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Clostridium difficile: an overview of the changes in our understanding the organism over the last 30 years

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Recognition of the pathogenic role of *Clostridium difficile*

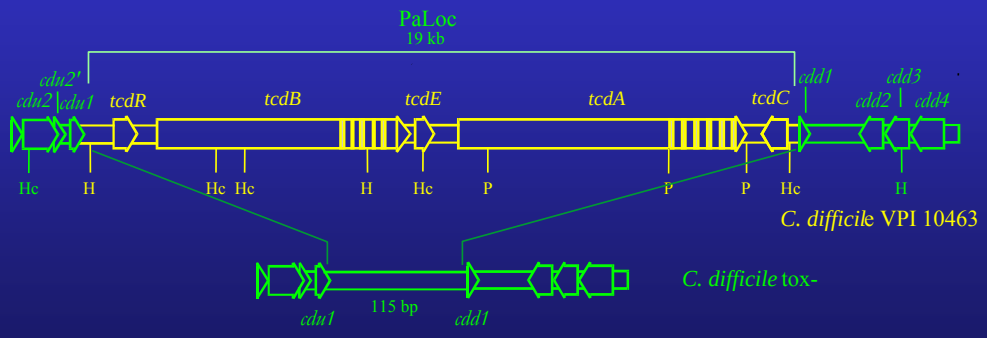
- 1977 First description that a *Clostridium* sp. caused AAD in hamsters (subsequently identified as *C. difficile*). Bartlett *et al*
- 1978 Identification of *C. difficile* as the cause of PMC in man *Bartlett et al*
- 1979 Development of a selective medium for *C.difficile*. George *et al*
- 1981 Demonstration that *C. difficile* produces two toxins. Banno *et al* and Taylor *et al*

- Disease
  - disease is toxin mediated
  - CDI is only a disturbance and easy to treat
  - importance of normal gut flora
    association with antibiotics
- Toxins and pathogenesis
  - two toxins are main virulence factors
  - synergistic action
- Epidemiology
  - *C. difficile* is human nosocomial pathogen
    (typing systems, diagnostics)

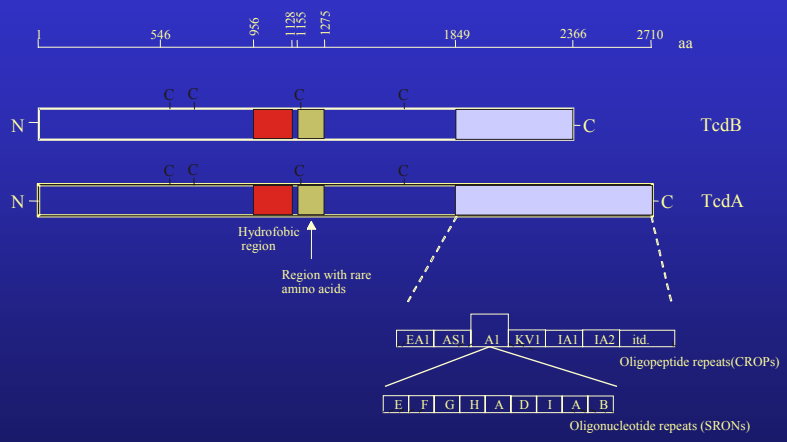
C. difficile toxins

- Toxin A and Toxin B
- purification
- cytotoxicity, effects on animals
- antibodies
- molecular biology

C. difficile pathogenicity locus (PaLoc)



C. difficile toxins A and B share structural properties

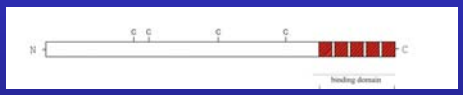


C. difficile toxins act on intracellular targets

- Bacterial protein toxins act on
  - Membrane
  - Receptors
  - Intracellular targets
    - Binding
    - Internalization
    - (Specific) modification of (specific) targets

