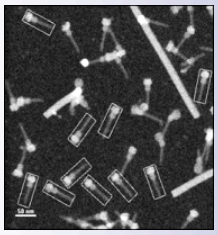
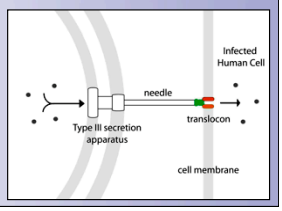
Type III Secretion - Injectisome

Lecture 19





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Learning Objectives

- Understand diversity among prokaryotic secretion machinery.
- Understand overall structure of T3SS apparatus.
- Understand classification of T3SS machinery.
- Define specific components of T3SS machinery.
- Understand hypothesis of needle length control.

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What defines protein secretion?

- Export
  - Localization of non-cytoplasmic proteins to the cell envelope
- Secretion
  - Extracellular proteins that are entirely outside of the outer most lipid bilayer
    - Includes soluble (free) proteins, surface associated proteins, surface appendages

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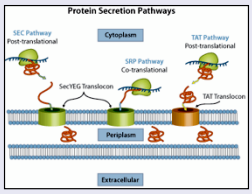
Where Do Secretion Systems Exist?

- Bacterial secretion systems exist at membranes
  - Inner membrane
  - Outer membrane (gram negatives)

A bacterial cell often expresses multiple and distinct secretion systems to traffic specific proteins



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Why do Secretion?

- Strict requirement for function
  - Increase growth, Adherence, Virulence, Pores, Pumps, Periplasmic proteins, cell surface structures
- Manipulate environment
  - Coordinate cell movement, Competition, Invade etc.
    - Toxins, Proteases, signaling molecules

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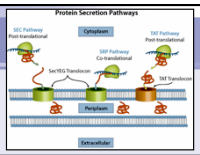
Protein Secretion General Requirements

1. Energetics.
2. Selectivity. Only certain proteins are chosen for secretion.
3. Substrate folding/unfolding
4. Amphipathic channel - pushing hydrophilic residues across hydrophobic layers.
5. Tight seal.

Not absolutely required:

- Regulation
- Complex assembly

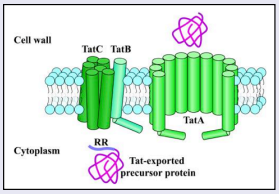
Sec Translocase

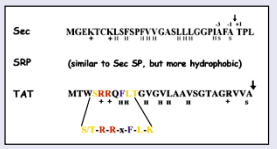


- Translocase is the term used to describe the complex of proteins that serve to 'translocate' substrate proteins
  - SecYEG is termed the protein-conducting channel
    - Exists as a complex in the membrane
      - Trimer (3 SecYEG) is functional to move polypeptides although some evidence for dimer and other oligomers in SecYEG function
- Considered as the 'core' secretion system
  - Homologues in all domains of life
    - Suggests that this system was used to traffic proteins to extracellular locations in a primitive (early) organism
  - Some secretion systems require Sec to assemble its components
    - e.g. Type III secretion system

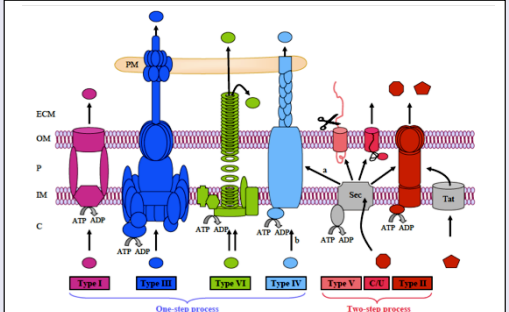
TAT translocase

- Exports FOLDED proteins!
- Does not require nucleotide hydrolysis
- Uses Proton gradient for energy source
- First described for targeting of proteins in plants





Prokaryotic Secretion Systems



Sec dependent

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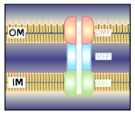
Type I Secretion

- Secretion of proteins in a single step without stable periplasmic intermediates
  - The simplest of the so called 'type' secretion systems
  - transports various molecules, from ions, drugs, to proteins of various sizes
  - Examples: metalloproteases, hemolysins, toxins



