Toxins of *Clostridium difficile*

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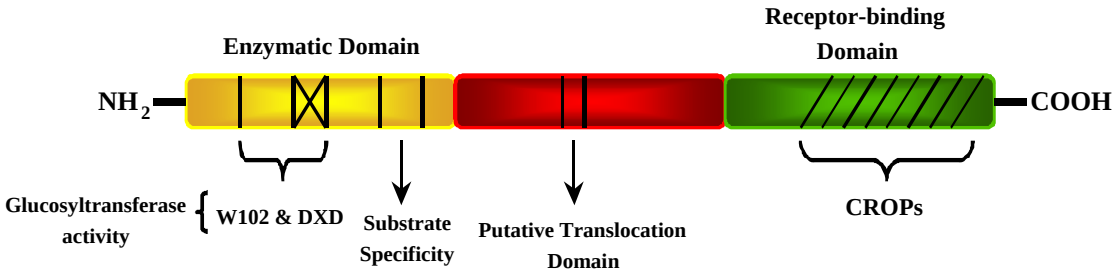
A Brief History

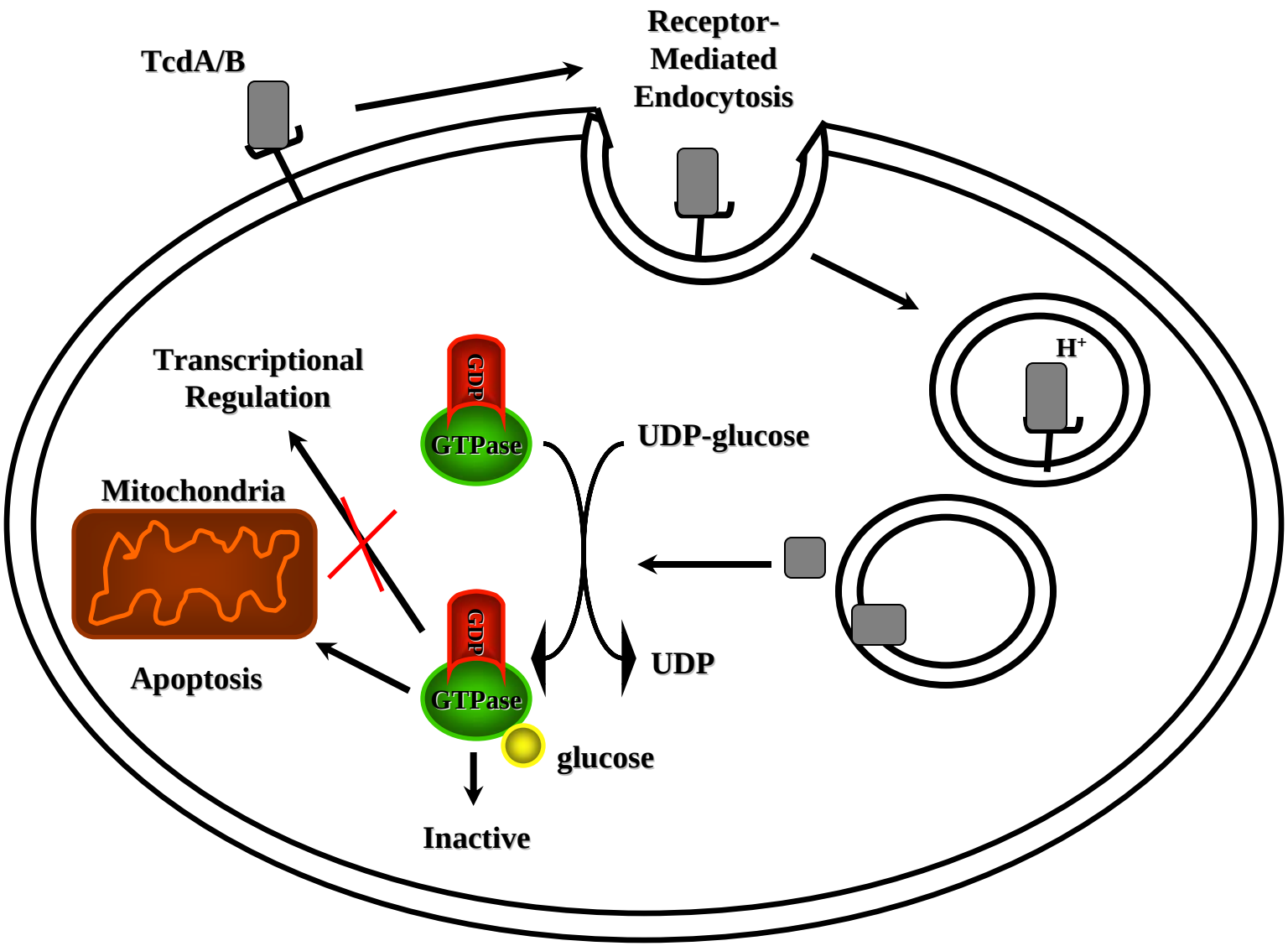
- Hall and O'Toole (1938) Intestinal Flora in New-Born Infants, *Am. J. Dis. Child.* 49:390-402
- Taylor et al. (1981) Comparison of two toxins produced by *Clostridium difficile*, *Infect. and Immun.* 34:1036-1043
- Banno et al. (1981) Two toxins (D-1 and D-2) of *Clostridium difficile* causing antibiotic associated colitis: purification and some characterization. *Biochem Int.* 2:629-635
- Barroso et al. (1990) Nucleotide sequence of *Clostridium difficile* toxin B gene. *Nucleic Acids Res.* 18:4004
- Dove et al. (1990) Molecular characterization of the *Clostridium difficile* toxin A gene. *Infect. and Immun.* 58:480-488
- Just et al. (1995) Glucosylation of Rho proteins by *Clostridium difficile* toxin B. *Nature* 375:500-503
- Reinert et al. (2005) Structural basis for the function of *Clostridium difficile* toxin B. J Mol. Biol. 351(5): 973-81