C-terminal receptor binding domain

- Repetitive structures involved in carbohydrate binding

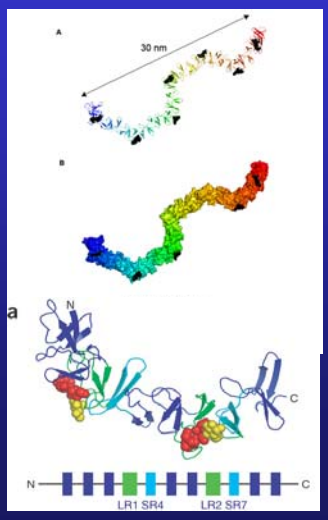


- Receptors

poorly known
carbohydrate structures containing Galβ1-4GlcNAc (TcdA)
carbohydrate linkage to protein or lipid is unknown

TcdA – apical side (of T-84 cells)
TcdB – basolateral side (of T-84 cells)

Structure of TcdA repetitive regions



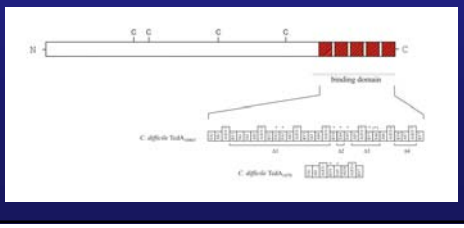
•crystal structure of a 127 aa fragment within repetitive region
(Ho et al., PNAS, 2005)

•solenoid structure of 3 to 5 SRs connected with 1 LR

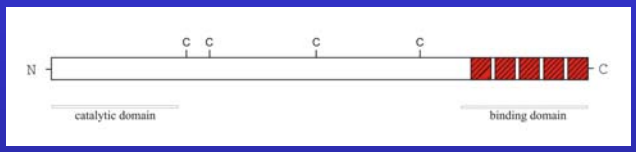
•carbohydrate recognition

(Greco et al., Nature Struct Mol Biol, 2006)

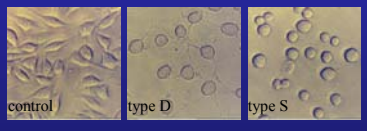
•optimal for multivalent binding to the cell

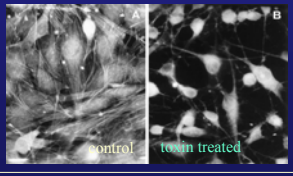


Catalytic domain and molecular mechanism of action



- cytotoxic effect of LCTs
- disruption of actin filaments



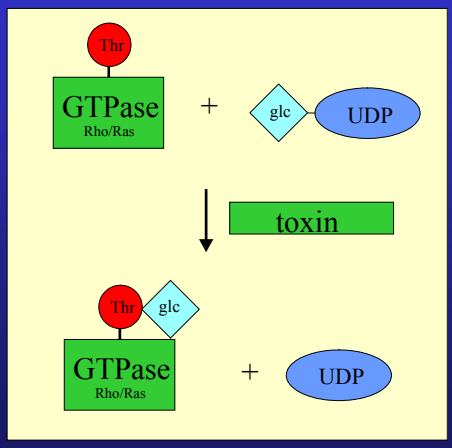


Clostridial toxins acting on actin cytoskeleton:

- actin ADP-ribosylating toxins
  - clostridial binary toxins
  - C3 like toxins
- glycosyltransferase activity
  - large clostridial toxins

new and unique mechanism of action for bacterial protein toxins
(Just et al., Nature, 1995)

Clostridial toxins - glycosylation of small GTPases



Substrate (small GTPases)

Rho subfamily (Rho, Rac, Cdc42)

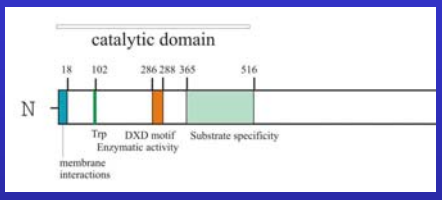
Ras subfamily (Ras, Rap, Ral)

