TEAM: SHENZHEN
ABOUT THE 8-SCHOOL CONSORTIUM
BACKGROUND
PROJECT BRAIN STORMS
ONLY MEMBRANE?
ORGANELLE!
CELL

YAO

PRODUCT

NUCLEUS
BANG
A genome (YAO.Genome)
A transport system (YAO.Channel)
A sensor (YAO.Sensor)
A suicider (YAO.Suicider)

A YAO (YAO.Factory)
Highlights
Foundational Advances
Foundational Advances

- Access to organelles for iGEMers
- Sending signals in or out organelles
  - Transcriptional regulation in organelle
- A series of signal peptides
- Simulations for bio-synthesis in YAO
YAO. GENOME
ARTIFICIAL GENOME IN YAO
Key Features for YAO.Genome

• Self-replication
• Genetical Stability
• High capacity
Stages towards YAO. Genome

1. DNA Fragment Recombination
2. Shuttle Plasmid
3. YAO.Genome
DNA Fragment Recombination

gene gun

mitochondria

yeast

ρ-

ρ+
DNA Fragment Recombination
Positive Clones

Control

Date: 2012年10月6日 星期六
Shuttle Plasmid

Transform the shuttle plasmid
Shuttle Plasmid

Transform the shuttle plasmid

>COB_Terminator
ATTATAGTTCCGGGGGCCCCTACGGGAAGCCGGAACCCCGCAAGGAGATT

>COX3_Terminator
AAAAACTCCTAACGGGGTTCCCAGCGAAGCGGGAACTAATAATAATATAAT

>Q0255_Terminator
ATATTAATTAAGTTTGCAGGGTACGGGACCCGGAACCCCGAGAGG
AGTTATTATATTTA

>AI1_RBS
ATGAATATAATAATAATAATATTAAAATTAATATATAAAAAAATGAAAA

>OLI1_RBS
ATGTTAATTATAATAATAATAATATTAAAATTAATATATAAAAAAATGAAAA

12年10月6日星期六
Shuttle Plasmid

Transform the shuttle plasmid

BioBricks
Positive Clones

Control
Feature Required for YAO.Channel

- Specificity

Natural Mitochondrion

YAO.Channel

YAO
Stages towards YAO. Channel

I. Standardize Signal Peptides (SP)

- SP to outer membrane
- SP to inner membrane
II. Assemble TOC and TIC Complex

Red alga chloroplast
Stages towards YAO. Channel

II. Assemble TOC and TIC Complex
Stages towards YAO. Channel

II. Assemble TOC and TIC Complex
Stages towards YAO. Channel

III. Test the New Transit Machinery

SP specific to Toc
Stages towards YAO. Channel

III. Test the New Transit Machinery
Stages towards YAO. Channel

IV. Kick out Original Transit Machinery
Achievements

- Standardization of signal peptides

![BioBricks]

Inaccurate information in PartsRegistry leads to frame shift

Primers used to solve this problem:

- Common: 5'-GTTCCTTCGAATTCGCGGCCGCTTCTAG
- ZIM17-R: 5'-GGGCTTCTTCCTGCAGCGGCCGCTATACTAGTATGAGATGCGATGAT
- FER-R: 5'-GTTCCTTCCTGCAGCGGCCGCTATACTAGTAGGATTTTACTTG
- TIM21-R: 5'-GTTCCTTCCTGCAGCGGCCGCTATACTAGTAGGGATTTTACTTG
- TOM22-R: 5'-GTTCCTTCCTGCAGCGGCCGCTATACTAGTAGGCAAGTATAG
- TOM70-R: 5'-GTTCCTTCCTGCAGCGGCCGCTATACTAGTAGGTTGTAATAATA
- TOM20-R: 5'-GGGCTTCTGGCAGGGCGCCGCTATAGTAGGCTAACGAG
- TOM40-R: 5'-GTTCCTTCCTGCAGCGGCCGCTATAGTAGCAATTGAGGAAG
Achievements

- Designs for composite BioBricks
Functions

• Receive Signals
• Report State
  • Qualitatively
  • Quantitatively

Example:

Redox state

Redox state is a mitochondria related state, which is important to the cell.

