Chapter 13

In Silico Control of Biomolecular Processes

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Abstract

By implementing an external feedback loop one can tightly control the expression of a gene over many cell generations with quantitative accuracy. Controlling precisely the level of a protein of interest will be useful to probe quantitatively the dynamical properties of cellular processes and to drive complex, synthetically-engineered networks. In this chapter we describe a platform for real-time closed-loop control of gene expression in yeast that integrates microscopy for monitoring gene expression at the cell level, microfluidics to manipulate the cells environment, and original software for automated imaging, quantification, and model predictive control. By using an endogenous osmo-stress responsive promoter and playing with the osmolarity of the cells environment, we demonstrate that long-term control can indeed be achieved for both time-constant and time-varying target profiles, at the population level, and even at the single-cell level.

Key words Model predictive control, Gene expression, High-osmolarity glycerol (HOG) pathway, Computational biology, Quantitative systems and synthetic biology

1 Introduction

Understanding the information processing abilities of biological systems is a central problem for systems and synthetic biology [1–6]. The properties of a living system are often inferred from the observation of its response to perturbations. Currently it is not possible to control protein levels in a precise and time-varying manner, even though this would be instrumental in our understanding of gene regulatory networks. To deal with this problem, we present a novel experimental strategy to gain quantitative, real-time control on gene expression in vivo. We see the problem of manipulating gene expression to obtain given temporal profiles of protein levels as a model-based control problem. More precisely, we investigate the effectiveness of computerized closed-loop control strategies to control gene expression in vivo. In model based closed-loop control, a model of the system is used to constantly update the control strategy based on real-time observations.

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We propose an experimental platform that implements such an *in silico* closed-loop in the budding yeast *Saccharomyces cerevisiae*. We show that gene expression can be controlled by repeatedly stimulating a native endogenous promoter over many cell generations for both time-constant and time-varying target profiles and at both the population and the single-cell levels.

2 Results

2.1 The Controlled System

We based our approach on the well-known response of yeast to an osmotic shock, which is mediated by the HOG (high osmolarity glycerol) signaling cascade. Its activation leads to the phosphorylation of the protein Hog1 (Fig. 1) which orchestrates cell adaptation through glycerol accumulation. Phosphorylated Hog1 promotes glycerol production by activating gene expression in the nucleus as well as by stimulating glycerol producing enzymes in the cytoplasm. Once adapted, the cells do not sense the hyperosmotic environment anymore, the HOG cascade is turned off and the transcriptional response stops [7–9]. In control terms, yeast cells implement several, short-term (non-transcriptional) and long-term (transcriptional) negative feedback loops (*see* Chapter 10) which ensure their perfect adaptation to the osmotic stress [10]. Because of these adaptation mechanisms, it is a priori challenging to control gene expression induced by osmotic stress. It is thus an excellent system to demonstrate that one can robustly control protein levels even in the presence of internal negative feedback loops. Several genes are

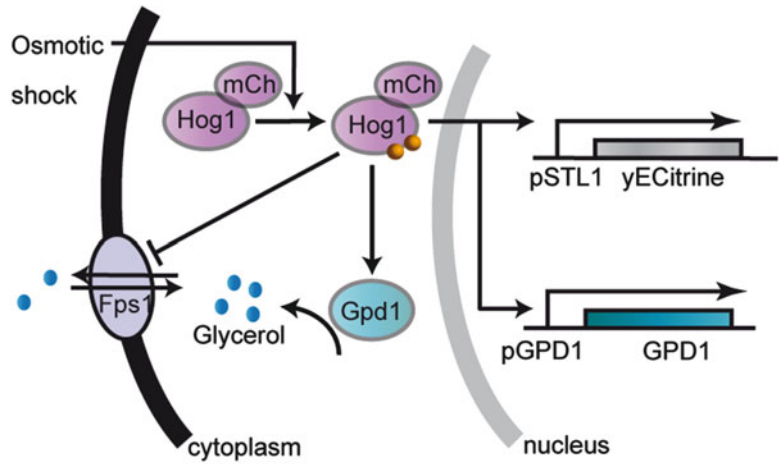

Fig. 1 Natural and engineered cell response to hyperosmotic shocks [14]. A hyperosmotic stress triggers the activation and nuclear translocation of Hog1. Short-term adaptation is mainly implemented by cytoplasmic activation of the glycerol-producing enzyme Gpd1 and closure of the aqua-glyceroporin channel Fps1. Long-term adaptation occurs primarily through the production of Gpd1. For our application, the expression of the protein of interest, yECitrine, is controlled by the osmo-responsive promoter pSTL1

