Coupled positive and negative feedback circuits form an essential building block of cellular signaling pathways

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Summary
Cellular circuits have positive and negative feedback loops that allow them to respond properly to noisy external stimuli. It is intriguing that such feedback loops exist in many cases in a particular form of coupled positive and negative feedback loops with different time delays. As a result of our mathematical simulations and investigations into various experimental evidences, we found that such coupled feedback circuits can rapidly turn on a reaction to a proper stimulus, robustly maintain its status, and immediately turn off the reaction when the stimulus disappears. In other words, coupled feedback loops enable cellular systems to produce perfect responses to noisy stimuli with respect to signal duration and amplitude. This suggests that coupled positive and negative feedback loops form essential signal transduction motifs in cellular signaling systems. BioEssays 29:85–90, 2007. © 2006 Wiley Periodicals, Inc.

Introduction
Cellular circuits have multiple positive and negative feedback loops to maintain homeostasis during the regulation of cell growth, proliferation and differentiation in response to external stimuli. Various studies have been conducted on the role of positive and negative feedback loops.(1–10) In general, it is known that positive feedback induces a switch-like behavior and bistability,(2,3,5,9) and that negative feedback suppresses noise effects.(6,7,9) It has also been revealed that a signaling system with multiple feedback loops is more robust than one with a single feedback loop.(1,10) However, studies on individual feedback loops do not enable us to understand the complex regulatory mechanism of cellular signaling systems caused by feedback loops. Most of all, it is intriguing that positive and negative feedback loops exist in many cases in coupled structures (Fig. 1). In particular, these coupled structures consist of fast positive and delayed negative feedback loops. During calcium spike regulation, IP3R and RYR constituting positive feedback loops of calcium signaling in cytosol are activated rapidly to increase cytoplasmic calcium (Ca2+cyt) (Fig. 1i). Then, the SERCA ATPases forming a delayed negative feedback loop pump Ca2+cyt out into the endoplasmic reticulum lumen.(11) We note that many positive feedbacks are activated via fast reactions like phosphorylation in budding yeast polarization,(12) eukaryotic chemotaxis(13) and various other signaling pathways, whereas negative feedback operates via slow reactions like protein synthesis in budding yeast polarization,(12) muscle cell fate specification,(14) PDGF signaling,(15) and various other processes. Taken together, these findings raise questions concerning the functional roles of such coupled positive and negative feedback loops with different time delays.

Motivation and hypothesis
To investigate the distinct functional roles of coupled positive and negative feedback loops, we have surveyed various experimental studies on the prominent phenomena that occur...
when either a positive or a negative feedback loop is removed from cellular signaling systems. When a positive feedback loop is removed, signaling systems often exhibit delayed or unstable responses, whereas systems deprived of a negative feedback loop display sustained or excessive responses (see Table 1 for summary).

From these previous experimental results, we hypothesized that the foremost activated positive feedback in a coupled feedback circuit can rapidly induce the "on" state transition of the signaling system, and that then another
delayed positive feedback robustly maintains this on state. Finally, the most delayed negative feedback reinstates the system in the original “off” state and prevents any further excessive response to the applied stimulus.

**Simulation**

To test this hypothesis computationally, we created four models, \([P], [PP], [PN] \) and \([PPN]\) (the upper part of Fig. 2; see Appendix). The loop component \(A\) interacting with \(Output\) without any delay, forms a positive feedback loop and the loop component \(B\) forms another positive feedback loop that is delayed a little. However, the loop component \(C\) forms a negative feedback loop that is delayed further than both positive feedback loops. \([P]\) consists of one positive feedback loop formed by \(A\) and \([PP]\) is composed of two positive feedback loops formed by \(A\) and \(B\). In contrast, \([PN]\) and \([PPN]\) are formed by adding the negative feedback loop to \([P]\) and \([PP]\), respectively. We assume that the *stimulus* influences only \(Output\) in all of the models to reflect the real features of many signaling pathways, as shown in Fig. 1 (see Appendix).

