CASE 1 PATH 417

Bacterial Pathogenesis

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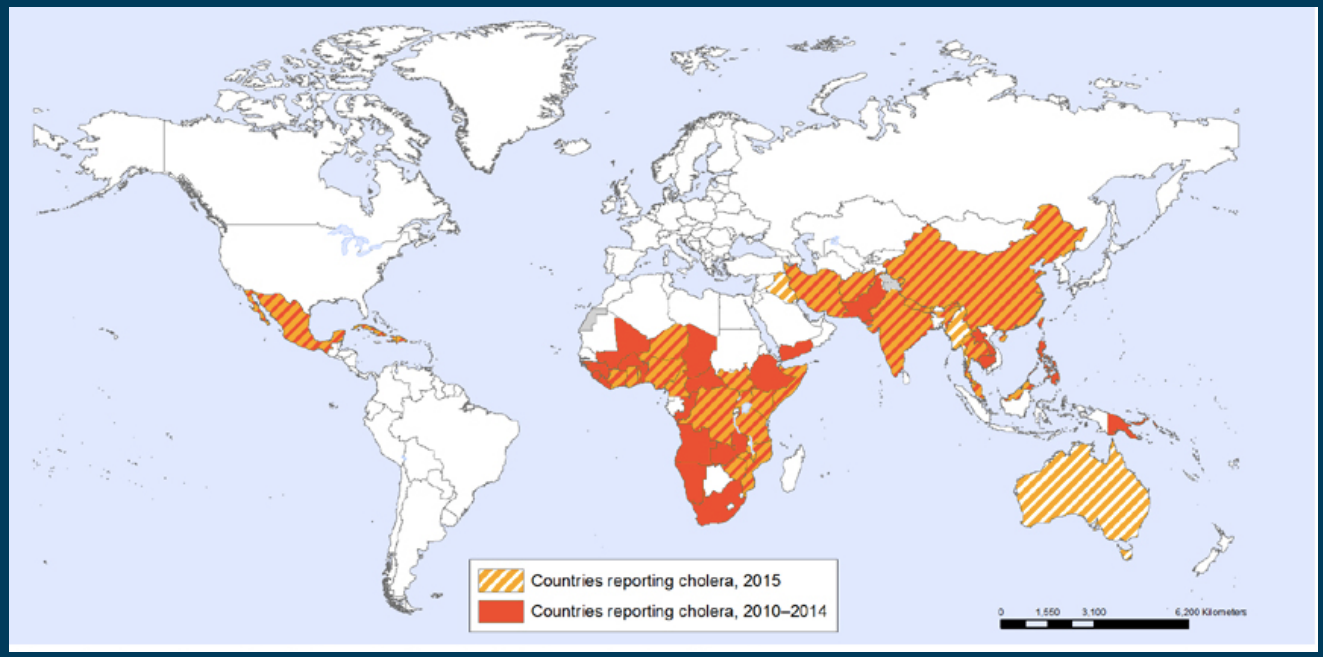
CASE 1

Fulfilling a long held travel dream, Robert has taken six months off work and is making his way through India taking in the sights, experiencing local festivals and making time to get to know the people. He is cautious in his hygiene, eating and drinking habits but despite this he contracts a diarrhea with voluminous outpouring of fluid accompanied by vomiting. He suspects cholera and with the help of a fellow traveler gets himself to a local hospital where a stool sample is examined and his presumptive diagnosis is confirmed. He stocks up on appropriate fluids and stays put at the hostel he has booked into for a few days, experiencing some minor leg cramping along with the diarrhea. His curiosity about his illness has him reading up on the organisms when he returns to North America and he is left wondering what serotype of Vibrio cholerae he might have contracted, should he have been prescribed antibiotics, was there anything more he could have done to prevent contracting the organism and might he now be a carrier?

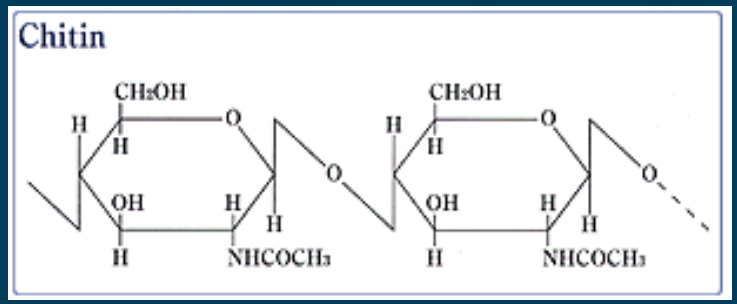
Where does the bacteria normally reside?