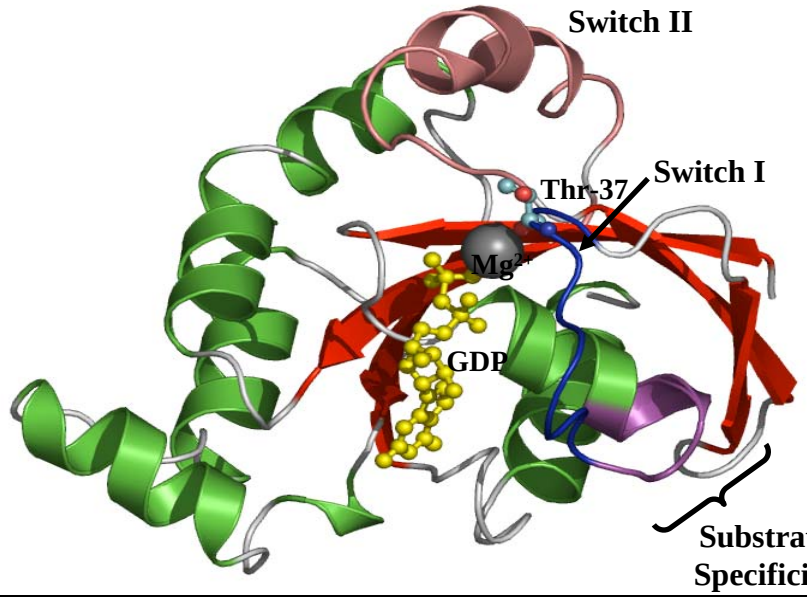
Toxin A (TcdA) and Toxin B (TcdB)

