Interlinked Fast and Slow Positive Feedback Loops Drive Reliable Cell Decisions
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mounting a substantial TSE interference effect. No immune system cells were necessary for this protection, and stable interfering infections were reproducibly achieved without cloning. Interference did not depend on the presence or absence of abnormal PrP. Only persistent infection protected target cells from superinfection. Additionally, only particular agent-strain combinations showed positive interference, and these could not be predicted from cellular PrPres amounts or banding patterns. Moreover, despite continuous replication in cells with PrPres band patterns very different from those found in brain tissue, SY and FU CJD agents each breed true when reinoculated into mice, as does rodent-passaged scrapie reinoculated in sheep (10). The stability of the BSE agent also contrasts with the many different PrPres patterns seen in various affected species. Together, these results are not compatible with the common assumption that TSE strains are encoded by some unresolved type of PrPres folding (16, 17). Indeed, there is still no conclusive evidence that any recombinant or amplified form of abnormal PrP can infect normal animals directly, reproduce meaningful levels of infectivity, or encode all the strain differences observed in mice infected with scrapie, CJD, and BSE agents.

Unlike heterogeneous aggregates of pathologic PrP, infectious TSE particles have a discrete viral size of ~25 nm and 10^7 daltons (as assessed by field flow fractionation and high-pressure liquid chromatography, respectively) (18), and releasing their tightly bound nucleic acids destroys infectivity (19). Thus, some TSE agents such as SY may produce defective interfering particles, as found in many persistent viral as well as noncoding human viroid infections (20, 21). Unlike pathologic host PrP, TSE agents can also provoke innate cellular defenses, including intracellular and diffusible factors that are not restricted to immune system cells (7, 8), and such factors are likely to be involved in interference. Small interfering RNAs with extensive secondary structure may also be evoked by TSE agents, and these can provide even greater strain specificity (22). Notably, several small RNAs with extensive secondary structure have been identified in TSE-infected but not in normal brain tissue (23), and such motifs deserve further study in TSE culture models.

Cocultures were more efficient than mouse bioassays and can be useful for rapid assessment of agent purification and recovery (24). Additionally, they may provide a sensitive test for cells that are infected but show no PrPres (such as white blood cells), and they may be useful for evaluating a wide range of evolving TSE agents that have become important epidemiologically, such as those that cause BSE and chronic wasting disease (CWD). The resistance of cells infected with a prototypic sporadic CJD agent (SY) to two scrapie strains supports the suggestion that a commensal but rarely pathogenic TSE agent may help protect people against infection by sheep TSE strains in nature (4), and may explain why so few people have developed BSE-linked CJD (25). The clustering of sporadic CJD cases is also consistent with an environmental agent of low virulence (26).

Interlinked Fast and Slow Positive Feedback Loops Drive Reliable Cell Decisions

Onn Brandman,1,2* James E. Ferrell Jr.,1 Rong Li,2,3,4 Tobias Meyer1,2

Positive feedback is a ubiquitous signal transduction motif that allows systems to convert graded inputs into decisive, all-or-none outputs. Here we investigate why the positive feedback switches that regulate polarization of budding yeast, calcium signaling, X. laevis oocyte maturation, and various other processes use multiple interlinked loops rather than single positive feedback loops. Mathematical simulations revealed that linking fast and slow positive feedback loops creates a "dual-time" switch that is both rapidly inducible and resistant to noise in the upstream signaling system.

Studies in many biological systems have identified positive feedback as the key regulatory motif in the creation of switches with all-or-none "digital" output characteristics (1). Although a single positive feedback loop (A activates B and B activates A) or the equivalent double-negative feedback loop (A inhibits B and B inhibits A) can, under the proper circumstances, generate a bistable all-or-none switch (1–5), it is intriguing that many biological systems have not only a single but multiple positive feedback loops (Table 1). Three examples of positive feedback systems are shown in more detail in Fig. 1.

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References and Notes
11. See supporting data on Science Online.
Table 1. Examples of interlinked positive feedback loops in biological regulation.