High prevalence in developing countries with poor sanitation and minimal sources of clean water. Cholera epidemics reported in several African countries, most notably Kenya, DRC, Malawi, Nigeria, Somalia, South Sudan, and Tanzania. Also found in Haiti and the Dominican Republic. Incidence of disease higher in areas prone to floods and spread of fecal matter.

What are the bacterial characteristics?

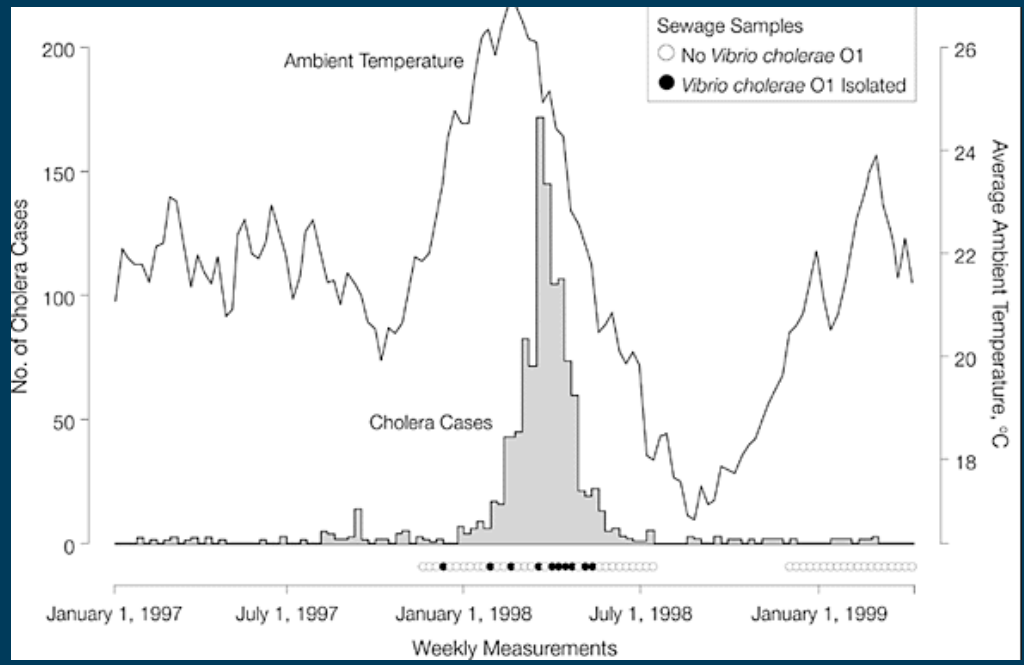
- waterborne pathogen
- gram negative bacterium, comma-shaped
- no known animal hosts
- *V. cholerae uses* chitin, which forms the exoskeleton of copepods and crustaceans, as a carbon source
- TCP is an adherence factor that promotes biofilm formation on chitin
- Formation of biofilm helps promote bacterium survival and provide protection from toxic compounds such as antibiotics
- Adaptive attachment to environmental surfaces such as green algae, zooplankton, insects and crustaceans



Optimal temperature

- Optimal growth occurs @ 37°C and pH 7.6
- Possible growth between pH 5.0-6.0, 10-43°C

Intestinal pH ranges from 5.7-7.4



- To withstand low pH, *V. cholerae* engages in ACID TOLERANCE RESPONSE:
- *inorganic ATR and organic ATR (regulated by *toxR*)
- *mediated by the *cad* system
- *under this system *V. cholerae* are pre-adapted to mild acid conditions (pH 5. exhibit increased resistance to highly acidic conditions