=> NAD+/NADH
Genetic Circuit

• Redox Sensor:

Rex can sense NAD+/NADH, bind on ROP(Rex Operator) when NAD+/NADH is high and prevent GFP from transcribing.
Genetic Circuit

- Redox Sensor:
  Rex can sense NAD+/NADH, bind on ROP (Rex Operator) when NAD+/NADH is high and prevent GFP from transcribing.

Promoter ROP MT GFP Terminator
Genetic Circuit

- **Redox Sensor:**
  
  Rex can sense NAD+/NADH, bind on ROP(Rex Operator) when NAD+/NADH is high and prevent GFP from transcribing.
Genetic Circuit

BioBricks
Modeling

Division of Parts

1. Transport of REX into mitochondrial matrix
2. Activation of REX as a transcription factor with NAD+ and NADH
3. Control of promoter activity by REX
4. Synthesis and degradation of mRNA
5. Synthesis, dilution and degradation of mtGFP

Finally, the model’s response to dynamic state are tested.

\[ C_{\text{rex}} = \frac{K_{sp} \times X_{SR}}{N_{m}} \]

\[ R_f = 1 - \frac{1}{1 + \frac{K_h}{H} + \frac{K_h}{K_n} \frac{N}{H}} \]

\[ P_f = \frac{\alpha \times \frac{\text{IF}}{K_{if}} + \beta \times \frac{C_{\text{rex}} \times R_f}{K_{rp}} + 1}{\frac{\text{IF}}{K_{if}} + \frac{C_{\text{rex}} \times R_f}{K_{rp}} + 1} \]

\[ X_{\text{mRNA}} = \frac{V_{mRNA}^{\text{max}} \times N_{p} \times P_f}{K_{dm}} \]

\[ X_{\text{GFP}} = \frac{K_p \times N_{\text{rib}} \times X_{\text{mRNA}}}{K_{dGFP} + \mu} \]
Modeling

Division of Parts

1. Transport of REX into mitochondrial matrix
2. Activation of REX as a transcription factor with NAD+ and NADH
3. Control of promoter activity by REX
4. Synthesis and degradation of mRNA
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Finally, the model’s response to dynamic state are tested.
YAO.SUICIDER
HOLIN BASED BIOSAFETY DEVICE
Cast & Drama

YAO = BOMB
Yeast = FIRE PLAYER

GET BURNT TOGETHER
Changes of Design

I. Input signaling

II. YAO sacrifices

III. Cascade reaction

IV. Yeast repression
Changes of Design

I. Input signaling
   - Signal from nucleus
     - Imitation

II. YAO sacrifices

III. Cascade reaction

IV. Yeast repression

PROJECT::YAO#1.0  TEAM::SHENZHEN  BGI·college

12年10月6日星期六
1. T7 Imitation Signaling

2. Mitochondrion Sacrifice
1. T7 Imitation Signaling

2. Mitochondrion Sacrifice

NUCLEUS

GALI Promoter

Signal Peptide to MT matrix

Engineered MT

DNA Fragmentation

T7 Promoter

DNase I
3. Retrograde Signaling
4. Holin Destruction
5. Yeast Suppression

NUCLEUS

RTG Pathway

Engineered MT

ER Ca^{2+}

DLD3 Promoter

Holin

MT
3. Retrograde Signaling

4. Holin Destruction

5. Yeast Suppression

NUCLEUS

DLD3 Promoter

Holin

RTG Pathway

Engineered MT

MT Disintegration & Cellular Vacuolization

ER

MT

Dead Draft
Yeast cell is suppressed
(non-apoptotic cell death)
## Tests

<table>
<thead>
<tr>
<th>Part</th>
<th>Scope</th>
<th>Main BioBricks</th>
<th>Result</th>
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<td>Signal Imitation</td>
<td>Nucleus</td>
<td><img src="image" alt="Signal Imitation Nucleus BioBricks" /></td>
<td>mtGFP fluorescence</td>
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<td>Suppression</td>
<td>Nucleus</td>
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<td>Lower growth rate</td>
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<td><img src="image" alt="Suppression Mitochondrion BioBricks" /></td>
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12年10月6日星期六
Verification of Partial Result

PgaI Engineering

Holin Adaptor Replacement
Isoprenoid biosynthetic pathways
Modeling

Production of terpenes will increase with the concentration of enzymes.