up-regulated in response to a hyper osmotic stress. This includes the nonessential gene STL1 which codes for a glycerol proton symporter gene [12]. We decided to use its native promoter to drive the expression of yECitrine, a fluorescent reporter. Applying an osmotic stress transiently activated the HOG cascade and yECitrine levels reached modest values. Importantly, when short but repeated stresses were applied, pSTL1 could be repeatedly activated and much higher levels could be reached [14].

2.2 The Experimental Platform

To observe the cells and control their environment, we designed a versatile platform made of standard microscopy and microfluidic parts. The microfluidic device contained several 3.1 μm high chambers which were connected by both ends to large channels through which liquid media could be perfused (Fig. 2). Since the typical diameter of a *S. cerevisiae* cell is 4–5 μm, the cells were trapped in the chamber as a monolayer and their motion was limited to slow lateral displacement due to cell growth. This design allowed for long-term cell tracking (>15 h) and for relatively rapid media exchanges (~2 min). The HOG pathway was activated by switching between normal and sorbitol enriched (1 M) media.

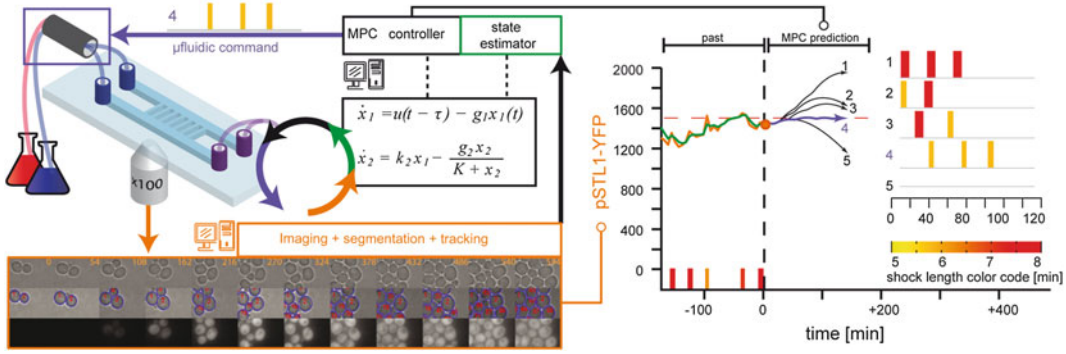

Fig. 2 A platform for real-time control of gene expression in yeast [14]. (*Left*) Yeast cells grew as a monolayer in a microfluidic device which was used to rapidly change the cells' osmotic environment (valve, *blue frame*) and to image their response. Segmentation and cell tracking were done using a Hough transform (*orange frame*). The measured yECitrine fluorescence, either of a single cell or of the mean of all cells, was then sent to a state estimator connected to an MPC controller. A model (*center*, *black frame*) of pSTL1 induction was used to find the best possible series of osmotic pulses to apply in the future so that the predicted yECitrine level follows a target profile. (*Right*) At the present time point (*orange disk*), the system state is estimated (*green*) and the MPC searches for the best input (pulse duration, number of pulses) whose predicted effect (*blue* and *black curves*) minimizes its distance to the target profile (*red dashed line*) for the next 2 h. Here, the osmotic series of pulses that corresponds to the *blue curve* (#4) was selected and sent to the μfluidic command. This control loop is iterated every 6 min unless a stress is applied. *Solid lines* and their envelopes are the experimental means and standard deviations of the cells fluorescence

2.3 Model of pSTL1 Induction

To decide what osmotic stress to apply at a given time, we used an elementary model of pSTL1 induction. Many models have been proposed for the hyperosmotic stress response in yeast [10, 15–19]. We used a generic model of gene expression written as a two-variable delay differential equation system where the first variable denotes the recent osmotic stress felt by the cell and the second the protein fluorescence level (Fig. 2). Since our goal was to demonstrate robust control despite the presence of un-modeled feedback loops, the adaptation mechanisms described above were purposefully neglected. The choice of this model was also motivated by the trade-off between its ability to quantitatively predict the system's behavior (favors complexity) and the ease of solving state estimation problems (favors simplicity). Despite its simplicity, we found a fair agreement between model predictions and calibration data corresponding to fluorescence profiles obtained by applying either isolated or repeated osmotic shocks of various durations [14].

