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TWEAKING BIOLOGICAL SWITCHES THROUGH A BETTER UNDERSTANDING OF BISTABILITY BEHAVIOR

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Abstract

Many biological events are binary. The switch between mutually exclusive OFF to ON state in response to a stimulus is frequently mediated by a control circuit with a positive and/or a negative feedback. Such a system typically exhibits hysteresis with its switching ON and OFF stimulus levels dependent on the current state of the system. The system can be shown to be bistable both experimentally and mathematically. Work to synthesize such switches by combining natural or engineered components has begun to illustrate the potential of such control circuits in many areas of applications.

Keywords

Bistability; hysteresis; gene networks; switch; stimulus; feedback

INTRODUCTION

Many biological processes incur “states” which are binary, cells or the organisms exist only in one of the mutually exclusive states. For example, a microbial cell may be either a vegetative cell or a spore at a given time, but not both. A cell can be either in or not in an apoptotic state; a pluripotent stem cell may be at an undifferentiated state capable of differentiating to multiple lineages and replicate, or at a state committed to differentiating to a particular lineage. At biochemical levels, glycolysis and gluconeogenesis, with their reaction fluxes in opposite directions, are mutually exclusive metabolic states. Numerous such biological switches occupy critical decision-making points in the process of life. Failure in their control action is often catastrophic. In the course of evolution, these switches have become highly robust. However, from a technological perspective, it might be desirable to alter the control characteristics of some switches such as in the case of directed differentiation of stem cells. In other applications such as gene therapy one may wish to exert control on the on-set of gene delivery through a synthetic switch. A better understanding of the mechanism of the switches will lead to their wider applications in biotechnology.

For instance, mathematical models have been used to demonstrate the role of bistability in sonic hedgehog (Shh) signaling pathway, which plays an important role during development

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[1]. The positive feedback loop and negative loop embedded in the system endows it with two distinct states in response to Shh. Stochastic simulations have demonstrated that the feedback loop may dampen the fluctuations of the positive autoregulator (Gli) and render the system robust. In another study, the behavior of Oct4-Sox2-Nanog, the three transcription factors important in determining the fate of embryonic stem cells, was examined theoretically with a number of putative network structures [2]. The stability behavior of various network structures may aid in the experimental investigation of stem cell fate determination.

NETWORK DYNAMICS AND BISTABILITY BEHAVIOR

A key characteristic of a genuine switch is that, in response to a concentration change of the stimulus over a threshold value, the system turns from one state to the other; the system does not reside in a state that is in between the two states. The machinery that makes up a biological switch usually entails a network of genes and their transcriptional, translational and biochemical products. The participation of those constituents in a series of protein-protein, protein-DNA and other molecular interactions as well as biochemical reactions results in a switch behavior (Figure 1). The response of individual reactions or molecular interactions with respect to the concentration of the participating component is continuous and is usually graded (Figure 1(b)). However, the combination of those individual ramping responses gives rise to the sharp, binary (ON-OFF) type of switch behavior.

In principle, an ON-OFF type of response can also be accomplished by a single chemical reaction such as one with a highly cooperative behavior (one whose kinetics are described by a Hills function with a large exponent). However, such a system is extremely sensitive to fluctuations in stimulus concentration. Upon switching to an ON state after crossing a threshold stimulus concentration, the system will quickly return to an OFF state if the stimulus concentration fluctuates below the threshold level. Such a behavior is unacceptable for a robust switch. Studies in the past few years have led to the emergence of some general features for such ON-OFF switches in various biological systems. The network of these switches generally consists of a positive feedback and a negative feedback (Figure 1(a)) that can be shown mathematically to exhibit a bistable behavior. These general features and bistability are described below.

A robust switch behaves as though it possesses a memory of the signal. Once at an ON state, even if the stimulus concentration is reduced to below the threshold level, it continues to be at the ON state. In some special cases, the system stays ON even if the stimulus is completely removed. Xiong et. al. [3] demonstrated such irreversibility in cell fate decision making during the maturation of *Xenopus* oocytes. The maturation of *Xenopus* oocytes is mediated by the phosphorylation and dephosphorylation of p42 mitogen activated protein kinase (MAPK) and cell-division protein kinase Cdc2. These kinases are activated during oocyte maturation and are responsive to steroid stimulus. It was elegantly demonstrated that after being turned on upon exposure to a high stimulus concentration, p42 MAPK and Cdc2 activities are maintained even if stimulus was removed.