Production of IPP  Production of GPP  Production of FPP
HUMAN PRACTICES
CONTRIBUTION TO SYNTHETIC BIOLOGY
Eight lectures or courses on synthetic biology and iGEM have been given to:

- BGI Research Institute & BGI College
- 4 universities in China
- 3 high schools in Shenzhen
Teamwork with SUSTC

- Two co-instructors
- Laboratory sharing
- Two workshops among Shenzhen team, SUSTC’s two teams and HKUST team
iGEM in High School

At least four high schools in Shenzhen now have the plan to participate in an localized iGEM HS like competition in 2013, initialed by BGI College.
ACKNOWLEDGEMENT
THANKS FOR SUPPORTING OUR TEAM
Thanks to

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KANG KANG

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Dr. Junguang XU
Dr. Ming Ni

12年10月6日星期六
YAO.GENOME
ARTIFICIAL GENOME IN YAO
Genetic Transformation of Saccharomyces cerevisiae Mitochondria

- Transfer of mitochondria by protoplast fusion in Saccharomyces cerevisiae (1977)
- Mitochondrial transformation in yeast by bombardment with microprojectiles (1988)
- Genetic transformation of Saccharomyces cerevisiae mitochondria (2007)
Physical analysis of the mitochondrial DNA (mtDNA) from the transformant and control strains.
Schematic diagram of recombination events that allow identification of nonrespiring recombinant cytoductants by marker rescue in yeast.

Optical imaging of a yeast strain JSC350X containing a mitochondrially expressed cox3::GFPm reporter.
Fig. 2. Fluorescence microscopy of cells expressing cox3 :: GFPm-3. Cells were photographed under fluorescence (A, C, and E) or DIC light (B, D, and F) microscopy (Section 2). cox3 :: GFPm-3 (JSC350X) cells were examined after stationary phase (A and B) or exponential phase (E and F) growth in complete medium containing raffnose. cox3D cells (GW22) lacking a gene for GFP were examined after stationary phase growth (C and D) as a negative control.
Conjugation between E.coli and mitochondrion containing T7 RNA polymerase (T7RNAP).
Promoter Annotation

The promoter sequences for YAO project are found in literatures[6]:

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<th>Natural variants</th>
<th>-8</th>
<th>-7</th>
<th>-6</th>
<th>-5</th>
<th>-4</th>
<th>-3</th>
<th>-2</th>
<th>-1</th>
<th>+1</th>
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<td>T</td>
<td>A</td>
<td>T</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>T</td>
<td>A</td>
<td>A</td>
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<td>COX II</td>
<td>T</td>
<td>A</td>
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<tr>
<td>tRNA(fMet), tRNA(Phe), tRNA(Ala)</td>
<td>T</td>
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<td>tRNA(Glu)</td>
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<td>tRNA(Thr-CUN)</td>
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<td>tRNA(Thr-ACN)</td>
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<td>tRNA(Cys)</td>
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<td>RPM1</td>
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</table>
RBS Annotation

- The RBS sequence for mitochondria is discerned from the mito-chromosome based on following principles:
  - 1) RBS are on up-stream of CDS sequence and down-stream of promoter sequence in 5’-UTR sequence.
  - 2) The ribosome binds on RBS sequence and starts translation when coming across the first AUG codon.

- So the sequence between the last ATG codon and CDS on the DNA sequence is believed to contain RBS. With further modifications, we made those sequences BioBricks.