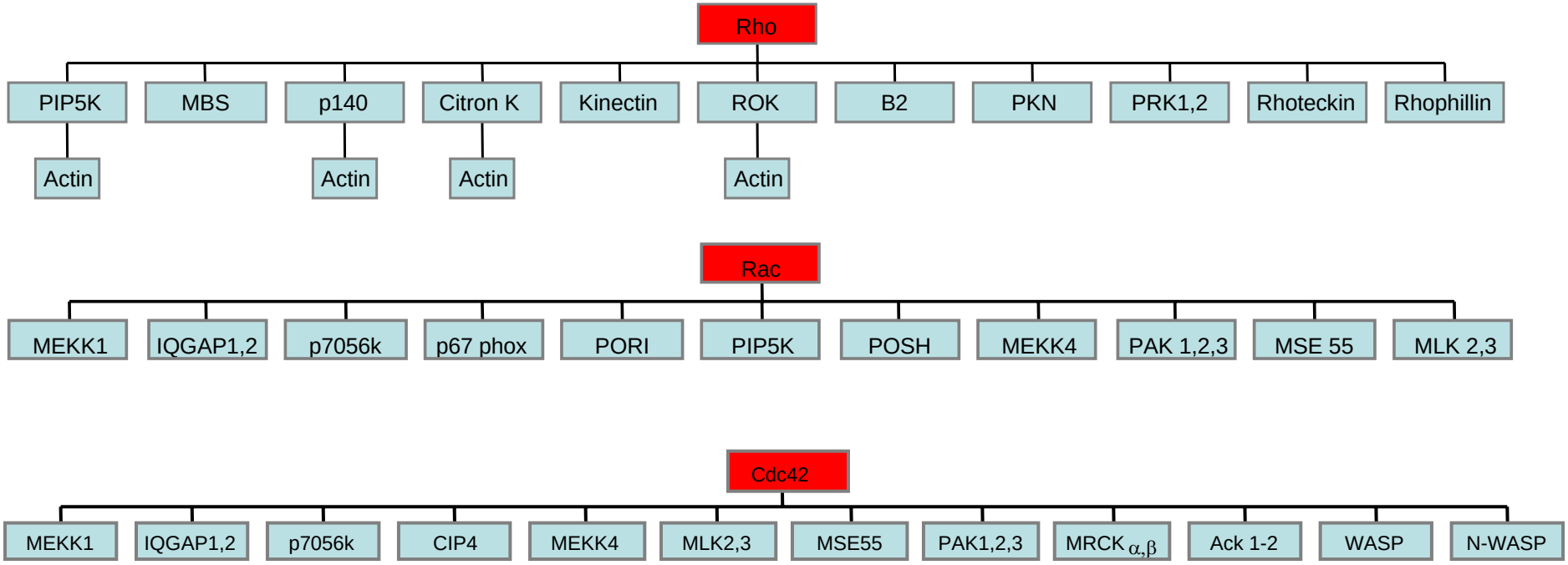
- Large Clostridial Toxins
  - TcdA ~ 308 kDa
  - TcdB ~ 270 kDa
- Intracellular Bacterial toxins
- Glucosyltransferases
  - Type A subfamily of glycosyltransferases
- Major virulence factors
  - Found in almost all clinically relevant isolates
  - Immunity to the toxins provides protection from CDAD



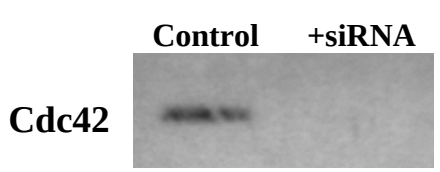
Mechanism of Cellular Intoxication

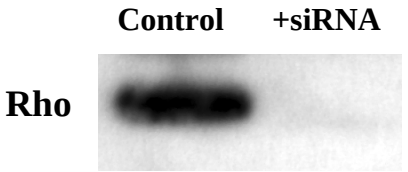


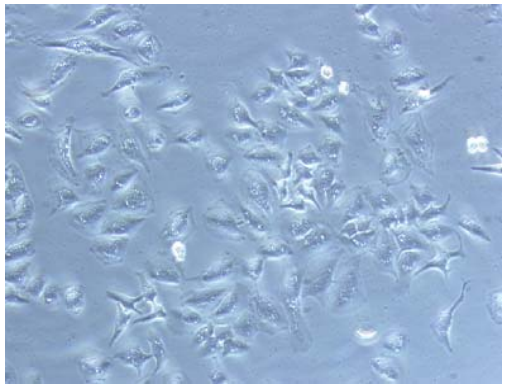

Switch II
Switch I
Thr-37
Mg^{2+}
GDP
Substrate
Specificity

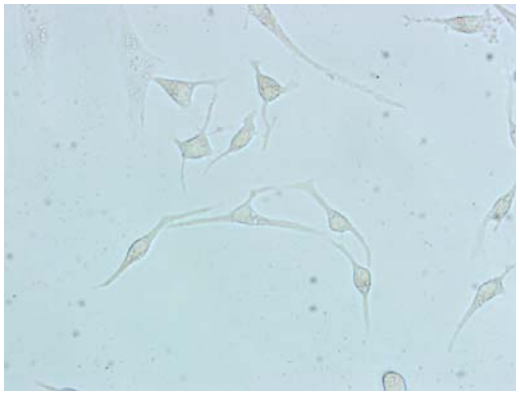

Rho
PIP5K
MBS
p140
Citron K
Kinectin
ROK
B2
PKN
PRK1,2
Rhoteckin
Rhophillin
Actin
Actin
Actin
Actin
Rac
MEKK1
IQGAP1,2
p7056k
p67 phox
PORI
PIP5K
POSH
MEKK4
PAK 1,2,3
MSE 55
MLK 2,3
Cdc42
MEKK1
IQGAP1,2
p7056k
CIP4
MEKK4
MLK2,3
MSE55
PAK1,2,3
MRCK α,β
Ack 1-2
WASP
N-WASP

