

Analysis of Feedback Mechanisms in Cell-biological Systems

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Abstract: A major issue in systems biology, which is well studied in control theory, is the analysis of feedback circuits in a dynamical system. These circuits endow biological systems with required functional properties. We give an overview on recent research results for the analysis of feedback circuits in cell-biological systems using methods from control theory. Starting from the known functional roles of feedback circuits, we summarize the biological questions that motivate a control theoretical analysis and perspective. Then, suitable methods for such an analysis are presented. We discuss how to apply these methods to biological research problems by summarizing different research projects that deal with feedback circuits in cell-biological systems. *Copyright © 2008 IFAC.*

1. INTRODUCTION

The complex behavior of a living cell is generated by the interactions among thousands of different components. The complexity and precision of cellular behavior rely heavily on the existence of feedback circuits in the underlying interaction networks (Araujo and Liotta, 2006). Thus, understanding the role and functions of feedback circuits in cellular biological systems is a major issue in post-genomic biology.

In this review, we summarize recent research results that use mathematical methods, partly supported by experimental data, to show that and how feedback circuits in cell-biological systems generate many of the diverse functions that a living organism requires. Moreover, we want to show with these results that control theory provides useful theoretical tools to derive relevant conclusions about the function of feedback circuits in cellular systems.

We limit the scope of this review to intracellular, non-spatial processes, where cellular compartments are essentially considered as well-mixed chemical reactors. Mathematical modeling of these processes still remains a difficult and time-consuming task, although one can typically make use of some established methodological approaches. The resulting models are usually given by systems of ordinary differential equations (ODEs). Although other modelling methods are used, ODEs are by far the most common for intracellular processes.

There is a large number of systems within living cells that fall into the scope of this paper. Metabolic reaction networks contain a lot of feedback circuits, and in particular the interactions between the levels of mass flow and information flow often go beyond classical studies conducted in the 1970s and 80s.

Another important domain is cellular signalling systems, ranging from simple environmental response systems that

bacteria use to adapt to their environment to the complex gene regulation systems involving intercellular communication in higher organisms. Also, a number of internal cellular mechanisms make intensive use of feedback circuits. Prominent examples are the circadian clock or the cell-cycle oscillator.

The paper is structured as follows. In Section 2, we first introduce special properties of the models that are used in computational cell biology. Then, the problem of how to define feedback circuits is discussed. Finally, the roles of feedback circuits in the cell and related analysis goals from a theoretical perspective are summarized. In Section 3, we give an overview on control-theoretic methods that have been used to analyse feedback circuits. Due to the wide range of topics, the overview is certainly not exhaustive. Citing four research projects in the area, we show how biological questions motivated the application of control-theoretic methods. We conclude with a short outlook.

2. PROPERTIES OF BIOLOGICAL FEEDBACK

2.1 Typical problem setups

As discussed in the introduction, mathematical models for a cell-biological system often take the form of an ordinary differential equation

$$\dot{x} = f(x, p), \quad (1)$$

where x is the concentration vector, f describes the reaction rates, and p is a vector of reaction parameters like kinetic constants.

A different formulation of the same model is given by the differential equation

$$\dot{x} = Sv(x, p), \quad (2)$$

which is based directly on the reaction network that underlies the considered system. In this formulation, the

structural and dynamical parts are separated: S is the stoichiometric matrix and is based solely on the structure of the chemical reactions, whereas $v(x, p)$ is a vector of reaction rates, corresponding to the dynamics of the system (Heinrich and Schuster, 1996).

Models for cell-biological feedback systems share special properties that are relevant for the application of analysis tools. Due to the inherent nonlinearities in the reaction rates $v(x, p)$, the classical methods for linear systems are typically insufficient to solve a given problem. Yet, not any type of nonlinearity needs to be considered, as the reaction rates are generally described by polynomial or rational functions. Adding further complexity, time delays are often used when modelling gene expression processes such as transcription or translation.

