. The equilibrium of the system is defined by the fixed points of the differential equations (2). These fixed points satisfy

\[
\frac{dG}{dt} = \frac{dG_a}{dt} = \frac{dM}{dt} = \frac{dP}{dt} = \frac{dS}{dt} = \frac{dC}{dt} = 0 \quad [8].
\]

Then, we have a system of equations and the solution for the number of mRNAs can be written as

\[
M = \frac{1}{k_3} \left( \frac{k_2\alpha + k_3M}{\alpha + M} - k_9 \frac{M}{\beta + M} \right),
\]

(3)

where the parameters are as follows: \(\alpha = k_{-1}k_6/k_1k_5\), \(\beta = k_{10}(k_{-7} + k_8)/k_7k_8\). There is only one gene in the system, then we have assumed \(G + G_a = 1\) molecule. A method to visualize the
position of the fixed points consists on plotting the two members of the equation (3) as different functions in the same graph (Fig. 1B). The function for left and right members of the equation are denoted by \( f = M \) and \( g = \frac{1}{k_4} \left( \frac{k_{20}+k_3 M}{a+M} - k_9 \frac{M}{a+M} \right) \), respectively. The points where the function \( f \) intersects the function \( g \) are the fixed points of the differential equations (2). The Fig. 1B shows three intersections, then there are three fixed points in the system. The middle point is an unstable fixed point and its value is \( M_{unst}^0 = 4.1 \) molecules. The other two points are stable fixed points and their values are \( M_{low}^0 = 0.1 \) and \( M_{high}^0 = 129.7 \) molecules. Therefore, the system exhibits bistability, i.e., two stable steady states: high state and low state. Depending on the initial conditions the system evolves toward the high or low state. The value of the parameters used for plotting are shown in the caption of the Fig. 1. We have used standard values for the reaction rates [9, 10].

The positions of the fixed points showed in the Fig. 1B depend on the values of the reaction rates. There is a dependency of the steady state on the direction of the parameter changes. The system responds differently to increase or decrease of the reactions rates due to there is a region of bistability. This phenomenon is known as hysteresis [8]. In the Fig. 2 are shown the bifurcation diagrams for all the reaction rates of the model. These reaction rates are able to control the transitions between the high and low states. When the value of the rates \( k_1, k_2, k_3, k_5, k_{-7} \) and \( k_{10} \) increase the bistable switches from the low to the high state, and when the rates decrease the bistable switches from the high to the low state (Figs. 2A,C,D,F,I,L). On the other hand, when the rates \( k_{-1}, k_4, k_6, k_7, k_8, \) and \( k_9 \) increase the bistable switches from the high to the low state, and and when the rates decrease the bistable switches from the low to the high state (Figs. 2B,E,G,H,J,K). The rate \( k_9 \), that is the synthesis rate of ncRNA molecules, is specially interesting for engineering new biomolecular devices in synthetic biology. This rate can be interpreted as the input of the bistable and can be easily adjust to switch the bistable between the two states.

3. Conclusions

In conclusion, we have shown that a self-activating gene repressed by ncRNA molecules can produce bistability in a simple way. We have studied how the reaction rates involved in the model can control the transitions between the high and low states. This simple model can be useful for clarifying the role of ncRNAs in cells and human diseases and for designing new genetic devices like, for example, memories in synthetic biology.

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