- Consists of three proteins located in the cell envelope
  - 1) ATP binding cassette protein (ABC)
    - Recognizes substrate and secretion signal
  - 2) Membrane fusion protein (MFP)
    - Forms links between inner (where it is anchored) and outer membrane assembly
  - 3) Outer membrane protein (OMP)
    - Forms a barrel in the outer membrane
- Substrates have a C-terminal secretion signal
  - Signal is part of the protein and is NOT cleaved
  - Substrate proteins often have a conserved glycine rich repeat (GGXGXDXXX)
    - calcium binding motif
    - Proteins vary greatly in size (and length)
      - 80 aa to 8000aa

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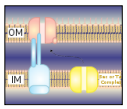
Type II Secretion

- Secretion occurs in 2 distinct steps
  - Initially proteins use the Sec-system, then enter the T2SS pathway as a terminal branch
  - Substrates enter from periplasm
  - Examples: cholera toxin, phospholipases, proteases



- The system is believed to span the entire gram-negative cell envelope
- An inner membrane ATPase provides ATP hydrolysis believed to drive the secretion process
- Some components share similarity with type IV pilins (termed pseudopilins)
  - Believed to form a pilus-like structure as part of the T2SS
  - Some believe that this may act as a 'piston' to push protein substrates out
    - Extension/retraction is dependent on energy from ATP hydrolysis (driven by cytoplasmic ATPase)

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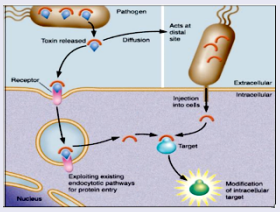
Type III Secretion (T3SS)

- **Contact dependent delivery system!**
  - Mainly found in pathogenic or symbiotic Gram- bacteria
  - Key factor for virulence in pathogens
  - Spans both bacterial membranes and delivers (translocates) effector molecules to host cell cytoplasm
  - Nanomachine called the Injectisome

Type I and Type II

Secrete active proteins into bacterial exoenvironment



Type III

Translocates effectors

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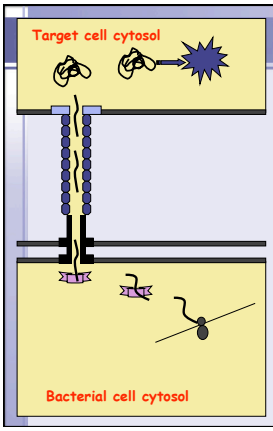

T3SS Secretion

- **Five Major Components**
  - Regulators
  - Chaperones
  - Secretion Apparatus
  - Translocators
  - Effectors
- **Five functions**
  - Export proteins across bacterial envelope
  - Bring bacterial & host cells close together
  - Translocate proteins between bacterial and host cells
  - Translocate proteins across host cell membrane
  - Translocated proteins subvert host cell functions

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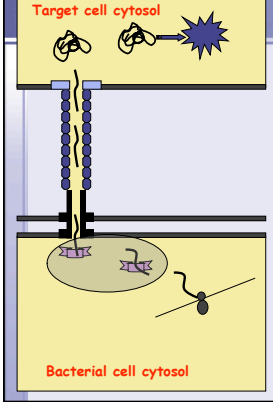
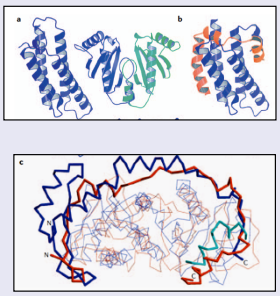


T3SS Chaperones



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T3SS Chaperones

- Type III secretion depends on cytosolic molecular chaperones
  - bind specifically to the translocators and effectors
  - chaperone loss results in rapid degradation, aggregation or reduced secretion of its cognate secretion substrate(s)
- Sequence identity low but common features
  - similar small size (100-150 residues)
  - C-terminal amphipathic helix
  - tendency towards an acidic pI
- 3 main structural classes
  - Class IA: dedicated chaperone for an effector
  - Class IB: chaperone can bind many effectors
  - Class II: chaperone translocator proteins (neutralize)
  - Class III: chaperone binds and masks proteins of polymerization