2.4 Closing the Loop

The fluorescence intensity either of a single cell, arbitrarily chosen at the start of the experiment, or averaged over the cell population, was sent to a state estimator (extended Kalman filter), connected to a model predictive controller (MPC) [14]. MPC is an efficient framework well adapted to constrained control problems. Schematically, given a model of the system and desired temporal profiles for system's outputs, MPC aims at finding inputs so as to minimize the deviation between the outputs of the model and the desired outputs. The control strategy is applied for a (short) period of time. Then the new state of the system is observed and this information is used to compute the control strategy to be applied during the next time interval. This receding horizon strategy yields an effective feedback control. In practice, every 6 min, given the current estimate of the system state, past osmotic shocks, and our model of gene expression, the controller searched for the optimal number of osmotic pulses to apply within the next 2 h and their optimal start times and durations (Fig. 2). If a shock had to be applied within the next 6 min, then it was applied. Otherwise, the same computation was reiterated 6 min later based on new observations. We dealt with short term cell adaptation by imposing a maximal stress duration of 8 min and a 20-min relaxation period between consecutive shocks. Under such conditions cells stay responsive to osmotic stress at all times.

2.5 Closed-Loop Population Control Experiments

First, we demonstrate that one can maintain the average fluorescence level of a cell population at a given constant value (set-point experiment) and force it to follow a time-varying profile (tracking experiment). Both types of experiments lasted at least 15 h, starting with a few cells and ending with 100–300 cells in the field of view. The control objective was to minimize the mean square deviations (MSD) between the mean fluorescence of the population of cells and the target profile. We succeeded in maintaining the average

