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Regulating Oscillation Dynamics of A Synthetic Genetic Circuit through Positive Feedback

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Abstract: In cellular systems, sustained oscillations of mRNAs and proteins can be generated by negative feedback mechanism. However, the negative feedback is often accompanied by the positive feedback, raising the intriguing question of what the extra function the positive feedback imparts. In this work, we investigate how the positive feedback affect the systems-level oscillation dynamics (e.g., frequency, amplitude and robustness) of a synthetic repressilator circuit. We show numerically that the original repressilator can produce oscillations with a tunable frequency and near-constant amplitude. But adding a self-positive feedback loop to the repressilator is found to simultaneously weaken its frequency's tunability and amplitude. Moreover, this positive feedback reduces robustness of the repressilator to environmental perturbations. Combining with previous results on the negative-plus-positive feedback design principle, our findings indicate that the positive feedback may play different functions in different network structures of the cellular circuits.

Key Words: Tunability, Robustness, Synthetic Genetic Circuit, Repressilator, Amplitude/Frequency Curve

1 Introduction

Biological oscillators widely exist in many contexts, ranging from metabolic to signaling processes [1–3]. Until now, many examples of oscillations in protein-interaction networks and gene-regulatory networks have come to light, such as the period protein in circadian oscillators [4], the cyclic protein in eukaryotic cell-cycle systems [5, 6]. It is well known that the systems-level properties of these oscillators (e.g., periodicity, robustness) transcend those of individual molecules and involve the full topology of the reaction network [7]. However, the complexity of natural biological systems has hindered our full understanding of its structure and function. Fortunately, synthetic genetic oscillators offer us an alternative approach by providing a well-controlled test bed in which the function of natural genetic oscillators can be isolated and well characterized, independent of the rest of the cellular machinery [8–12].

Now based on the engineering-based approach of synthetic biology, several fundamental gene circuits have been constructed. For instance, Gardner *et al.* first presented the construction of a genetic toggle switch (a synthetic, bistable gene regulatory network) in *Escherichia coli* and provided a simple theory that predicts the conditions necessary for bistability [13]. Then, using three transcriptional repressor systems that are not part of any natural biological clocks, Elowitz and Leibler successfully builded an oscillating network in *Escherichia coli*, which was termed as the repressilator [14]. Later, other synthetic oscillatory gene circuits have also been constructed, such as the synthetic genetic circuitry exhibiting toggle switch or oscillatory behavior [15], the synthetic gene-metabolic oscillator [16], and the fast, robust and tunable synthetic gene oscillator [17].

Generally speaking, in biological systems, there are two basic circuits responsible for oscillatory behavior, i.e. those containing only negative feedback loop and those containing both positive and negative feedback loops. In [7], four general requirements (including negative feedback) for biochemical oscillations were discussed and the positive feedback was considered as a mechanism to delay the negative-feedback signal. On the other hand, both experimental and theoretical studies have demonstrated that simple negative feedback loop of interacting genes or proteins has the potential to generate self-sustained oscillations. However, the negative feedback loop is often companied by the positive feedback loop in many natural biological oscillators, raising the intriguing question of what advantages the extra positive feedback loop imparts. In [18], Tsai *et al.* found that it was generally difficult to adjust a negative feedback oscillator frequency without compromising its amplitude, whereas with positive-plus-negative feedback, one can achieve a widely tunable frequency and near-constant amplitude. Positive-plus-negative oscillators also appear to be more robust and easier to evolve, rationalizing why they are found in contexts where an adjustable frequency is unimportant. To understand how these models are built and why they work the way they do, one must develop a precise mathematical description of molecular circuitry and some intuition about the dynamical properties of regulatory networks [19–23].

In this paper, we further investigate the impact of the positive feedback on the oscillation dynamics of the repressilator. By numerically analyzing its systems-level characteristics, we find that the original repressilator can produce oscillations with a tunable frequency and near-constant amplitude. But introducing a self-positive feedback to the repressilator is found to simultaneously weaken its frequency's tunability and amplitude. Moreover, we also show that the added positive feedback reduce the robustness of the repressilator to environmental perturbations.