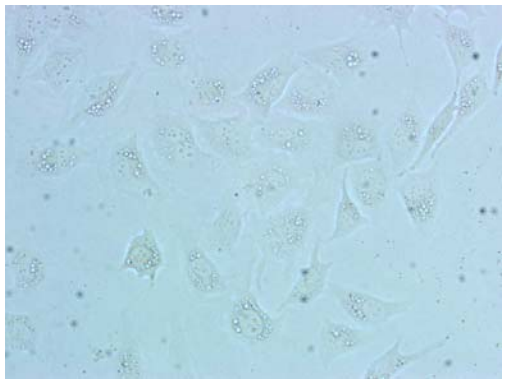
Relevance of Target Substrates











Effects of TcdA

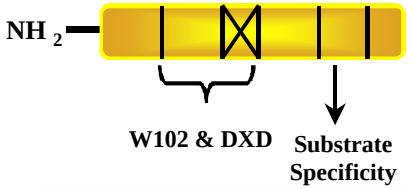
- In vitro (Cellular Level)
  - Cell Rounding
  - Caspase Activation (caspase 3, 6, 8 and 9)
  - Modulation of the mitochondria ?
  - MAPK activation (e.g. p38)
  - Apoptosis and Cell Death
  - Increased permeability of epithelial barrier
- In vivo (Host Level)
  - Lethal
  - Fluid accumulation in rabbit ileal loops
  - Neutrophil recruitment
  - Macrophages and Mast cells (TNF-α)
  - Reactive oxygen intermediates
  - Chemokines
  - Cytokine production (e.g. IL-8)
  - Neuronal Effects

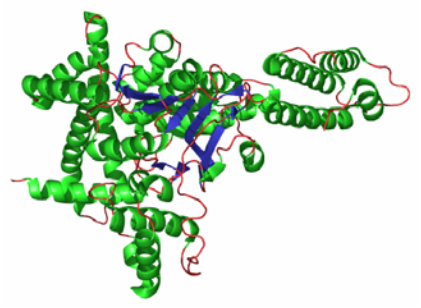
Effects of TcdB

- In vitro (Cellular level)
  - Intoxicates a wide variety of cell-types
    - fibroblasts, neuronal cells, epithelial cells, endothelial cells, lymphocytes, hepatocytes
  - Cell rounding (actinomorphic effects)
  - Modulation of intracellular signaling pathways
  - Caspase activation
  - Numerous effects related to Rho proteins
  - Disrupts tight junctions
- In vivo (Host level)
  - Lethality
  - Systemic effects?
  - Enterotoxic?

Functional Domains

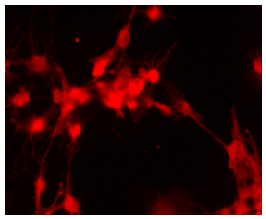
Enzymatic Domain



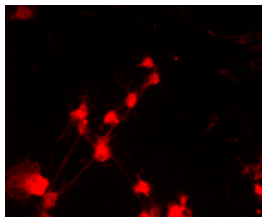


Reinert et al. (2005) J. Mol. Biol.