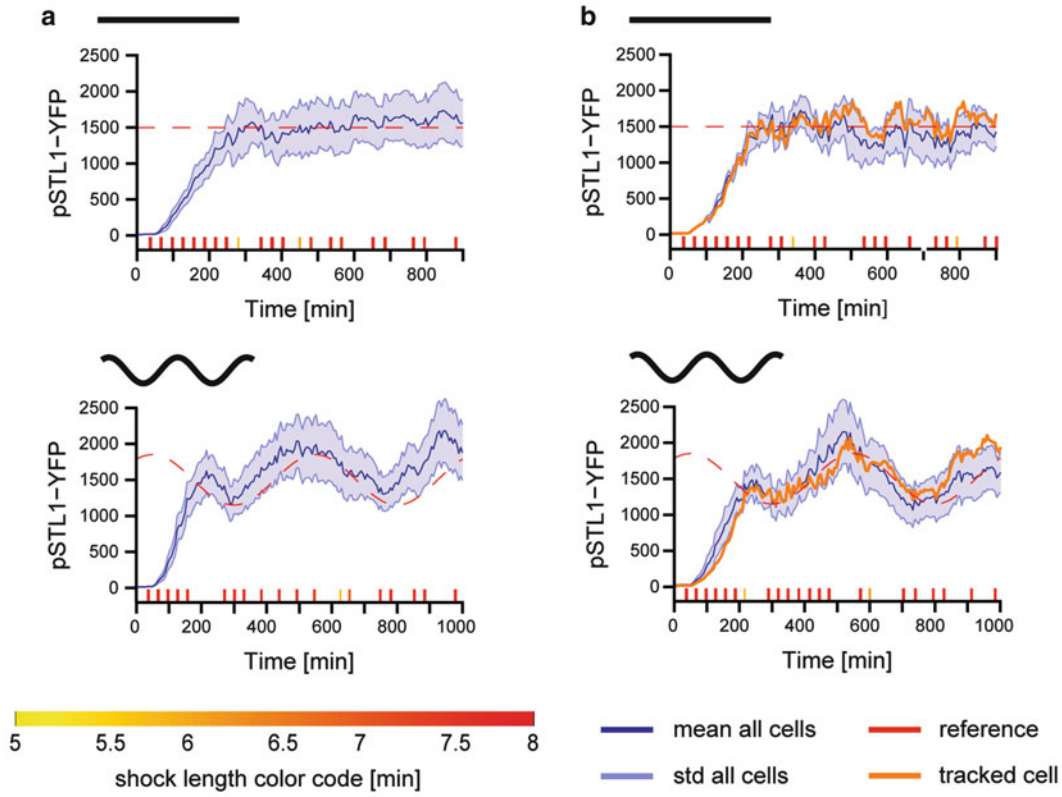

Fig. 3 Real-time control of gene expression [14]. (**a**) Control at the population level. Representative set-point control experiments and tracking control experiments are shown. Shock starting times and durations (see *color code*) were computed in real time. The measured mean cell fluorescence is shown as *solid blue* lines. The envelopes indicate standard deviation of the fluorescence distribution across the yeast population. (**b**) Control at the single cell level. The yECitrine fluorescence of the controlled cells are shown as *orange lines*. Note that the population follows the target profile but with less accuracy than the controlled single cell

fluorescence level at a given constant value, or in forcing it to follow time-varying profiles (Fig. 3) [14]. Admissible time-varying target profiles were obviously constrained by the intrinsic timescales of the system such as the maximal protein production and degradation rates. The effective control range spans an order of magnitude: set-point control can achieved between 200 and 2,000 fluorescence units [14]. Quantitative limitations of our experimental platform can originate from the model, the state estimator, the control algorithm and the intrinsic biological variability of gene expression. *In silico* analysis showed that applying the proposed control strategy to the (estimated state of the) system resulted in control performances that were significantly better than those obtained experimentally [14]. Therefore the control algorithm performed well, and future improvements should focus on system modeling and state estimation to better represent the experimental state of the system.

2.6 Closed-Loop Single-Cell Control Experiments

In a second set of experiments, we focused on the real-time control of gene expression at the single-cell level. We tracked one single cell over at least 15 h and used its fluorescence to feed the MPC controller. As shown in Fig. 3, we obtained results whose quality is out of reach of any conventional gene induction system, both for constant and for time-varying target profiles. Because of intrinsic noise in gene expression, single cell control was a priori more challenging than population control. And indeed, when compared with the mean fluorescence levels in population control experiments, the fluorescence levels of controlled cells in single-cell control experiments showed larger fluctuations around the target values. However, at the cell level, the mean square deviations of controlled cells obtained in single-cell control experiments were significantly smaller than that of a cell in population control experiments [14]. This shows that real-time control effectively improves control quality and counteracts the effects of noise in gene expression when performed at the single-cell level.

3 Discussion

3.1 Summary

We demonstrated that gene expression can be controlled in real-time with quantitative accuracy at both the population level and the single-cell level by interconnecting conventional microscopy, microfluidics, and computational tools. Importantly, we provided evidence that real-time control can dynamically limit the effects of biological noise when applied at the single-cell level. The fact that good control results can be obtained in a closed-loop setting with a relatively coarse model of an endogenous promoter suggests that extensive modeling will not be required to transpose our approach to other endo- and exogenous induction.

3.2 Related Works

The actual use of *in silico* feedback loops to control intracellular processes has been proposed only recently. In 2011, we showed that the signaling activity in live yeast cells can be controlled by an *in silico* feedback loop [20]. Using a proportional-integral (PI) controller we controlled the output of a signal transduction pathway by modulating the osmotic environment of cells in real time. A similar framework has been proposed by Menolascina et al. to control a large synthetic gene network [21]. More recently, Toettcher et al. used elaborate microscopy techniques and optogenetics to control in real time and at the single-cell level the localization and activity of a signal transduction protein (PI3K) in eukaryotic cells [22]. Interestingly, they were able to buffer external stimuli by clamping PIP_3 for short time scales. With this approach, the authors were able to reduce cell-to-cell variability of the cells output by applying different inputs to each cell (Fig. 4a). The most closely-related work is that of Milias-Argeitis et al. [23].

