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Bistable responses in bacterial genetic networks: Designs and dynamical consequences

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ABSTRACT

A key property of living cells is their ability to react to stimuli with specific biochemical responses. These responses can be understood through the dynamics of underlying biochemical and genetic networks. Evolutionary design principles have been well studied in networks that display graded responses, with a continuous relationship between input signal and system output. Alternatively, biochemical networks can exhibit bistable responses so that over a range of signals the network possesses two stable steady states. In this review, we discuss several conceptual examples illustrating network designs that can result in a bistable response of the biochemical network. Next, we examine manifestations of these designs in bacterial master-regulatory genetic circuits. In particular, we discuss mechanisms and dynamic consequences of bistability in three circuits: two-component systems, sigma-factor networks, and a multistep phosphorelay. Analyzing these examples allows us to expand our knowledge of evolutionary design principles networks with bistable responses.

1. Introduction

Living cells react to external stimuli by mediating specific responses that are governed by the dynamics of underlying biochemical and genetic networks. Evolutionary design principles have been well studied in networks that display graded responses, with a continuous relationship between input signal and system output. Alternatively, biochemical networks can exhibit bistable responses such that the network possesses two stable steady states over a range of signals.

The possibility of bistability in simple genetic and metabolic networks has been realized for quite some time. One of the first experimental observations of bistability dates back more than 50 years to Novick and Weiner, who characterized induction of the lactose (*lac*) operon with a gratuitous inducer [1]. They showed the existence of a range of inducer concentrations for which cells can be in either an 'off' state, in which the *lac* operon is not expressed, or an 'on' state, in which the *lac* operon is fully induced. In this intermediate range of inducer concentrations, the composition of the cell population will depend on its history: initially fully induced cells will remain in the 'on' state for many generations, whereas initially uninduced cells will remain mostly 'off" and will have a small probability of switching to the 'on' state. Later, single-cell experiments confirmed the conclusions of Novick and Weiner [1] and related stochastic switching between states to the underlying stochasticity in bacterial gene expression [2–5].

As classical mechanisms of gene regulation were being discovered, researchers realized that certain circuits can display multiple steady states. In 1961, Jacob and Monod [6] proposed several such circuits based on the known regulatory elements contained within a positive feedback, which can either be direct or result from a combination of two negative interactions (a double-negative feedback). Even without experimental evidence, Jacob and Monod realized that these or similar circuits might explain cell differentiation. Since these early studies, many examples of bistable developmental switches have been identified. Among these genetic switches are those controlling the alternative 'lifestyles' of phage λ [7–9], the induction of maturation in *Xenopus laevis* oocytes [10,11], cell cycle progression [12–14], and cell fate determination in the sea urchin [15–17] and hematopoietic stem cells [18,19]. In addition, several synthetic bistable switches have been constructed [20–24].

What are the characteristics of a bistable switch? First, the steady-state signal–response curve (mathematically speaking, a one-parameter bifurcation diagram of the underlying dynamical system) contains a range of signals at which two different steady-state

Abbreviations: AA, SpoIIAA; AB, SpoIIAB; *lac*, lactose; RR, response regulator; SHK, sensor histidine kinase; TCS, two-component system; TF, transcription factor.

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responses are possible. This curve consists of three branches; two of them represent the stable steady states, and the intermediate branch represents the unstable steady state (Fig. 1a). As the intermediate branch is unstable, a signal corresponding to Point 2 (which lies within the range of bistability) will result in either of the two stable branches, depending on the initial conditions. Such curves can be easily computed from a deterministic mathematical model of the underlying network. In the case of the *lac* operon, the two steady states correspond to two levels of *lac* operon expression (response) at the same level of extracellular inducer (signal). At the boundaries of the bistable signal range, the steady-state response of the system discontinuously jumps from one state to the other (arrows in Fig. 1a). Note that this discontinuous jump in the steady state does not indicate a fast dynamic response to a signal that crosses the threshold. In fact, the second characteristic of a bistable switch is a slow response to a signal near the switching threshold (Fig. 1b). In addition, stochastic models of bistable switches can reveal other dynamic properties. In single cells, slow switching in response to an above-threshold signal will lead to a very noisy response with heterogeneous switching times in the population (Fig. 1b). This heterogeneity may manifest as a transient bimodal distribution in the population. A bimodal distribution is also expected in populations responding to a signal in the bistable range (Fig. 1c).

In this review, we discuss some conceptual network designs that produce bistable behavior. Later, we present examples of how these designs are used in bacterial master-regulatory circuits. We discuss mechanisms of bistability in two-component systems, sigma-factor networks, and a multistep phosphorelay. For each example, we point out physiologically relevant dynamical consequences of bistability. Analyzing these examples allows us to expand the knowledge of evolutionary design principles of biochemical networks with bistable responses.

