E. coli The Clock

--- a TA system to prolong the lifespan
• How we generate our idea

Our Inspiration, Design & Background, and practical Significance

• How we realize our idea

The Mathematic Model, experimental Result & Discussion, as well as a Summary

• Specialties

Special Acknowledgement & remarkable Display!
Can we create a circadian rhythm in simply organisms to execute the plan we want to do?

Can the regular dormancy we generated affect the lifespan of the organism as we want hibernation of animals?
The Toxin Module
- What is relE?
- Why is E.coli dormant?

The Rescue Module
- tRNA? mRNA? tmRNA!
- E.coli starts to work.

The Suicide Module
- Cleavage rate makes sense.
- relE actually inhibits itself!

The Antitoxin Module
- Hey, I thought it’s relB.
- Get up, E.coli!
**RelE toxic protein**

An RNase that preferentially cleaves mRNAs in the ribosome between the 2nd and 3rd nucleotide of stop codons. Expression of the *relE* gene has been shown to severely inhibit translation and prevent colony formation, leading to bacteria dormancy.

*right fig:* Structure Of E. Coli Toxin RelE

**Lon protease**

Lon proteases are ubiquitous, multidomain, ATP-dependent enzymes. It's the protease that cleavage RelB protein, and thus enhance the dormant effect of RelE protein.

*left fig:* Catalytic Domain Of E.Coli Lon Protease
RelE represses expression of genes by cleavage of mRNA at stop codons on transcriptional level.

This leads to stalled ribosome and unfinished peptide and the cell goes to bed.
Genetic circuit of toxin module

placI lacI plac OlacI relE lon
RelB antitoxin protein
An antagonist on RelE protein by binding it to inhibit its effect of translation inhibition and colony formation.

right fig: Structure Of The Dbd Domain Of E. Coli Antitoxin Relb
This tetrameric is too big to fit into A site!!!

RelB can form a heterotetrameric \((\text{RelB-RelE})_2\) structure when binding with \(\text{relE}\).
Genetic circuit of antitoxin module

pcI  CI  pc  Oci  relB
Rescue module

- **tmRNA**
  A hybrid RNA molecule that combines the functions of both transfer and messenger RNAs, rescues stalled ribosomes, and targets aberrant, partially synthesized, proteins for proteolytic degradation.

  Right fig: Nmr Structure Of The Aquifex Aeolicus Tmrna Pseudoknot Pk1

- **SmpB Protein**
  Small protein B is required for trans-translation, binding specifically to tmRNA and essential for its biological functions.

  Right fig: Solution Structure Of A Tmrna-Binding Protein, Smpb, From Thermus Thermophilus
A stalled ribosome

The stalled ribosome can be put into use again.
Genetic circuit of rescue module

paraC  araC  pBAD  Oara  tmRNA  smpB
**Suicide module**

**RelE cleavage rate:**
UAG > UAA > UGA

<table>
<thead>
<tr>
<th>GENE</th>
<th>Original stop codon</th>
<th>Current stop codon</th>
<th>Current cleavage rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>relE</em></td>
<td>UGA</td>
<td>UAA</td>
<td>2.2</td>
</tr>
<tr>
<td><em>lon</em></td>
<td>UAG</td>
<td>UAG</td>
<td>26</td>
</tr>
<tr>
<td><em>relB</em></td>
<td>UGA</td>
<td>UGA</td>
<td>0.078</td>
</tr>
</tbody>
</table>
The Overall System

Fig: Time dependent concentration of RelE, RelB and Lon

expression

dormant awake
We develop a model to prove our project is feasible to guide our experiments to regulate the length of E.coli’s sleeping time.

Factors taken into consideration:
- single gene expression
- RelE cleavage process
- Hydrolysis of RelB by Lon
- RelE & RelB polymerization

Expression of RelE, Lon, and RelB over time.
Interaction relationships
MATHEMATICAL MODEL

\[
\frac{d[\text{relE}]}{dt} = \alpha_1 [\text{mRNA,relE}] - k[\text{relE}][\text{relB}] - \beta_1[\text{relE}]
\]

\[
\frac{d[\text{Lon}]}{dt} = \alpha_2 [\text{mRNA,Lon}] - \beta_2[\text{Lon}]
\]

\[
\frac{d[\text{relB}]}{dt} = \alpha_3 [\text{mRNA,relB}] - \beta_3[\text{relB}] - k[\text{relE}][\text{relB}] - \frac{\text{kat}_1[\text{Lon}][\text{relB}]}{[\text{Lon}] + K_m}
\]

\[
\frac{d[\text{mRNA,relE}]}{dt} = \alpha_4 - \frac{k\text{kat}_1[\text{relE}][\text{mRNA,relE}]}{[\text{mRNA,relE}] + K_m} - \beta_4[\text{mRNA,relE}]
\]

\[
\frac{d[\text{mRNA,relE}]}{dt} = \alpha_5 - \frac{k\text{kat}_1[\text{relE}][\text{mRNA,Lon}]}{[\text{mRNA,relE}] + K_m} - \beta_5[\text{mRNA,Lon}]
\]

\[
\frac{d[\text{mRNA,relE}]}{dt} = \alpha_6 - \frac{k\text{kat}_1[\text{relE}][\text{mRNA,relB}]}{[\text{mRNA,relE}] + K_m} - \beta_6[\text{mRNA,relB}]
\]