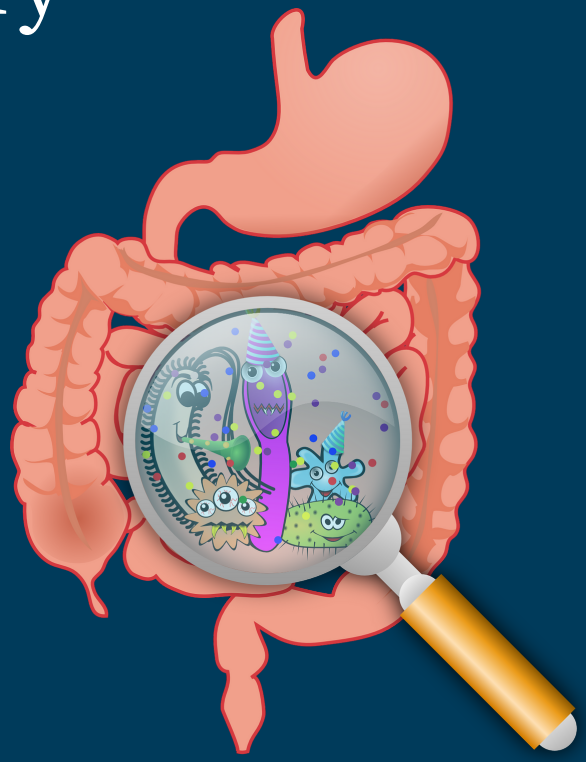
Host Residence

- Type IV- Pilus needed for intestinal colonization
- Colonization factor, GbpA helps mediate bacterial attachment to the epithelial intestinal cells
- surface proteins that correspond to intestinal receptors, many of these surface proteins have not yet been identified
- presence of sodium carbonate in the intestine increases susceptibility to cholera
- *V. cholerae* in biofilms are more resistant to acid inactivation and may also be more effective in competing for nutrients in the small intestine
- Late in infection, virulence factor expression is down-regulated and *vibrios* detach in order to spread through out the small intestine and pass into the aquatic environment
- Bacterium colonizes the intestine for 12 to 72 hours
- Infected individuals can continue to shed *V cholerae* in their feces weeks after infection

How would Robert have come into contact with the bacteria?

- Drinking water or eating food prepared with water contaminated by the feces of a person with cholera
- water contamination via soil that has been exposed to *V. cholerae* O1 or O139

Route of entry



Ingestion of the contaminated water or food

Enters the Gastrointestinal tract

- Flagella helps the vibrios reach the epithelia of the small intestine
- Proteases called mucinases help penetrate mucus covering epithelia

Adherence

Adheres to microvilli in the small intestine:

Factors needed for colonization:

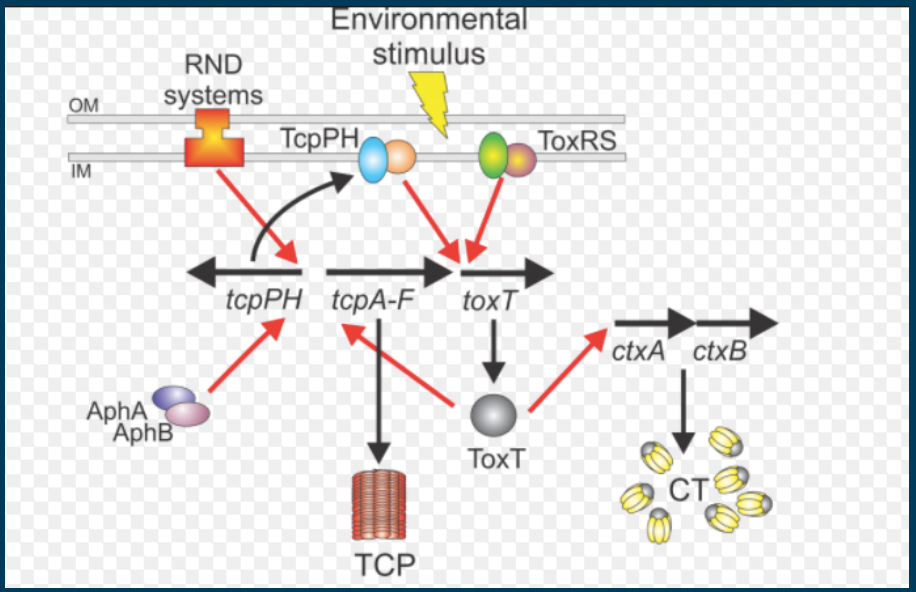
- Most important colonization factor: Toxin-corregulated pilus (TCP), responsible for micro colony formation, facilitates interactions between bacteria
- GbpA binds to residues of mucous in the small intestine and works to increase the production of mucin

Factors that enhance colonization:

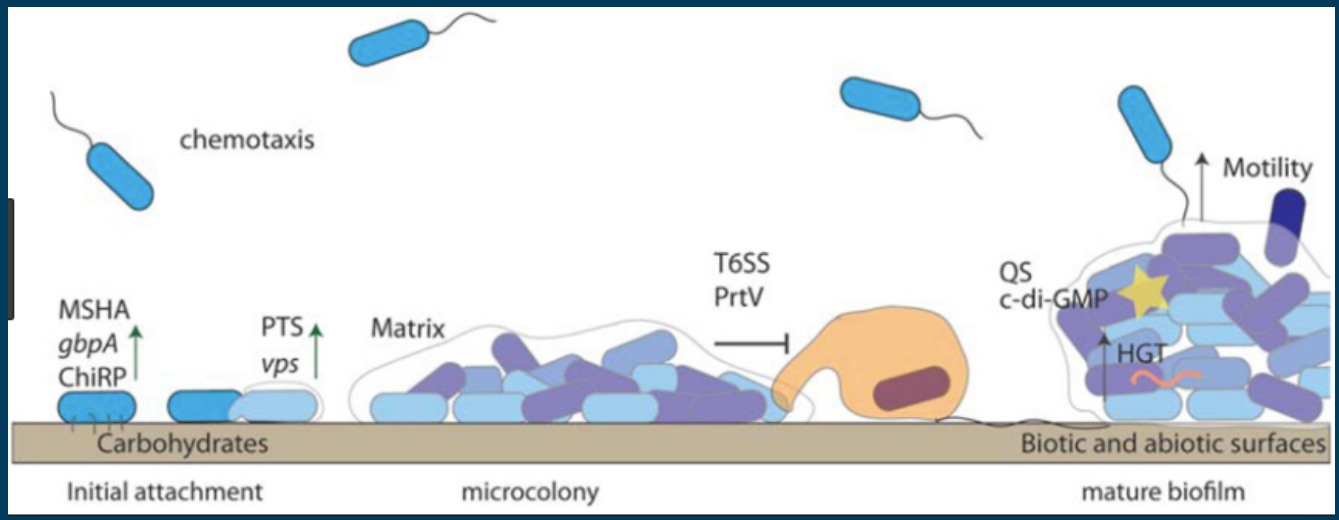
- Surface protein RbmA can enhance cell-cell adhesion to grow biofilm
- Flagellar motility can help mediate penetration

Toxin-coregulated pilus (TCP)

- Most important colonization factor
- Encoded by *tcp* operon
- Has a crystal structure
- TcpF, soluble colonization factor secreted by TCP can accommodate a fairly large substrate due to its flexibility in the linker segment



Penetration



BIOLFILM FORMATION

- 1. Flagella helps mediate penetration: improves adhesion by grasping to substructures not accessible to bacterial cells
- 2. Non-motile penetration: mucous is too thick for flagella to act, translocation aided by wild-type LPS< activity of mucolytic enzyme expression and Zn-dependant metalloprotease hemagglutinin (HA)/protease

Biofilm formation

Why is biofilm important?

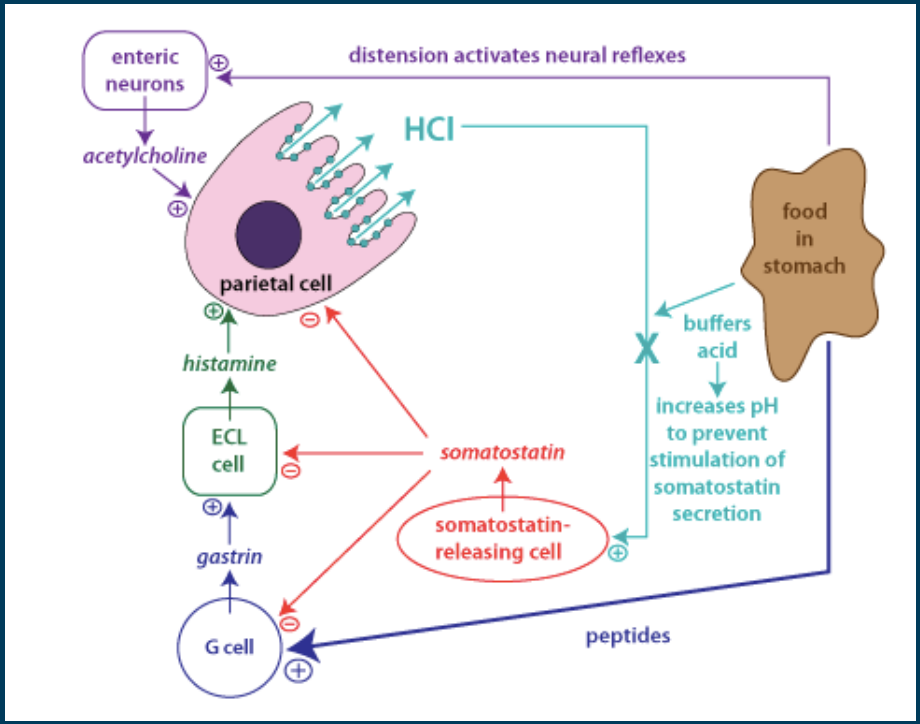
*resistant to bile, antimicrobial peptides and aids in mechanical clearance