A frequent situation for biochemical reaction networks in cells is that their structure, represented by the stoichiometric matrix S , is much better known than the exact reaction mechanism, which determines the reaction rate $v(x)$. Also, reaction parameters are uncertain, often up to several orders of magnitude. Thus, large uncertainties have always to be taken into account in the analysis of a biological system.

A structural feature that can be exploited for analysis is that cell-biological systems are often made up of interconnections of smaller input-output systems (or modules) (Saez-Rodriguez et al., 2004). Techniques from control theory that make use of the modular composition are therefore often beneficial for analysis (Angeli et al., 2004; Kurata et al., 2006).

2.2 How to define feedback in cell biology

Research results in computational cell biology often lack a precise mathematical definition of what is meant by feedback, even if specifically studying feedback effects in biological systems. The traditional biochemist's view on feedback arises from metabolic pathways, where the end product often influences the activity of an enzyme that catalyzes an upstream reaction (Tyson and Othmer, 1978). In that view, feedback is restricted to information flow that modifies upstream mass flow. However, the distinction between mass and information flow is often not possible, in particular in signal transduction, and the term feedback is often used quite vaguely in systems biology. The lack of a precise definition of feedback may easily induce misunderstandings. Circuits that are actually present in a system might not be apparent in cartoon models, and this may lead to wrong conclusions about the effects of feedback circuits.

A precise definition of feedback that is useful in our framework builds upon the ODE model $\dot{x} = f(x)$ describing the biological system. Feedback circuits in such a system are identified with circuits in the interaction graph of the Jacobian $\partial f / \partial x$ (see e.g. Cinquin and Demongeot (2002) for the technical definition). This definition of feedback has proven very useful in several theoretical investigations of biological systems. A potential drawback is the large number of feedback circuits that the definition gives already for small to medium size systems. Therefore methods to evaluate the relevance of individual feedback circuits or

to find a subsystem decomposition that hides irrelevant feedbacks are required (Angeli et al., 2004; Schmidt and Jacobsen, 2004).

2.3 Analysis goals for biological feedback circuits

In this section, we outline some frequent biological questions that mainly concern systems with feedback circuits. Later in this paper, they are used as examples to show how methods from control theory can help to gain biological insight. We focus on five frequent functional roles of biological feedback: stability, complex behavior, signal sensitivity, robustness and optimality. An illustration of these functional roles is shown in Figure 1. The questions arising typically concern the functional role of feedback circuits: which mechanisms ensure the system's function, and how various external or internal perturbations may influence this function.

One role of feedback circuits in cell-biological systems is very familiar to the control engineer: it is the stabilization of a steady state, in particular the ability to counteract disturbances without directly measuring them. In fact there are several biological functions that require a steady state to be maintained despite external perturbations. Also among biologists, it is widely accepted that feedback mechanisms can be used to achieve or improve stability.

A special case is the maintenance of the steady state under the influence of noise. This is of particular importance for biological systems, which typically face considerable noise both from the environment and from the discrete nature of the underlying biochemical reactions (Paulsson, 2004). The effects of feedback in this case have been shown in a nice experiment, where a negative feedback circuit has been constructed in the genetic regulation of a cell by Becskei and Serrano (2000). This feedback circuit could efficiently reduce fluctuations from internal noise.

In view of the diverse functions that biological systems may have, stability can not be the only relevant dynamical property. More complex patterns of qualitative behavior like multistability or oscillations are also of interest. Feedback circuits in the definition discussed in Section 2.2 have been shown mathematically to be required for a system to display complex dynamical behavior (Cinquin and Demongeot, 2002). Two frequently encountered examples are bistability, i.e. the existence of two stable equilibrium points, and sustained (limit cycle) oscillations. This observation has also been made in various biological systems. Bistable switches based on a positive feedback circuit have been found for example in the maturation of frog eggs (Ferrell and Machleder, 1998) and the process of programmed cell death (Eißing et al., 2004). Also, several types of oscillatory behavior in biological feedback systems have been studied extensively (Goldbeter, 2002).

