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-hazardous
- 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

- TnrA
- TnrA
- GlnR
- GlnR
- GlnA - Glutamine Synthetase

- ureA
- ureB
- ureC

- gltAB
- gabP
- nuaA
- pucR
- yklB-ykoL
- nasABCDEF
- nrgAB
- ansZ
- yes-hip
- pucABCDE

- glnR
- glnA
wildtype versus mutant in the same medium

wildtype versus wildtype in different media

Allantoin

Glutamate

Glutamine

TnrA

GlnA

GlnR

$\text{NH}_4/\text{glutamate}$
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 [γ]-galactosidase activity than the wildtype

... and two putative binding sites
ureP3

gagcgataaatagcagttttatattttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
Verified new discoveries:

*ureABC* (urease operon) is activated by PucR

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<th>+ N</th>
<th>- N</th>
<th>allantoine</th>
<th>glutamine</th>
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<td><strong>wildtype:</strong></td>
<td>700</td>
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<td><strong>TnrA-mutant:</strong></td>
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<td><strong>PucR-mutant:</strong></td>
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*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>
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<tr>
<th>Gene</th>
<th>TnrA</th>
<th>GlnA</th>
<th>GlnR</th>
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Redundant

A → B
C → D

Parsimonious

A → B
C → D