Transcriptome analysis of the TnrA regulon in *Bacillus subtilis*

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Bacillus subtilis

- Bacillus means rod-shaped bacteria. The rod in the gut was *Bacillus coli* and the rod found in rotting hay was *Bacillus subtilis*
- Louis Pasteur used heat-attenuated *Bacillus anthracis* as the first anti-bacterial vaccine
- Subtilis means: highly sensitive – weak – mild (subtle)
- Soil bacteria typically found in association with plants
Bacillus subtilis

- *B. subtilis* has been used for studying different systems: gene regulation, metabolism and differentiation
- The second most extensively studied bacteria
- The first to be transformed in the laboratory. This process was optimized by Anagnostopoulos and Spizizen in 1961
Bacillus subtilis

- Fully sequenced and has around 4,225 predicted genes (SubtiList, R16.1)
- Non-hasardous
- Easy to grow and manipulate genetically
- Sporulates
- Secretes proteins
- Of extensive interest to industry
Glutamine synthetase

\[ \text{NH}_4^+ + \text{glutamate} \rightarrow \text{glutamine} \]
Access to nitrogen through alternative pathways

- \( \text{glnR} \)
- \( \text{glnA} \)
- \( \text{glnB} \)
- \( \text{gabP} \)
- \( \text{nasA} \)
- \( \text{pucR} \)
- \( \text{ys2B-ykoL} \)
- \( \text{nasABCDEF} \)
- \( \text{nrgAB} \)
- \( \text{ansZ} \)
- \( \text{yes-hip} \)
- \( \text{pucABCDE} \)

- \( \text{tnrA} \)
- \( \text{ureA} \)
- \( \text{ureB} \)
- \( \text{ureC} \)

GlnA - Glutamine Synthetase
Allantoin

Glutamate

Glutamine

TnrA

GlnA

GlnR

\( \text{NH}_4/\text{glutamate} \)

wildtype versus wildtype in different media

wildtype versus mutant in the same medium

wildtype versus mutant in the same medium

wildtype versus wildtype in different media
Purine catabolism
B. subtilis microarrays

- PCR products from ~97% of all genes
- spotted onto poly-L-lysine coated glass microscope slides using a 16-pin microarrayer
- Hybridized to Cy3 and Cy5 labeled first-strand cDNAs
- Scanned
NUMBERS:

2 different growth experiments
3 hybridizations (arrays) per culture
2 spots per gene on each array

$2 \times 3 \times 2 = 12$ intensities for each gene
Normalization

.... is it worth it?
Known positives versus the total number of significantly affected at 5 different cutoffs in the GlnA experiment.
RESULT: 6 lists

- Allantoin: 132
- Glutamine: 60
- Glutamate: 91
- TnrA: 239
- GlnA: 159
- GlnR: 67
Extraction - Visualization

- Extraction: The gene should be significantly affected in at least two of the six experiments (except “Glutamine”)

- List of ~100 genes highly likely to be involved in the TnrA-GlnA system

- Visualization: ClustArray
Opposite expression profile
– common TnrA regulated regulator?
What is the function of these genes?

- **yrbD**: sodium/proton-dependent alanine carrier
- **hxlA**: 3-hexulose-6-phosphate synthase
- **ywpH**: single-strand DNA-binding protein
- **ywfM**: unknown
- **yhdG**: amino acid transporter
- **dppA**: D-alanyl-aminopeptidase
- **ysnE**: similar to acetyltransferase
- **cotZ**: spore coat protein
TnrA box = TGT.A......T.ACA

- Built a Weight Matrix from known sites
- searched the upstream regions
- sorted and extracted
- clustered and displayed
Repressed by TnrA?

Repressed by GlnR?
TnrA activated genes?

**yrbD**: similar to sodium/proton-dependent alanine carrier protein

**yhdG**: similar to amino acid transporter

**yyaF**: similar to GTP-binding protein

*yrbD* and *yhdG* have the TnrA box in a plausible position

Experimental investigations
TnrA repressed genes?

yuiA: ??

yycB: similar to permease

yodF: similar to proline permease

hisI: phosphoribosyl-AMP cyclohydrolase/phosphoribosyl-ATP pyrophosphohydrolase

yocR: similar to sodium-dependent transporter

bioA: adenosylmethionine-8-amino-7-oxononanoate aminotransferase

Experimental investigations
GlnR repressed gene?

*braB:* branched-chain amino acid transporter

Experimental investigations

A GlnR knockout with a *braB-lacZ* fusion do have higher \( \beta \)-galactosidase activity than the wildtype

.... and two putative binding sites
ureP3

braB
Verified new discoveries:

**ureABC (urease operon)** is activated by PucR

<table>
<thead>
<tr>
<th>ACTIVITY OF yhdG</th>
<th>+ N</th>
<th>– N</th>
<th>allantoine</th>
<th>glutamine</th>
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<tr>
<td>wildtype:</td>
<td>700</td>
<td>3.000</td>
<td>700</td>
<td>700</td>
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<tr>
<td>TnrA-mutant:</td>
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<tr>
<td>GlnA-mutant :</td>
<td></td>
<td></td>
<td></td>
<td>2.000</td>
</tr>
<tr>
<td>PucR-mutant :</td>
<td>3.000</td>
<td>2.000</td>
<td></td>
<td></td>
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</tbody>
</table>

**yrbD and yhdG:**

activated by TnrA
repressed by PucR
...more verified new discoveries

- Competence is induced in glutamate medium
  = one-step transformation procedure

- A TnrA mutant has reduced competence
  ...only 2% of wildtype level

- TnrA somehow regulates the development of competence
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Today's exercise
inferring regulatory networks
<table>
<thead>
<tr>
<th>Gene</th>
<th>TnrA</th>
<th>GlnA</th>
<th>GlnR</th>
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</tr>
<tr>
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<tr>
<td>glnR</td>
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<td>1</td>
<td>1</td>
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