* promoted by TCP

*regulated by c-di-GMP-- changes in its concentration are sensed by three different regulators

1. FLrA, binds to c-di-GMP to inhibit *V. cholerae* flagella
2. Under high concentrations of c-di-GMP VpsR and VpsT enhance expression of genes (vps) essential for biofilm formation

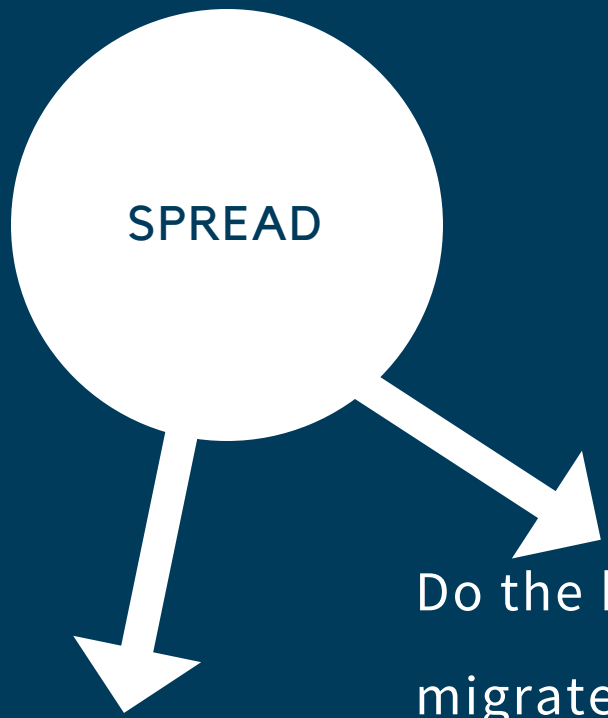
Host defence



- IgA in the small intestines acts to prevent attachment of vibrios and enterotoxins to the mucosal surface
- Stomach gastric acid secretion (ranges from pH 1.5 to 3.5)- *V. cholerae* is sensitive to the acidic environment of the stomach

Why do they prefer certain environments?

Do they enter cells?



Do the bacteria migrate?

Are there secondary sites of infection?

Cell location

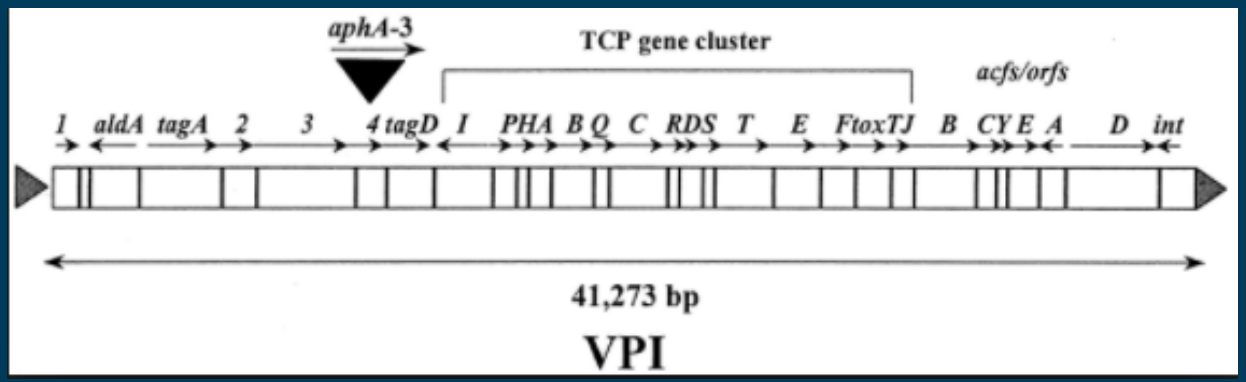
How does this gram negative bacteria invade the host?