Co-substrate (activated sugars)

UDP-Glc (*C. difficile, C. sordellii*)

UDP-GlcNAc (C. novyi)

Crystal structure of the catalytic domain of toxin B



Jank, T. et al. Glycobiology 2007

- Enzymatic activity (DXD and Trp)
- Substrate specificity
  sugar molecule – Ile383, Gln 385
  (Jank et al., JBC, 2005)
  GTPase – not known
- Crystal structure of 543 fragment
  (Reinert et al., JMB, 2005)
  Catalytic core (234 residues)
  type A family of glycosyltransferases
  Additional subdomains
  unknown function
  4 N terminal helices
  interaction with membrane?
  C. sordelii TcsL 18 aa
  binding to phosphatidylserine

Pathways of internalization of secreted bacterial toxins

binding to the receptor

↓

endocytosis

↙ late endosomes → membrane translocation

diphteria toxin
antrax toxin
C. difficile toxins

↘ retrograde transport to ER → membrane translocation

cholera toxin
shiga toxin

LCT entry into host cell – pore formation

- endosomes, pH↓ → conformational change
- insertion of hydrophobic regions into endosomal membrane
- pore formation on cells and artificial membranes

TcdA – cell type specific; strictly cholesterol dependent
(Giesemann, JBC, 2006)
TcdB – not cholesterol dependent
(Barth et al., JBC, 2001)

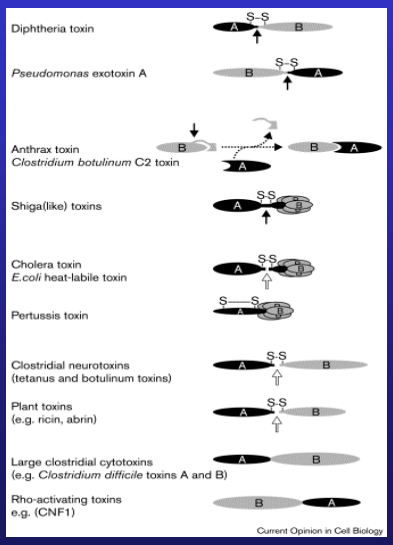
Entry of catalytic domain into the cell

- Toxins with two or more subunits

  subunit B – binding
  subunit A – enzymatic

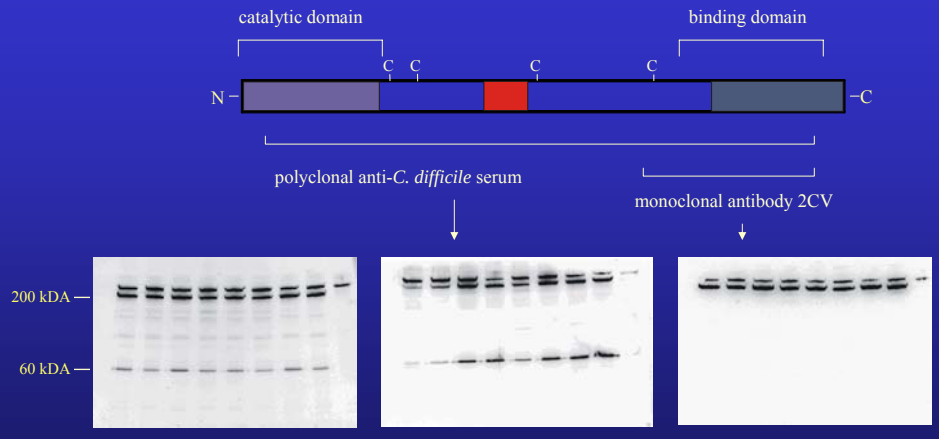
- Single chain proteins

  cleavage of catalytic domain?



