Perfect and Near-Perfect Adaptation in Cell Signaling

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Adaptation is an important basic feature of cellular regulation. Previous theoretical work has identified three types of circuits—negative feedback loops, incoherent feedforward systems, and state-dependent inactivation systems—that can achieve perfect or near-perfect adaptation. Recent work has added another strategy, termed antithetic integral feedback, to the list of motifs capable of robust perfect adaptation. Here, we discuss the properties, limitations, and biological relevance of each of these circuits.

In cell signaling, adaptation can be defined as a process where a system initially responds to a stimulus, but then returns to basal or near-basal levels of activity after some period of time. Adaptation limits the duration of a response and allows for the basal activity of the system to be homeostatically maintained. It can also permit the detection of changes in a stimulus over a wider than usual range of basal stimulus levels. If the final level of response is the exactly same as the pre-stimulus level, the behavior is termed perfect adaptation. Adaptation is common in sensory signal transduction—think about vision, hearing, olfaction, touch, and pain—and it is a feature of many other signaling systems as well. For example, in mitogen signaling, a sustained dose of a mitogen like EGF generally leads to transitory activation of Ras, the downstream MAP kinases ERK1 and ERK2, and a variety of further-downstream transcriptional targets (Figure 1). The transitory nature of these responses is important for keeping cells from undergoing uncontrolled proliferation or apoptosis.

The question then is what types of signal transduction circuits would work for generating perfect or near-perfect adaptation and which of the possible circuits are found in natural adapting systems. One approach to this question is to look to biology for guidance: take a well-studied system known to exhibit adaptation, model it, and see if the model adapts. Examples of this approach include the Barkai and Leibler model of adaptation in bacterial chemotaxis (Barkai and Leibler, 1997) and Friedlander and Brenner's model of ion channel activation and inactivation (Friedlander and Brenner, 2009).

A second approach is to look to nonlinear dynamics for guidance: think about what it means for a system to adapt perfectly (e.g., the steady-state level of output should not depend upon input), and then try to devise some simple modifications to a standard input-output circuit would be that could achieve this. We suspect that this may be how Tyson, Chen, and Novak generated their simple, parameter-independent, perfectly-adapting "sniffer" circuit (Tyson et al., 2003).

Both of these approaches leave open the possibility that some important adaptation strategies will be missed. Is there a more systematic way of determining which of the almost infinitely many possible signal transduction circuits can generate a good adaptive response?

Chao Tang and colleagues took on this challenge by implementing a computational high-throughput strategy. In collaboration with Wendell Lim and Hana El-Samad, the Tang group generated ordinary differential equation (ODE) models of 16,038 possible two- and three-protein circuits, ran time course simulations for each model with 10,000 random sets of kinetic parameters, and determined how frequently each model exhibited adaptation (Ma et al., 2009). A model was considered to be a potentially useful adaptation circuit if: first, its response to a stimulus was reasonably high in amplitude; second, the response eventually returned to reasonably near its starting level; and third, the system responded and returned for some not-infinitesimal fraction of the random parameter sets. They identified several hundred circuits that fit the bill and, gratifyingly, each of the circuits proved to be a variation on one of two common elementary signaling motifs, the negative feedback loop and the incoherent feedforward system.

Negative Feedback

The simplest version of an adapting negative feedback loop consists of two proteins organized into a simple negative feedback loop (Figures 2A and 2B). Barkai and Leibler's model of bacterial chemotactic responses falls into this general class (Barkai and Leibler, 1997; Yi et al., 2000). Given the right parameter values, a negative feedback circuit can respond to a step in input stimulus with a reasonably high amplitude response followed by a return to near its initial response level—sometimes monotonically, and sometimes with damped oscillations (Figure 2C). Adaptation can arise if the feedback regulation by protein B lags behind the input stimulus. This allows the output of the system (A) to first rise and then fall.