2. Conceptual network designs of bistable mechanisms

2.1. Positive feedback with cooperativity

One of the most widely accepted and studied mechanisms through which bistability can be attained in a genetic circuit is a direct or indirect transcriptional positive feedback characterized by a kinetic order greater than one (cooperativity), so that the dependence of the expression rate on the transcription factor (TF) is superlinear. This mechanism is sufficient to produce bistability for a wide range of parameter values. Fig. 2a illustrates one of the simplest examples of such a mechanism. Protein **A** is expressed from a promoter autogenously regulated by its own homodimer, $\mathbf{A_2}$. A simple model for this system has the following kinetic equations:

$$\frac{dA}{dt} = \beta + \frac{vA_2}{K + A_2} - 2k_aA^2 + 2k_dA_2 - k_{deg}A \tag{1}$$

and

$$\frac{dA_2}{dt} = 2k_aA^2 - 2k_dA_2 - k_{deg}A_2, \tag{2}$$

where A and A_2 are the concentrations of monomer **A** and activator dimer $\mathbf{A_2}$, respectively; β and v are the basal and maximal synthesis rates of monomer **A**, respectively; K is the equilibrium dissociation constant of dimer $\mathbf{A_2}$ from the promoter; k_a and k_d are the rate constants for dimer association and dissociation, respectively; and k_{deg} is the protein degradation rate (for stable proteins in bacteria, this degradation is dominated by dilution due to growth and thus reflects the doubling time).

Assuming the quasi-steady-state approximation for the kinetics of dimer formation in Eq. (2) and using the result obtained in Eq. (1), the rate of change of A (dA/dt) can be plotted as a function of A (Fig. 2b). The quasi-steady-state assumption is justified biologically as protein production and degradation processes are slower than the post-translational reactions. This assumption is used here to graphically illustrate the existence of bistability, but the resulting conclusions can be generalized beyond this approximation. The intersections with the dashed line ($dA/dt = 0$) define the steady states of the network. The two filled circles represent the stable steady states, and the open circle represents the unstable steady state. The existence of bistability depends on the kinetic parameters of the network: for some parameter values, the inflection points of the curve fall on opposite sides of the dashed line, whereas for others, this is not the case and the system possesses only one (physically meaningful) steady state.

2.2. Positive feedback without cooperativity: post-translationally generated ultrasensitivity

In the previous example, dimerization of the activator is necessary to produce the superlinear transcriptional input that is required for bistability. However, for TFs that do not undergo dimerization and therefore function as monomers, positive transcriptional feedback does not lead to bistability in the system (dashed gray curve in Fig. 2d). Not all transcriptional activators function as high-cooperativity multimers; what mechanisms can provide superlinearity in these cases? One way to achieve superlinearity is by activating the TF via a post-translational network that is ultrasensitive, in which a sharp transition occurs between inactive and active forms of the TF. For example, 'zero-order ultrasensitivity' can be observed in multistep or reversible covalent modification cascades as long as one of the enzymes involved operates near saturation (zero kinetic order) [25–27].

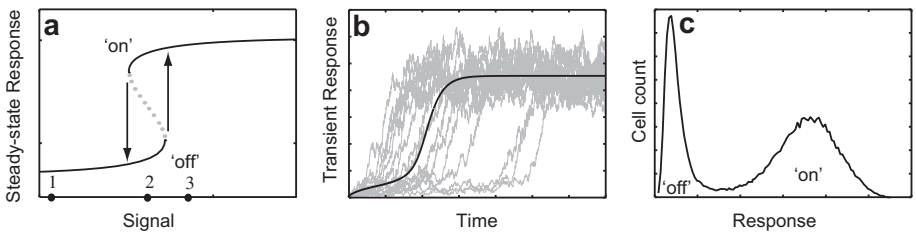

Fig. 1. Characteristics of a bistable switch. (a) The steady-state signal–response curve shows a range of signals for which two different steady-state responses are possible. At the boundaries of the range of bistability, the steady-state response of the system discontinuously jumps from one state to the other (arrows). The two solid curves represent the stable steady states, which are separated by the unstable steady state (dotted curve). (b) An above-threshold signal (starting at Point 1 and increasing to Point 3 in panel a) results in a noisy response with switching-time heterogeneity in the population. The black curve corresponds to the deterministic response, whereas the gray curves correspond to simulations of the stochastic model. (c) Deterministic bistability in the system gives rise to a bimodal population distribution at steady state. Distributions are computed from the long-time limit of the Gillespie simulations at the signal corresponding to Point 2 in panel a. The two peaks correspond to the low ('off') and high ('on') steady-state responses of panel a, respectively.