Interlinked mutual inhibitory positive feedbacks induce robust cellular memory effects

Tae-Hwan Kim\textsuperscript{a}, Sung Hoon Jung\textsuperscript{b}, Kwang-Hyun Cho\textsuperscript{c,*}

\textsuperscript{a} Interdisciplinary Graduate Program in Genetic Engineering, Seoul National University, Seoul 151-747, Republic of Korea
\textsuperscript{b} Department of Information and Communications Engineering, Hansung University, Seoul 136-792, Republic of Korea
\textsuperscript{c} Department of Bio and Brain Engineering and KI for the BioCentury, Korea Advanced Institute of Science and Technology, 335 Gwahangno, Yuseong-gu, Daejeon 305-701, Republic of Korea

Received 7 May 2007; revised 7 August 2007; accepted 10 September 2007

Available online 19 September 2007

Edited by Robert B. Russell

Abstract Mutual inhibitory positive feedback (MIPF), or double-negative feedback, is a key regulatory motif of cellular memory with the capability of maintaining switched states for transient stimuli. Such MIPFs are found in various biological systems where they are interlinked in many cases despite a single MIPF can still realize such a memory effect. An intriguing question then arises about the advantage of interlinking MIPFs instead of exploiting an isolated single MIPF to realize the memory effect. We have investigated the advantages of interlinked MIPF systems through mathematical modeling and computer simulations. Our results revealed that interlinking MIPFs expands the parameter range of achieving the memory effect, or the memory region, thereby making the system more robust to parameter perturbations. Moreover, the minimal duration and amplitude of an external stimulus required for off-to-on state transition are increased and, as a result, external noises can more effectively be filtered out. Hence, interlinked MIPF systems can realize more robust cellular memories with respect to both parameter perturbations and external noises. Our study suggests that interlinked MIPF systems might be an evolutionary consequence acquired for a more reliable memory effect by enhancing robustness against noisy cellular environments.

Keywords: Cellular memory; Coupled positive feedback; Double-negative feedback; Mutual inhibition; Robustness; Simulation

1. Introduction

Some biological systems often need to remember their current states for various reasons. For instance, the progress of cell cycle should be ordered and directional, $G_1 \rightarrow S \rightarrow G_2 \rightarrow M \rightarrow G_1$ [1]. In cellular differentiation, cells should never revert to their original states once they are terminally differentiated [2]. We also note that cells respond differently depending on their status history as observed in the yeast galactose-signaling network [3]. To produce such history-dependent responses, cells should remember their current states for future decisions. In these respects, a capability of maintaining transited states caused by transient stimuli is required. Positive feedbacks, the well-known key regulatory motifs for bistable switch-like behavior, can induce such an effect by producing sustained responses to transient stimuli [4].

A single positive feedback loop can be realized by either a mutual activation (A activates B and B activates A) or inhibition (A inhibits B and B inhibits A). Their steady-state characteristics are however different. In a positive feedback formed by a mutual activation, two molecules A and B show the same expression states (both A and B are on or off). Such positive feedbacks can be found in irreversible biological processes. For instance, once Xenopus oocytes are matured by transient progesterone, they cannot be reverted to the pre-matured states [5–8]. On the other hand, in a mutual inhibitory positive feedback (MIPF), two molecules A and B show different expression states (one is on while the other is off). Such MIPFs can be found in biological processes where transitions between two different stable states are necessary. A typical example is the bacteriophage $\lambda$ switch (Fig. 1A) in which two repressors (cI and Cro) form a mutual inhibition and make a lysis-lysogen decision [9–12]. In this paper, such a capability of maintaining two opposing states which can be reversed by transient stimuli is defined as the memory effect (Fig. 2B and D).

Interestingly, the structure of MIPF is also found in an electrical system. RS-latch is a well-known digital memory circuit in electrical systems, and shows a similar memory effect as in the single MIPF system (Fig. 2A and C). Note that the core structure of RS-latch is also composed of a mutual inhibition where the output $Q$ is determined by the inputs $S$ (Set) and $R$ (Reset). Hence, MIPF is a key regulatory motif of the memory effect in both electrical and biological systems. It is intriguing however that various biological systems make use of interlinked MIPFs instead of an isolated single MIPF. In summary, MIPFs are present as asymmetrically-interlinked (Fig. 1E–H) or symmetrically-interlinked (Fig. 1I) structures as well as single structures (Fig. 1A–D).

In this paper, we explored the advantages of interlinked MIPF systems in realizing the memory effect despite it can also be acquired by only a single MIPF. To this end, we modeled the three types of MIPF systems – single, asymmetrically-interlinked and symmetrically-interlinked MIPF systems – and simulated them under a broad range of parameter sets. Within this
framework, we first investigated the parameter range of having the memory effect, which is defined as a memory region, for the three MIPF systems. Second, we examined the minimal amplitude and duration of an external stimulus required for off-to-on state transition. The results suggest that many biological systems might have evolutionarily acquired interlinked MIPF systems.
systems to enhance the robustness of their memory effect with respect to both internal parameter perturbations and external noise disturbances.