*V. cholerae enters using the toxin co-regulated pilus (TCP)

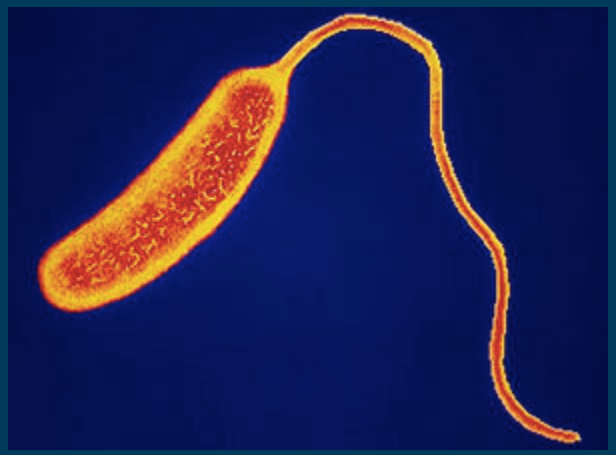
*TCP helps colonize the host

*genes encoding TCP and cholera toxin are found on the Vibrio pathogenicity island (VPI)

*these encoded genes regulate virulence gene expression



Cell location



V. cholerae prefers:

*oxygenated environment

*pH 7.6

*uses glucose as carbon energy source

*can also use chitin as carbon source (outside of the body)

*can migrate as it has a flagellum, making it highly motile - translocation is inhibited in the presence of thick mucus

Bacterial Multiplication

Does *V. cholerae* enter the cells of the body?

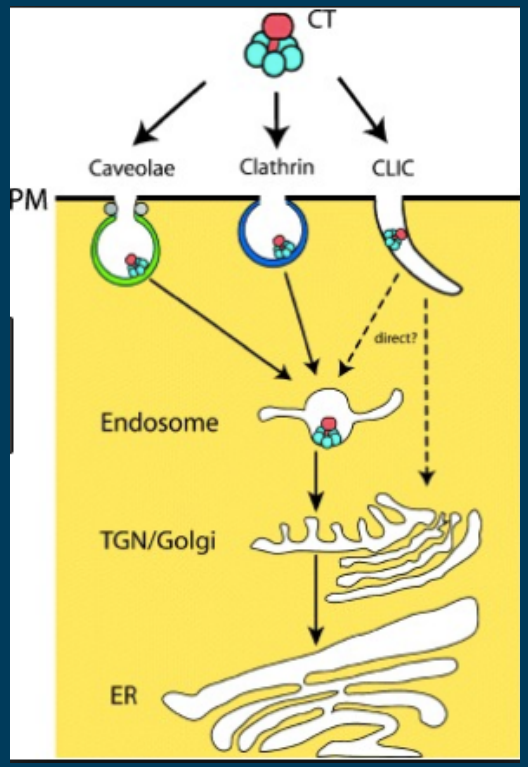
*The bacterium does not enter the cell itself

*CT enterotoxin accumulates and this component enters the cell

CT2 TOXIN (0139 SEROGROUP)

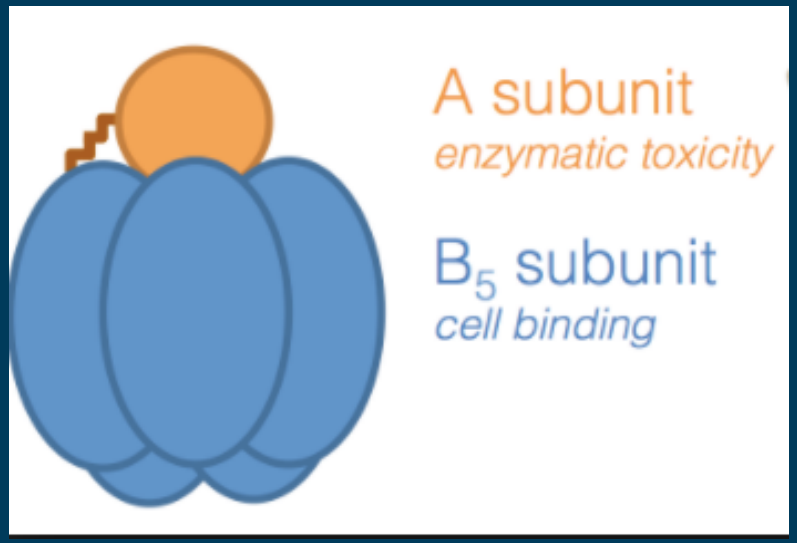
CT1 TOXIN (EL TOR & 01 SEROGROUP

Cholera toxin accumulation is essential for pathogenesis



Cholera Toxin Structure I

- Has five binding B subunits and one enzymatic A subunit
- Mucinases help toxin penetrate mucous layer