Falnes and Sandvig, 2000

Toxin TcdB is cleaved *in vitro* in the presence of cell lysate

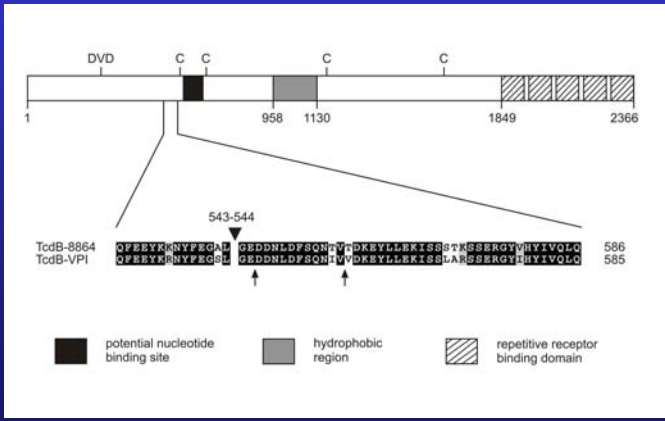


cleaved labeled toxin TcdB
detected on SDS PAGE gel

Western blots with two different antibodies

Rupnik et al., Microbiology, 2005

Cleavage site defines catalytic domain



holotoxin

Binding/translocationdomain

Catalytic domain

Characteristics of proteolytic activity

In vitro cleavage reaction:

Cy3 labeled toxin

cell lysate

pH dependent

low at pH 5.0, optimal at pH 8.0

not inhibited by

EDTA, EGTA,

various protease inhibitors

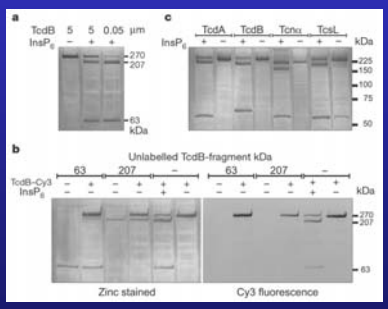
heating, protease K (later studies)

inhibited by

Ca^{2+}, pepstatin A

Autocatalytic cleavage of *C. difficile* toxin B

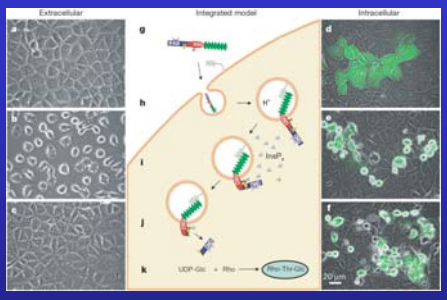
- novel mechanism of toxin processing
- cleavage of TcdB is catalyzed by proteolytic activity within the toxin molecule
- only required factor is host cell IP6



Reineke et al., Nature, 2007

Autocatalysis is found in several large bacterial toxins

cysteine protease



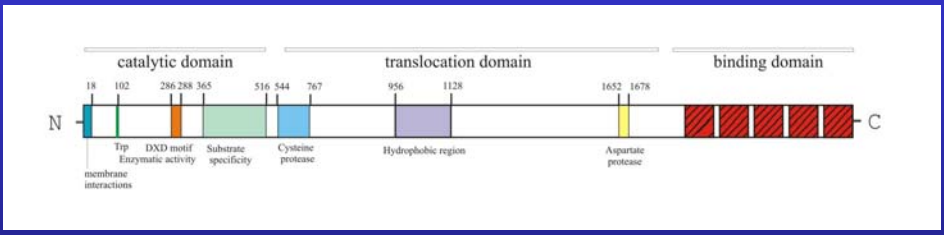
aspartat protease

Vibrio RTX toxins
Sheahan et al., EMBO J 2007

C. difficile toxins A and B
Egerer et al., J Biol Chem 2007

C. difficile toxin B
Reineke et al., Nature, 2007

Structure and function of LCTs

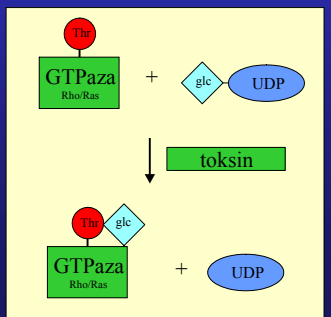


- toxins – main virulence factors
- basis for new therapeutic targets
- non-antibiotic treatment