- Annotated RBS:
  >AI1
  ATGAATATAATAATAATAATATTATTAAAATTAATATATAAAAAAAAAGTAAAA
  >OLI1
  ATGTTAATTATATATAATATATTTATATATAATTATATATATATATATATAAATAATAAATATATATATAATATAAAAATAAGAATAGATTAAATATTTAATAAATAAATAAATATT
Terminator Annotation

• Mitochondria transcription systems are similar to bacterial transcription system and the RNA polymerase in yeast mitochondria is very similar in sequence and structure to bacteriophage T7 RNA polymerase. With DNA secondary structure analysis of 5’UTR sequence of mitochondrial genes, we found the stem-loop structure in these sequences which are very similar to secondary structure of bacterial Rho-independent terminator.

• Annotated Terminators:

>COB
ATTATAGTTCCGGGGCCCGGTCAACGGAAGCCGGAACCCCCGCAAGGAGATT

>COX3
AAAACTCCTAACCGGGTTCGCCGCGAAGCGGGGAACCTAATAATAATATAAT

>Q0255_2
ATATTAATTAAGTTTCGGGTACCAGGAACCCCGGAGAGGGAGTTATTATATTTTA
References


Annotation of Signal Peptide

- **SP of Zim17**
  Zim17 is a zinc finger protein located in mitochondrial matrix. The signal peptide of Zim17 is amino-terminal segments rich in positively charged residues that form two to three turns of a helix with amphipathic character, and it directs the proteins imported into mitochondrial matrix.[1]

- **SP of Tim21**
  Tim21 is one component of the translocase of the inner mitochondrial membrane (TIM complex), and the signal peptide of Tim21 contains 239 amino acids with a N-terminally single transmembrane segment showing the characteristics of a positively charged mitochondrial presequence with a predicted cleavage site after residue 42.[2]
• **SP of Tom70**
  Tom40 is the core component of the translocase of the outer mitochondrial membrane (TOM complex), presenting an $\beta$-barrel structure. The signal peptide of Tom40 is a conserved $\beta$-signal motif, located near the C-terminus[3]

• **SP of Tom70**
  Tom70 is also one component of TOM complex, but only contain a single transmembrane segment at their N terminus. The signal peptide of Tom70 is the transmembrane domain comprising a hydrophilic, positively charged segment[4]

• **SP of Tom22**
  Tom22 is similar with Tom70, but it’s a tail-anchored protein. The signal peptide of Tom22 is also similar with the one of Tom70: moderately hydrophobic, relatively short, with positive charges at the flanking regions[5]
Transport machinery of chloroplast of *Cyanidioschizon merolae*

*Cyanidioschizon merolae* is the most primary red alga and possesses the versatile chloroplast transport machinery.

There are only
2 Toc proteins in TOC complex
4 Tic proteins in TIC complex[6]
Frame Shift

- The adapter of reporter protein:
  GAATTC GCGGCCGC T TCTAGA [ATG ... AATAA] ACTAGT A GCGGCCG CTGCAG
The adapter of signal peptides:
  GAATTC GCGGCCGC T TCTAG [ATG ... TAATAA] TACTAGT A GCGGCCG CTGCAG
When the reporter gene and signal peptide were linked together, the fusion locus is:
  GAATTC GCGGCCGC T TCTAG [part1] TACTAGAG [part2] TACTAGT A GCGGCCG CTGCAG

- No frameshift and stop codon should be found. While accidentally “GA” was found in BioBricks backbone of RFP by sequencing.
References


YAO SENSOR
REDOX STATUS BIOSENSOR
I. Transportation of REX into Mitochondrial Matrix

- The transcription of SP-REX is constant, SP-REX concentration in cytoplasm is constant, the probably for REX to be transported into each mitochondrion is equal, and mitochondria are of similar size. So with different number of mitochondria in yeast (Nm), the concentration of REX in each mitochondria (Ctf) might differ. Ksp is the transport coefficient of signal peptid which links the XR/Nm with Ctf. For different kinds of signal peptid, Ksp might be different.

\[
C_{\text{rex}} = \frac{K_{sp} \cdot X_{SR}}{Nm} \quad (1)
\]