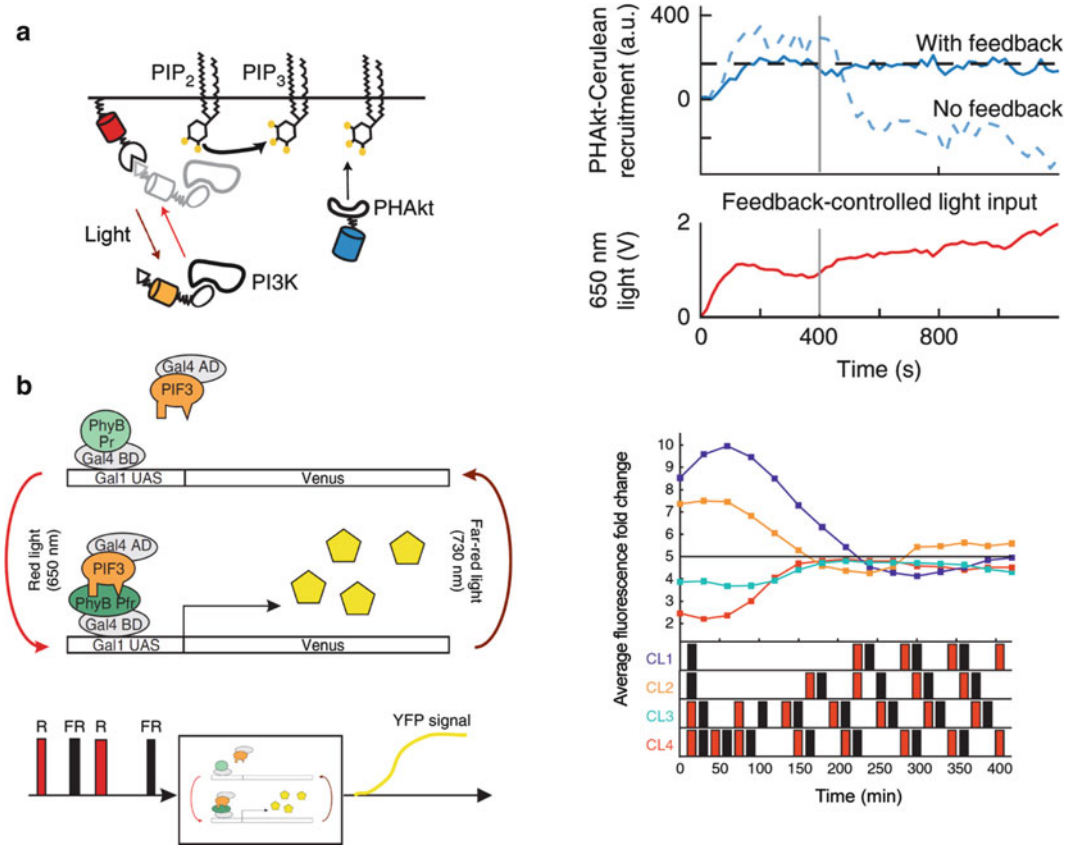

Fig. 4 Other real-time control platforms (**a**) Optogenetics control of localization and activity of PI3K in mammalian cells [22]. The amount of PI3K products, PIP3, was assayed by measuring PHAkt-cerulean recruitment to the plasma membrane. *Gray line* indicates the addition of a PI3K inhibitor at 400 s. (**b**) Optogenetics control of gene expression in chemostat using Venus as reporter protein in yeast [23]. Set-point control was achieved irrespectively of the initial state of the cell population

Using optogenetic techniques, they managed to control the expression of a yeast gene to a constant target value over several hours (Fig. 4b). In particular they are able to control the system to a fixed set point after they have sent a random series of pulses. Their approach is based on a chemostat culture and is therefore promising for many biotechnological applications such as the production of biofuels or small-molecule drugs, even if scaling up laboratory experiments to industry scale has proven difficult. However, because it does not allow for single-cell tracking and single-cell control, it is less adapted to probe biological processes in single-cell quantitative biology applications. These works have been reviewed in more depth by Chen et al. [24].

3.3 Perspectives

Connecting living cells to computers is a promising field of research both for applied and fundamental research. By maintaining a system around specific operating points or by driving it out of its standard

operating regions, real-time control approaches offer unprecedented opportunities to investigate how gene networks process dynamical information at the cell level. We also anticipate that such platforms will be used to complement and help the development of synthetic biology via the creation of hybrid systems resulting from the interconnection of in vivo and *in silico* computing devices.

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