However, the parameter values and initial conditions compatible with near-perfect adaptation are restricted. By looking at which random parameters worked best for adaptation, Ma et al. (2009) found that the protein mediating the feedback (B in Figure 2A) needed to respond in an extremely ultrasensitive fashion to changes in the activity of protein A. This could be accomplished by having the enzymes acting on B operate very close to saturation, resulting in zero-order ultrasensitivity (Figure 2B) (Ferrell and Ha, 2014; Goldbeter and Koshland, 1981). In addition, the circuit's other kinetic parameters must be such that the

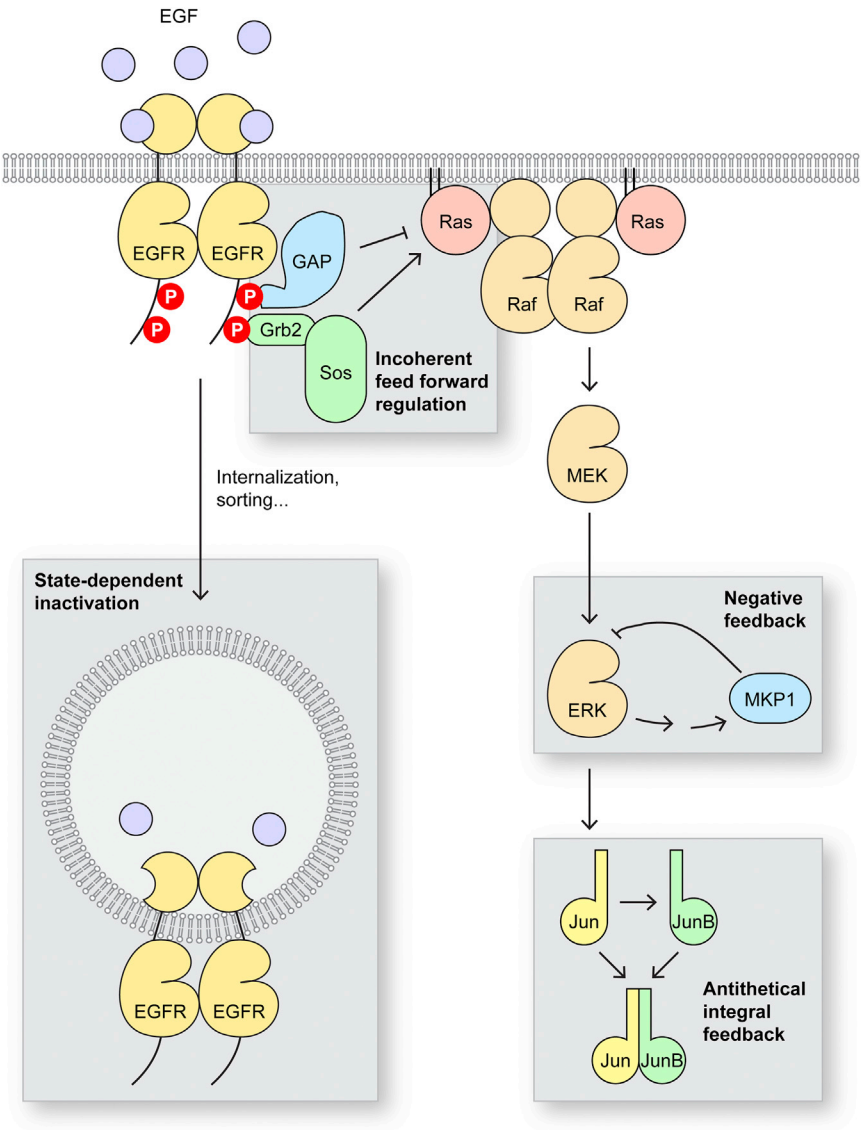

Figure 1. Adaptation in Receptor Tyrosine Kinase Signaling
Schematic view of the signaling downstream of the EGF receptor (EGFR). Four motifs that could contribute to adaptation—incoherent feedforward regulation, negative feedback, state-dependent inactivation, and antithetical integral feedback—are highlighted.

steady state or set point of the system has protein B poised on the upstroke of this ultrasensitive response curve in order to generate near-perfect adaptation (Ma et al., 2009). If these two constraints are satisfied, then adaptation becomes fairly robust with respect to variation in the system's other parameters.