II. Activation of REX as a Transcription Factor with NAD+ and NADH

\[ \frac{K_h}{REXH} = \frac{REX \cdot H}{REX} \]

\[ \frac{K_n}{REXN} = \frac{REX \cdot N}{REX} \]

\[ REX + REXH + REXN = C_{rex} \]

\[ REX' = REXN + REX \]

\[ R_f = \frac{REX'}{C_{rex}} \]

\[ R_f = 1 - \frac{1}{1 + \frac{K_h}{H} + \frac{K_n}{N}} \]

Figure 1. Rf responding to different NAD+/NADH ratio. For plotting of this picture the following parameters were used: assuming K_H = 0.02 μM and K_N = 2 μM since REX NADH affinity > REX NAD affinity [2].
III. Control of promoter activity by REX

\[ K_{rp} = \frac{RP \cdot P}{PRP} \]
\[ K_{if} = \frac{P \cdot IF}{PIF} \]
\[ RP = REX = C_{rex} \cdot R_f \]

\[ P_f = \frac{P'}{PIF + PRP + P} \]
\[ P_f = \frac{\alpha \cdot IF + \beta \cdot \left( \frac{RP}{K_{rp}} + 1 \right)}{K_{if} + \frac{RP}{K_{rp}} + 1} \]
\[ P_f = \frac{\alpha \cdot IF + \beta \cdot \left( \frac{C_{rex} \cdot R_f}{K_{rp}} + 1 \right)}{K_{if} + \frac{C_{rex} \cdot R_f}{K_{rp}} + 1} \]

Figure 2. Pf with different β and changing NAD+/NADH ratio. X axis: NAD+/NADH ratio, range from 0 to 500, Y axis: β, range from 0 to 0.2, Z axis: Pf.

Figure 3. Pf with different Crex and changing NAD+/NADH ratio. X axis: NAD+/NADH ratio, range from 0 to 500, Y axis: Crex, range from 0mM to 5mM, Z axis: Pf.

Figure 4. Pf with different NAD+/NADH ratio. β=0.05, Crex= 2mM was used to plot this picture.
IV. Synthesis and degradation of mRNA

\[
\frac{dX_{mRNA}}{dt} = V_{mRNA} - K_{dm} \cdot X_{mRNA}
\]

\[
V_{mRNA} = V_{\text{max}}^{mRNA} \cdot N_p \cdot P_f
\]

\[
\frac{dX_{mRNA}}{dt} = V_{\text{max}}^{mRNA} \cdot N_p \cdot P_f - K_{dm} \cdot X_{mRNA}
\]

\[
X_{mRNA} = \frac{V_{\text{max}}^{mRNA} \cdot N_p \cdot P_f}{K_{dm}}
\]

Figure 5. XmRNA with different NAD+/NADH ratio. $\beta = 0.05, V_{\text{max}} = 180 \text{ U/h}$ (assuming 75 bases per second, and 1500 bases in gene), $N_p = 10$ (1 micron plasmid is used), $K_{dm} = 8.3 \text{ h}^{-1}$ (half life = 5 min) was used to plot this picture.
V. Synthesis, dilution and degradation of GFP

\[
\frac{dX_{GFP}}{dt} = V_{GFP} - (K_{dGFP} + \mu) \cdot X_{GFP}
\]

\[
\frac{dX_{GFP}}{dt} = K_p \cdot N_{rib} \cdot X_{mRNA} - (K_{dGFP} + \mu) \cdot X_{GFP}
\]

\[
X_{GFP} = \frac{K_p \cdot N_{rib} \cdot X_{mRNA}}{K_{dGFP} + \mu}
\]

Figure 6. XGFP with different $\beta$ and changing NAD+/NADH ratio. X axis: NAD+/NADH ratio, range from 0 to 500, Y axis: $\beta$, range from 0 to 0.2, Z axis: XGFP unit.

Figure 7. XGFP with different Crex and changing NAD+/NADH ratio. X axis: NAD+/NADH ratio, range from 0 to 500, Y axis: Crex, range from 0mM to 5mM, Z axis: XGFP unit.

