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The role of dynamic stimulation pattern in the analysis of bistable intracellular networks

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Abstract

Bistable systems play an important role in the functioning of living cells. Depending on the strength of the necessary positive feedback one can distinguish between (irreversible) "one-way switch" or (reversible) "toggle-switch" type behavior. Besides the well- established steady-state properties, some important characteristics of bistable systems arise from an analysis of their dynamics. We demonstrate that a supercritical stimulus amplitude is not sufficient to move the system from the lower (off-state) to the higher branch (on-state) for either a step or a pulse input. A switching surface is identified for the system as a function of the initial condition, input pulse amplitude and duration (a supercritical signal). We introduce the concept of bounded autonomy for single level systems with a pulse input. Towards this end, we investigate and characterize the role of the duration of the stimulus. Furthermore we show, that a minimal signal power is also necessary to change the steady state of the bistable system. This limiting signal power is independent of the applied stimulus and is determined only by systems parameters. These results are relevant for the design of experiments, where it is often difficult to create a defined pattern for the stimulus. Furthermore, intracellular processes, like receptor internalization, do manipulate the level of stimulus such that level and duration of the stimulus is conducive to characteristic behavior.

Keywords: Systems biology; Cell signalling; Multistability; Transient behavior; Bounded autonomy

1. Introduction

Living cells must continually sense their external and internal environment and induce changes on the basis of this information. In this way they are able to adapt to their environment, continue or stop their development, and form more complex structures through intercellular communication (Wolkenhauer et al., 2005b). This processing of information in living cells is carried out by signalling networks (Downward, 2001; Wolkenhauer and Mesarović, 2005). The character of information and the corresponding responses include a wide range of physical and chemical quantities, changes in temperature, pressure, water balance, concentration gradients, and pH-level.

Within these networks, information is transmitted by dynamic changes in protein concentrations. Besides continuously varying signals, some cellular processes lead to discontinuous, switch-like responses (Bhalla and Iyengar, 1999; Ferrell and Xiong, 2001; Huang and Ferrell, 1996; Melen et al., 2005). Such a bistable system toggles between two discrete, alternative stable steady states, in contrast to monostable systems (Ferrell, 1998; Ferrell and Machleder, 1998; Markevich et al., 2004). Their separated branches of the steady-state response allow the implementation of switches in biochemical networks. Other examples include cell cycle oscillations and mutually exclusive cell cycle phases (Pomerening et al., 2003; Tyson, 1991; Tyson et al., 2002) as well as the generation of biochemical "memory" (Eißing et al., 2004; Lisman, 1985; Xiong and Ferrel, 2003). Due to their properties, bistable systems also play an important role in development (Melen et al., 2005), cell dif-

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ferentiation, and evolution of biological systems (Laurent and Kellershohn, 1999; Thomas and Kaufman, 2001).

Bistability may arise in signalling pathways that contain at least one feedback loop or a combination of feedback loops whose sum of signs is positive with respect to the considered response component (Cinquin and Demongeot, 2002; Thomas, 2004). The existence of positive feedback is a necessary but not a sufficient condition for bistability (Angeli et al., 2004). A standard graphical test in the phase plane can be used to analyze these conditions, especially the parameter values, under which the system is bistable. Nevertheless, the analysis of complex positive-feedback systems is difficult. In (Angeli, 2006; Angeli et al., 2004), a method to investigate systems with arbitrary order was presented within the framework of monotone systems. In this so-called 'open-loop approach', the feedback loop is cut and treated as an additional input. The system can then be treated as a simple input/output system. A generalization to more complex feedback structures is possible, if the feedback can be summed up in one single apparent feedback loop.

Positive feedback in signalling pathways has to be highly nonlinear in order to create two asymptotically stable steady states in the system. In biological signalling networks such behavior is often realized through ultrasensitive structures, such as covalent modification cycles (Goldbeter and Koshland, 1981; Tyson et al., 2003), protein cascades with multiple steps (Asthagiri and Lauffenburger, 2001; Bhalla and Iyengar, 1999; Heinrich et al., 2002; Huang and Ferrell, 1996), or inhibitor ultrasensitivity (Ferrell, 1996; Thron, 1994). A simple autocatalytic reaction can also bring about bistability (Schlögl, 1972).