2. Materials and methods

2.1. Mathematical models of MIPF systems

We have developed mathematical models for the three types of MIPF systems – single, asymmetrically-interlinked and symmetrically-interlinked MIPF systems – to quantitatively compare their memory effects. Although a single MIPF system can be realized by only two molecules inhibiting each other, post-translational modifications such as dimerization and phosphorylation are further required for a translated protein to function as an activator or inhibitor [13]. Hence, we implemented a sequential process of mRNA(R) → protein(P) → modified protein(P') in our model (Fig. 2B). In the RS-latch, an equivalent electrical system, the time required to produce a modified protein from mRNA can be considered as the time delay taken for each external stimulus (“S” or “R”) to arrive at the input terminal of cognate NOR gate (Fig. 2A).

We have employed three commonly-used assumptions in developing our mathematical models [13–15]. First, each molecule constituting an MIPF system is linearly self-degraded depending on its concentration. Second, the synthesis and modification rates of a protein are linearly proportional to the concentration of the cognate mRNA and protein, respectively. Third, all cooperative regulatory interactions such as activations and inhibitions are described with Hill functions. Under these assumptions, a single MIPF system (Fig. 2B) is modeled as follows (see Table 1 for the description of parameters):

\[
\begin{align*}
\frac{dR}{dt} &= -i_1 - d_R R_1, \\
\frac{dP}{dt} &= s_{R,P} R_1 - d_P P_1, \\
\frac{dP'}{dt} &= s_{R,P'} P_1 + \frac{s_3}{1 + P_2^4} - d_{P'} P_1', \\
\frac{dR_2}{dt} &= i_3 - d_R R_2, \\
\frac{dR_3}{dt} &= s_{R,P} R_2 - d_R R_2, \\
\frac{dP_2}{dt} &= s_{R,P} P_2 + \frac{s_3}{1 + P_3^4} - d_P P_2, \\
\frac{dP_3}{dt} &= s_{R,P'} P_2 + \frac{s_3}{1 + P_3^4} - d_{P'} P_3'.
\end{align*}
\]  

(1)

Interlinked MIPF systems can be classified into two categories: asymmetrically-interlinked (Fig. 1E-H) and symmetrically-interlinked (Fig. 1I). The asymmetrically-interlinked MIPF system (Fig. 3A) is constructed by adding a P'-mediated inhibitory link to the single MIPF system. The mathematical model of the asymmetrically-interlinked MIPF system can be developed from (1) by modifying the ordinary differential equation (ODE) of \( P'_i \) and adding the ODE of \( P_i' \) as follows:

\[
\begin{align*}
\frac{dP_i}{dt} &= s_{R,P} P_i + \frac{s_3}{1 + P_2^4} + \frac{s_3}{1 + P_3^4} - d_{P_i} P_i', \\
\frac{dP_i'}{dt} &= s_{R,P'} P_i + \left( \frac{P_i'}{1 + P_2^4} \right) - d_{P_i} P_i'.
\end{align*}
\]  

(2)

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nominal value*</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( d_R, d_R' )</td>
<td>0.23521</td>
<td>Self-degradation rate of mRNA (R)</td>
</tr>
<tr>
<td>( d_P, d_P' )</td>
<td>0.22367</td>
<td>Self-degradation rate of protein (P)</td>
</tr>
<tr>
<td>( s_{R,P}, s_{R,P'} )</td>
<td>0.37048</td>
<td>Translation rate from ( R ) to ( P )</td>
</tr>
<tr>
<td>( s_{R,P}, s_{R,P'} )</td>
<td>0.47305</td>
<td>Protein modification rate from ( P ) to ( P' )</td>
</tr>
<tr>
<td>( s_{R,P}, s_{R,P'} )</td>
<td>0.28687</td>
<td>Activation rate of interlinking molecules</td>
</tr>
<tr>
<td>( n )</td>
<td>9</td>
<td>Hill coefficient</td>
</tr>
</tbody>
</table>

The asymmetrically-interlinked MIPF system (Fig. 3B) is constructed by adding a \( P'_i \)-mediated inhibitory link to the asymmetrically-interlinked MIPF system. The mathematical model of the symmetrically-interlinked MIPF system can be developed from (1) and (2) by modifying the ODE of \( P'_i \) and adding the ODE of \( P_i' \) as follows:

\[
\begin{align*}
\frac{dP_i}{dt} &= s_{R,P} P_i + \frac{s_3}{1 + P_2^4} + \frac{s_3}{1 + P_3^4} - d_{P_i} P_i', \\
\frac{dP_i'}{dt} &= s_{R,P'} P_i + \left( \frac{P_i'}{1 + P_2^4} \right) - d_{P_i} P_i'.
\end{align*}
\]  

(3)

These MIPF systems exhibit the memory effect depending on parameter values as follows: \( P_i' \) is turned on and \( P_i' \) is turned off if \( i_3 \) is on whereas \( P_i' \) is turned on and \( P_i' \) is turned off if \( i_3 \) is on. The switched states are maintained even after the stimulus is removed (Fig. 2D).