TcdB



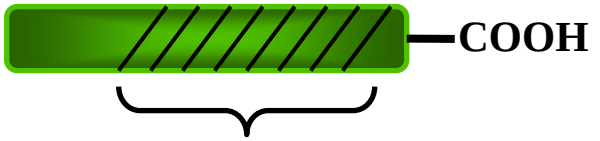
TcdB1-556



Spyres et al. (2001) Infection and Immunity

Translocation Domain

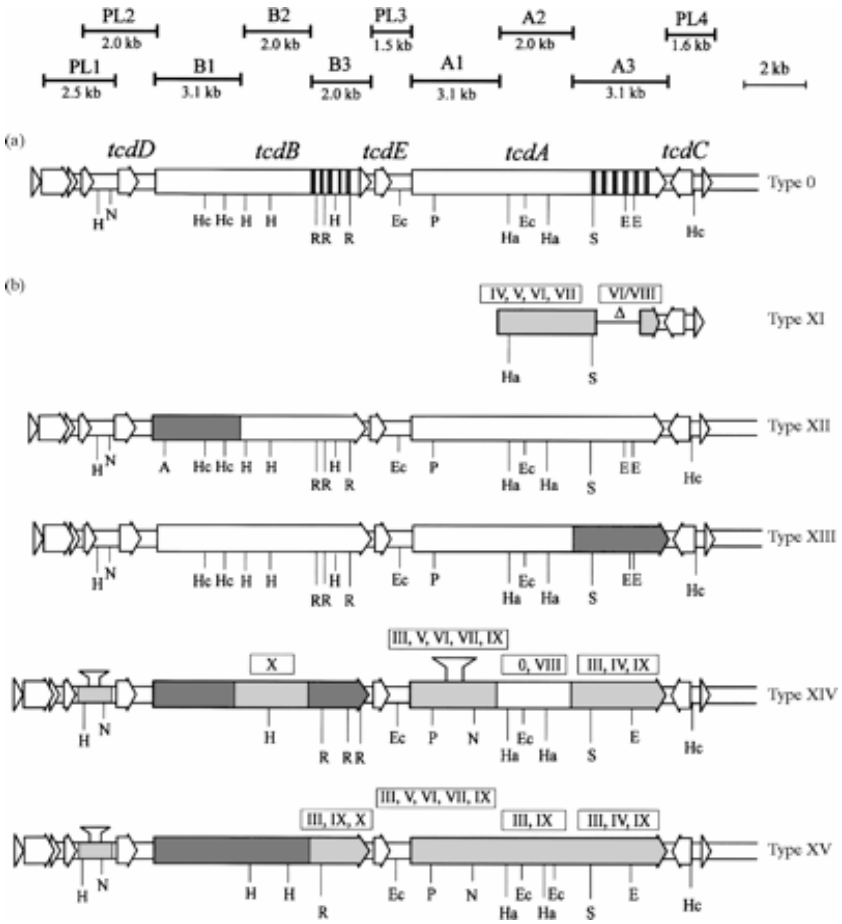
Receptor-binding Domain



CROPs

Greco et al. (2006) Nat. Struct. & Mol. Biol

Toxin Variants (toxinotypes)



$TcdA^+$, $TcdB^+$

$TcdA^-$, $TcdB^+$

$TcdA^-$, $TcdB^-$

Rupnik et al. (2001) Microbiology 147, 439-447

C. difficile Binary Toxin

CDTa

- Enzymatic
- ADP-Ribosyltransferase
- Modifies actin
- Similar to Ia

CDTb

- Entry Component
- Proteolytic activation
- Similar to Ib

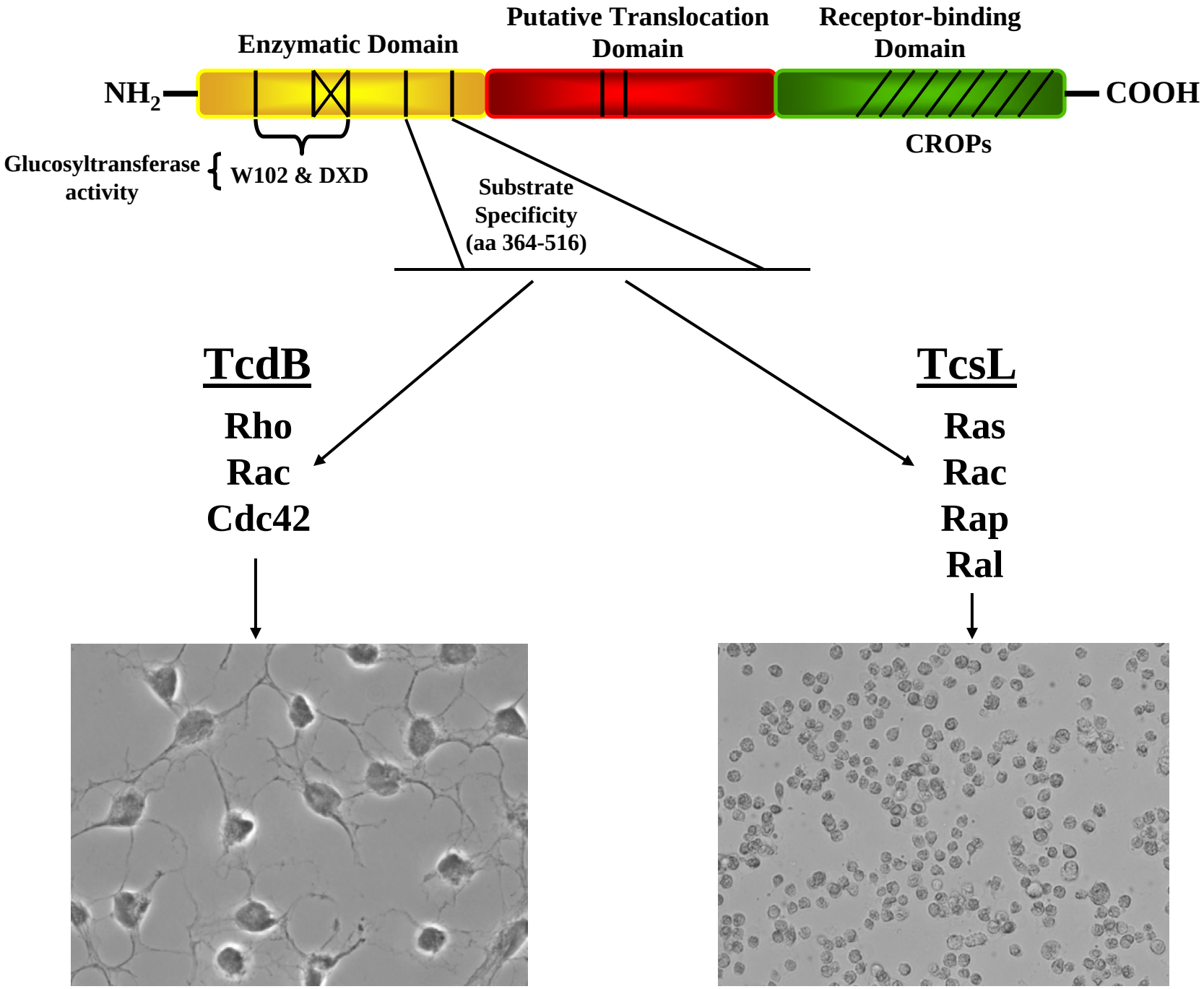
- Found in 6-12% of isolates
- Associated with recent epidemic strains
- Possible role in colonization
- Overall contribution to disease?