Negative feedback loops are common in signal transduction, and there are several negative feedback loops in the EGFR system shown in Figure 1. For example, active ERK1/2 can bring about the activation of a variety of transcription factors, among whose targets are the MAP kinase phosphatase MKP1 (Sun et al., 1993), which can shift the balance of ERK1/2 phosphorylation to low levels and thereby inactivate the ERK1/2. In some cell types, notably mouse NIH 3T3 fibroblasts, inhibiting protein synthesis converts ERK1/2 responses from pulses to more sustained responses (Sun et al., 1993), consistent with the idea that the ERK-MKP1 negative feedback loop, or at least some protein synthesis-dependent consequence of ERK1/2 activation, is critical for adaptation. In many other cell lines this negative feedback loop appears not to be critical; the pulsatile character of ERK1/2 activation is unaffected by cycloheximide treatment, implying that some other mechanism suffices for adaptation (Alessi et al., 1995). This could mean that other negative feedback loops are more important for adaptation than the ERK-MKP1 loop is, and indeed there are several other negative feedback loops. Active ERK1/2 can phosphorylate and thereby feed back upon its upstream regulators MEK, Raf, Sos, and the EGFR itself, and some or all of these loops may help generate the canonical pulse of ERK activity (Brunet et al., 1994; Fritsche-Guenther et al., 2011; Li et al., 2008; Matsuda et al., 1993; Shin et al., 2009). Or it could be that some other type of motif provides the adaptation.

Incoherent Feed-Forward Regulation

The second type of adaptation circuit identified by Ma and his coworkers (Ma et al., 2009) is an incoherent feed-forward system (Mangan and Alon, 2003) (Figure 3A). In an incoherent feed-forward system, the input influences the downstream output in two opposite ways: positively, through the direct effect of the input on protein A, and negatively, through the activation of protein B, which promotes the inactivation of protein A (Figures 3A and 3B). However there is no feedback in this model; the downstream components are assumed not to influence the upstream ones.

It is relatively easy to make an incoherent feed-forward system adapt. All that is needed is a delay between when the positive signal and the negative signal arrive at protein A, which allows the activity of protein A to first rise and then fall. This delay can be generated by putting more intermediaries in the negative leg than in the positive leg, as is the case in Figure 3, or by making the kinetics of the activation leg be faster than the kinetics of the inactivation leg, or by a combination. So adaptation is not difficult to obtain.

Perfect, or near-perfect, adaptation is harder. Ma et al.'s random parameter sets showed one way of accomplishing near-perfect adaptation: have the activation of B by the input be operating close to saturation and the inactivation of B by a constitutive inactivase be operating far from saturation (Figures 3B and 3C) (Ma et al., 2009). Tyson and co-workers came up with another simple way of achieving this in their "sniffer" model, where they assumed that