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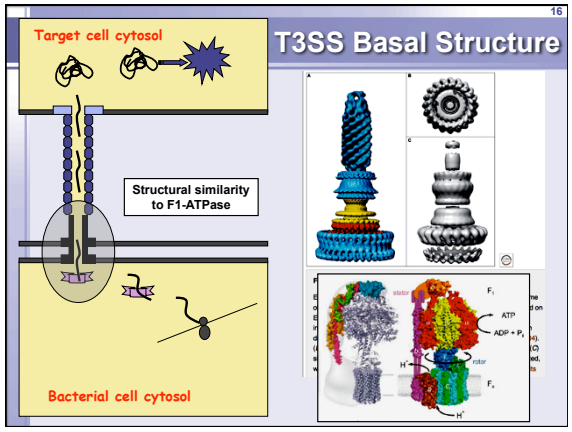
T3SS Basal Structure



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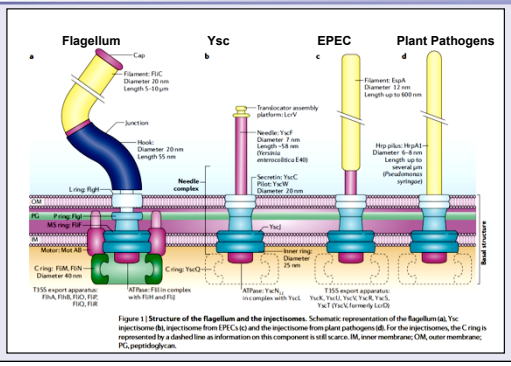
Structural and Functional Similarities



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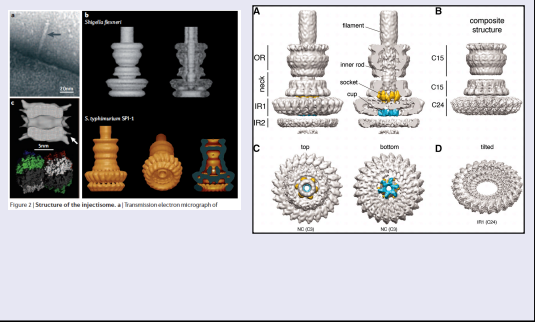
T3SS Basal Structure



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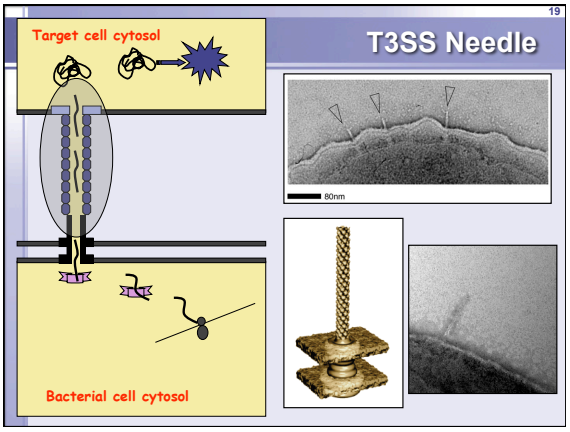
T3SS Needle



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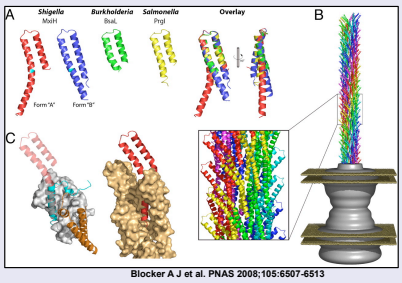
Complex of Needle Proteins