Large Clostridial Toxins (LCTs)

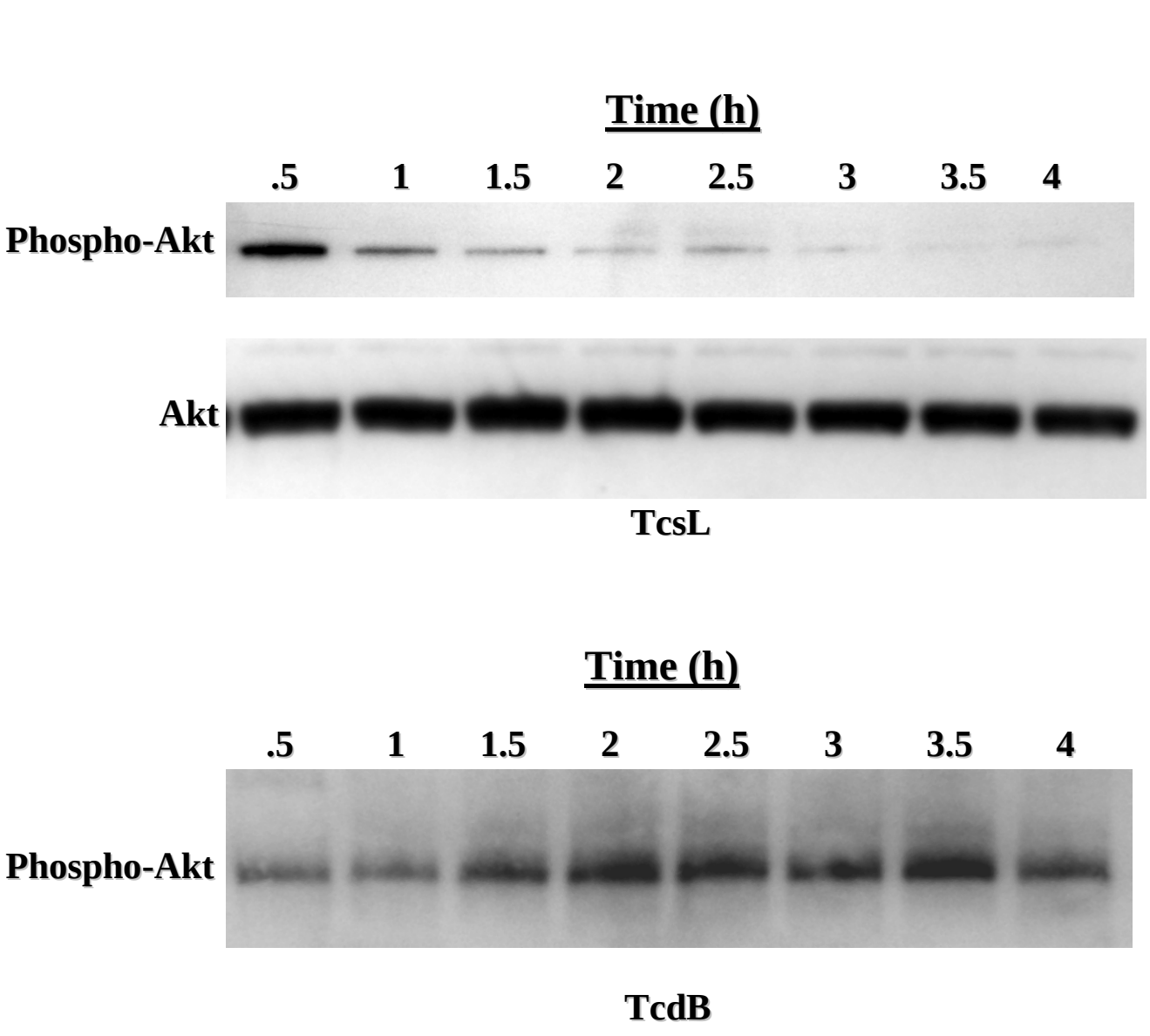
- **Five members**
  - ***C. difficile* TcdA (304 kDa)**
  - ***C. difficile* TcdB (280 kDa)**