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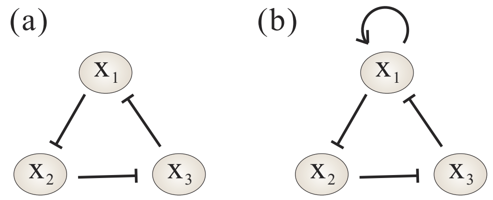

Fig. 1: Schematic diagram of genetic oscillators: (a) negative feedback-only, namely, repressilator; (b) negative-plus positive-feedback version of repressilator.

2 The Repressilator: A Negative Feedback only Genetic Circuit

Recently, by making use of three transcriptional repressor system, Elowitz and Leibler synthesized a genetic oscillator in *Escherichia coli*. That is the namely repressilator [14]. The idea for the oscillatory mechanism of the repressilator is based on connecting an odd number of inverters (negative control elements) in a ring. Its genetic implementation uses three proteins that cyclically repress the synthesis of one another by inhibition of corresponding mRNA production (as shown in Fig. 1 (a)). The following mathematic model and parameters are just as that given in [14]:

$$\begin{aligned}
\frac{dm_1}{dt} &= \alpha_0 + \frac{\alpha}{1+P_3^n} - m_1, \\
\frac{dm_2}{dt} &= \alpha_0 + \frac{\alpha}{1+P_1^n} - m_2, \\
\frac{dm_3}{dt} &= \alpha_0 + \frac{\alpha}{1+P_2^n} - m_3, \\
\frac{dP_1}{dt} &= \beta(m_1 - \phi P_1), \\
\frac{dP_2}{dt} &= \beta(m_2 - P_2), \\
\frac{dP_3}{dt} &= \beta(m_3 - P_3).
\end{aligned} \tag{1}$$

where α is dimensionless transcription rate in the absence of activator. The parameters are set to $n = 2, \alpha = 300, \alpha_0 = 0, \beta = 0.2$. The model consists of three mRNAs, m_1, m_2, and m_3, which give rise to three proteins, P_1, P_2 and P_3. Each protein inhibits the transcription of the next message. The frequency of the oscillator could be changed by varying one of the translation rates by a factor ϕ.

To see how the varied translation rate affect the oscillation regions, oscillation amplitude and frequency of repressilator through the above model, we solve the differential equations numerically and plot the bifurcation curves with the help of Matlab and XPPAUT. By using of XPPAUT, we identify the range of the parameter ϕ over which the system exhibits limit cycle oscillations by locating the Hopf bifurcations at which oscillations are born and extinguished, see Fig. 2. From Fig. 2, we easily find that there are two supercritical Hopf bifurcation. That is to say, oscillation appears only in proper parameter regions. In examining the above refined model, the region of parameter space that could support oscillatory behaviour could be discovered. Varying the parameter ϕ in [0.1957, 26.6346], this negative feedback-only synthetical network exhibits oscillation through supercritical Hopf bifurcation, as shown in Fig.2. To construct amplitude vs. frequency curves, we choose ϕ as a bifurcation parameter and then identify the range of the parameter. Meanwhile, we obtain the amplitude and frequency of the limit cycle solution in the oscillation regions, shown in Fig. 3.

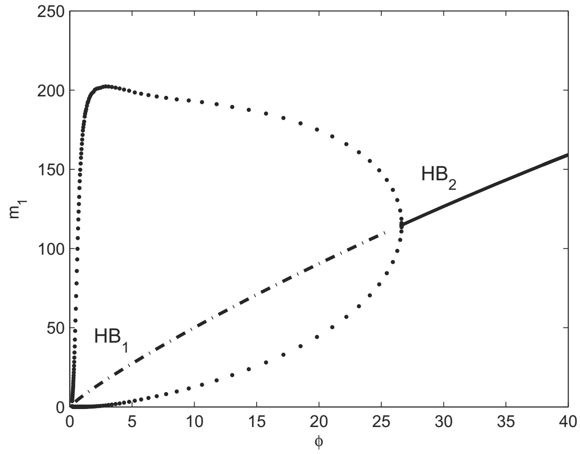

Fig. 2: The supercritical Hopf bifurcation diagram of component m_1 versus parameter ϕ. Solid line stands for stable steady states, dash-dotted line for unstable steady states; solid dotted lines for the maximum and minimum values of m_1 of stable periodic solution. The meaning of the lines are all the same throughout the paper.

