Structures and functions of 
*Clostridium difficile* TcdA and TcdB

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Clostridium difficile Toxin B causes epithelial cell necrosis through an autoprocessing- and glucosyltransfer-independent mechanism

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Mechanism of Host Cell Entry

- **TcdA**
  - Glucosyl Transferase
  - Cysteine Protease
  - Delivery
  - CROPs

- **TcdB**

- **InsP6**
X-ray crystal structures of individual functional domains
EM structures of TcdA at neutral and low pH

The TcdA holotoxin at neutral and low pH
Future directions for structure

Mechanism of action
Antibody recognition
Structure based drug design
Two enzymes that can be targeted from small molecule inhibitor discovery
Can we identify small molecule inhibitors of discrete steps in the mechanism of action?
Can we use caspase-3/7 activation as an indicator of TcdB-induced apoptosis?
No caspase 3/7 activation but cells are rounding at 10 pM and exploding at 10 nM
TcdB induces necrosis as judged by loss of cellular ATP and release of LDH.
Generating an autoprocessing deficient mutant
Autoprocessing mutants are cytotoxic
TcdB autoprocessing mutants are delayed in cytopathic effects
TcdB and TcdB autoprocessing mutants have the same cytotoxicity kinetics.
Mutants are deficient in autoprocessing in cells.

Is the glucosyltransferase activity required?
GTD mutants are deficient in GT activity *in vitro*.
TcdB point mutants with defects in glucosyltransferase activity

A

<table>
<thead>
<tr>
<th></th>
<th>TcdB</th>
<th>D270N</th>
<th>D270A</th>
<th>Y284A</th>
<th>W520A</th>
<th>N384A</th>
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B

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<thead>
<tr>
<th></th>
<th>TcdB 100nM</th>
<th>D270N 100nM</th>
<th>TcdB 500nM</th>
<th>D270N 500nM</th>
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<tr>
<td>UDP-glucose (μM)</td>
<td>20 40 80</td>
<td>20 40 80</td>
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C

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<th>TcdB</th>
<th>D270N</th>
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<th>Mock</th>
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<td>Unglucosylated Rac1</td>
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<td>Total Rac1</td>
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<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
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</tbody>
</table>
Glucosyltransferase domain mutants are cytotoxic
What toxin concentration is physiologically relevant?

Ryder A. B., et. al. Assessment of Clostridium difficile infections by quantitative detection of tcdB toxin by use of a real-time cell analysis system. JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2010, p. 4129–4134 Vol. 48, No. 11

<table>
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<tr>
<th># Toxin Positive specimens</th>
<th>CDI severity</th>
<th>Minimum TcdB Conc.</th>
<th>Maximum TcdB Conc.</th>
<th>Mean TcdB Conc.</th>
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<tr>
<td>9</td>
<td>None</td>
<td>0.1 pM</td>
<td>3.3 pM</td>
<td>1 pM</td>
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<td>10</td>
<td>Mild to severe</td>
<td>4.9 pM</td>
<td>413 pM</td>
<td>146 pM</td>
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</table>

What concentration of toxin is required to cause a lesion in the colon?
TcdB and C698A cause epithelial damage to porcine colonic explants
Conclusions

• TcdB induces necrosis in epithelial cells.
• The autoprocessing and glucosyltransferase activities are not required for TcdB induced epithelial cell necrosis.
• Cytotoxicity and cytopathicity are distinct phenomena in the cell.
• The concentrations required to cause cytotoxicity in tissue culture correlate with concentrations that cause damage in tissue explants and with concentrations expected in people experiencing CDI.
Questions for future study

• What is the mechanism of TcdB mediated necrosis?

• Why are the autoprocessing and glucosyltransferase activities maintained?

• Are the autoprocessing and glucosyltransferase domains good therapeutic targets?

• Are glucosyltransferase-deficient mutants safe for vaccine development?
Acknowledgments

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NIH NIAID
The cysteine protease domain of TcdA

- Cysteine protease domain
- Glucosyl transferase domain
- Pore-forming domain
- Binding domain

**Gels:**
- InsP6
  - 37 kDa
  - 30 kDa
  - 7 kDa

**Table:**

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<th></th>
<th>wt</th>
<th>c700s</th>
<th>h655a</th>
<th>d589n</th>
<th>d590n</th>
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X-ray crystal structures of individual functional domains

The proposed GTPase binding surface differs in TcdA

Pruitt RN, et. al.
X-ray crystal structures of individual functional domains

TcdA

TcdB

Glucosyl Transferase
Cysteine Protease
Delivery
CROPs
X-ray crystal structures of individual functional domains

TcdA

TcdB

Glucosyl Transferase
Cysteine Protease
Delivery
CROPS
Structural homology between the cysteine protease domains of TcdA and TcdB


CROPs: Combined repetitive oligopeptides

CROPs: Combined repetitive oligopeptides

The TcdA CROPs bind carbohydrates

InsP6 binds a positively charged pocket
Defining the GTPase binding surface of the TcdB glucosyltransferase domain

Several *C. difficile* strains have mutations in the TcdB GTD that allow them to modify Ras family substrates

- TcdB (630) – RhoA, Rac1, Cdc42
- TcdB (8864) – Rac1, Cdc42, **Ral**, Rap, Ras
- TcdB (1470) – Rac1, Cdc42, **Ral**, Rap, Ras

**Variant TcdB GTDs**

Homology model of TcdB from strain 1470