Even more than maintaining steady state values, living systems need to maintain complex functionality, as e.g. represented by their qualitative dynamical behavior, under environmental and internal perturbations. Thus, robustness is a highly important property of all cellular functions (Kitano, 2004). Generally, a system is said to be robust if it does not lose its function under any allowed perturbation. Note that both the function and the allowed

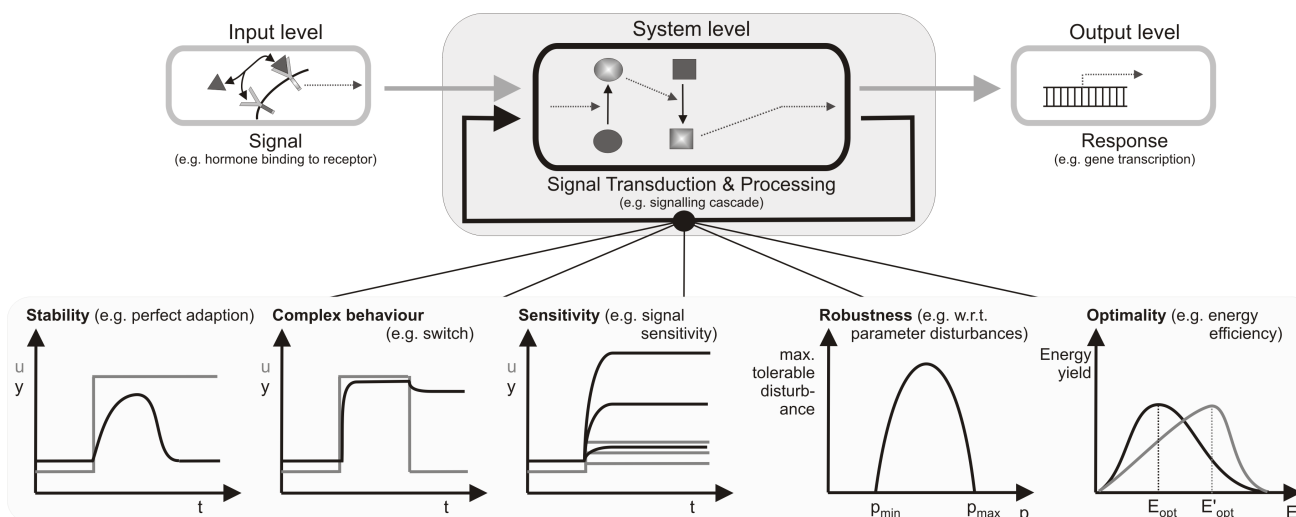


Fig. 1. Functional roles of feedback circuits in cell-biological systems

perturbations have to be well defined for any reasonable robustness analysis, which is often challenging for biological systems. One of the basic functions for which robustness can be studied is stability, such as in the adaptation of a signalling system to a basal stimulus of uncertain and varying magnitude. This plays a role in cellular sensing of chemical concentration gradients (Barkai and Leibler, 1997). An example for a more sophisticated function is the signalling network involved in apoptosis, where the decision whether to initiate programmed cell death should be taken reliably under noisy external signals and internal perturbations. In this case, the biological function can be traced to bistability in the system, a property for which robustness measures can be formulated in a straightforward way (Eiing et al., 2005). In most cases, perturbations are defined such that parameter values are uncertain within a given range. However, also dynamic perturbations are relevant in several cases (Jacobsen and Cedersund, 2005). For many cellular functions, feedback circuits are used to provide or increase robustness (Araujo and Liotta, 2006). Relevant research questions are how robust important properties of the system are against different perturbations, which specific mechanisms provide robustness, and what modifications in the system might change the robustness.