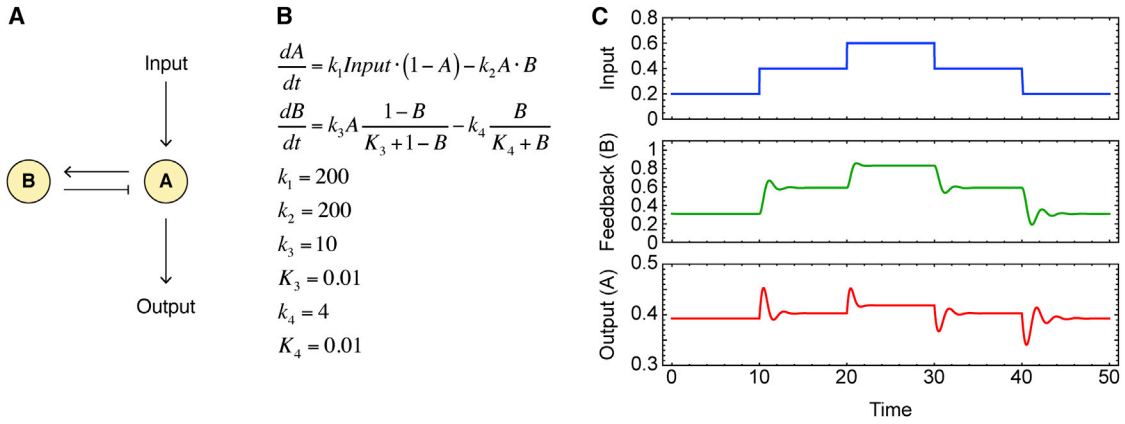

Figure 2. Near-Perfect Adaptation from Negative Feedback
(A) A simple negative feedback system capable of generating near-perfect adaptation.
(B) Rate equations and parameters for the system. Note that Ma and co-workers (2009) used Michaelis-Menten terms for all four rate terms. For simplicity we have assumed mass action kinetics for the first rate equation.
(C) The response of the system to steps up and down in the input level. The negative feedback (middle panel, green curve) lags behind the input stimulus (top panel, blue curve), allowing the output (bottom panel, red curve) to go up and then come back down. Note that the return toward baseline is not always monotonic; the system sometimes exhibits damped oscillations as it is adapting.

A and B were being synthesized and degraded rather than activated and inactivated (Tyson et al., 2003). The resulting model adapts perfectly to any step in stimulus, irrespective of parameter choice.

Incoherent feedforward regulation is important for adaptation in receptor tyrosine kinase (RTK) signaling. The relevant proteins are the famous Ras, Sos, and GAP proteins (Figure 1). Activated EGFR dimers autophosphorylate to produce docking sites for the guanine nucleotide exchange factor Sos via the intermediacy of the Grb2 adaptor protein. The docking of the Grb2-Sos complexes at the phosphorylated EGFR brings Sos into proximity of its target, the plasma membrane-bound Ras protein, allowing Ras to drop its bound GDP, pick up a GTP, and become activated. EGFR–>Grb2-Sos–>Ras represents the positive leg of the incoherent feedforward system. Autophosphorylated receptors also recruit the GTPase activating protein GAP; EGFR–>GAP–|Ras constitutes the negative leg of the feedforward system. The recruitment of GAP appears to be delayed relative to the recruitment of Grb2-Sos (Sasagawa et al., 2005), resulting in a sharp pulse of Ras activation and a return to basal levels of activity in minutes.

The importance of the feedforward Ras-Sos-GAP system is underscored by the fact that Ras mutations that render Ras resistant to GAP, compromising the negative leg of the incoherent feedforward system, result in oncogenic transformation (Trahey and McCormick, 1987). Evidently the various negative feedbacks described above are not sufficient to allow RTK signaling to be terminated normally without an intact Ras-Sos-GAP incoherent feedforward system.

State-Dependent Inactivation

As mentioned, Ma et al. identified hundreds of circuits that could generate adaptive pulsatile responses. However, all of these generally more complicated circuits turned out to be elaborations of either a negative feedback loop or an incoherent feedforward system. This suggested that the two motifs might be the only simple mechanisms for generating near perfect adaptation (Artyukhin et al., 2009; Ma et al., 2009).

This proved not to be the case. Shortly after the publication of the comprehensive Ma et al. study, Friedlander and Brenner proposed an even simpler way of achieving perfect adaptation inspired by the biophysics of the voltage-dependent sodium channel (Catterall, 2014), which they called "state-dependent inactivation" (Friedlander and Brenner, 2009).

Voltage-gated sodium channels become activated in response to depolarization, and then auto-inactivate and only slowly return to their original responsive state. Similar principles govern the activation followed by desensitization of G protein coupled receptors (Kang et al., 2014) and the activation followed by internalization of receptor tyrosine kinases (Goh and Sorkin, 2013).