Each model responded to a noise-free stimulus (Fig. 2, lower-left) and a noisy stimulus (Fig. 2, lower-right). The single positive feedback model turned on and off rapidly and thereby it was greatly susceptible to noise (Fig. 2, \([P]\)). The model with two positive feedbacks also turned on rapidly and showed a robust response to a noisy stimulus. However, it did not turn off rapidly since the sufficiently activated positive feedbacks maintain a high basal activation of \(Output\) without any stimulus (Fig. 2, \([PP]\)). The problem is that it stays in the on state even after the stimulus disappears. Moreover, the model with a positive feedback and a negative feedback showed almost the same response as the single positive feedback model until the negative feedback began to operate (Fig. 2, \([PN]\)). The negative feedback loop suppresses the activation of \(Output\) after a time delay, \(\tau_C\) (see Appendix) and triggers the *off* response. The activated negative feedback loop lasted for about \(\tau_C\) time scales and the system did not respond to the stimulus over this period. In other words, the key regulator did not respond to any other stimulus while the negative feedback was activated. A similar phenomenon was observed in an experiment on MAPK activation in the PDGF pathway.\(^{15}\) We reason that such a function is required not to respond too often in noisy environments. However, the model with two positive feedbacks and one negative feedback showed almost the same result as the model with two positive feedbacks until the negative feedback loop was activated and made the system silent for a stimulus as in the model with a positive feedback and a negative feedback (Fig. 2, \([PPN]\)). Consequently, coupled positive and negative feedback circuits induced a noise-robust response while preventing any unintentional sustained response after the stimulus disappeared.

These computational studies help us to understand the role of coupled positive and negative feedback loops that are ubiquitously present in many cellular signaling circuits. This coupled feedback motif can rapidly turn on in response to a stimulus and robustly maintain the on state owing to the involvement of two positive feedback loops. This is in accord with an experiment on the PDGF signaling pathway, in which the system without a positive feedback shows a much shorter response than a normal system.\(^{15}\) In contrast, the negative feedback in this coupled feedback motif suppresses the excessive response caused by multiple positive feedback loops. This explains the observation that SERCA null mutants cause hyper-contracture injury by maintaining high concentration of \(\text{Ca}^{2+}\) during calcium signaling.\(^{11}\)

**Conclusions**

Cellular signaling circuits have developed feedback structures that elicit robust responses to communications with noisy external environments. We discovered that a particular

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**Table 1. Examples of dysfunction in cellular dynamics caused by inhibition of feedback circuits**

<table>
<thead>
<tr>
<th>Roles</th>
<th>Experimental conditions</th>
<th>Experimental results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle cell fate specification (Fig. 1g)</td>
<td>CDO mutation — loss of positive feedback</td>
<td>The expression of MyoD decreases and this results in delayed activation.(^{17})</td>
</tr>
<tr>
<td>Start of cell cycle in budding yeast (Fig. 1d)</td>
<td>cln(cln1, cln2) mutation — loss of positive feedback</td>
<td>The delayed S phase entry is observed in yeast cell cycle.(^{16})</td>
</tr>
<tr>
<td>PDGF signaling (Fig. 1l)</td>
<td>cPLA2 mutation — loss of positive feedback</td>
<td>MAPK activity has a much shorter duration than that of a normal system.(^{15})</td>
</tr>
<tr>
<td>Budding yeast polarization (Fig. 1e)</td>
<td>Bem1 mutation — loss of positive feedback</td>
<td>cdc42 is slowly activated.(^{12})</td>
</tr>
<tr>
<td>Blood clotting (Fig. 1h)</td>
<td>APC mutation — loss of negative feedback</td>
<td>Thrombosis occurs due to the activated thrombin.(^{16})</td>
</tr>
<tr>
<td>Muscle cell fate specification (Fig. 1g)</td>
<td>Myostatin mutation — loss of negative feedback</td>
<td>Muscle is excessively generated.(^{19})</td>
</tr>
<tr>
<td>PDGF signaling (Fig. 1l)</td>
<td>Decreasing the MKP expression — weakening the negative feedback</td>
<td>Sustained MAPK activity is observed.(^{15})</td>
</tr>
<tr>
<td>Budding yeast polarization (Fig. 1e)</td>
<td>Inhibition of actin — loss of single positive feedback and negative feedback</td>
<td>CDC42 activity is unstable.(^{12})</td>
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</table>
coupled feedback structure composed of fast-responding positive feedback loops and a slow-reacting negative feedback loop can guarantee a desired response of uniform duration and amplitude to noisy external cues. These coupled positive and negative feedback loops are ubiquitously found in many cellular signaling pathways. Moreover, various experimental observations indicate that cellular systems devoid of one of the coupled feedback loops show some physiological dysfunctions. Hence, we infer that the coupled positive and negative feedback circuits form an essential building block of signaling pathways.