2.2. Parameters and initial conditions

Based on the mathematical models, we have compared the memory effect of the three types of MIPF systems. As we are only interested in MIPFs, we have perturbed those parameters that determine the strength of inhibitory regulations (i.e., \( s_1, s_2 \)), and \( s_3 \) while fixing the others as shown in Table 1. The initial condition of each molecule in the MIPF systems was basically set as \( (R, P, P') = (0.01, 0.1, 0.1) \). To achieve fast convergence to steady states, the initial conditions of type 2 molecules \( (R_2, P_2, P'_2) \) were however set 10 times greater than those of type 1 molecules \( (R, P, P') \). Hence, the initial conditions were set as \( (R_1, P_1, P'_1, R_2, P_2, P'_2, P_3, P'_3) = (0.01, 0.1, 0.1, 0.1, 0.1, 0.1, 0.1, 0.1) \).

3. Results

3.1. Enhanced robustness to parameter perturbations by interlinked MIPFs

To examine the memory effect of the three types of MIPF systems, we first compared the parameter range of achieving the memory effect, called a memory region. The memory region in \( (s_1, s_2) \) parameter space is bounded by the left and lower boundaries which move as \( s_3 \) changes (Fig. 3C and D). The area of this memory region was largest for the symmetrically-interlinked MIPF system (Fig. 3D), followed by asymmetrically-interlinked (Fig. 3C) and single \( (s_3 = 0 \) in Fig. 3C and D) MIPF system. Moreover, for fixed \( s_1 \) and \( s_2 \), the symmetrically-interlinked MIPF system (Fig. 3F) showed a larger \( s_3 \) margin of having the memory effect than the asymmetrically-interlinked MIPF system (Fig. 3E). As the memory region expands, the possibility of acquiring the memory effect under an arbitrary parameter set becomes higher. Hence, an MIPF system is more likely to acquire the memory effect as MIPFs are being interlinked.

As cellular biochemical networks are subject to incessant environmental noises [16], cellular memories should be robust to various perturbations. We investigated the robustness of each cellular memory circuit to both internal parameter pertur-
bations and external noise disturbances. The robustness of a cellular memory circuit with respect to parameter perturbations can be measured by the area of its memory region. For instance, let us assume that both asymmetrically- and symmetrically-interlinked MIPF systems have the memory effect at position X, where \( s_3 \) is 0.2 (Fig. 3C and D). In this case, if \((s_1, s_2)\) is perturbed to position Y, then the asymmetrically-interlinked MIPF system loses its memory effect by escaping from the memory region (Fig. 3G) while the symmetrically-interlinked MIPF system still preserves its memory effect (Fig. 3H).

Fig. 3. The comparison of asymmetrically- and symmetrically-interlinked MIPF systems. (A) Asymmetrically-interlinked MIPF system. (B) Symmetrically-interlinked MIPF system. (C) The memory region of the asymmetrically-interlinked MIPF system on \((s_1, s_2)\) parameter space. (D) The memory region of the symmetrically-interlinked MIPF system on \((s_1, s_2)\) parameter space. The inset figure represents the corresponding area of memory region. The memory region can be classified into three parts: the line equidistant from both the left and lower boundaries (dash-dot line inside each memory region), and the lower and upper memory region divided by this equidistant line. At each \((s_1, s_2)\) on the equidistant line, the features of \( P_0^1 \) and \( P_0^2 \), such as the on/off state level and the minimally required external stimulus for off-to-on state transition, are exactly the same. If \((s_1, s_2)\) is located at the lower memory region, the inhibition strength acting on \( P_0^1 \) exceeds that acting on \( P_0^2 \). Hence, the on state level of \( P_0^2 \) is smaller than that of \( P_0^1 \), implying that the off-to-on state transition of \( P_0^1 \) is easier than that of \( P_0^2 \). On the contrary, if \((s_1, s_2)\) is located at the upper memory region, the off-to-on state transition of \( P_0^2 \) is easier than that of \( P_0^1 \).
interlinked MIPF system at position X while the memory effect will be preserved for the symmetrically-interlinked MIPF system. Hence, we found that the memory effect of an MIPF system becomes more robust to parameter perturbations as the memory region expands.