In sensitivity analysis, the main question is how the response of the cell is modulated by variations in parameters or external signals. Although the basic function may be robust with respect to these variations, cells typically show a quantitative change in their behavior in response to the variations. In fact, feedback circuits in signaling pathways can be used by cellular systems to modulate responses and to distinguish between different external signals (Brightman and Fell, 2000). The type of feedback that may be used ranges from simple phosphorylation cycles as studied by Levine et al. (2007) to complex combinations of different feedback connections that ensure both precise and robust signaling (Kurata et al., 2006).

Even more than being robust and yet sensitive to external signals, biological systems always need to optimize their behavior with respect to some performance criteria. Although the exact criteria are typically not easy to decipher

(Doyle III and Stelling, 2006), it is clear that the process of natural selection drives living organisms to some form of optimality. However, there are very often conflicting aims for a biological system, which makes multi-objective optimization essential. Again, feedback circuits play a crucial role in this optimization task. Biological systems from the organism level down to the level of individual cellular pathways (El-Samad et al., 2005) make use of feedback circuits to increase their performance in a multi-objective surrounding. Supporting this view, theoretical studies for simple cellular systems show the efficiency of feedback to increase performance (Liebermeister et al., 2004).

3. ANALYSIS OF BIOLOGICAL FEEDBACK

3.1 Suitable analysis methods from control theory

The problem setup that one typically faces when dealing with the analysis of cell-biological systems calls for an appropriate selection of control theoretical methods. In this section, several methods that are suitable for analysis of feedback circuits in cell-biological systems are discussed.

We have argued in Section 2.1 that the models we deal with often use polynomial or rational equations. Therefore, polynomial-type methods such as the sum of squares decomposition are sometimes useful for studying cell-biological systems. In a stationary framework, this approach can be applied to obtain bounds on steady state values under highly uncertain parameters (Waldherr et al., 2008). For dynamical properties, polynomial methods may help to construct a Lyapunov function and to analyze robustness of stability with respect to parameter variations in biological systems (El-Samad et al., 2003).

Several cellular signalling pathways can be modelled as monotone systems, or systems that are composed of a monotone control system with a positive or negative feedback interconnection. There are sound mathematical methods that can be used to study monotone systems (Angeli and Sontag, 2003). A particular relevant application for cell-biological systems is the analysis of complex dynamical behavior like bistability arising from positive feedback (Angeli et al., 2004) or oscillations arising from

negative feedback (Wang et al., 2005; Angeli and Sontag, 2008). These methods can also be applied to systems that are monotone after singular perturbation (Wang and Sontag, 2007), thus extending the methods to systems where non-monotone effects act on a fast time scale.

Several types of cell-biological systems contain only few nonlinearities. One example are regulatory networks for gene expression, where constitutive transcription, translation and degradation of mRNA and proteins are often modelled linearly, and only regulatory interactions are represented by nonlinear functions. The regulatory interactions are conveniently considered as feedback path, and the model is easily transformed to a so-called Lur'e system. Existing methods of stability analysis for these systems can be combined with powerful computational tools to study stability of gene regulatory networks (Li et al., 2006). Concerning complex dynamical behavior, Sepulchre and Stan (2005) presented a Lur'e system based approach to study oscillations in feedback systems, which is also useful for the analysis of biological oscillators (Stan et al., 2007).

Considering the importance of robustness analysis for cell-biological systems, it is not surprising that the sophisticated approaches that have been developed in control theory are applied to these problems. One issue is the robust stability of an equilibrium point with respect to parametric uncertainties. Polynomial methods have already been quoted as possible tools, but also approaches which consider only a linear approximation of the system close to an equilibrium point can provide valuable insight. Moreover, often also structural uncertainties need to be taken into account. For this setup, μ -analysis is an efficient tool that has been applied successfully to establish robustness results for cell-biological systems under both parametric uncertainty (Kim et al., 2006) and structural uncertainty (Jacobsen and Cedersund, 2005). Interestingly, in both cases the authors extend the classical μ -analysis to check for robustness of complex behavior, in that case limit cycle oscillations.