In a bistable system, the threshold concentration of the signal required for the system to be switched from OFF to ON state is different from that for transitioning in the reverse direction. If one performs an experiment by adding stimulus to the system in small increments, and assesses the state of the system, one would obtain a response curve (C-D-A in Figure 1 (c)). Reversing the direction of the experiment, one generates another response curve. The downward threshold concentration differs from that of the upward. Such a behavior is referred to as hysteresis. In such a system, the switch of the state is not sensitive to fluctuations in stimulus concentration. Many experimental systems have shown hysteresis ([4-9]).

At a population level, the switch between mutually exclusive states is often graded in time as well as in stimulus concentration. However, at a single cell level the response is still ON-OFF. Stochastic behavior among different individuals in a population is inevitable, resulting in different individual cells responding at somewhat different concentrations and time. When observed at a population level, the response is thus graded. Such contrast between population level and single cell level has been illustrated experimentally in a number of systems, including *Xenopus levis* oocytes [8,9], and oxidative stress induced apoptosis mediated by ERK pathway and p53 pathway [10].

The control circuit or network structure of a number of systems with switch behavior has been formulated in mathematical forms and their behavior analyzed. Typically, the system of interest is confined to a small segment of cellular reactions, and the system is considered as isolated, thus neglecting the interactions with other cellular regulatory circuitry and material flows. Usually, a stability analysis is performed by allowing the system to reach a steady state and then the system is allowed to bifurcate. After the introduction of perturbation, if the system returns to the original steady state, then the point is considered stable. If it drifts out of the original steady state point, the system is not stable. If the system is in a stable region introducing a small change of the stimulus concentration to a new level this will lead the system to a new steady state point corresponding to the new stimulus concentration. The steady state analysis of the switch system often leads to a behavior depicted in Figure 1 (d). The two segments, the lower curve containing points C and D and the upper curve containing points B and A, are stable steady states, whereas the region of the curve between B and D is unstable. In the region enclosed by ABCD thus there are three steady states for each stimulus concentration, two are stable and one is unstable. If the system is originally at an ON state (Point A, Figure 1 (d)), and the concentration is decreased gradually, the system moves gradually along the steady state line. Upon reaching Point B, further decrease in stimulus concentration will shift the system to the new point C in the other stable region which is at an OFF state. Further decrease in stimulus will continue in the stable OFF region. Reversing the trend of stimulus concentration change will cause the system to return to point C.

EXPERIMENTAL OBSERVATION OF BISTABILITY

Bistability has also been observed during cellular differentiation in bacteria. A notable example is antibiotic production in *Streptomyces coelicolor* [11]. Many members of streptomycetes species use a pheromone, γ-butyrolactone to synchronize the population for antibiotic production and sporulation. The regulatory cascade in *coelicolor* includes two genes and their products, an autorepressor protein (ScbR) and a signal amplifier protein (ScbA) which synthesizes the signaling molecule. The repression by ScbR is neutralized by the formation of ScbA/ScbR complex and the binding of signaling molecule Scb1 to ScbR. Thus Scb1 suppresses the negative feedback of ScbR by removing the active form of ScbR (indicated by dash line in Figure 1(b)). ScbA/ScbR complex is postulated to be positive feedback for ScbA. The delicate balance of ScbA and ScbR affects their own expression (Figure 2(a)). As a result, when a few cells in a population start releasing Scb1, the derepression of ScbA causes rapid synthesis of signaling molecules. The accumulation of Scb1 triggers the production of antibiotics. It also triggers others in the population to synthesize Scbl, thus synchronizing the entire population for antibiotic production. In *S.coelicolor* the corresponding genes of ScbA and ScbR, namely *scbA* and *scbR*, were shown to transiently increase in their expression and then subside to a low level[11]. A mathematical model for the butyrolactone system in *Streptomyces* species that describes the dynamics of the system was shown to exhibit a bistable switch behavior, wherein a threshold concentration of signaling molecule switches the system from a stable OFF (no antibiotic) state to a stable ON state (antibiotic production). It was articulated that antibiotics are not only growth inhibitory to other microbes in their surroundings, but also to the producing cells. Producers express antibiotic resistance machinery

before the on-set of antibiotic production. Thus, a switch type of control that also synchronizes the population is critical for the producing cells.