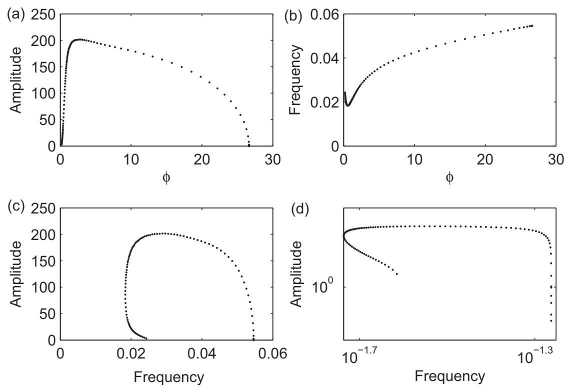

Fig. 3: Amplitude/frequency curves. Negative feedback only provides an oscillator with a tunable frequency and near-constant amplitude. The frequency of the oscillator is changed by varying one of the translation rates by a factor ϕ.

Figs. 3(a) and (b) show the amplitude and frequency curves for different values of parameter ϕ, respectively. It is obviously seen that there is a maximum amplitude and a minimum frequency during the oscillation region. Figs. 3(c) and (d) exhibit amplitue/frequency curves for various values of parameter ϕ. In the negative feedback-only version of the model (shown in Fig. 1(a)), a finite range of ϕ values yielded oscillations. Plotting the amplitude and frequency of the oscillations on a log-log plot yielded a tight, inverted U-shaped curve (Fig. 3(d)). The range of frequencies over which the oscillator functioned is small ([0.0184, 0.0546]). However, within this range, the frequency could be adjusted without compromising the amplitude substantially.

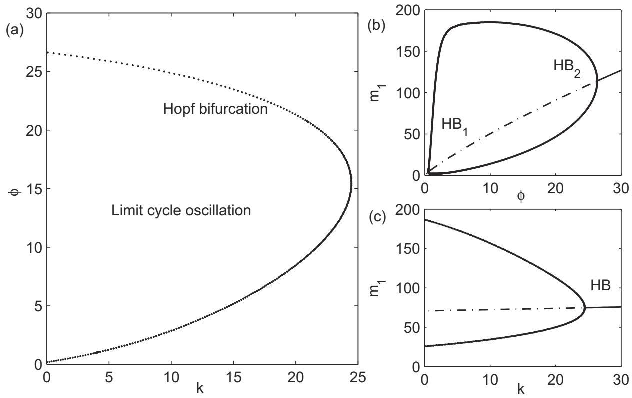

Fig. 4: Oscillation regions and bifurcation of repressilator with positive feedback loop. (a) Oscillation regions in the parameters plane (k, ϕ) through supercritical Hopf bifurcation. (b) The supercritical Hopf bifurcation diagram by varying parameter ϕ varying and taking $k = 2$. (c) The supercritical Hopf bifurcation diagram by varying parameter k and taking $\phi = 15$. Other parameters are the same as Fig. 3.

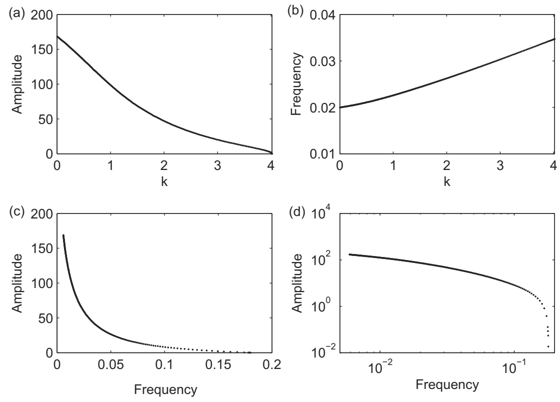

Fig. 5: Amplitude/frequency curves. Negative plus positive feedback provides an oscillator without a tunable frequency and nearly constant amplitude. The strength of positive feedback is specified through a parameter k. Frequency of the oscillator is changed by varying k, however, the amplitudes are changed too. Parameter ϕ is chosen to be 15.

