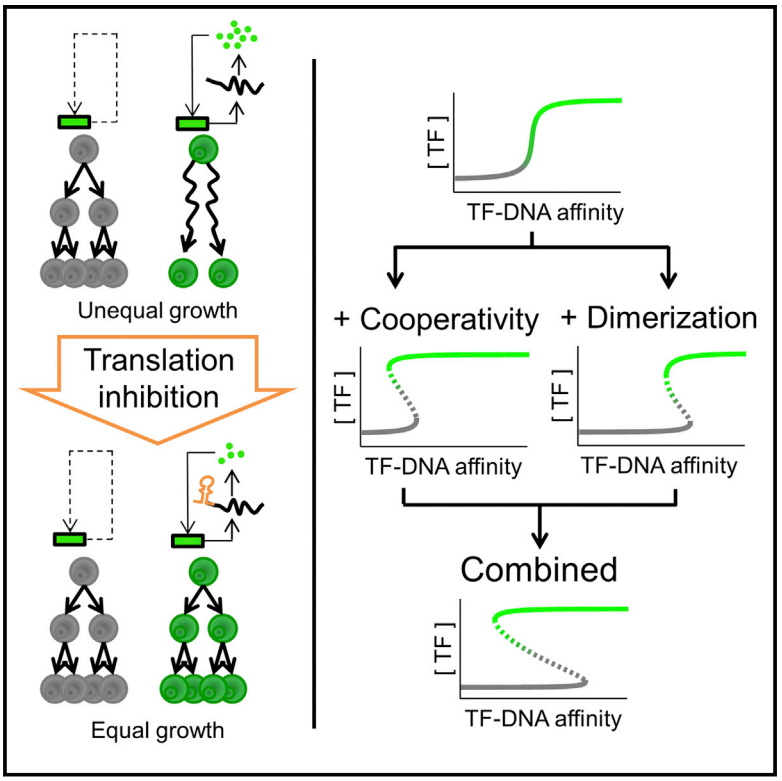
Report

Cell Reports

Protein Dimerization Generates Bistability in Positive Feedback Loops

Graphical Abstract



Authors

Chieh Hsu, Vincent Jaquet, Mumun Gencoglu, Attila Becskei

Correspondence

attila.becskei@unibas.ch

In Brief

Using RNA stem loops to attenuate translation rates, Hsu et al. designed synthetic feedback loops in yeast to study the sources of bistability. They show that cooperative binding of a transcription factor to its promoter or its dimerization generates bistability. Bistability is particularly robust when the dimerizing transcription factor binds to the promoter cooperatively.

Highlights

- RNA stem loops tune translation rates over two orders of magnitude
- Positive feedback loops with reduced translation generate bistable cell fates
- Dimerizing transcription factors generate bistability without cooperative binding

Hsu et al., 2016, Cell Reports *16*, 1204–1210
August 2, 2016
http://dx.doi.org/10.1016/j.celrep.2016.06.072

OPEN ACCESS
CellPress

Cell Reports
Report

Protein Dimerization Generates Bistability in Positive Feedback Loops

Chieh Hsu,[1,2,3] Vincent Jaquet,[1,3] Mumun Gencoglu,[1] and Attila Becskei[1,*]
[1]Biozentrum, University of Basel, Klingelbergstrasse 50/70, 4056 Basel, Switzerland
[2]School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, UK
[3]Co-first author
*Correspondence: attila.becskei@unibas.ch
http://dx.doi.org/10.1016/j.celrep.2016.06.072

SUMMARY

Bistability plays an important role in cellular memory and cell-fate determination. A positive feedback loop can generate bistability if it contains ultrasensitive molecular reactions. It is often difficult to detect bistability based on such molecular mechanisms due to its intricate interaction with cellular growth. We constructed transcriptional feedback loops in yeast. To eliminate growth alterations, we reduced the protein levels of the transcription factors by tuning the translation rates over two orders of magnitude with designed RNA stem loops. We modulated two ultrasensitive reactions, homodimerization and the cooperative binding of the transcription factor to the promoter. Either of them is sufficient to generate bistability on its own, and when acting together, a particularly robust bistability emerges. This bistability persists even in the presence of a negative feedback loop. Given that protein homodimerization is ubiquitous, it is likely to play a major role in the behavior of regulatory networks.

INTRODUCTION

Bistability, the persistence of two alternative stable-activity states under identical conditions, can uphold alternative cell fates and differentiation states, store cellular memory of past stimuli, and enhance adaptation in organisms ranging from bacteria to mammals (Angel et al., 2011; Arnoldini et al., 2014; Bouchoucha et al., 2013; Chickarmane et al., 2009; Park et al., 2012).

Positive feedback is a necessary, but not sufficient, condition for bistability in a gene regulatory network. The second requirement is that the feedback loop contains reactions such as cooperative binding, sequestration by inhibitor molecules, and multiple phosphorylation of a protein by a kinase (Chen and Arkin, 2012; Ferrell and Ha, 2014; Májer et al., 2015; Shopera et al., 2015; Thomson and Gunawardena, 2009). These reactions display a sigmoidal, switch-like nonlinear response, also termed ultrasensitive response. Without ultrasensitive responses, a feedback loop can have only a single steady-state expression level, i.e., the system is monostable.

In transcriptional regulation, dimerization and cooperative binding of a transcription factor are expected to be common sources of ultrasensitivity (Buchler and Louis, 2008). Most transcription factors bind to DNA as dimers, and binding can be cooperative when more than one binding site is present in a promoter (Becskei et al., 2005). Despite the ubiquity of protein homodimerization, its ability to generate bistability remained elusive.

The difficulty to identify the sources of bistability may be explained by the effect of the feedback loop on cell growth. In positive feedback loops, the transcription factors are often expressed at high levels; therefore, they can sequester mediators of transcription (Becskei et al., 2001; Kelleher et al., 1990). This results in squelching of global gene expression, which reduces cellular growth and alters the behavior of networks. Even more, growth alterations rather than ultrasensitivity in the feedback can generate bistability (Brophy and Voigt, 2014; Tan et al., 2009).

In this work, we illustrated a design principle to tackle this difficulty with synthetic feedback loops. We show that alteration of the cell growth caused by overexpression of the transcription factor can be circumvented by using RNA stem loops to adjust translation rates. After translation rate adjustment, we show that either of the two ultrasensitive reactions, cooperative binding to the promoter or homodimerization, can support bistability. When they were both present, a particularly robust bistability emerged.

RESULTS

Design of Synthetic Loop and Control Elements

Synthetic positive feedback loops were created by placing the gene encoding the transcription factor rtTA (reverse tetracycline transactivator) under the control of a promoter containing *tet* operators and inserted into the chromosome of the yeast *S. cerevisiae* (Table S1). rtTA is composed of the bacterial rTetR DNA-binding domain and the VP16 activation domain; rtTA binds to the *tet* operators only in dimeric form (Kamionka et al., 2006). The ligand doxycycline enables rtTA to bind to *tet* operators; thus, the affinity of rtTA binding to DNA was adjusted by the ligand concentration (Figure 1A).