Figure 8. XGFP with different NAD+/NADH ratio.
Response for Dynamic states

Figure 9. Dynamic change of NAD+/NADH

Figure 10. Response in GFP production
YAO SUICIDER
HOLIN BASED BIOSAFETY DEVICE
Holin to eukaryon

L-holin protein locates in the ER and in mitochondria of eukaryotic cells.

Immunofluorescence laser scanning microscopy and subcellular fractionation

Organelle-specific marker proteins calnexin and cytochrome c oxidase IV
Holin to eukaryon

Mitochondria were dilated, rounded or damaged; a substantial number of double membrane-bound vesicles.
T7 promoter & T7 RNAP links NU and MT
References


YAO.FACTORY
MODEL FOR FPP PATHWAY
Modeling of MVA Pathway in YAO

- For acetoacetyl-CoA thiolase, the reaction is
  \[ [AcCoA] + [AcCoA] + [H_2O] = [AcacCoA] + [CoASH] \]

  The rate expression is defined as
  \[ J_{ACAT} = \frac{V_{\text{max}} [AcCoA]^2}{K_{mA} K_{iA} + 2 * K_{mA} [AcCoA] + [AcCoA]^2} \]

  Table 1. Parameters for acetoacetyl-CoA thiolase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value (mM)</th>
<th>Organism</th>
</tr>
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<tbody>
<tr>
<td>K_{mA}</td>
<td>binding constant of AcCoA</td>
<td>0.33</td>
<td>Zoogloea ramigera</td>
</tr>
<tr>
<td>K_{iA}</td>
<td>Inhibit constant of AcCoA</td>
<td>0.0014</td>
<td>Rattus norvegicus</td>
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</table>

- For 3-hydroxy-3-methylglutaryl coenzyme A synthase, the reaction is
  \[ [AcacCoA] + [AcCoA] + [H_2O] = [HMG - CoA] + [CoASH] \]

  The rate expression is defined as
  \[ J_{HMS} = \frac{V_{\text{max}} [AcacCoA][AcCoA]}{K_{mA} [AcacCoA] (1 + \frac{[AcacCoA]}{K_{IB}} + \frac{[CoA]}{K_{IC1}}) + K_{mA} [AcCoA] (1 + \frac{[CoA]}{K_{IC2}}) + [AcacCoA] [AcCoA]} \]

  Table 2. Parameters for 3-hydroxy-3-methylglutaryl coenzyme A synthase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value (mM)</th>
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<td>0.0014</td>
<td>Rattus norvegicus</td>
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</table>
• For 3-hydroxy-3-methylglutaryl coenzyme A synthase, the reaction is

\[ [\text{AcacCoA}] + [\text{AcCoA}] + [\text{H}_2\text{O}] = [\text{HMG} - \text{CoA}] + [\text{CoASH}] \]

The rate expression is defined as

\[
J_{\text{HMGS}} = \frac{V_{\text{max}} [\text{AcacCoA}][\text{AcCoA}]}{K_m\text{A}[\text{AcacCoA}]\left(1 + \frac{[\text{AcacCoA}]}{K_i\text{B}} + \frac{[\text{CoA}]}{K_i\text{C}_1}\right) + K_m\text{B}[\text{AcCoA}]\left(1 + \frac{[\text{CoA}]}{K_i\text{C}_2}\right) + [\text{AcacCoA}][\text{AcCoA}]}
\]

Table 2. Parameters for 3-hydroxy-3-methylglutaryl coenzyme A synthase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value (mM)</th>
<th>Organism</th>
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<tr>
<td>$K_m\text{A}$ (mM)</td>
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<tr>
<td>$K_m\text{B}$ (mM)</td>
<td>binding constant for AcacCoA</td>
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<td>$K_i\text{B}$ (mM)</td>
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</tr>
<tr>
<td>$K_i\text{C}_1$ (mM)</td>
<td>inhibit constant for CoA on AcCoA</td>
<td>0.038</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>$K_i\text{C}_2$ (mM)</td>
<td>Inhibit constant for CoA on AcacCoA</td>
<td>0.06</td>
<td>Saccharomyces cerevisiae</td>
</tr>
</tbody>
</table>