<table>
<thead>
<tr>
<th>System</th>
<th>Positive feedback loops</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitotic trigger</td>
<td>Cdc2 → Cdc25 → Cdc2 → Cdc2</td>
<td>(12, 13)</td>
</tr>
<tr>
<td>p53 regulation</td>
<td>p53 → PTEN → Akt → Mdm2 → p53</td>
<td>(14)</td>
</tr>
<tr>
<td>Budding yeast traversal of START</td>
<td>Cdc28 → Cln transcription → Cdc28 → Cdc42 → Cdc24 → Cdc42 → Cdc42 → actin → Cdc42</td>
<td>(6, 17)</td>
</tr>
<tr>
<td>Eukaryotic chemotaxis</td>
<td>PIP3 → Rac/Cdc42 → PIP3 → Rac/Cdc42 → actin → PIP3</td>
<td>(18)</td>
</tr>
<tr>
<td>Muscle cell fate specification</td>
<td>MyoD → MyoD → Myogenin → MyoD → Cdc25 → Mos → Cdc2 → Myt1 → Cdc2 → actin → Cdc42 → MyoD</td>
<td>(19–21)</td>
</tr>
<tr>
<td>B cell fate specification</td>
<td>IL-7 → EBF → IL-7 → EBF → Pax-S → Notch-1 → E2A → Notch-1 → E2A → Pax-S → Notch (cell A)</td>
<td>(22, 23)</td>
</tr>
<tr>
<td>Notch/delta signaling</td>
<td>Notch (cell A) → Delta (cell A) → Notch (cell A) → Notch (cell B) → Delta (cell B) → Notch (cell A)</td>
<td>(24)</td>
</tr>
<tr>
<td>EGF receptor signaling</td>
<td>EGFR → PTP → EGFR → SOS → Ras → Sos → ERK2 → arachidonic acid → ERK2 → EGFR → sheddases → EGFR</td>
<td>(25–28)</td>
</tr>
<tr>
<td>S. cerevisiae galactose regulation</td>
<td>Gal2 → galactose → Gal80 → Gal2 → Gal3 → Gal80 → Gal3</td>
<td>(29)</td>
</tr>
<tr>
<td>Blood clotting</td>
<td>thrombin → Xa:Va → thrombin → XIIIa → XIIa → Xa:VIIa → Xa → Xa:VIIa</td>
<td>(30)</td>
</tr>
<tr>
<td>Platelet activation</td>
<td>activation → ADP secretion → activation → S-HT secretion → activation → TxA activation → activation → aggregation → activation</td>
<td>(31)</td>
</tr>
<tr>
<td>Ca^{2+} spikes/oscillations</td>
<td>Ca^{2+}<em>{ER} → PLC → IP</em>{3} → Ca^{2+}<em>{cyt} → Ca^{2+}</em>{ER} → IP_{3} → Ca^{2+}<em>{cyt} → IP</em>{3} → Ca^{2+}<em>{ER} → SOC → Ca^{2+}</em>{cyt}</td>
<td>(7, 8)</td>
</tr>
</tbody>
</table>

ADP, adenosine 5′-diphosphate; CDK, cyclin-dependent kinase; cyt, cytochrome; CDO, a component of a cell surface receptor; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; S-HT, serotonin (5-hydroxytryptamine); IL-7, interleukin-7; IP_{R}, inositol 1,4,5-trisphosphate receptor; PIP_{2}, phosphatidylinositol 3,4,5-trisphosphate; PLC, phospholipase C; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PTP, protein tyrosine phosphatase; S. cerevisiae, Saccharomyces cerevisiae; TXA_{2}, thromboxane A_{2}

Fig. 1. Schematic views of positive feedback loops in three systems. (A) Establishment of polarity in budding yeast. (B) Mammalian calcium signal transduction. (C) Xenopus oocyte maturation.
the dual-time switch provides the ability to transit rapidly from the off state to the on state together with robust stability of the on state \((10)\).

These computational studies help understand the yeast phenotypes described above and provide a rationale for the existence of dual-time positive feedback systems in Ca\(^{2+}\) signaling, oocyte maturation, and other biological systems. In the case of Ca\(^{2+}\) signaling, the dual-time system enables rapid Ca\(^{2+}\) responses from IP3-induced Ca\(^{2+}\) release, while also enabling long-term robust Ca\(^{2+}\) signals once the store-operated Ca\(^{2+}\) influx is triggered. Although weak stimuli or noise have been shown to trigger IP3-mediated Ca\(^{2+}\) spikes, more persistent stimuli are needed to induce Ca\(^{2+}\) influx and prolonged Ca\(^{2+}\) responses \((7)\). These long-term Ca\(^{2+}\) signals are required for T-cell activation and differentiation and many other cellular processes \((7, 8)\). *Xenopus* oocyte maturation includes a period termed interkinesis, during which Cdc2 becomes partially deactivated \((11)\). We conjecture that the slow positive feedback loop helps prevent a transition to the off state during this critical interkinesis period.

Our study suggests that many biological systems have evolved interlinked slow and fast positive feedback loops to create reliable all-or-none switches. These dual-time switches have separately adjustable activation and deactivation times. They combine the important features of a rapid response to stimuli and a marked resistance to noise in the upstream signaling pathway.

References and Notes

9. The ordinary differential equations for the one- and two-loop positive feedback switches are

\[
\frac{dx}{dt} = \frac{dx_{\text{out}}}{dt} = k_{\text{out, min}} \times \left((B) \times (1 - OUT) - k_{\text{out, off}} \times OUT + k_{\text{out, min}}\right)
\]

\[
\frac{da}{dt} = \frac{da_{\text{out}}}{dt} = \left[(\text{stimulus}) \times \frac{(OUT) + k_{\text{out, min}}}{OUT + ec_{50}}\right] \times (1 - A) - A + k_{\text{min}} \times \tau_A
\]

\[
\frac{db}{dt} = \frac{db_{\text{out}}}{dt} = \left[(\text{stimulus}) \times \frac{(OUT) + k_{\text{out, min}}}{OUT + ec_{50}}\right] \times (1 - B) - B + k_{\text{min}} \times \tau_B
\]

\[
k_{\text{out, min}} = 2 - k_{\text{out, off}} - 0.3, k_{\text{out, min}} = 0.001, k_{\text{min}} = 0.01, n = 3, ec_{50} = 0.35. \text{ For a fast loop, } t = 0.5. \text{ For a slow loop, } t = 0.008. \text{ The equations were solved numerically with Matlab 7.0.}

10. An interesting variation on this scheme can be envisioned by assuming that A and B have distinct effects on the output, and that both effects are required to activate the output. For example, A and B could phosphorylate different sites on the output protein, so that the protein is only activated when both sites are phosphorylated. The behavior of this dual-time AND switch is essentially the mirror image of the dual-time system shown in Fig. 2E: It turns on slowly, turns off rapidly, and acquires noise resistance when it has been in the off state for a period of time determined by the slow loop.

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