Although this summary shows that several approaches from control theory have already been used for biological research, suitable general analysis methods in control theory have not yet emerged for the issues concerning signal sensitivity and optimality. Therefore, one can anticipate that research motivated from biological questions will also contribute new theoretical results to the field of control. The case studies which we present in the next section show how questions that are specific to a biological system may also provide general theoretical results.

3.2 Applications of feedback circuit analysis

In this section, we describe four research projects that use methods from control theory to answer biologically relevant research questions. Each research project considers a specific biological system, and the different systems are not directly related. However, one of the first steps in each of the research projects was to perceive feedback circuits as the major reason for the observed behavior of the biological system under consideration. Moreover, theoretical methods as described in Section 3.1 have been selected or even newly developed to obtain biologically meaningful results and insights. Table 1 shows schematically which

feedback circuit related issues arise in each of the four research projects and which methods were used to address them.

The heat shock response. A problem where stability is of major concern is the heat shock response of bacteria. Increased temperature is a major stress factor for living organisms, as it makes proteins unfold and lose their functionality. Cells use so called chaperones to stabilize proteins, and the signalling system that controls the activity of chaperones is very sophisticated, containing several feedback circuits. Based on a mathematical model, one of the first steps in the analysis was to establish robust stability of the folded protein state with respect to parameter uncertainties (El-Samad et al., 2003). A Lyapunov function and sum of squares techniques have been used as theoretical tools.

There are several results concerning the role of different feedback circuits in the heat shock system. A modular decomposition identified different feedback circuits and showed how they act together to provide robustness, noise rejection and fast convergence to equilibrium (Kurata et al., 2006). Moreover, a dynamic optimization revealed that the complex feedback structures are used to obtain Pareto-optimal performance for the conflicting objectives of keeping proteins folded and minimizing energy costs (El-Samad et al., 2005).

Chemotaxis. Living cells can direct their movement according to concentration gradients of substrates in their environment by chemotaxis. A particular feature of the chemotactic system is that its response has to adapt to the basal substrate concentration in order to provide a high sensitivity to gradients over a wide range of basal concentrations. Since the system adapts exactly to the basal concentration, this feature is called perfect adaptation. It has been shown that this feature is robust: despite variations in reaction rates and enzyme concentrations, adaptation remains perfect (Barkai and Leibler, 1997). A control-theoretic interpretation based on a mathematical model of the chemotactic system has been given by Yi et al. (2000). They showed that under suitable assumptions, the mechanism by which cells achieve this can be formulated as integral feedback control. Cells thus exploit an effect that is well known in control engineering as the internal model principle (Sontag, 2003).

Apart from perfect adaptation, the high signal sensitivity in chemotaxis is an important issue on its own. Cells are able to respond to very weak concentration gradients, and the mechanism by which they transform such a weak signal to a reliable response are not clear (Bray, 2002). Control theoretical approaches are however promising to contribute to the elucidation of that mechanism (Paliwal et al., 2004).

Programmed cell death. The third research project deals with programmed cell death. As a mechanism that is present in every cell, it gives multicellular organisms the means to remove unneeded or malfunctioning cells by applying appropriate cellular signals. There are conflicting requirements in that the living state should be stable against small signal fluctuations, whereas cell death should be executed quickly upon a stimulus that exceeds some

	Heat shock response	Chemotaxis	Cell death	Circadian clock
Stability	Lyapunov function	linear approximation		
Dynamical behavior			Bifurcation analysis	Monotone systems
Robustness	Sum of squares	Internal model principle (integral feedback)	μ -analysis	parameter sensitivity

Table 1. Properties of feedback circuits relevant to the research projects under consideration and methods that have been used to evaluate them. For signal sensitivity and optimality, generally applicable methods have not yet emerged and this topics are dealt with on an individual basis for each biological system.

threshold. Bifurcation analysis showed that a bistable switch based on a positive feedback circuit can provide this behavior (Eiřing et al., 2004). Due to the lack of appropriate methods from control theory, robustness of bistability with respect to parameter uncertainties was first studied via statistical methods (Eiřing et al., 2005). Later on, μ -analysis was applied to evaluate robust stability of either the state where the cell is alive (Shoemaker and Doyle III, 2007), or alternatively of the state where the cell is dying (Shoemaker and Doyle III, 2006).

