A GENETIC LIMITER CIRCUIT IN S. CEREVISIAE

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An Ideal Limiter

- Protects against signal overload
- Limits input to a given threshold
- Leaves subthreshold signal unchanged
An Ideal Limiter

Subthreshold input ➞ Output equals input
Suprathreshold input ➞ Output equals threshold

Input ➞ Limiter ➞ Output

Threshold setting

Input setting

Signal Level

Time
We need a device to:

- Repress excessive gene expression
- Permit normal gene expression
A Problem of Scale

Too much of transcriptional activator “A” causes overexpression of the gene of interest “G”

Constitutive expression of an introduced repressor “R” may repress G too much when [A] is low and repress G too little when [A] is high
Scaling repression

With R under the control of G’s endogenous promoter pG, R’s repression of G is scaled to A’s activation of G

Still, repression occurs when “G” is expressed at a low level, with no threshold response
Transcription factors are mutually inhibitory, forming a bistable toggle switch: the factor with a stronger promoter represses the other, relieving its own inhibition and entering a situation of stable expression.
Design for the Limiter Device

\[ \alpha, \tau \] – bistable repressor pair

\[ R \] – competitive repressor for G

\[ p\text{Const.} \] – constitutive promoter sets threshold

\[ G \] – gene of interest

\[ A \] – activator for G

\[ pG \] – endogenous promoter for G
Design for the Limiter Device

Subthreshold activity of A (normal expression)

Constitutively active τ represses α and R

Relatively LOW levels of A cannot overcome repression from τ

Gene expression of G is same as endogenous case
Design for the Limiter Device

Suprathreshold activity of A (overexpression)

Constitutively active τ represses α and R

Relatively HIGH levels of A overpower τ repression
α maintains new stable state

Newly formed R competes with A to repress G
R feedback to α and to itself prevent excessive repression
Modeling Approach

- Hill equation for transcription factors
- Used to model cooperativity
- Parameter values based upon experimentation
(A)  \( \frac{dA}{dt} = 0 \)

(G)  \( \frac{dG}{dt} = \frac{M_G}{1 + \left( \frac{R}{K_{RG}} \right)^N} \cdot \frac{A^N}{K_{AG} + A^N} + \beta_G - \mu_G G \)

(τ)  \( \frac{d\tau}{dt} = \frac{M_\tau}{1 + \left( \frac{\alpha}{K_\alpha \tau} \right)^\beta} + \beta_\tau - \mu_\tau \tau \)

(R)  \( \frac{dR}{dt} = \frac{M_R}{1 + \left( \frac{R}{K_{RR}} \right)^N} \cdot \frac{1}{1 + \left( \frac{\tau}{K_{\tau R}} \right)^M} \cdot \frac{A^N}{K_{AR} + A^N} + \beta_R - \mu_R R \)

(α)  \( \frac{d\alpha}{dt} = \frac{M_\alpha}{1 + \left( \frac{R}{K_{R\alpha}} \right)^N} \cdot \frac{1}{1 + \left( \frac{\tau}{K_{\tau \alpha}} \right)^M} \cdot \frac{A^N}{K_{A\alpha} + A^N} + \beta_\alpha - \mu_\alpha \alpha \)
Modeling

Compare expression levels of G

Gene G with endogenous activation as the only input

Endogenous system

Gene G under control of the limiter device

Synthetic regulation
Results

Suprathreshold: expression is repressed

Subthreshold: endogenous expression level
Implementation

From theory to practice

- The Chassis
- The Parts and Components
Yeast Chassis

Benefits to yeast over bacteria

- Regulation: diversity of transcription factors
- Dynamics: inherent cooperativity of transcription
- Research: a model organism for other eukaryotic systems
Synthetic Transcription Factors


- **Fluorescent tag x2** tracks expression and stabilizes protein
  - RFP, CFP, or YFP

- **DNA binding domain** specifies target DNA sequence
  - LexA, Gli1, Zif268-HIV, or YY1

- **Regulatory domain** determines effect of transcription factor (+/-)
  - VP64 activator or Sin3 repressor

- **Nuclear localization sequence** translocates protein to nucleus
Building a repressor

Endogenous Transcription Factor

- Activation Domain
- Binding Domain

Synthetic Repressor

- Repression Domain
- Binding Domain
Building promoters

DNA-binding domains on transcription factors target specific binding sites

Introduced binding sites can add regulation to existing promoters

Promoters we built:

- **Minimal** - all regulation comes from synthetic factors
- **Constitutive** - regulation added to a basal strength
- **Regulated** - regulation added to a variable strength

DNA-binding domains  |  Binding Sites
---|---
LexA  |  Gli1  |  Zif-HIV  |  YY1  |  mCYC  |  ADH  |  TEF  |  MET25
Proof of Principle Design

Introduced to cell

Endogenous to cell

Representation of endogenous elements
Manifestation
All ligations propagated in E. coli

- Final ligation onto yeast shuttle vectors
  - 4 Sikorski genomic integration vectors
  - 4 auxotrophic loci and selection markers
5 constructs to integrate, only 4 loci
- Transform into haploids of different mating type
- Mate with complimentary selection markers

α mating type

Gli
lex
mCYC
Zif Repressor
zif
TEF
Gli Repressor

a mating type

GAL
Lex Activator

lex
mCYC
YFP x2
Competitive Binding Test

1000 µM Methionine
RFP repressor repressed
YFP reporter expressed

0 µM Methionine
RFP repressor expressed
YFP repressed
Expected limiter behavior

Compare a control strain with just A and G to the entire construct
Submitted over 100 useful parts and devices to the Registry of Standard Biological Parts

Plasmids

- **BBa_K165061**: Plasmid, pRS304* yeast shuttle vector, TRP1 selection

Basic Parts

- **BBa_K165037**: Regulatory, TEF2 yeast constitutive promoter

Intermediates

- **BBa_K165018**: Terminator, SV40 nuclear localization sequence + ADH1 transcription terminator
- **BBa_K165028**: Intermediate, Kozak + Sin3 Repression Domain

Devices

- **BBa_K165096**: Device, Zif268-HIV bs + MET25+ Gal1 repressor (CFPx2 tagged) on pRS304*

About 40% of these parts came straight from:
Dr. Caroline Ajo-Franklin, Lawrence Berkeley National Lab
Dr. David Drubin, Harvard University

Thanks!
Accomplishments

- Designed and modeled a novel limiter network
- Complete construction of limiter prototype
- Transformation of limiter into yeast
- Showed a number of devices to work in yeast
- Demonstrated functionality of competitive binding
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