To model this process, Friedlander and Brenner assumed that signal transduction protein A can exist in three states: an initial off state (A_{off}), which is ready to receive a stimulus; an on state (A_{on}), where it produces an output; and an inactivated state (A_{in}) that is incapable of responding to a stimulus (Figure 4A). The inactivation is assumed to be either intrinsic to the activated protein (as is the case for the sodium channel) or to depend only upon constitutively active downregulators (which in some cases is true in GPCR desensitization and RTK internalization).

Initially, we assume that the conversion back to the A_{off} form is relatively slow compared to the activation and inactivation reactions, and we assume simple mass action kinetics for the activation and inactivation reactions (Figure 4B) (Friedlander and Brenner, 2009). The result is a 2-ODE model that exhibits perfect adaptation (Figure 4C), irrespective of parameter choice. The peak height and the time course of the adaptation depend upon the ratio of k_1 to k_2, but in all cases the response is a pulse of activity with a return to the initial A_{on} = 0 state. Like the Tyson sniffer model, here the process of perfect adaptation is robust with respect to changes in the model's parameters.

The perfect adaptation is even robust with respect to the functions used to describe the kinetics of the two reactions. Here, for simplicity, we assumed mass action kinetics, but the system adapts equally well if Michaelis-Menten kinetics

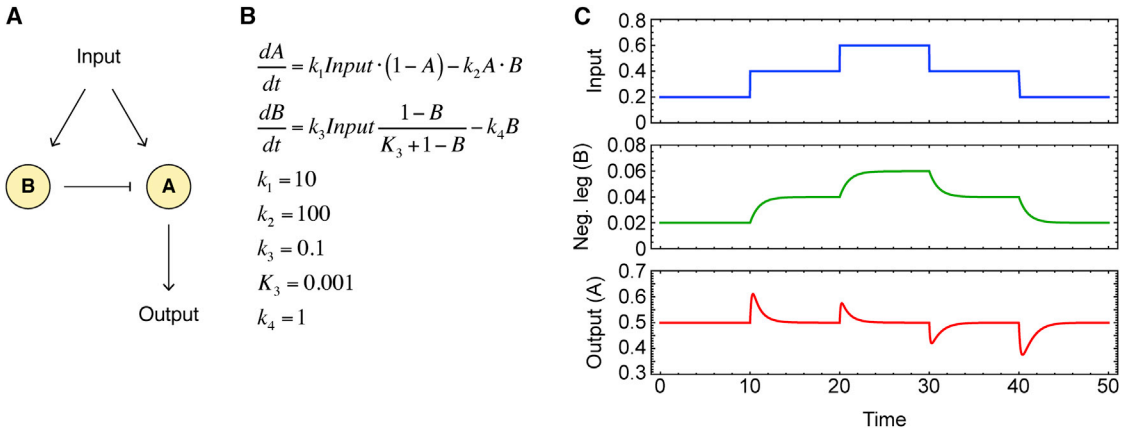

Figure 3. Near-Perfect Adaptation from an Incoherent Feedforward System

(A) Schematic view of the system.

(B) Rate equations and parameters for the system. Again we have simplified the systems present by Ma and co-workers (2009), decreasing the number of rate equations from three to two and using mass action kinetics for all but one of the reactions.

(C) The response of the system to steps up and down in the input level. The negative leg of the response (middle panel, green curve) lags behind the input stimulus (top panel, blue curve), allowing the output (bottom panel, red curve) to go up and then come back down. In this model, the return toward baseline is always monotonic.

or Hill functions are assumed. Indeed, as long as the rate of the first step is zero when $A_{off} = 0$ and greater than zero otherwise, and the rate of the second step is zero when $A_{on} = 0$ and greater than zero otherwise, the system will respond to an input stimulus with a pulse of A_{on} that then falls back toward zero.

Note that at the set point or steady-state of this system, *A* has zero activity, with *A* in either the off-state or in the inactive state. This type of mechanism could therefore work for signaling systems with low basal activities, but is not suitable for most homeostatic systems that maintain an appreciable basal output. If a slow step that allows the A_{in} species to be converted back to the A_{off} form is added to the system, or if resynthesis of A_{off} is permitted, the basal level of signaling can be increased and it becomes possible to obtain multiple response pulses from multiple input pulses. However the quality of the adaptation is compromised.