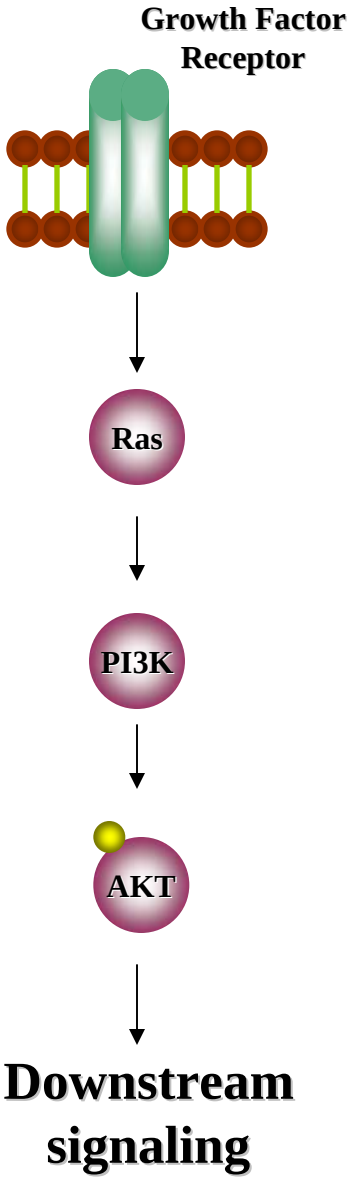
  - ***C. novyi* Tcnα (260 kDa)**

  - ***C. sordellii* TcsH (300 kDa)**
  - ***C. sordellii* TcsL (270 kDa)**

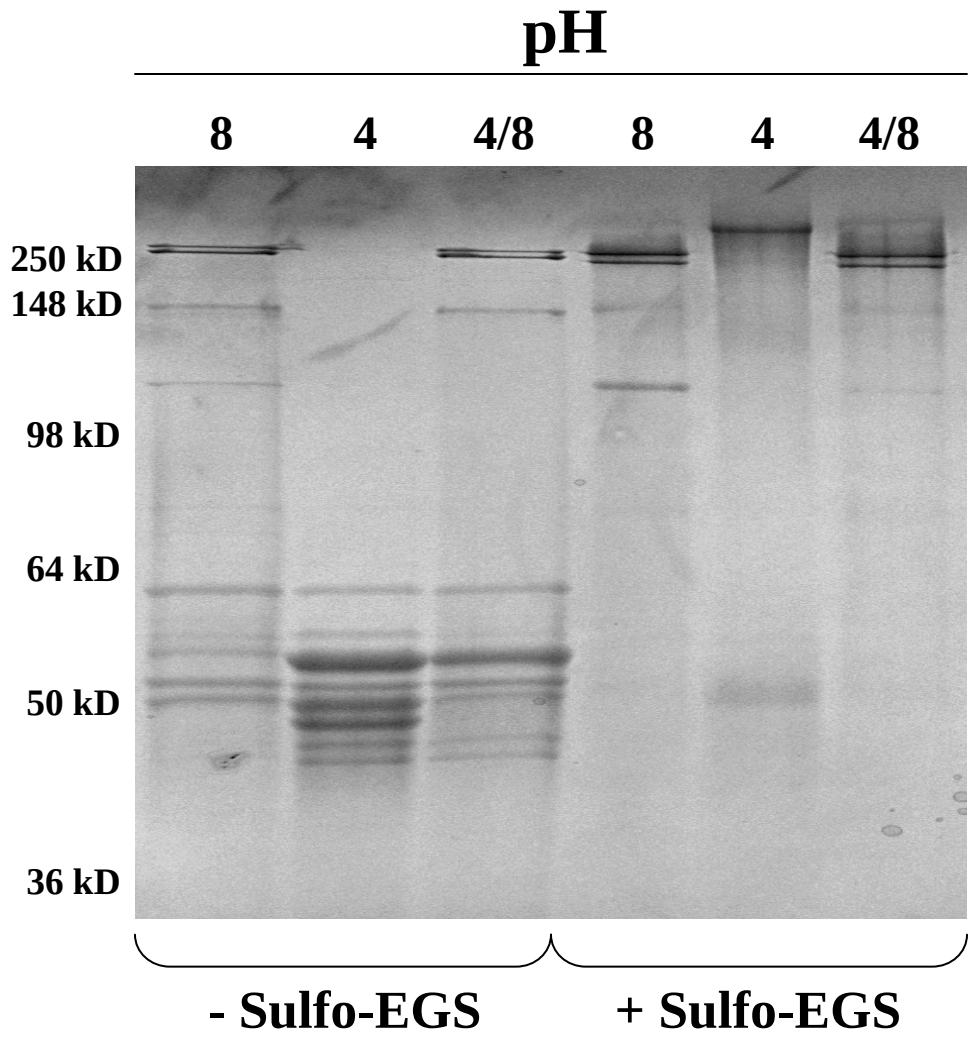

Enzymatic Domain
Putative Translocation Domain
Receptor-binding Domain
NH2
COOH
CROPs
Glucosyltransferase activity
W102 & DXD
Substrate Specificity (aa 364-516)
TcdB
Rho
Rac
Cdc42
TcsL
Ras
Rac
Rap
Ral

Akt Modulation is TcsL-Specific





pH-Induced Dissociation Profile of TcsL



4/8 = acid pH sample shifted back to neutral pH

(Voth, et al. 2004)

Do All Disease-Causing Isolates of *C. sordellii* Produce TcsL?

Ribotype & Strain	Characterization
Ribotype I	
ATCC9714	*tcsl*$^{+}$, *cdc*$^{+}$, TcsL^{+}, Neu^{+}, CDC^{-}, Csp^{+}, GEN^{I}, CLI^{R}, CIP^{I}
Ribotype II	
UMTB2	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{+}, Csp^{+}, CLI^{R}, CIP^{I}
UMC178	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{-}, Csp^{+}, CLI^{I}, CIP^{I}
Ribotype III	
UMTB1	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{+}, Csp^{+}, CLI^{I}
Ribotype IV	
UMC164	*tcsl*$^{+}$, *cdc*$^{+}$, TcsL^{+}, Neu^{+}, CDC^{-}, Csp^{+}, GEN^{I}, CLI^{R}, CIP^{I}, LVX^{I}
UMC193	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{-}, Csp^{+}, CLI^{R}
UMC212	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{-}, Csp^{+}, CIP^{I}
PS4423	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{-}, Csp^{-}
PS4475	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{+}, Csp^{+}
Ribotype V	
PS4401	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{+}, Csp^{+}, CLI^{R}, CIP^{I}