• For HMG-CoA Reductase, the reaction is

\[ [\text{HMG} - \text{CoA}] + [\text{NADPH}] + [\text{H}^+] = [\text{CoASH}] + [\text{NADP}^+] + [\text{MVA}] \]

The rate expression is defined as

\[
J_{\text{HMGR}} = \frac{V_{\text{max}} [\text{HMG} - \text{CoA}][\text{NADPH}]}{K_m\text{A}[\text{NADPH}] + K_m\text{B}[\text{HMG} - \text{CoA}] + [\text{HMG} - \text{CoA}][\text{NADPH}]} \]

Table 3. Parameters for HMG-CoA Reductase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value (mM)</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m\text{A}$</td>
<td>binding constant for HMG-CoA</td>
<td>0.045</td>
<td>Sulfolobus solfataricus</td>
</tr>
<tr>
<td>$K_m\text{B}$</td>
<td>Binding constant for NADPH</td>
<td>0.023</td>
<td>Sulfolobus solfataricus</td>
</tr>
</tbody>
</table>
• For mevalonate kinase, the reference reaction is

\[ [MVA] + [ATP] = [MVA-5P] + [ADP] \]

The rate expression is defined as

\[ J_{MK} = \frac{V_{\text{max}} [MVA][ATP]}{K_{mA}[ATP] + K_{mB}(1 + \frac{[ADP]}{K_{iADP}})[MVA] + [ATP][MVA]} \]

Table 4. Parameters for mevalonate kinase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value(mM)</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_{mA}</td>
<td>binding constant for MVA</td>
<td>0.0408</td>
<td>Homo sapiens</td>
</tr>
<tr>
<td>K_{mB}</td>
<td>binding constant for ATP</td>
<td>7.4</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>K_{iADP}</td>
<td>inhibit constant for ADP</td>
<td>2.7</td>
<td>Enterococcus faecalis</td>
</tr>
</tbody>
</table>

• For phosphomevalonate kinase, the reference reaction is

\[ [MVA-5P] + [ATP] = [MVA-5PP] + [ADP] \]

The rate expression is defined as

\[ J_{PMK} = \frac{V_{\text{max}} [MVA-5P][ATP]}{K_{mA}[ATP] + K_{mB}(1 + \frac{[ADP]}{K_{iADP}})[MVA-5P] + [ATP][MVA-5P]} \]

Table 5. Parameters for phosphomevalonate kinase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value(mM)</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_{mA}</td>
<td>binding constant for MVA-5P</td>
<td>0.034</td>
<td>Homo sapiens</td>
</tr>
<tr>
<td>K_{mB}</td>
<td>binding constant for ATP</td>
<td>0.107</td>
<td>Homo sapiens</td>
</tr>
<tr>
<td>K_{iB}</td>
<td>inhibit constant for ATP</td>
<td>0.137</td>
<td>Streptococcus pneumoniae</td>
</tr>
<tr>
<td>K_{iADP}</td>
<td>Inhibit constant for ADP</td>
<td>0.41</td>
<td>Streptococcus pneumoniae</td>
</tr>
</tbody>
</table>
• For mevalonate pyrophosphate decarboxylase, the reference reaction is

\[ [MVA-5PP] + [ATP] = [ADP] + [Pi] + [IPP] + [CO_2] \]

The rate expression is defined as

\[ J_{DMD} = \frac{V_{\text{max}} [ATP][MVA-5PP]}{K_{mA}[ATP] + K_{mB}[MVA-5PP] + [ATP][MVA-5PP]} \]

Table 6. Parameters of mevalonate pyrophosphate decarboxylase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value (mM)</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_{mA}</td>
<td>binding constant for MVA-5PP</td>
<td>0.123</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>K_{mB}</td>
<td>binding constant for ATP</td>
<td>0.061</td>
<td>Saccharomyces cerevisiae</td>
</tr>
</tbody>
</table>