The Toilet Flush Phenomenon

Since the adaptation to the input stimulus results from depletion of A_{off}, once the system has adapted it will not respond again to successive increases in *Input* (Figure 4C). A second response requires that the stimulus be removed so that the system can recharge through the re-accumulation of A_{off}. Thus the system functions like a toilet, which, once flushed, cannot be flushed again until the handle is released and the tank refills with water. In this respect, the state-dependent inactivation model differs from the incoherent feed-forward and negative feedback models, which can be parameterized to respond to a staircase of inputs with a series of output pulses that successively decrease in amplitude.

However, multiple output pulses can be generated by a variant of Friedlander and Brenner's original model. We assume now that the signal transduction protein A_{off} is activated by binding to an input protein *B*, and that both *A* and *B* are inactivated or consumed in the second step of the cycle (Figures 4D and 4E). This is, indeed, the way EGF receptor responds to ligand binding; receptor activation is followed by internalization and sometimes degradation of both the receptor and the ligand (Figure 1A) (Goh and Sorkin, 2013). The system still responds to an input stimulus (B_{tot}) with a pulse of activation of *A*, and then returns back toward a steady state with $AB_{on} = 0$. However, now the adaptation of the system to a sub-maximal level of *B* does not deplete all of the A_{off} species, and so the system responds to a staircase of input steps with a succession of output pulses, with each pulse decreasing in amplitude as the remaining concentration of A_{off} falls (Figure 4F).

So why were these simple and effective mechanisms for generating perfect adaptation not discovered in Ma et al.'s systematic search for adaptation motifs? Ma et al. only considered signaling proteins that can toggle between two states. Friedlander and Brenner's state-dependent inactivation system depends on the assumption that the (solitary) signaling protein can toggle between three states.

Antithetical Integral Feedback

This brings us to the work by Briat, Gupta, and Khammash (Briat et al., 2016). They begin with a negative feedback system, like that shown in Figure 2, and then ask how they could modify the reactions in the feedback loop to produce integral negative feedback, that is, to have the strength of the feedback be proportional to the integral over time of the difference between the steady state output (the "set point") of the system and the current output of the system. Integral feedback is often utilized in control theory to achieve effective and reliable adaptation, and Ma et al.'s simple negative feedback system actually can function as an integral feedback system, but only for a relatively restricted range of many of its parameters. In particular, if the set point were changed, the system's ability to adapt would generally be severely degraded. Briat et al. set out to see if they could design a system that would produce integral negative feedback and perfect adaptation over a wide range of set points and system parameters.

Their solution was a stoichiometric depletion scheme, similar in concept to that found in the state-dependent inactivation mechanism described in Figures 4D–4F. They implemented this feedback on a model of gene expression: a gene is transcribed in response to a transcription factor (D) to yield an mRNA (A) that is translated to