Growing cells oscillate between interphase and mitosis of the cell cycle. The switch from interphase to mitosis is controlled by an oscillatory regulatory network, consisting of positive and negative feedback loops. In the early embryo of *Xenopus*, cdc2-cyclin B functions as an oscillator (Figure 2(b)). Cdc2 activates the Anaphase promoting complex (APC), which then degrades cyclin B. The cyclin B is involved in activating Cdc2 by forming the Cdc2/cyclin B complex. The active Cdc2/cyclin B activates the cdc25 which further activates Cdc2. It is also involved in inactivating the inhibitors wee1 and Myt1. The system thus entails negative feedbacks, positive feedbacks, and a mechanism to remove the negative feedback like in the ScbA-ScbR system of *S. coelicolor*.

Pomerening et. al. [7] demonstrated that these positive feedback loops impart the system bistability. They demonstrated that the activation of Cdc2 with respect to cyclin B exhibits hysteresis. This allows the cell to settle in either the M-phase or interphase but not in any intermediate state. Using extracts from unfertilized *Xenopus eggs* in M-phase that can be forced into interphase by destruction of cyclin B, and then manipulating the concentrations of cyclin B has shown that two distinct levels of cdc2 activity exit in the 45-60 nM range of cyclin concentration. The level of cdc2 activity is dependent on whether the system was going up from interphase to M-phase or vicecersa. This provided experimental evidence that the system exhibits hysteresis, a characteristic of bistability.

Bistable switch is also frequently observed in determining cell fate during development. Before lineage commitment, multipotential hematopoietic progenitors express low levels of multi-lineage genes [4]. The fate of the multi-potent cell is determined mainly by the actions of two primary transcription factors, PU.1 and C/EBPα, (Figure 2(c)). The expression of PU.1 causes an increase in transcription of Egr/Nab which activates the expression of genes that lead to macrophage development. On the other hand, expression of C/EBPα activates the transcription of Gfi which activates the transcription of genes that lead to a neutrophil lineage. Both Egr/Nab and Gfi repress each other. Expression of PU.1 prior to C/EBPα, leads to macrophage cell fate. However, induction of C/EBPα was before PU.1, results in neutrophil cell fate. The hysteresis in the system prevents the differentiated cell to switch to the other state. Through a mathematical model for the gene regulatory network that successfully simulated the experimental observation the system was shown to be bistable and is robust to perturbations. The bistable state can potentially be exploited to obtain a single (monostable) differentiated state by shifting the balance between Egr/Nab-2 and Gfi-1 thereby guiding the cell to commit to a particular lineage.

MATHEMATICAL ANALYSIS OF BISTABILITY

The existence of bistable steady states can give rise to switch behavior. However, it is not clear that all biological switches are explained by bistability. For many systems, detailed experimental studies on their bistable behavior is difficult. Many have taken a mathematical approach to demonstrate the possible existence of bistability. It is often argued that a biologically viable system should not be sensitive to parameter value change. Since biologically cells reside in an environment where virtually all biological parameters fluctuate around a mean value, any biological switch must function in spite of those noises. There is no consensus on how best to evaluate the robustness of the response to multiple parameters. Testing each parameter over a range of value independently does not predict the behavior when a number of parameters change simultaneously. The large combination of different parameters over their biologically feasible range of values can be a daunting task. Nevertheless,

demonstration of the robustness of a system's bistable behavior can give credence to its being a biological switch.

The decision of a cell to undergo apoptosis has been thought to behave like a switch [12]. A mathematical model was used to demonstrate the role of pro-apoptotic Bax and anti-apoptotic Bcl-2 in determining the apoptotic cell fate [13]. A highly cooperative formation of apoptosome was shown to give a sudden increase in the concentration of the apoptotic signal (caspase-3) in response to a slight increase in the concentration of the input stimuli. The ratio of Bax to Bcl-2 is critical in deciding the cell fate. Therefore, controlling the ratio of Bax: Bcl-2 by manipulating their degradation or synthesis rate could direct the cell to either survival or apoptosis. With the help of reduced models that included cooperative interactions and a positive feedback loop, the cooperative binding and the formation of the apoptosome was shown to play a key role in imparting bistability to the model. To assess the robustness of the system, different combinations of values of rate constants were tested using the reduced model. Twenty-six of the 81 combinations tested showed bistable behavior. However, with the feedback loop alone and without cooperative effect no bistable behavior was observed.

