

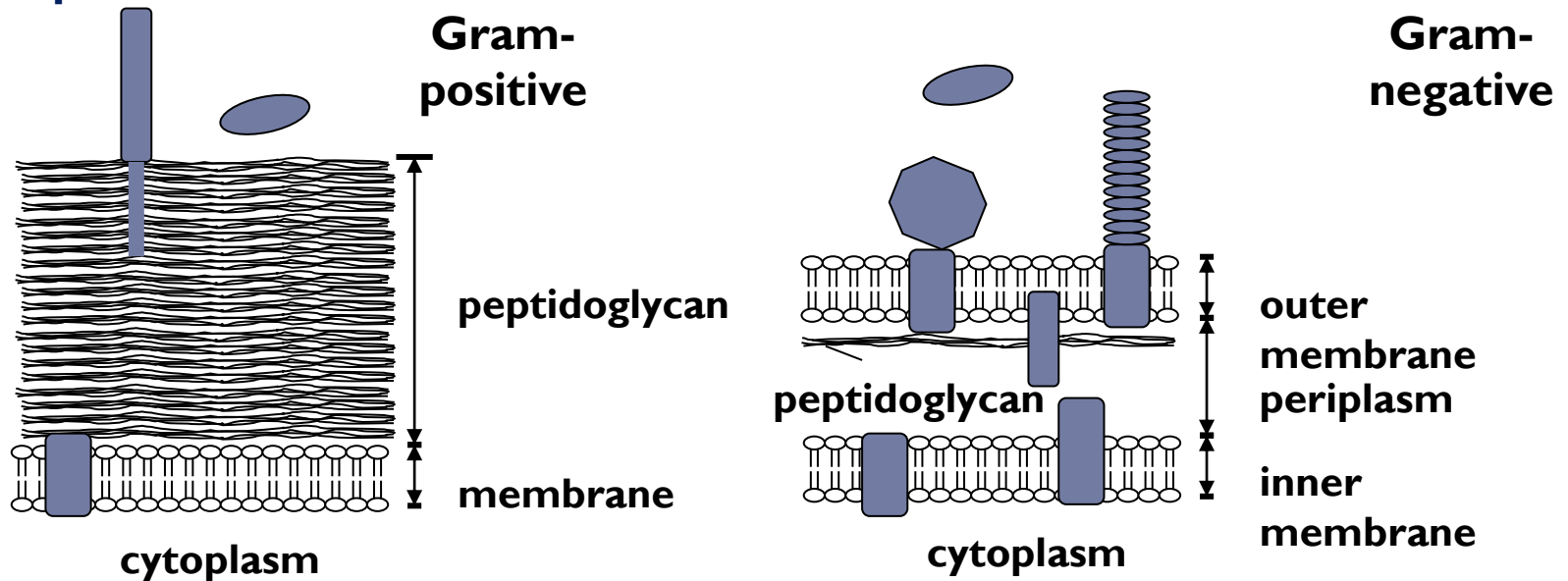
Chapter 4: Bacterial secretion systems

a.a. 2017-18

Translocation and secretion

Protein translocation (and secretion) plays a central role in modulating interactions with the environment and in biotic associations with larger host organisms.

Approximately 20% of the polypeptides synthesized by bacteria are located partially or completely outside of the cytoplasm. Gram-negative bacteria possess several **distinct compartments**