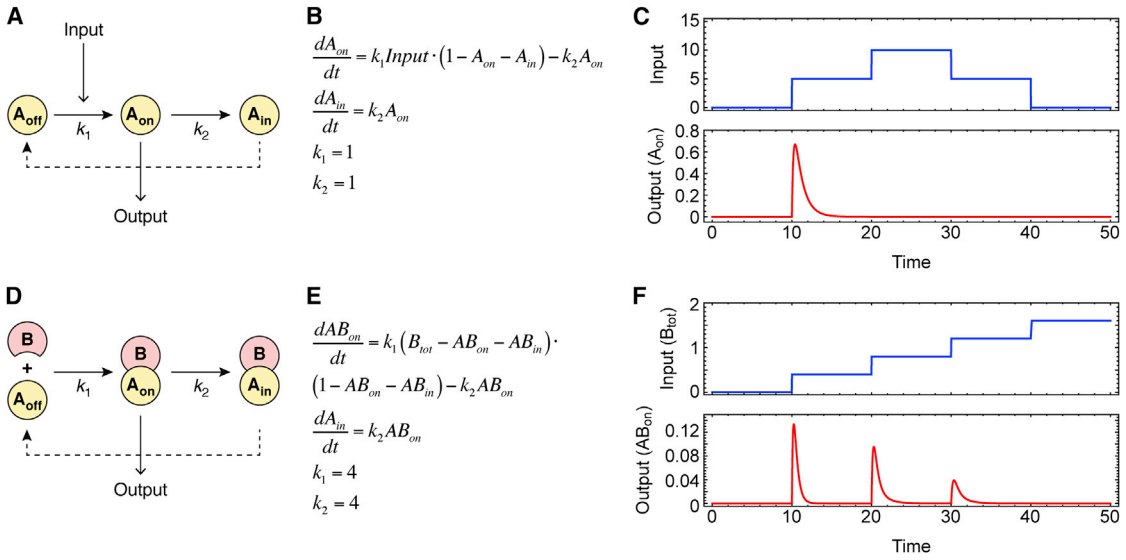

Figure 4. Perfect Adaptation from State-Dependent Inactivation

(A) Schematic view of the system.

(B) Rate equations and parameters for the system. We have assumed that the conversion of inactivated A back to Aoff is slow relative to the activation and inactivation steps. Changing the values of the two parameters affects the amplitude and duration of the response, but in all cases the system exhibits perfect adaptation.

(C) The response of the system to steps up and down in the input level. This system responds to the first step up in the input stimulus, but not to subsequent steps.

(D) Stoichiometric activation of A by B, with subsequent inactivation of the resulting AB complex.

(E) Rate equations and parameters for the system. Again the system adapts perfectly irrespective of parameter choice.

(F) The response of the system to a staircase of input levels. We have not shown steps back down since the irreversible binding of B to A would make it impossible to decrease B^{tot} below the sum of the concentrations of AB_{on} and AB_{in}.

produce a transcription factor, protein B (Figure 5A). However, one of protein B's transcriptional targets is a transcriptional repressor, protein C, that can bind D stoichiometrically and irreversibly inhibit it, possibly through degradation (Figure 5A). They termed this scheme "antithetical integral feedback" because of the opposing roles of the two transcription factors.

Assuming mass action kinetics, the result is a model with four ordinary differential equations and six kinetic parameters (Figure 5B). They then made the rate of one step in the circuit (the translation step) include a dependence on an input stimulus, and put the model through its paces. As shown in Figure 5C, for any step change in the input (the translation rate), the output of the system rises and then falls back toward an unchanging steady state. The same is true if the input is applied to three of the system's other parameters. Thus the system produces perfect adaptation, and the adaptation is robust to changes in four of the system's parameters. The other two parameters (k_4 and k_6, the rate constants for the synthesis of the two antithetical transcription factors) determine the set point of the system.

Note that as the output of the system returns back toward the steady state, there is often an overshoot. As is commonly the case with negative feedback systems,