A different approach to robustness of bistability was used by Chaves et al. (2008). By assuming general uncertain activation and inhibition functions, they could establish robustness of bistability with respect to uncertainties in both parameters and kinetic mechanisms. Forward invariant sets in state space have been used as a theoretical tool in that study. An analysis based on the feedback structure of the system also allowed to reveal the relevant components and hinted at possible roles of feedback circuits (Waldherr et al., 2007).

The circadian clock. As a system where feedback circuits generate sustained oscillations, the next case study considers the circadian clock. This system is essential for most organisms to adopt efficiently to environmental changes that occur regularly each day. To date, several mathematical models have been constructed for this system. These have been used mainly for sensitivity analysis, which also allows to evaluate robustness of properties like oscillation period and amplitude (Stelling et al., 2004). Also based on sensitivity analysis, Rand et al. (2006) have defined the flexibility of a system as a measure of how system characteristics can be changed by changing system parameters. They find that flexibility is coupled to the complexity of feedback structures in the circadian clock.

Recently, Angeli and Sontag (2008) have applied the theory of monotone systems to the circadian clock. They could derive conditions for oscillatory behavior on parameter values, while also considering time delays. The method is applicable to a larger class of biological oscillators. A similar approach was taken by Wang et al. (2005), who in addition give an explicit formula for the value of the time delay at which sustained oscillations start to occur. Both approaches make direct use of the fact that the circadian clock can be decomposed into a monotone control system with a negative feedback interconnection.

4. DISCUSSION AND OUTLOOK

The ODE modelling framework considered in this paper is the most common road to mathematical models of cell-biological systems. The concept of a feedback circuit can

be defined in this approach such that both biologists and engineers can easily understand and use it. This definition can readily be applied to show that feedback circuits are required to generate complex dynamical behavior. Moreover, biological experiments and theoretical analyses have shown that cellular systems make use of feedback circuits to achieve a wide range of goals that are important for a living organism, such as robustness against perturbations and the closely connected signal sensitivity or optimality of their function with respect to relevant performance criteria.

Control theory has a successful history in the analysis and design of feedback circuits. Starting from biologically motivated research issues, we have discussed general methods and approaches from control theory that have been useful for the analysis of cell-biological feedback circuits. In summary, a number of biological research issues can be dealt with using established control theoretic methods and slight extensions. Also, several approaches used in control theory fit naturally to the problem setup one encounters in cell-biological systems.

Using four case studies, we have shown how these methods have been applied to study originally biological problems, and thus enabled deeper biological insights into the role of feedback circuits. Cell biology has a lot of problems which have not yet been studied with a control-theoretic analysis. Our expectation is that in the near future, research in cell biology will profit highly from the use of methods transferred from control engineering to systems biology.

However, there are also open problems in understanding cell-biological feedback systems for which suitable general methods in control theory are currently not available. Evaluating robustness of complex dynamical behavior like limit cycle oscillations, which is relevant e.g. for the circadian clock, remains difficult. Trade-offs between performance or signal sensitivity and robustness in nonlinear biochemical systems, as analysed by Levine et al. (2007), have not been studied within a formal framework. Noise in signals may be beneficial, or even required, for certain cellular functions (Rao et al., 2002). This observation is probably unlike anything that may be experienced in technical systems, and general system-theoretic methods to study this effect remain to be developed (Kim et al., 2007). Thus, open problems in cell biology are likely to incite the development of new general methods and approaches in the field of control theory.

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