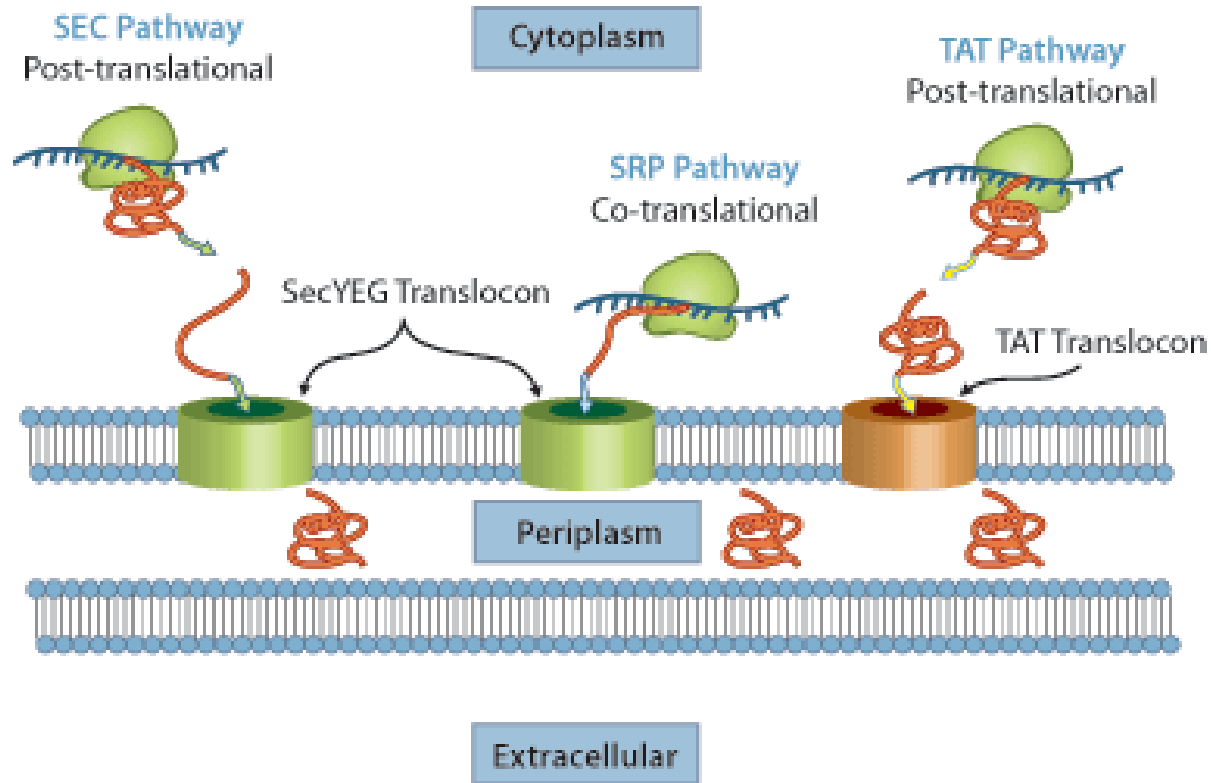


Most bacterial pathogens have evolved specialized protein secretion systems with the ability to transport adhesins, toxins, exoenzymes, proteases and other virulence factors for release into the medium or for direct delivery into host cells.

Bacterial secretion systems

Secretion Apparatus	Secretion Signal	Steps in Secretion	Folded Substrates?	Number of Membranes	Gram (+) or Gram (-)
Sec	N-terminus	1	No	1	Both
Tat	N-terminus	1	Yes	1	Both
T1SS	C-terminus	1	No	2	Gram (-)
T2SS	N-terminus	2	Yes	1	Gram (-)
T3SS	N-terminus	1-2	No	2-3	Gram (-)
T4SS	C-terminus	1	No	2-3	Gram (-)
T5SS	N-terminus	2	No	1	Gram (-)
T6SS	No known secretion signal	1	Unknown	2-3	Gram (-)
SecA2	N-terminus	1	No	1	Gram (+)
Sortase	N-terminus (Sec) C-terminus (cws)	2	Yes	1	Gram (+)
T7SS	C-terminus	1	Yes	1-3	Gram (+)