For many other signaling pathways known to have only a single positive or negative feedback loop, we can conjecture that there might be another undiscovered feedback loop that eventually forms a coupled feedback structure based on the present study. On the other way around, it can also help us to design robust cellular circuits under external stimulus and noise in synthetic biological applications. Interestingly, we note that the underlying mechanism of the coupled fast positive and slow negative feedback loops is similar to the Alan Turing’s reaction–diffusion mechanism describing the formation of various growth and pigmentation patterns in animals.\(^{(20)}\)

**Acknowledgments**

Adam Wilkins and anonymous reviewers contributed significantly to the improvement of a first draft of this paper.

**Appendix**

**Model description**

Brandman et al.\(^{(1)}\) investigated the mechanism of interlinked positive feedback loops where a stimulus influences every
loop component; however, their model could not represent the bistable characteristic, which is a typical property of the positive feedback. Hence, we propose here a more generalized model in which a stimulus affects only Output as observed in many cellular signaling systems (see Fig. 1). In addition, we describe the effect of Output on each component as a Hill-type function to represent the real dynamics of signaling cascades.\(^{(21)}\) The resulting ordinary differential equations of the feedback circuits are as follows:

\[
\frac{dA}{dt} = k_1 \cdot h(\text{OUT}, \tau_A) \cdot (10 - A) - k_2 \cdot A
\]

\[
\frac{dB}{dt} = k_1 \cdot h(\text{OUT}, \tau_B) \cdot (10 - B) - k_2 \cdot B
\]

\[
\frac{dC}{dt} = k_1 \cdot h(\text{OUT}, \tau_C) \cdot (10 - C) - k_2 \cdot C
\]

\[
[P]: \frac{d\text{OUT}}{dt} = \left( k_{\text{off}} \cdot \text{sti} + f_A \cdot A \right) \cdot (10 - \text{OUT}) - k_{\text{min}} \cdot \text{OUT}
\]

\[
[PP]: \frac{d\text{OUT}}{dt} = \left( k_{\text{off}} \cdot \text{sti} + f_A \cdot A + f_B \cdot B \right) \cdot (10 - \text{OUT}) - k_{\text{min}} \cdot \text{OUT}
\]

\[
[PN]: \frac{d\text{OUT}}{dt} = \left( k_{\text{off}} \cdot \text{sti} + f_A \cdot A \right) \cdot (10 - \text{OUT}) - f_C \cdot C - k_{\text{min}} \cdot \text{OUT}
\]

\[
[PPN]: \frac{d\text{OUT}}{dt} = \left( k_{\text{off}} \cdot \text{sti} + f_A \cdot A + f_B \cdot B \right) \cdot (10 - \text{OUT}) - f_C \cdot C - k_{\text{min}} \cdot \text{OUT}
\]

where \(k_{\text{off}} = 0.04, \ k_{\text{min}} = 0.4, \ h(\text{OUT}, t) = \frac{\text{OUT}(t-t_0)^2}{\text{OUT}(t-t_0)^2 + \tau^2}, \ f_A = 0.012, \ f_B = 0.008, \ f_C = 1.5, \ k_1 = 0.2, \ k_2 = 0.25, \ \tau_A = 0, \ \tau_B = 5, \ \tau_C = 100.

The equations were solved numerically using Matlab 7.1.

In our model, we assumed feedback loops with different time delays. On the one hand, a delayed positive feedback helps to induce a noise-robust response. Hence, before its operation, the signaling system is susceptible to noise. This is the reason why the time delay of the positive feedback is set to a relatively small value. On the other hand, the negative feedback loop is controlled to become activated further after the operation of the positive feedback such that the signaling system can properly respond to a stimulus before the negative feedback triggers the off response.

The model proposed by Brandman et al. consists of interlinked fast and slow positive feedback loops resulting in a rapidly turning-on switch which is also resistant to noise.\(^{(1)}\) However, this switch cannot be properly turned-off when the stimulus disappears and thereby it causes a difficulty in regulating a pertinent response to the given stimulus. To resolve this problem, we have considered an additional delayed negative feedback loop coupled with the positive feedback loops and also proved its existence in various cellular networks. This is the most prominent difference between the two models.

### References


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