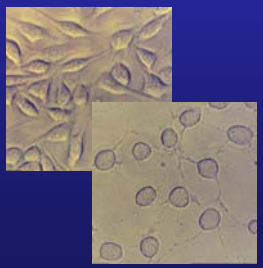
inhibition of binding, inhibition of pore formation, inhibition of catalytic centre, inhibition of proteolysis

Large clostridial toxins (LCT)

C. difficile (TcdA, TcdB)
C. sordellii (TcsH, TcsL)
C. novyi (Tcnα)
C. perfringens (TcpL)



- size (250-300 kDa)
- cytotoxicity
- glycosyltransferases
- autoproteolytic activity



Role of the toxins in the pathogenesis

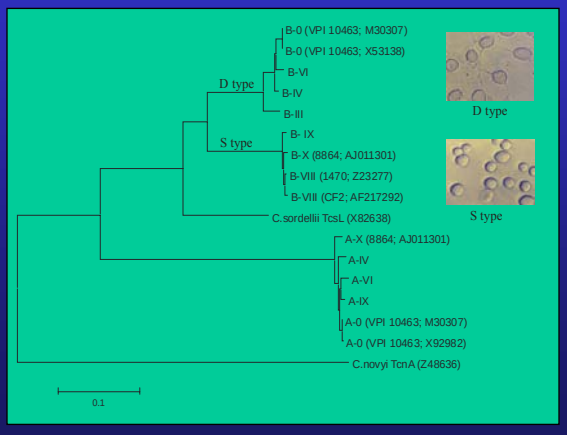
C. difficile always produces both toxins (A+B+ strains)

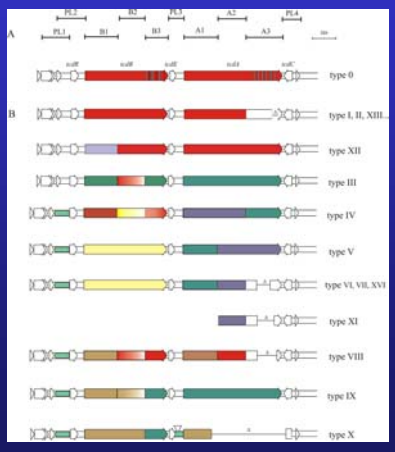
Early hamster experiments (Lyerly et al., 1986)
- purified A – symptoms (local)
- purified B – no symptoms
- purified B with low concentr. A – symptoms (systemic)

K/O mutants
- *tcdB* and *tcdA* - hamster ↓
- *tcdB* only - hamster ↓
- *tcdA* only - hamster ↑

C. difficile strains can produce up to 3 toxins
A-B+ strains – high virulence for humans

Variability in *C. difficile* toxin genes





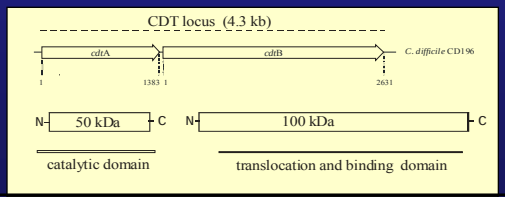
Rupnik, FEMS Rev. 2008

C. difficile binary toxin CDT

- Additional virulence factor
- Prevalence of binary toxin producing strains is increasing
  (Spigagalia and Mastrantonio, JMM, 2004)

time interval	before 1990	1991-1999	2000-2001
% of CDT+ str.	0	24	45

- Binary toxin positive strains more likely associated with severe disease
  (Barbut t al., JMM, 2005; Terhes et al., JCM, 2004)



