Regulation of *Clostridium difficile*

Toxin Gene Expression

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Relationships between \textit{C. difficile} sporulation and pathogenesis:

- Spores act as the reservoir of disease-causing organisms

- Germination in the GI tract is essential for pathogenesis

- Toxins A and B are only synthesized during stationary phase/sporulation
Motility and chemotaxis
Secretion of degradative enzymes
Transport of secondary nutrients
Intracellular catabolic pathways
Genetic competence
Antibiotic and toxin production

Sporulation
Figure 2  The stages of sporulation and germination.

R. Losick and P. Youngman
Transcriptional regulation during sporulation

Spo0A~P - a major transcription factor for early sporulation genes

RNA polymerase sigma factors - dissociable subunits that direct RNA polymerase to specific promoter sites
Regulation of Sporulation Gene Expression in *Bacillus subtilis*
RNA polymerase Sigma Factors of \textit{C. difficile}

$\sigma^{70}$ Family

Primary $\sigma^A, \sigma^A$-like

Sporulation $\sigma^H, \sigma^F, \sigma^E, \sigma^G, \sigma^K$

Alternative

Stress $\sigma^B$

Toxin gene expression TcdR

ECF $\sigma^W, \sigma^V, \sigma^X$

$\sigma^{54}$ Family $\sigma^L$
Toxin Gene Locus (PaLoc) in *C. difficile*

- **PtcdR**
- **PtcdB**
- **PtcdA**

Gene products: **tcdR**, **tcdB**, **tcdE**, **tcdC**, **tcdA**

- **tcdB**: Toxin B
- **tcdE**: Holin?
- **tcdA**: Toxin A
TcdR is a sigma factor for toxin gene transcription
C. difficile Paloc locus

C. botulinum toxin locus

C. perfringens bacteriocin locus

C. tetani toxin locus
Questions:

1) What is the metabolic signal for nutrient deprivation?

2) What regulatory protein senses this signal?

3) By what mechanism does this regulatory protein control expression of toxin genes?
CodY protein

- a dimeric DNA binding protein first discovered in *Bacillus subtilis*.

- Helix-Turn-Helix region (HTH), located between residues 202-223, is required for DNA binding.

- represses mostly strongly in vivo in cells growing in a complex medium or a medium containing glucose and a mixture of amino acids.

- homologs are found in almost all low G+C gram-positive bacteria.
CodY is activated additively by two different effectors
CodY Homologs

- Nearly ubiquitous in low G+C Gram-positive bacteria

- Strong conservation in putative motifs
Table 4. CodY HTH motifs

<table>
<thead>
<tr>
<th>Organism</th>
<th>Helix 1 Turn Helix 2</th>
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<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>ASKIADRVGITRSVIVNALR</td>
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<tr>
<td>Bacillus stearothermophilus</td>
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<tr>
<td>Bacillus halodurans</td>
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CodY represses \textit{tcdA-gusA} expression in \textit{B. subtilis}
Comparison of putative GTP binding motifs in CodY homologs.

<table>
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<th>Small GTPases</th>
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CodY

- **B. subtilis**
  - GGERLGLTL DRVG VLNNKFL
- **B. stearothermophilus**
  - GGERLGLTL DRVG VLNDKFL
- **B. halodurans**
  - GQQRLGLTL DRVG VLNDKFL
- **B. anthracis**
  - GGERLGLTL DRVG VLNDKFL
- **B. cereus**
  - GGERLGLTL DRVG VLNDKFL
- **S. aureus**
  - GGERLGLTL DRVG VKEKFL
- **S. epidermidis**
  - GGERLGLTL DRVG VKKDKFL
- **L. innocua**
  - GGERLGLTL DRVG VLNDKFL
- **L. monocytogenes**
  - GGERLGLTL DRVG VLNDKFL
- **C. hydrogenoformans**
  - GGQRLGLTL DRVG VLNDKFL
- **D. hafniense**
  - GGERVGLTL DRVG DLYNDLL
- **S. pneumoniae**
  - SGERGLGLSL DRIG VLSIDIF
- **S. equi**
  - GGMRLGLTL DRIG VINEGIF
- **S. mutans**
  - GGMRLGSL DRIG VINEGIF
- **S. pyogenes**
  - GGMRLGSL DRIG VINEGIF
- **E. faecalis**
  - AGKRLGTL DEIG VLNQQFI
- **L. lactis**
  - SGMLRLGTF DKIG VNTGLF
- **C. difficile**
  - SGQRLGLTL DRVG ILNDKLT
- **C. perfringens**
  - NGDRLGTL DKVG ILNEKLM
- **C. acetobutylicum**
  - NRRRLGTL DKVG ILNDRLL
- **C. botulinum**
  - NRRRLGTL DKVG ILNDKLK
Crystal Structure of CodY with Ile

V. Levdikov
E. Blagova
A. J. Wilkinson
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</table>
C. difficile CodY binds tcdR promoter

No effectors

2 mM GTP

10 mM ILV

2 mM GTP + 10 mM ILV
A Model for Toxin Regulation in *C. difficile*

- **GTP**: Influences CodY
- **Ile-Val**: Activates PtcdR
- **PtcdR**: Activates tcdR
- **PtcdB**: Activates tcdB
- **PtcdA**: Activates tcdA
- **TcdR**: Activates TcdR
- **tcdE**: Activates tcdC

**CodY**

**GTP**

**Ile-Val**

**PtcdR**

**PtcdB**

**PtcdA**

**tcdR**

**tcdB**

**tcdA**

**tcdC**

**TcdR**
Is TcdC an Inhibitor of TcdR?
CodY interacts with the *tcdC* regulatory region

<table>
<thead>
<tr>
<th></th>
<th>no effectors</th>
<th>+ 2 mM GTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ 10 mM BCAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ 2mM GTP, 10 mM BCAA</td>
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</table>

[Graph showing the interaction of CodY with the tcdC regulatory region under different conditions]
A Model for Toxin Regulation in *C. difficile*

- **CodY**
  - GTP
  - Ile-Val

- **PtcdR**
- **PtcdB**
- **PtcdA**
  - **tcdE**
- **PtcdC**

- **tcdR**
- **tcdB**
- **tcdA**
- **tcdC**

- **TcdR**
- **TcdC**

- **?**
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