3.2. Enhanced robustness to external noise disturbances by interlinked MIPFs

Reliable cellular memory circuits should prevent their states from being transited by external noises, but respond only to proper stimuli. External noises usually have relatively smaller amplitudes and shorter signal durations compared to proper signal inputs. Hence, sufficiently large thresholds to filter out such noises are required to achieve robustness to external noise disturbances. To compare the thresholds of the three MIPF systems, we have examined the minimal external stimulus \( i_1 \) (or \( i_2 \)) required for off-to-on state transition of \( P_0^1 \) and \( P_0^2 \) when \( s_3 \) is 0.2 (see Fig. 4). It turns out that both the minimal amplitude and duration of \( i_1 \) (or \( i_2 \)) required for off-to-on state transition of \( P_0^1 \) (or \( P_0^2 \)) increase as the distance from \((s_1,s_2)\) to the lower (or left) boundary increases. This means that the memory effect becomes more robust to external noises as the memory region expands. Hence, the minimum stimulus required for state transition can be estimated by the distance from \((s_1,s_2)\) to the boundary of the memory region.

4. Discussion

A positive feedback realized by mutual activation or inhibition is a key regulatory motif of cellular memory as it enables a perpetuating response to a transient stimulus. In particular, an MIPF can store current states and also induce switching between on and off states for proper stimuli. Such MIPFs are found in various biological systems and we noted that they are interlinked in many cases. In this paper, we have explored the advantages of interlinked MIPF systems with respect to the memory effect through mathematical modeling and simulations.

Interlinked MIPF systems can achieve larger memory regions compared to single MIPF systems. Moreover, the memory region of a symmetrically-interlinked MIPF system is larger than that of an asymmetrically-interlinked MIPF system. An MIPF system is more likely to acquire the memory effect as the memory region expands. In addition, the resulting memory effect is more robust to internal perturbations as it can maintain the property for larger parameter variations. The interlinked MIPF systems are also more robust to external perturbations since they can more effectively filter out external noises and thereby prevent undesirable state transitions by external noises.

Interlinked MIPF systems can topologically be classified into asymmetrically- and symmetrically-interlinked MIPF systems.
systems. As the inhibition strength through interlinking molecules gets increased, the asymmetry of the memory region becomes larger in an asymmetrically-interlinked MIPF system. This causes a preferred off-to-on state transition of one molecule to another. On the contrary, the memory region of a symmetrically-interlinked system gets just symmetrically extended. Such a difference depending on topology might represent the difference in the significance of each stable state of a biological system. For instance, in the motility of neutrophils, both stable states – the frontness and backness pathways – are equally significant. Thus, the symmetrically-interlinked MIPF structure is more advantageous in this case for effective transitions between the frontness and backness pathways (Fig. II). On the other hand, the B cell fate specification system (Fig. 1E) might have adopted the asymmetrically-interlinked MIPF structure so that B cells can prefer one stable state to another.

There were some previous studies investigating the robustness of different motifs that (nominally) produce equivalent behavior as we have studied in this paper. For instance, it was revealed that different topologies of two-gene circadian oscillator vary greatly in their robustness to parameter changes [13]. It was also shown that the topological difference of core regulatory networks leads to relatively slow (i.e., robust) entrainment to a light stimulus in animal circadian clocks compared to plants [17]. Moreover, the robustness of four different network topologies of bacterial chemotaxis was explored against gene expression noise and it was shown that the negative feedback mediated by CheB-phosphorylation might contribute to noise reduction [16]. Biological systems are subject to various incessant perturbations due to noisy cellular environments. Hence, robustness is a well-preserved property of biological systems to overcome such noises [18]. For instance, the fate-decision behavior of λ phage (Fig. 1A: single MIPF) was shown to be robust against point mutations, which correspond to internal perturbation, in the promoter region [18,19]. In _Xenopus_ oocytes, JNK responded to physiological and pathological stimuli in an all-or-none manner [5], implying the existence of a critical threshold to filter out external noises. If an MIPF system has a larger memory region, then it can more easily acquire the memory effect and the acquired memory effect is more robust to various perturbations. In this regard, the interlinked MIPF systems are more advantageous compared to single MIPF systems. Our study suggests that biological systems might have evolved to acquire the interlinked MIPF structures in order to realize more reliable cellular memories through enhanced robustness in noisy cellular environments.

_Acknowledgements:_ This work was supported by the Korea Science and Engineering Foundation (KOSEF) Grant funded by the Korea government (MOST) (M10503010001-07N030100112) and also supported from the Korea Ministry of Science and Technology through the Nuclear Research Grant (M20708000001-07B0800-00110) and the 21C Frontier Microbial Genomics and Application Center Program (Grant MG05-0204-3-40). It was also supported in part from the Korea Ministry of Commerce, Industry & Energy through the Korea Bio-Hub Program (2005-B000002). S. H. Jung acknowledges the financial support received from Hansung University in the year of 2007.

**References**