Blocker A J et al. PNAS 2008;105:6507-6513

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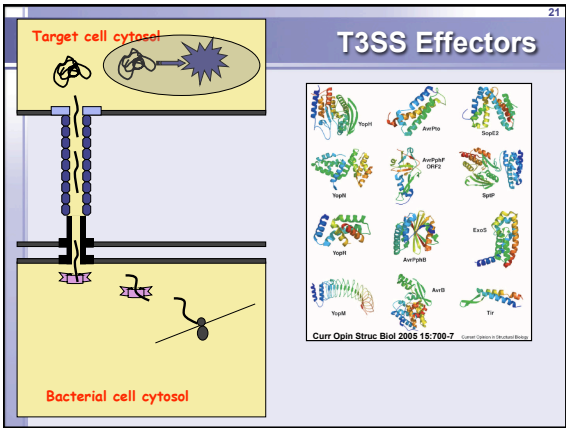
T3SS Effectors



Curr Opin Struc Biol 2005 15:700-7

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Injectisome Family Classification

Injectisome family	Species	Description	Taxon
Ysc	*Yersinia pestis*	Rodent and human pathogen (plague)	γ-proteobacteria
	Yersinia pseudotuberculosis	Rodent and human pathogen	γ-proteobacteria
	Yersinia enterocolitica	Human pathogen (gastroenteritis, mesenteric adenitis)	γ-proteobacteria
	Pseudomonas aeruginosa	Animal, insect and human (cystic fibrosis, burned, immunocompromised patients) pathogen	γ-proteobacteria
	Aeromonas salmonicida	Fish pathogen	γ-proteobacteria
	Photorhabdus luminescens	mutualistic with entomophagous nematodes	γ-proteobacteria
	Vibrio parahaemolyticus	Human pathogen (seafood-borne gastroenteritis)	γ-proteobacteria
	Bordetella pertussis	Human pathogen (whooping cough)	β-proteobacteria
	Desulfovibrio vulgaris	Sulphate reducing environmental bacteria	δ-proteobacteria
Hrp1	*Pseudomonas syringae*	Plant pathogen	γ-proteobacteria
	Erwinia amylovora	Plant pathogen	γ-proteobacteria
	Pantoea agglomerans (formerly *Enterobacter agglomerans*)	Environmental and human commensal, rarely pathogenic.	γ-proteobacteria
	Vibrio parahaemolyticus	Human pathogen (seafood-borne gastroenteritis)	γ-proteobacteria
Hrp2	*Burkholderia pseudomallei*	Human pathogen (melioidosis)	β-proteobacteria
	Ralstonia solanacearum	Plant pathogen	β-proteobacteria
	Xanthomonas campesitris	Plant pathogen	γ-proteobacteria
SPI-1	*Salmonella enterica*	Human pathogen (gastroenteritis)	γ-proteobacteria
	Shigella flexneri	Human pathogen (dysenteria)	γ-proteobacteria
	Burkholderia pseudomallei	Human pathogen (melioidosis)	β-proteobacteria
	Chromobacterium violaceum	Emerging human pathogen (evoking melioidosis)	β-proteobacteria
	Yersinia enterocolitica	Human pathogen (gastroenteritis, mesenteric adenitis)	γ-proteobacteria
	Sodalis glossinidius	Tse-tse fly symbiont	γ-proteobacteria
SPI-2	*Escherichia coli* EPEC	Human pathogen (gastroenteritis)	γ-proteobacteria
	Escherichia coli EHEC	Human pathogen (uremia, hemolysis)	γ-proteobacteria
	Salmonella enterica	Human pathogen (gastroenteritis)	γ-proteobacteria
	Citrobacter rodentium	Mouse pathogen, model for EPEC	γ-proteobacteria
	Chromobacterium violaceum	Emerging human pathogen (evoking melioidosis)	β-proteobacteria
	Yersinia pestis	Rodent and human pathogen (plague)	γ-proteobacteria
	Yersinia pseudotuberculosis	Rodent and human pathogen	γ-proteobacteria
	Edwardsiella tarda	Human pathogen (gastroenteritis)	γ-proteobacteria

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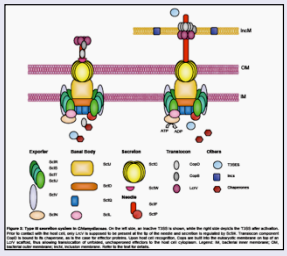
Injectisome Family Classification