In the present paper we do not focus on the investigation of possible mechanisms creating multistability but investigate the dynamics of a bistable system independent from the underlying mechanism. Hence, we assume, that the considered system is bistable or in general multistable. Furthermore, we are not interested in a general investigation of parameter dependencies but focus on stimulation patterns that influence the bistable behavior as this is relevant for the design of cell signalling experiments.

The paper is organized as follows. In Section 2, we introduce a minimal model to discuss dynamic properties of bistable systems and provide necessary definitions. In Section 3, steady-state properties of bistable systems are reviewed. In the following section we discuss the dynamic behavior of bistable systems, using the introduced model of a bistable system. The concept of "bounded autonomy" is defined for the system considered in Section 5. From the example we derive characteristic time scales, which are important for the signal duration of the stimulus and the system's low-pass filter characteristics. The results obtained in the previous sections are generalized to multistable systems. Finally, we summarize our results in the last section.

2. The Model

Throughout this study we use as an example the mutually-activated enzyme network, as described in Tyson et al. (2003) (Fig. 1). In this network, a linear system is coupled with a sigmoidal system through a positive feedback loop. The corresponding mathematical representation is given as

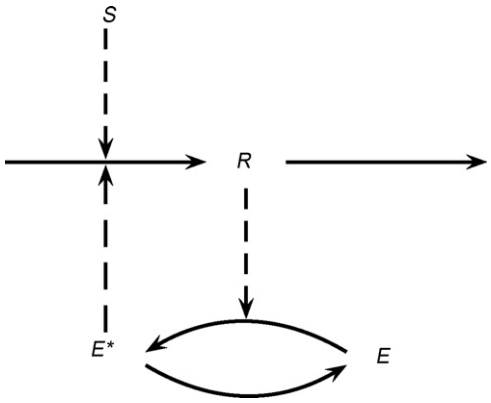

Fig. 1. Graphical representation of the system used as a case study to discuss multistability. The response component R is produced in a linear pathway, induced by an external stimulus S. The response component activates the enzyme E, which in turn facilitates the production of R through a positive feedback loop. This mechanism has been described as "mutual activation" (Tyson et al., 2003). The modification of the enzyme E follows a covalent reaction scheme (Goldbeter and Koshland, 1981; Tyson et al., 2003).

$$\frac{\mathrm{d}R}{\mathrm{d}t} = k_0 E^*(R) + k_1 S - k_2 R, \tag{1a}$$

$$E^*(R) = G(k_3 R, k_4, J_3, J_4), \tag{1b}$$

where the response component is $R(t)$ and the external stimulus or input is $S(t)$. For notational convenience, we do not show the dependence on time for these two variables from now on. The kinetic constants k_i and the Michaelis–Menten constants J_i (Segel, 1993; Cornish-Bowden, 2004) determine the chemical properties of the involved biochemical species. Further, $R(0) \equiv R_0$ denotes the initial condition, and $G(\cdot)$ the Goldbeter–Koshland function (Goldbeter and Koshland, 1981; Tyson et al., 2003) defined for system (1) as:

$$G(k_3 R, k_4, J_3, J_4) = \frac{2k_3 R J_4}{\mathcal{X} + \sqrt{\mathcal{X}^2 - 4(k_4 - k_3 R)k_3 R J_4}}, \tag{2}$$

where

$$\mathcal{X} = k_4 - k_3 R + k_4 J_3 + k_3 R J_4.$$

This function describes the concentration of the modified form $E^*(R)$ as a steady-state equation. It is frequently used considering (de)modification cycles, see Fig. 1 and Appendix A. The Goldbeter–Koshland function (3) has a typical sigmoidal shape, as shown in Fig. 2. Due to its highly nonlinear behavior it can cause multiple steady states in the investigated system (1). The use of the Goldbeter–Koshland function assumes that the modification of enzyme E is much faster than the change of the response component R. This allows us to use a quasi-stationary approximation (Millat et al., 2007). From Eq. (1), the production rate of R is separated into a stimulus dependent and enzyme dependent contribution. Due to the positive feedback loop the production rate is regulated in an autocatalytic fashion. More on modeling of activation/deactivation cycles in signaling pathways can be found in Salazar and Höfer (2006).