PS4404	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{+}, Csp^{+}, CLI^{I}, CIP^{I}
PS4422	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{-}, Csp^{+}, CIP^{I}
PS4451	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{-}, Csp^{+}, CLI^{R}, CIP^{I}
PS4477	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{-}, Csp^{+}, CLI^{I}, CIP^{I}
PS4490	*tcsl*$^{-}$, *cdc*$^{+}$, TcsL^{-}, Neu^{+}, CDC^{-}, Csp^{+}, CLI^{R}, CIP^{I}, TET^{I}

Strain	Source	*tcsl* PCR	Cytotoxic	Hemolytic
2005371	Blood	+	+	-
2005372	NA	-	-	-
2005373	Blood	+	+	-
2005374	Blood	+	+	-
2005375	Blood	-	-	-
2005376	Wound	+	ND	-
2006103	Liver	+	ND	-
2006104	Wound	-	-	-
2006105	Blood	+	ND	-
2006106	Blood	+	+	-
2006108	Wound	-	+	-
2006111	Blood	-	ND	-
2006115	Peritoneal fluid	-	-	+
2006116	Wound	-	+	-
2006117	Wound	+	+	-
2006119	Wound	-	+	-
2006120	Blood	-	-	-
2006123	Tissue	+	+	-
2006124	Tracheal aspirate	+	ND	-
2006125	Blood	-	-	-
2006127	Right toe	+	+	-
2006130	Blood	-	-	-
2006131	Wound	-	-	-
2006132	Abdomen	-	-	-
2006161	Cervical discharge	-	-	+

Questions

- What is the contribution of TcdB to CDAD? What are the specific in vivo effects of TcdB?
- What is the impact of increased expression of TcdA and TcdB during disease?
- What role does CDT play in CDAD?
- What is the overall profile of substrate targets in mammalian cells?
- Are there specific receptors for TcdA and TcdB?
- What is the contribution of TcsL (and TcsH) to *C. sordellii*-related illnesses?

Acknowledgements

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