3 The Modified Repressilator: A Negative-plus-Positive Feedback Genetic Circuit

In order to analyze the effect of positive feedback on the oscillation dynamics of original Repressilator, we introduce a self-positive feedback control mechanism through protein P_1, as shown in Fig. 1 (b). The corresponding mathematical model is given as follows:

$$\begin{aligned}
\frac{dm_1}{dt} &= \alpha_0 + \frac{\alpha}{1+P_3^n} - m_1 + \frac{kP_1^n}{(1+P_1^n)},\\
\frac{dm_2}{dt} &= \alpha_0 + \frac{\alpha}{1+P_1^n} - m_2,\\
\frac{dm_3}{dt} &= \alpha_0 + \frac{\alpha}{1+P_2^n} - m_3,\\
\frac{dP_1}{dt} &= \beta(m_1 - \phi P_1),\\
\frac{dP_2}{dt} &= \beta(m_2 - P_2),\\
\frac{dP_3}{dt} &= \beta(m_3 - P_3). \qquad (2)
\end{aligned}$$

where α is dimensionless transcription rate in the absence of activator. The parameter values are the same as above except with k and ϕ. Here we specified the strength of positive feedback through the parameter k.

Uncovering the design principles leading to the creation of robust gene circuits is a major goal in synthetic biology. In the context of biological networks, robustness broadly indicates that a system remains viable under different perturbations. Considering that robustness to different kinds of perturbations (e.g., environmental variation, molecular noise, changes due to mutations) might involve different features of an existing network , it is a very challenging task to define robustness in a precise form. In this paper, we analyze the robustness of oscillation through a parameter bifurcation. A parameter bifurcation diagram often characterizes how qualitative properties of a system, such as the stability of steady states or attractors, changes as some of the model parameters are varied. In order to investigate the composed effect of the varied translation rate and the strength of positive feedback, we demonstrate distributed regions of stable limit cycle oscillation in the (k, ϕ) parameter phase plane (shown in Fig. 4 (a). From Fig. 4 (a), it is obviously found that the more the strength of positive, the narrower the oscillation interval about parameter ϕ. What's more, once the strength of positive feedback is larger than the critical value 24.4728, the oscillation disappears. Figs. 4 (b) and (c) show the bifurcation diagram of component m_1 versus ϕ and k, respectively.

From Fig. 5, it is easily to find that the larger the values of parameter k (shown in Fig. 5 (a)), the smaller the amplitude. However, the larger the values of k, the larger the frequency (shown in Fig.5 (b)). During the oscillation region with k varying, when the amplitude is larger, the frequency is lower (shown in Fig. 5 (c) and (d), the amplitude/frequency curves). In the oscillation region for parameter $\phi = 1$, the amplitude is increasing as the value of parameter k is increasing. And it is just another case for the oscillation frequency.

We also analyze in detail the effect of the strength of positive feedback on oscillation amplitude and frequency. Choosing different values of parameter k, we will observe the changing of oscillation amplitude and frequency as parameter ϕ varying. From Fig. 6, we find that not only the oscillation parameter interval about ϕ is narrower, but also the amplitude is lower as k is chosen different values. In contrast, the oscillation frequency is increasing as k varying with ϕ fixed. And from the Fig. 6(d), we also see that the larger the value of k, the narrower the oscillation frequency. Moreover, we also analyze in detail the effect of parameter ϕ on oscillation amplitude and frequency, see Fig.7. We find that the oscillation amplitude/frequecny curves show similar characters: the amplitude is downing and the frequency is increasing as the values of parameter k is enlarging. From Fig. 5 (a) and Fig. 7 (a), it is not difficult to find that the oscillation intervals about parameter k is enlarging as ϕ is enlarging