C. difficile types

Phenotypic

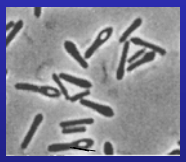
serotyping

Toxin production

toxin A, TcdA

toxin B, TcdB

binary toxin CDT



Entire genome

PCR ribotyping

PFGE

REA

MLVA

MLST

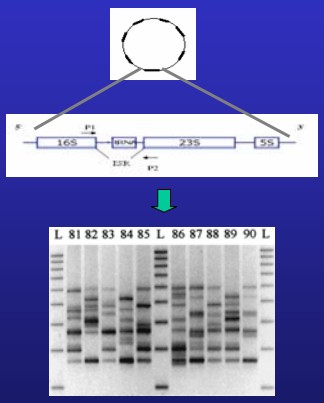
PaLoc region

toxinotyping

slpA

C. difficile – mostly used typing methods

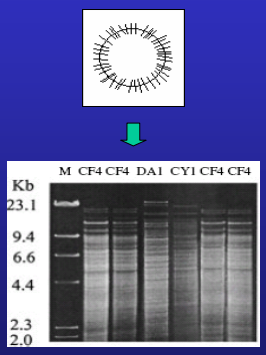
Ribotyping
(Europe)



PCR of 16S-23S rDNA intergenic spacer region
160 ribotypes

Stubbs, JCM 1999;Bidet, JCM 2000

REA
(USA)

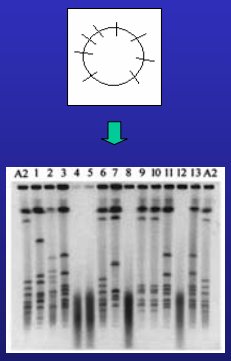


*Hind*III restriction of whole DNA

>100 REA groups
(Rea Types)

Gerding D., Chicago, USA

PFGE
(North America)



Sma1 restriction of whole DNA

no large international collection

Changes in human host

- Hospital-associated v.s. community-associated infections
- Increase in population previously at low risk
- Spread of a specific types
  toxinotype VIII (serogroup F; 017, A-B+CDT+)
  toxinotype III (type BI/NAP1/027; 027, A+B+CDT+)
  toxinotype V (type 078, A+B+CDT+)
- Emergence of hypervirulent types

C. difficile types in humans and animals

- diversity of *C. difficile* animal strains is lower than in human isolates
- same ribotypes are found in animals and humans
- different types are currently predominately associated with animals or with humans

C. difficile types in humans and animals

- cats and dogs, humans (Australia) (O'Neill et al., Epidemiol. Infect. 1993)
  no overlap between animal and human strains
  good correlation between animal and veterinary clinic environment strains
- calves (Canada) (Rodruigez-Palacios et al., Emerg.Infect.Dis., 2006)
  8 ribotypes
  7 of them also in human isolates (same time/geogr. area)
  078 (V), 017 (VIII), 027 (III), 033 (XI), 077 (0), 014 (0)
- calves and pigs (USA) (Keel et al., 2007)
  4 ribotypes
  all of them known in human isolates
  078 (V), 017 (VIII), 033 (XI), 002 (0), 126 (ND)

C. difficile toxinotype V/078 and animals

- 6 toxinotypes (III, IV, V, VIII, XI, XII) out of 27 known described in animal hosts
- toxinotype V/078 present in high proportion in different animal hosts worldwide and in food
  - horses
  - piglets
  - cattle
- current 'epidemic type' in animals?
- particularly adapted to animals?

Summary

- *C. difficile* is an important human and animal pathogen
- infection and transmition: hospital environment (direct or indirect contact), animals, food;
- changes in virulence and antibiotic susceptibility
- additional virulence factors to toxins TcdA and TcdB (CDT, adhesins, sporulation…)