SYNTHETIC GENE SWITCHES

The switching characteristic of gene expression in response to stimuli has aroused interest in synthetic biology, an emerging field that uses well-characterized gene modules to *de novo* construct gene networks with desirable system characteristics [14]. Genetic devices such as an epigenetic toggle switch and oscillators in *E.coli* have been reported [15-17]. Synthetic biologists are gradually turning their attention to building eukaryotic [18] and in particular mammalian synthetic gene networks [19-21]. By constructing a gene network containing two antibiotic inducible transcription control systems (streptomycin and macrolide responsive, respectively), that are mutually repressive to each other, a synthetic system was shown to toggle between HIGH and LOW states (Kramer et. al., 2004). It was also shown that once a state was achieved, it could be maintained even in the absence of antibiotics for several generations. A synthetic mammalian switch [20] was constructed with two opposing components. One provides a positive feedback with a self-inducing tetracycline responsive transactivator (VP16). The other component consists of a Erythromycin (EM) responsive transrepressor (E-KRAB), which represses the positive feedback in the presence of EM. These two opposing modules impart the system with its hysteretic behavior. Ajo-Franklin et. al. [22] constructed a high fidelity memory device in yeast based on a transcriptionally controlled positive feedback. There have been many theoretical approaches to synthetic biology [23]. Novel networks have been engineered by recombining existing genetic parts. In fact, by systematically investigating different components of the network one can rationally design novel gene networks.

OUTLOOK OF MANIPULATING STABILITY FOR METABOLIC ENGINEERING

With switch type of regulatory structures playing pivotal roles in development, cell fate determination and alteration of physiological state, it is natural that biotechnologists target those switches for metabolic engineering, especially for *in vivo* applications in higher organisms or even in humans. To date examples of such manipulation of natural control circuits are still few, however, synthetic switches that are capable of switching ON and OFF under different induction conditions have begun to emerge. Possible applications of such synthetic switches are numerous. Examples include, controllable delivery of medicines in gene therapy, cell fate control in cell therapy and event-triggered protein expression in plants for enhanced protection against adverse environmental conditions. As our understanding of the mechanism of switch behavior increases, the means of manipulating the bistability dynamics will also expand. It is conceivable that an antagonist or protagonist can be used to modulate the interactions of key control elements and change the bistability behavior in cell development

without resorting to genetic manipulation. So far the synthetic switches are largely based on genetic elements of bacterial origin. Employing control elements of higher organisms will be essential for many applications. More significantly, one needs to have a better assessment of the robustness of the natural and synthetic switches. How sensitive are natural switches to fluctuations in the levels of the components of the circuit? How sensitive is the switch behavior to alteration in the strength of molecular interactions? In most cases, the sensitivity or robustness of the system cannot be easily assessed experimentally.

The biological switches examined so far all have a relatively simple structure. For bacterium, they often consist of only a pair of genes, their gene expression and biochemical product. Yet the interactions of those few elements can give rise to robust and sophisticated switch behavior. A systems approach certainly offers the best way of understanding how the complexity of switches arises from the simplicity of gene structure and the best way of helping us develop means of tweaking the stability behavior. In marching toward the goal of manipulating biological switches, one sees the synergism between systems and synthetic biology. On one hand while systems biology emphasizes a top-down approach to elucidate regulatory components of particular gene network, synthetic biology on the other hand focuses on a bottom-up approach to construct artificial genetic circuits for producing desirable system characteristics. We hope this synergism will unlock many opportunities and create another fertile ground for innovation.