Injectisome family	Species	Description	Taxon
Chlamydiales	*Chlamydia trachomatis*	Obligate intracellular human pathogen (trachoma, genital infections)	Chlamydiaceae
	Chlamydia pneumoniae	Obligate intracellular human pathogen (acute respiratory disease)	Chlamydiaceae
Rhizobium	*Mesorhizobium loti*	Plant symbiont (Nitrogen fixation)	α-proteobacteria
	Rhizobium sp	Plant symbiont (Nitrogen fixation)	α-proteobacteria



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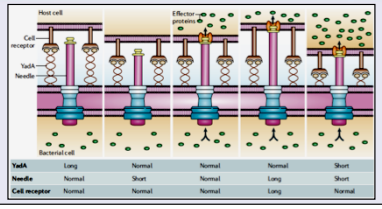
Triggering T3SS Export



YadA	Long	Normal	Normal	Normal	Normal	Short
Needle	Normal	Short	Normal	Long	Short	
Cell receptor	Normal	Normal	Normal	Long	Normal	

- T3SS Injectisome is tightly regulated
  - Specific signal triggers export and increase expression of T3SS genes
  - Direct contact - based an research with *Yersinia*
  - Chaperones are required for control - mutants result in constitutive export.
  - Needle may function as a sensor for transport

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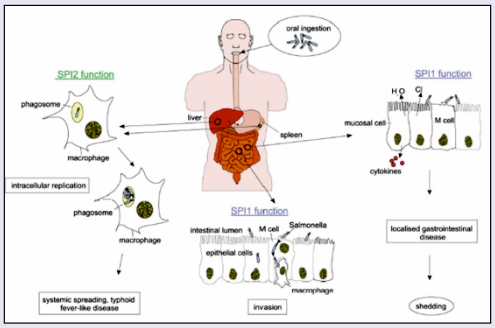
Salmonella T3SS - Two Types



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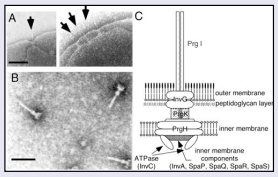
Injectisome Spi1: Invasion

- membrane ruffling
- depends on Spi1 T3SS
- Spi1 effectors
  - SopE affects actin cytoskeleton
  - SipA binds to actin, inhibits depolymerization
  - SopB inositol phosphate phosphatase
  - SptP: PTPase, disrupts the actin cytoskeleton



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Injectisome Spi1: Invasion

- Salmonella enters host cells by inducing host cell membrane ruffling
- membrane ruffles non-specifically wrap around the bacteria and pull them into the cell
- Salmonella end up in membrane -bound vesicles called Salmonella -containing vacuoles (SCV).
- SCVs are unique environments within the cell defined by the bacteria within them
- As they mature, SCVs do not follow the defined routes of cellular trafficking of vesicles and differ in their composition from normal phagosomes

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Injectisome Spi2: Survival

- Survival in Host Cell
  - expressed in cells
  - activated by acidic pH in phagosome
  - mutants severely attenuated in mice
  - effectors influence vesicular trafficking

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Quick Review

- Many Gram- bacteria use T3SS injectisome to translocate effectors directly to host cell
- Multiprotein complexes - more than 20 proteins
- Structure is related to flagellum
- Conserved structural proteins among the different pathogen T3SS classes
- Effector proteins are not conserved - subvert host cellular functions
- Chaperones “protect” proteins, help in regulation

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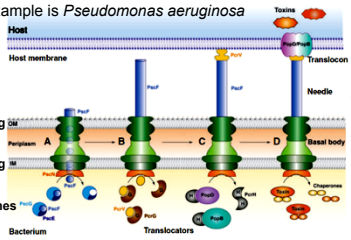
Translocon Formation

Specific example is *Pseudomonas aeruginosa*



Proteins :
2 hydrophobic and 1 hydrophilic

3.
2.
1.