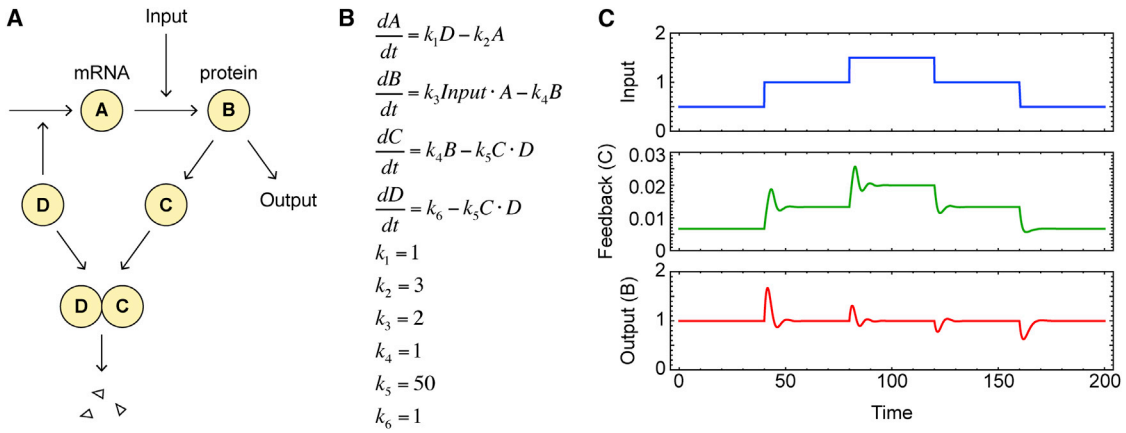

Figure 5. Antithetical Integral Feedback

(A) Schematic view of the system.

(B) Rate equations and parameters for the system. The parameters were taken from (Briat et al., 2016).

(C) The response of the system to steps up and down in the input level.

there is a possibility that the system will oscillate, and in this model the negative feedback loop is long enough that the oscillations can be sustained and never converge to a steady state. Oscillations are a feature in some biological contexts—think heart beats, cell cycles, and circadian rhythms—but they are a bug in a circuit intended to provide perfect adaptation. Briat et al. found that even when their ODE model did exhibit limit cycle oscillations, a stochastic implementation of the same reactions squelched the oscillations. This is a nice example of how noise in a system can sometimes improve the reliability of the system.

The question then is whether antithetical integral feedback motifs are present in natural signaling systems. One nice example, pointed out by Briat et al. (Briat et al., 2016), comes from prokaryotic transcription, where a positive transcription factor (σ^{70}) promotes the transcription and synthesis of an anti-sigma factor (Rsd), a transcriptional repressor that functions by stoichiometrically binding to and inhibiting σ^{70} (Jishage and Ishihama, 1999; Treviño-Quintanilla et al., 2013). An analogous process can be found in EGFR signaling. EGF brings about the induction of a number of immediate early genes, including the Jun oncoprotein, which is a component of the AP-1 transcription factor. AP-1 targets include JunB, a delayed early gene, which can bind stoichiometrically to Jun and block its ability to activate some AP-1 target genes (Figure 1) (Amit et al., 2007). Micro RNAs could well be utilized in the same way, if their targets include mRNAs that regulate their own production. A similar logic can also be found in NF-κB signaling, with NF-κB activation stimulating the transcription of IκBα, which feeds back to stoichiometrically inactivate NF-κB. Interestingly, in NF-κB signaling the oscillations in output described above as a potential hazard of the antithetical integral feedback scheme do occur (Nelson et al., 2004; O'Dea and Hoffmann, 2010; Tay et al., 2010), despite the fact that small numbers and the phenomenon of bursting are likely to make the system quite noisy. These oscillations are thought to be a physiological aspect of the response—a feature, not a bug.

From Adaptation to Homeostasis

So far we have considered it important for our motifs and biological systems to both respond and adapt—a change in input causes an increase in output followed by a return to baseline. Note though, that in some contexts—for example, the homeostatic maintenance of an mRNA, protein, or metabolite at constant concentration—the response phase may not be necessary or even desirable. Both the incoherent feedforward circuit (Figure 3) and the negative feedback circuits (Figures 2 and 5) can be effective in homeostatic adaptation. The only modification is to have the feedback be fast (in Figures 2 and 5) or the negative leg be fast (in Figure 3) so that there is a negligible delay between when the positive and negative influences converge upon the output. We suspect that this sort of homeostatic adaptation may be just as common and just as important as the pulsatile responses discussed here.

Conclusions

The basic question of how biological systems terminate responses is as fundamental and interesting as the question of how they initiate responses. Systems biologists have provided satisfying insights into the best strategies for generating adaptation, and the antithetical integral feedback model of Briat and coworkers (Briat et al., 2016) is a welcome new twist, improving the performance of negative feedback models. Already it is clear that negative feedback (with or without antithetical integral feedback), incoherent feed-forward loops, and state-dependent inactivation are recurring motifs in biological regulation. We suspect that as we begin to better understand the details of other signaling pathways, these old friends will visit us again and again.

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