Acknowledgments

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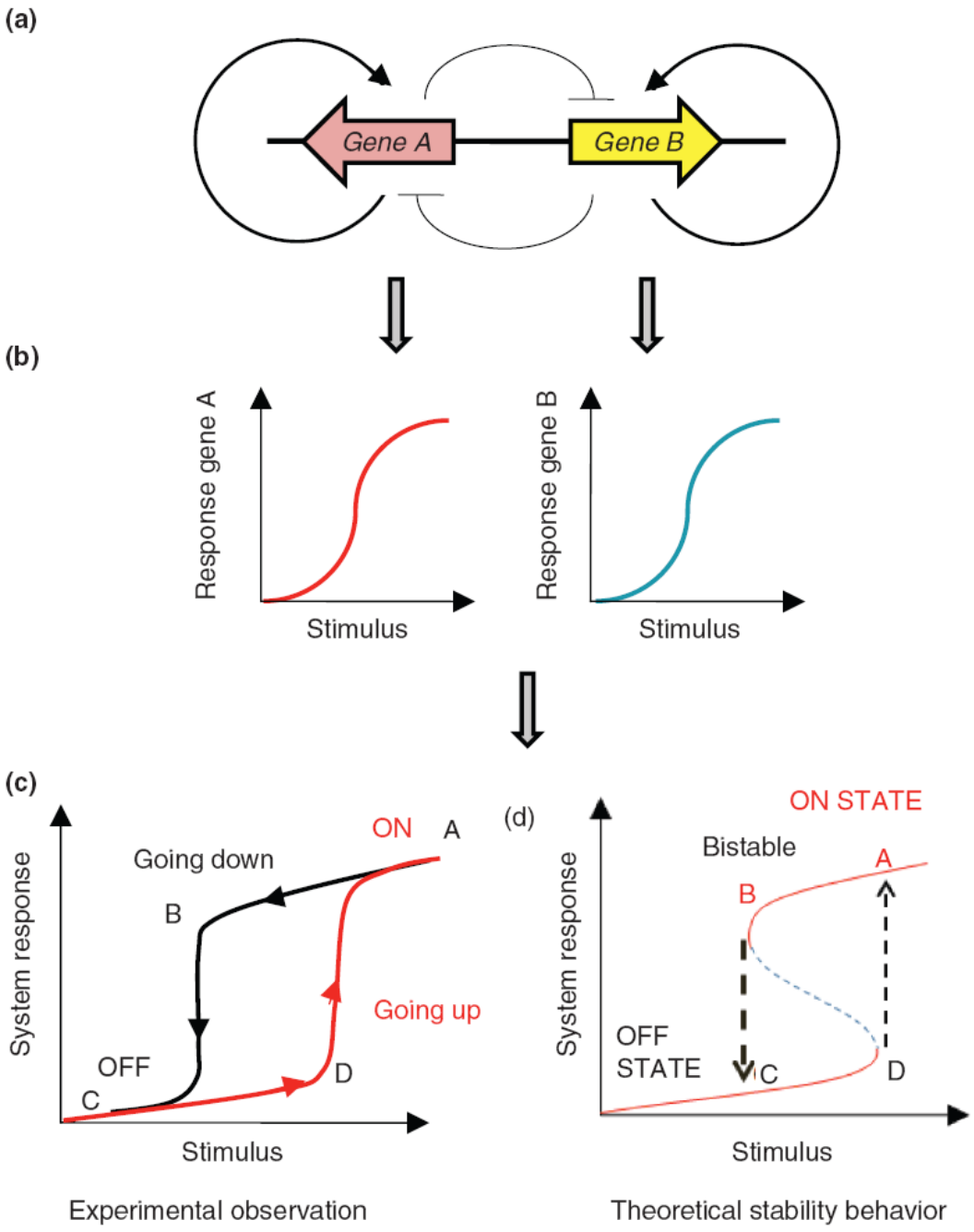

Figure 1.

(a) A two-gene network with positive (arrows) and negative (blunt arrows) feedback loops. (b) Graded response at individual gene level. (c) System demonstrating hysteresis, the threshold concentration for switching the system from OFF to ON state (point D) is higher than that required to switch the system from reverse direction (point B). (d) Bistable response at the system level. Region enclosed within ABCD has three steady states for each concentration of stimulus. Two are stable and experimentally observable. The one unstable one (dashed line) can be theoretically demonstrated.