• For isopentenyl diphosphate: dimethylallyl diphosphate isomerase, the reaction is

\[ [I-PP] = [DMA-PP] \]

The rate expression is defined as

\[ J_{IDI} = \frac{V_{\text{max}} [IPP]}{K_m + [IPP]} \]

Table 5. Parameters for isopentenyl diphosphate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value (mM)</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>K_m</td>
<td>Binding constant for IPP</td>
<td>0.035</td>
<td>Saccharomyces cerevisiae</td>
</tr>
</tbody>
</table>
• For farnesyl diphosphate synthase, the first reaction is:

\[ [DMA-PP] + [I-PP] = [GPP] + \text{[diphosphate]} \]

The rate expression is defined as

\[ J_{ERG20-1} = \frac{V_{max} [DMA-PP] [I-PP]}{K_{mA} [I-PP] + K_{mB} [DMA-PP] + [DMA-PP] [I-PP]} \]

The second reaction is:

\[ [I-PP] + [GPP] = [FPP] + \text{[diphosphate]} \]

The rate expression is defined as

\[ J_{ERG20-2} = \frac{V_{max} [I-PP] [GPP]}{K_{mB} [GPP] + K_{mC} [I-PP] + [GPP] [I-PP]} \]

**Table 7. Parameters for the ERG20**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value (mM)</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_{mA})</td>
<td>binding constant for DMA-PP</td>
<td>0.009</td>
<td>Abies grandis</td>
</tr>
<tr>
<td>(K_{mB})</td>
<td>binding constant for I-PP</td>
<td>0.0018</td>
<td>Abies grandis</td>
</tr>
<tr>
<td>(K_{mC})</td>
<td>binding constant for GPP</td>
<td>0.0153</td>
<td>Abies grandis</td>
</tr>
</tbody>
</table>
We set the concentration of Actyl-CoA to 1000μM, and consider it as a constant. For simplicity, we assume the concentration of Actyl-CoA is the same in YAO and cytoplasm.

The concentration of ATP, ADP and NADPH is set to 1500μM, 1500μM and 100μM respectively and considered as constants. Concentrations of all other metabolite are set to 0 in the beginning.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Turnover rate(1/s)</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetoacetyl-CoA thiolase</td>
<td>Acetyl-CoA</td>
<td>2.1</td>
<td>Zoogloea ramigera</td>
</tr>
<tr>
<td>3-hydroxy-3-methylglutaryl coenzyme A synthase</td>
<td>Acetyl-CoA</td>
<td>0.0667</td>
<td>Gallus gallus</td>
</tr>
<tr>
<td>hydroxymethylglutaryl-CoA reductase</td>
<td>hydroxymethylglutaryl-CoA</td>
<td>0.023</td>
<td>Rattus norvegicus</td>
</tr>
<tr>
<td>mevalonate kinase</td>
<td>(R)-mevalonate</td>
<td>21.9</td>
<td>Rattus norvegicus</td>
</tr>
<tr>
<td>phosphomevalonate kinase</td>
<td>phosphomevalonate</td>
<td>10.2</td>
<td>Sus scrofa</td>
</tr>
<tr>
<td>mevalonate pyrophosphate decarboxylase</td>
<td>5-diphosphomevalonate</td>
<td>4.9</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td>isopentenyl diphosphate:dimethylallyl diphosphate isomerase</td>
<td>isopentenyl diphosphate</td>
<td>0.2</td>
<td>Sulfolobus shibatae</td>
</tr>
<tr>
<td>farnesyl diphosphate synthase</td>
<td>isopentenyl diphosphate</td>
<td>0.49</td>
<td>Mycobacterium tuberculosis H37Rv</td>
</tr>
<tr>
<td>farnesyl diphosphate synthase</td>
<td>geranyl diphosphate</td>
<td>0.2</td>
<td>Mycobacterium tuberculosis H37Rv</td>
</tr>
</tbody>
</table>
References