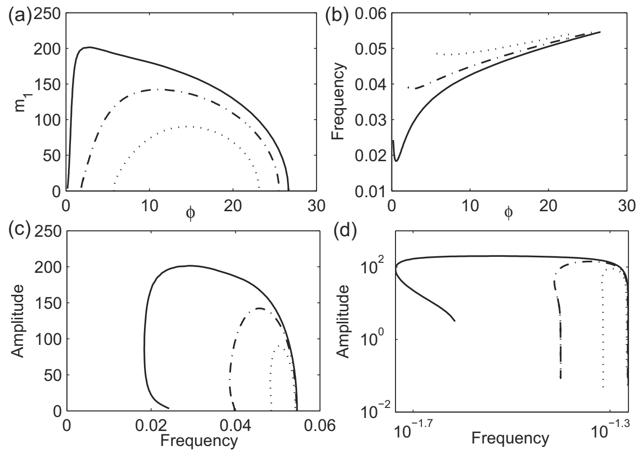

Fig. 6: Amplitude/frequency curves. Negative plus positive feedback provides an oscillator without a tunable frequency and near-constant amplitude. The strength of positive feedback is specified through a parameter k. Here ϕ is the regulatory parameter. Panels (c) and (d) show the frequency of the oscillator changing as parameter ϕ varying. Parameter k is chosen to be 0 (solid line), 7 (dash-dot line) and 16 (dotted line).

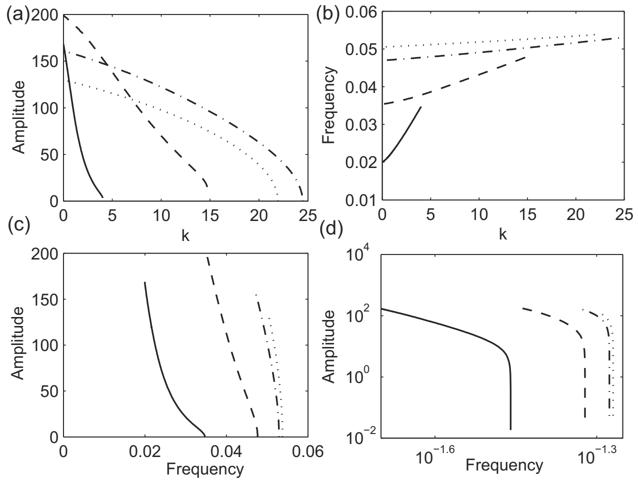

Fig. 7: Amplitude/frequency curves. Negative plus positive feedback provides an oscillator without a tunable frequency and nearly constant amplitude. The strength of positive feedback is specified through a parameter k. Here k is the regulatory parameter. Panels (c) and (d) show the frequency of the oscillator changing as parameter k varying. However, not only the frequency but also the amplitudes are changed. Parameter ϕ is chosen to be 1 (solid line), 5 (dashed line), 15 (dash-dot line) and 20 (dotted line).

at first. When ϕ exceeds the maximum value 15.468, the oscillation intervals about parameter k is decreasing. However, the frequency is increasing as ϕ enlarging. And from Figs. 6 (c) and (d), we also see that the amplitude/fequency curves do not show inverted U-shape.

4 Conclusions and Discussions

In summary, using the synthetic repressilator as the model, we analyzed the effect of positive feedback on the frequency's tunability and robustness of negative feedback loop. We found that negative feedback only version of repressilator exhibited oscillation through two Hopf bifurcation as parameter ϕ varying. The frequency of the oscillator could be changed by varying factor ϕ. Then by adding positive self-feedback to the negative feedback, we found that the stronger the positive feedback, the narrower the oscillation about parameter ϕ. What's more, once the strength of positive feedback is larger than a critical value, the oscillation of modified repressilator will disappear.

For the positive-plus-negative feedback structure, Tsai *et al.* [18] have reported that it can provide us oscillations with a widely tunable frequency and near-constant amplitude, which is especially suitable for biological rhythms like heartbeats and cell cycles that need to provide a constant output over a range of frequencies. However, by investigating the systems-level characteristics including frequency, amplitude and robustness of the oscillations in the synthetic repressilator circuit, we have shown that the original repressilator can produce oscillations with a tunable frequency and near-constant amplitude. But introducing additional self-positive feedback to the repressilator simultaneously weakens its frequency's tunability and amplitude. Moreover, the introduced positive feedback loop also reduce the robustness of the repressilator to model parameter variations. Combining with the previous results on the positive-plus-negative feedback design principle, the present study demonstrates that the positive feedback mechanism may play different functions depending on detailed network structure of cellular circuits.

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