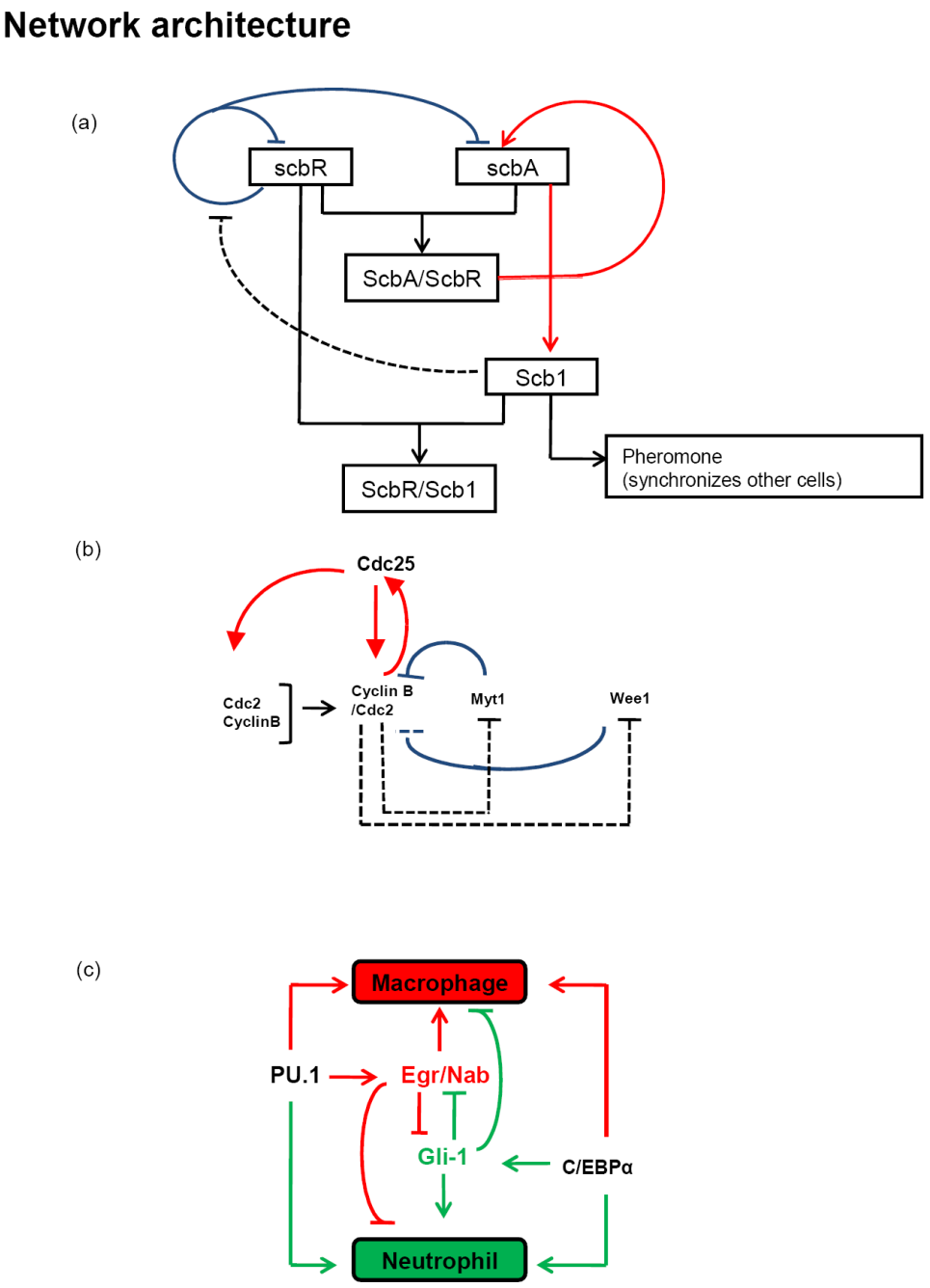

Figure 2.

(a) Schematic diagram of the ScbA/ScbR system regulating antibiotic production in *Streptomyces coelicolor*. Arrows represent positive regulation and blunt arrows represent repression. Red and blue colors denote positive an negative feedback respectively. The brown color indicates negation of repression control and the dash line indicates negative feedback directed toward a negative feedback. ScbR acts both as an autorepressor as well as a repressor of ScbA. The signaling molecule Scb1 (stimulus) derepresses ScbR repression of itself and ScbA by binding to ScbR. Depression of ScbA causes Scb1 synthesis, causing further synthesis of ScbA and Scb1. The response of ScbR to stimulus Scb1 has been shown to have a bistable behavior. (b) Cell cycle hysteresis observed in *Xenopus* oocyte cell maturation. The genetic

network involves a number of positive feedback loops which impart the system hysteresis. The positive feedback loops include activation by dephospohorylation of Cdc2/cyclin by Cdc25. Cdc2 is involved in the activation of Cdc25, which in turn activates Cdc2 and inactivates Myt1. Myt1 is an inhibitor of Cdc2. (Adapted from Pomerening et al, 2003 [7])(c) Differentiation of hematopoietic stem cells from a multi-lineage gene expression state to two different lineages, macrophage and neutrophil. The primary cell fate determinants are PU.1 and CEBP/α which are expressed constitutively. They are involved in induction of both macrophage specific and neutrophil specific genes. Secondary cell fate determinants Egr/Nab and Gfi-1 are responsible for inducing macrophage and neutrophil specific genes respectively. (Adapted from Laslo et al, 2006 [4])