Consideration:

a. lots of unknown parameters
b. three proteins we focus on
Goal: choose the proper rbs to make our system oscillate

\[
\frac{d[\text{relE}]}{dt} = \alpha_1 - \frac{k_1[\text{relE}]^n}{[\text{relE}]^n + K_M} - k[\text{relE}][\text{relB}] - \beta_1[\text{relE}]
\]

\[
\frac{d[\text{relB}]}{dt} = \alpha_2 - \frac{k_2[\text{relE}]^n}{[\text{relE}]^n + K_M} - \frac{k_{\text{cat}}[\text{Lon}][\text{relB}]}{[\text{Lon}] + K_{M0}} - k[\text{relE}][\text{relB}] - \beta_2[\text{relB}]
\]

\[
\frac{d[\text{Lon}]}{dt} = \alpha_3 - \frac{k_3[\text{relE}]^n}{[\text{relE}]^n + K_M} - \beta_3[\text{Lon}]
\]

Now just let Matlab handle this work...
MATHEMATICAL MODEL

Oscillate with periodicity

Damped oscillations

Found the majority of the systems are asymptotic stable

Built the oscillation criterion

Reduced the burden of Matlab

Equilibrium states
relative strength of the three rbs: 1:1:1
oscillating period: 1.29h

relative strength of the three rbs: 0.6:0.7:1
oscillating period: 1.50h

relative strength of the three rbs: 1:0.6:1
oscillating period: 2.25h

relative strength of the three rbs: 0.6:0.6:0.07
oscillating period: 0.66h

relative strength of the three rbs: 1:1:0.6
oscillating period: 1.10h

MATHEMATICAL MODEL

three rbs relative strength 1:0.6:0.07
RESULT & DISCUSSION

E. coli the Clock

- The TA system
- The Bioclock
- The Lifespan
Construct results of RelE+Double terminator and RelE+Rbs

Lane 2: BBa_K185000 RelE toxin+Rbs30  311bp
Lane 3: BBa_K185004 RelE toxin+Double terminator  443bp

RelE would generate the dormant state in *E. coli*.

*E. coli* BL21 were streaked on LB plates

Construct results of RelE+Double terminator and RelE+Rbs

+0.1% IPTG

No IPTG

pSB1A2(+relE)  Negative control  pSB1A2(+relE)  Negative control
Recovering Function of RelB Antitoxin

Fig: Western blotting results when RelB is induced.

RelB would revive from dormant state.

Plate colony observation of RelE+RelB+ and RelE+ RelB-.
So, we realized the dormancy state and revival state in *E. coli* the clock
IPTG-induced RelE strain growth curve

IPTG has a slight toxic effect. What exactly generate dormant state, IPTG or relE gene?
Therefore, we use arabinose to induce relE expression, and the result is ...
\[ N = N_0 \cdot 2^n \]
\[ \therefore \ln N = \ln N_0 + n \ln 2 \]
\[ n = \frac{t}{\tau} \]
\[ \therefore \ln N = \frac{\ln 2}{\tau} t + \ln N_0 \]

Parameters:
- \( N \) final cell number
- \( N_0 \) initial cell number
- \( n \) generation numbers during exponential growth
- \( \tau \) generation time

The result:
- IPTG+RelE \( \tau = 0.8795 \text{h} \)
- IPTG+NC \( \tau = 0.5125 \text{h} \)
Then, we bring forward:

We regard:

From the perspective of a single bacterium: splitting time

What is the definition of **lifespan** in prokaryotes?

<table>
<thead>
<tr>
<th>The result</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IPTG+RelE</strong></td>
<td>$\tau=0.8795h$</td>
</tr>
<tr>
<td><strong>IPTG+NC</strong></td>
<td>$\tau=0.5125h$</td>
</tr>
</tbody>
</table>

Of course, the prolongation of bacteria colony lifespan should be also observed. Actually, we have some primary results to define it, but it is required to confirm them further.
Logical Chain of Our Works

Original genetic part: TA system

Reprogram: E. coli Sleeping Bioclock

Experiments: Life span Prolongation

New Born Biobrick

A unique Bioclock controlling sleeping rhythm

Expanding in Eukaryotes

Life span: an amazing research direction
Fig: The immunofluorescent assay of RelB and RelE in the EcR-CHO cells
What We’ve Done

- Construction of toxin module
- Construction of antitoxin module
- Construction of rescue module
- Mathematic model
- Oscillation curve demonstration
- Extend longevity demonstration
- Standardize 42 plasmids, 6 are working and all are available!
- Contribute biobricks to the registry
- Characterize biobricks
- Wiki construction
- Soft tools for wiki
- Cultural artifact
- Team collaboration
- Have fun!
ACKNOWLEDGMENT

- Graduate & post-grad advisors
  - Cheng Lu, Bio-X
  - Haixia Zhao, Bio-X
  - Jingjing Cao, Bio-X
  - Jun Yang, Bio-X
  - Yong Wan, Bio-X
  - Wenrong Zhou, Bio-X
  - Huang Zhu, Bio-X
  - Kuanjun He, Bio-X
  - Yue Xiao, Bio-X
  - Xuming Zhu, Bio-X
  - Lingling Zhang, Bio-X
  - Sixia Huang, Bio-X

- Instructors
  - Lin He, Bio-X
  - Gang Ma, Bio-X
  - Shengying Qin, Bio-X

- Funding sources
  - Bio-X center
  - Shanghai Jiao Tong University
  - Shanghai Jiao Tong University Library

Special Thanks To:

- BIO-96 center
- Shanghai Jiao Tong University
- Shanghai Jiao Tong University Library
- MIT
- The MathWorks
- GENEART
- invitrogen
- The Gene of Your Choice
- Foundation
mascots
Our lovely University
Shanghai
thanks!