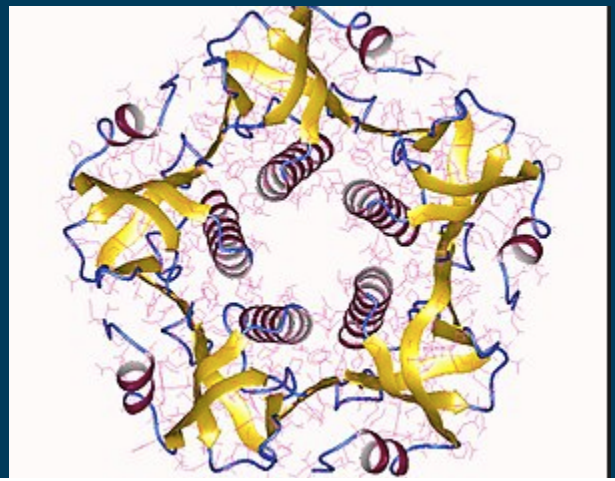


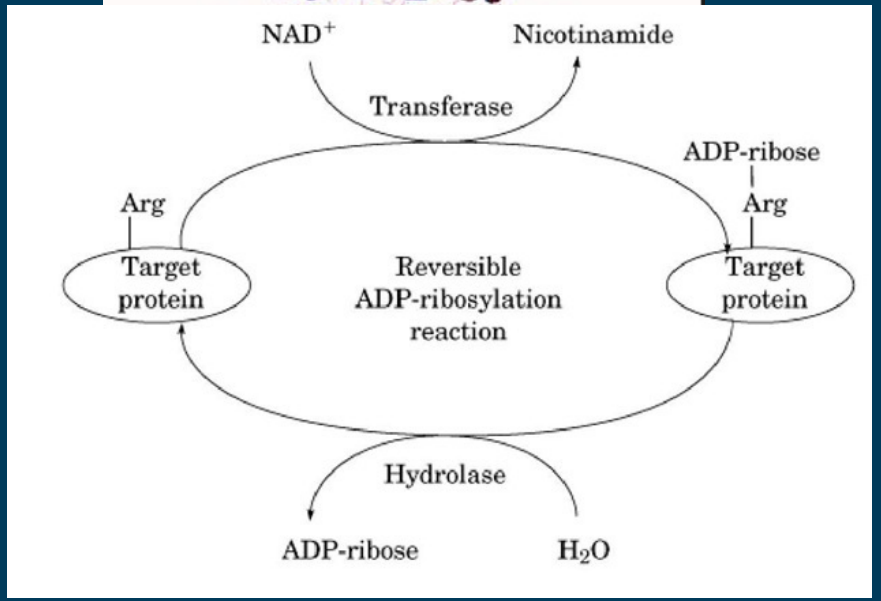
What does the subunit bind to?

- Gangliosides —(mucinases)→ monosialosyl gangliosides (acts as B subunit R)
- B subunits bind to monosialotetrahexosylgangliosides located on intestinal epithelial cells