Fig. 1. Schematic diagram illustrating needle and translocon formation, as well as toxin secretion steps, in the T3SS of *P. aeruginosa* (a representative of the Ysc T3SS family). (A) Upon formation of the base rings (green), PscF is released from its chaperones (PscG and PscE) and polymerizes to form the T3SS needle. (B) The V antigen PcrV is released from its cytoplasmic partner (PscG) and forms the cap of the PscF needle. (C) Translocator proteins PopB and PopD release PcrH. (D) Upon formation of the Pop translocon on the eukaryotic membrane, toxins produced in the bacterial cytoplasm release their cognate chaperones and are injected through the translocon pore and into the target cytoplasm. IM, inner membrane; MO, outer membrane.

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Three Translocon Components

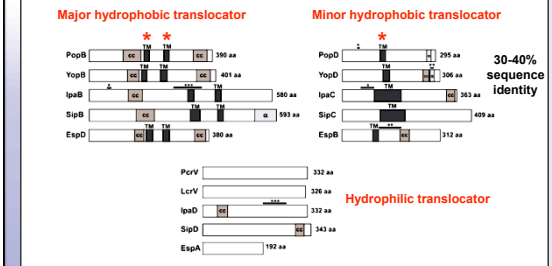

Fig. 2. Diagrammatic analysis of the translocator molecules of the Ysc, Ssa-Esc and Inv-Mxi-Spa systems. TM, predicted transmembrane region; CC, predicted coiled coil; *, chaperone interaction region; **, region predicted as interacting with the hydrophilic partner; ***, region predicted as interacting with the hydrophobic partner; α, predicted amphipathic helix. aa, amino acid.

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Tip Complex Formation

Higher order structure demonstrated

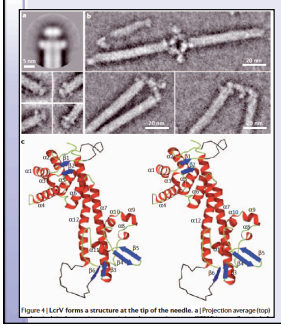

Figure 4 | LcrV forms a structure at the tip of the needle. a | Projection average (top)

Hypothesis:

The hydrophilic translocator functions as a scaffold/assembly platform for the pore proteins (major and minor translocators)

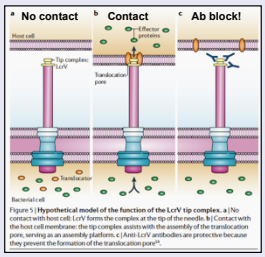

Figure 5 | Hypothetical model of the function of the LcrV tip complex. a | No contact with host cell: LcrV forms the complex at the tip of the needle. b | Contact with the host cell membrane: the tip complex assists with the assembly of the translocation pore, serving as an assembly platform. c | Anti-LcrV antibodies are protective because they prevent the formation of the translocation pore[34].

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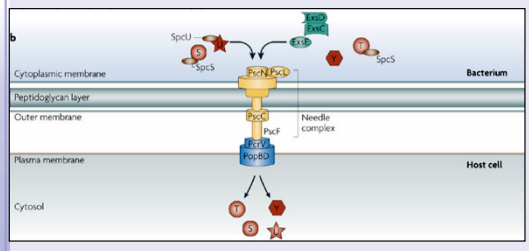
Three-tiered Ring Model



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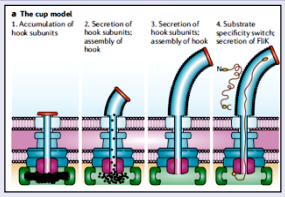
Assembly Process - Cup Model



- Flagellum assembly - general secretory system
  - Sequential process
    - Exists as a complex in the membrane
      - Trimer (3 SecYEG) is functional to move polypeptides although some evidence for dimer and other oligomers in SecYEG function

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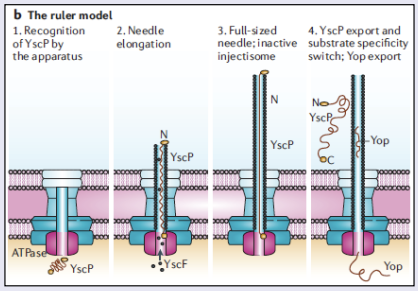
Assembly - Ruler Model



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Assembly - Alternative Ruler Model