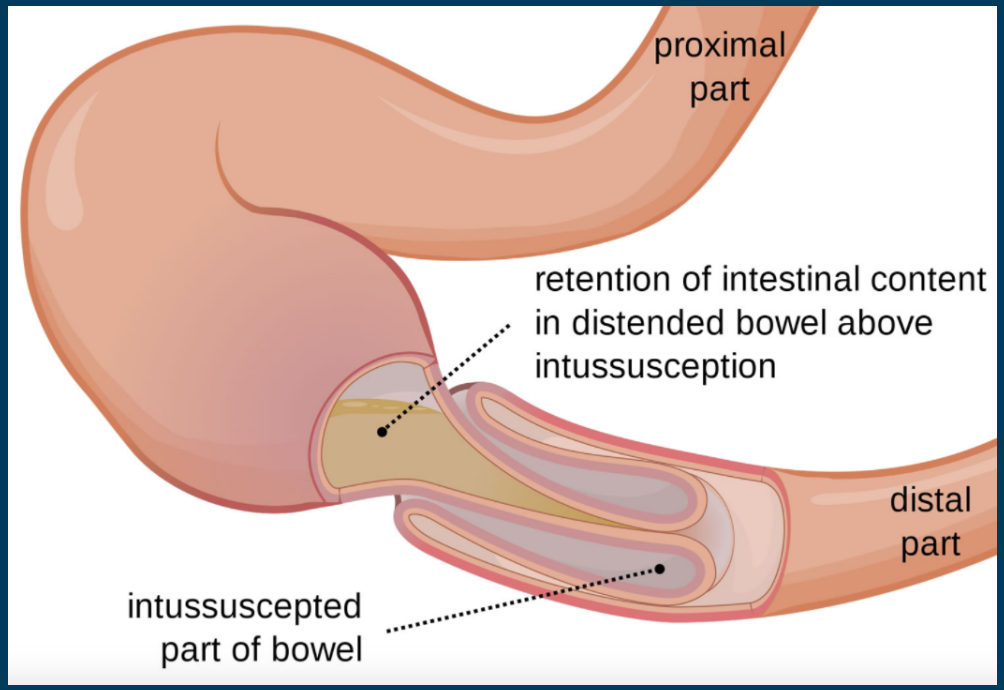
Cholera Toxin Structure II

- Binding induces endocytosis of the A subunit
- The A subunit regulates biological activity in host cells
- Production of cAMP is influenced by the activation G protein Gsa by subunit A1 through ADP-ribosylation reaction
- This cause GTP to remain in bound form
- High cAMP = prolonged opening of CFRT channels





Spread



Does *V. cholerae* spread throught the GI tract?

*Usually remains localized in the small intestine

*the bacterium preferentially colonizes the distal portion of the small intestine


WHAT ARE THE LONG-TERM AND SHORT-TERM EFFECTS OF DAMAGE?
CAN THE DAMAGE ACCOUNT FOR ANY SIGNS OR SYMPTOMS EXPERIENCED?
IS THERE ANY DIRECT DAMAGE TO THE HOST?

Damage to host- water filtration

Main site of damage: small intestine

* short-term consequence- addition of pumps in the cells of the small intestine drive excess water and eectrolytes into the lumen from blood and tissues

*cholera toxin increases cAMP which causes CFTR channels to stay open

*activity of the CFTR channels, which act to transport fluids and ions is increased, causes excess loss of chloride ions from tissues

*This accounts for the symptoms of voluminous watery diarrhea, dehydration, and leg cramps.

Damage to Host- loss of electrolytes

1. Loss of chloride ions: excessive loss due to an increase in CFTR channels
   * accounts for loss of electrolytes which leads to dehydration
   * loss leads to disruption of fluid regulation
   * may also have implications on blood pressure

2. Loss of potassium ions along with water:
   * help generate action potentials and aid in neurotransmission muscle contraction and heart function
   *loss may affect the circulatory system leading to collapse or circulatory failure

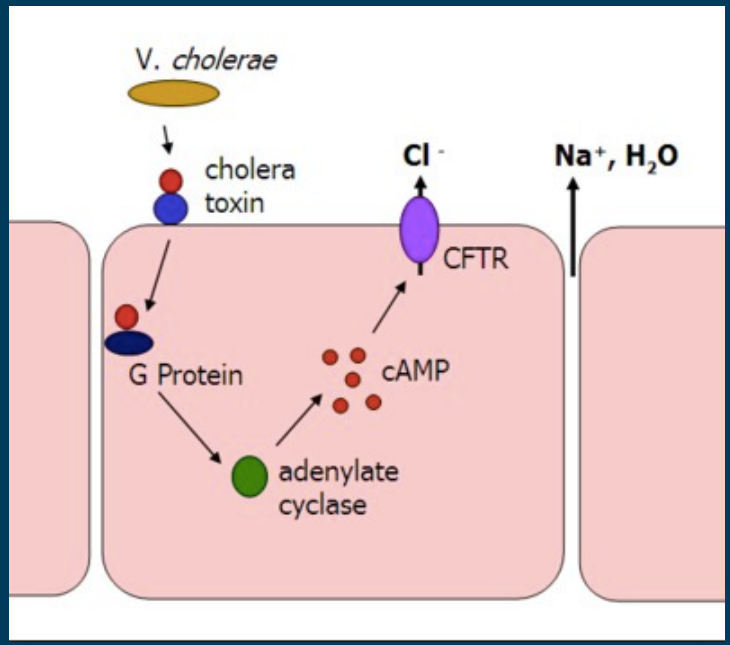
Damage to host- small intestine

During Infection:

*reduction in frequency, duration, and area of contractions

*the small intestine goes into compensatory mode

*increased transit time of substance passing through the small intestine



No long-term consequences, recovery leads to long-term protection against re-infection

THE END