Bacterial protein secretion pathways



Gram
negative
bacteria



Sec Secretion Pathway

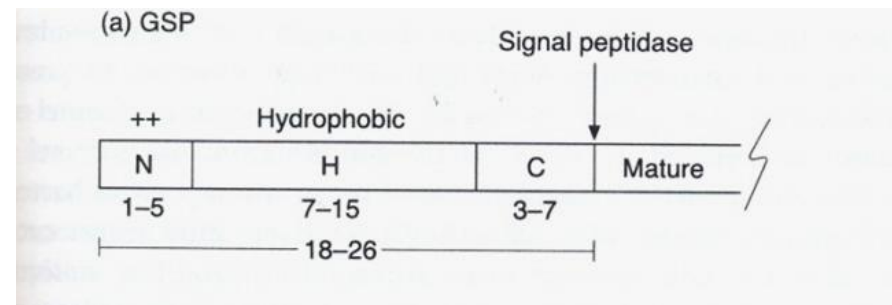
Sec secretion pathway is responsible for translocation into the periplasm of **most proteins** across the inner membrane of Gram-negative and secretion of proteins in Gram-positive bacteria; it is responsible for insertion of membrane proteins into the cytoplasmic membrane. It translocates precursors of proteins **in an unfolded state**.

It is a very conserved transport machinery which is homologous to the translocon in the eukaryotic endoplasmic reticulum.

The machinery of Sec pathway recognizes a specific hydrophobic **N-terminal leader sequence** on proteins destined for secretion.

The leader sequence is cleaved by a leader peptidase during the process of transport.

ATP hydrolysis is required as energy source.

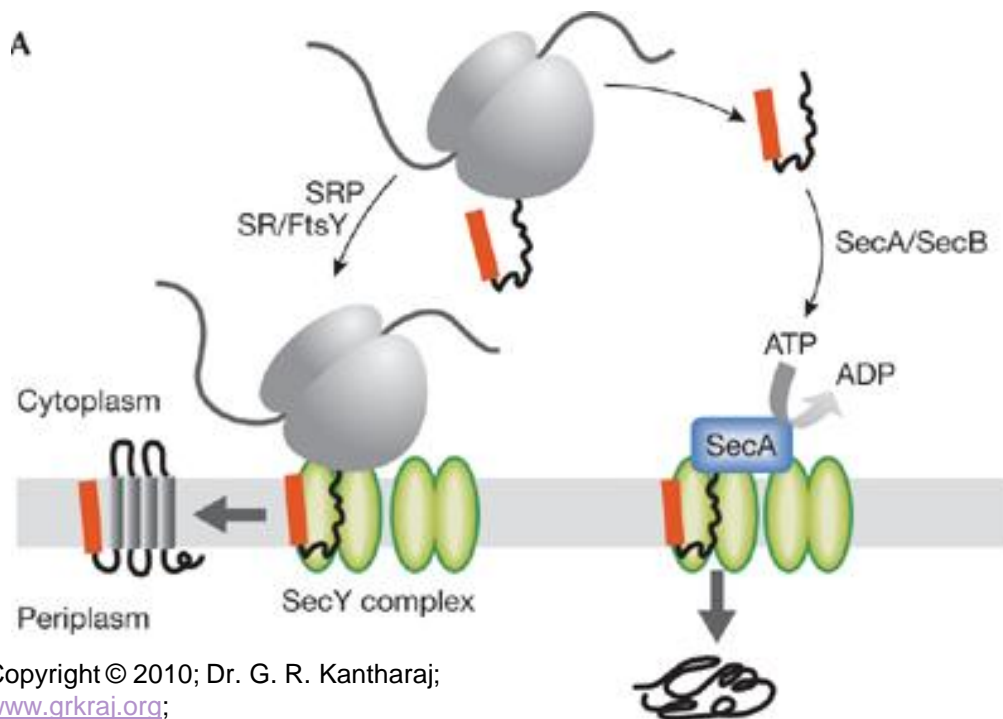


Signal sequence (leader sequence)

Proteins may be targeted to the Sec translocase via two routes.

The core of Sec machinery consists of a protein channel formed by the heterotrimeric **translocase complex SecY** (SecY SecE SecG Translocon)

The channel alone is a passive pore; it must associate with partners that provide a driving force for translocation.



Depending on the partner, there are 2 known routes in which the channel can function:

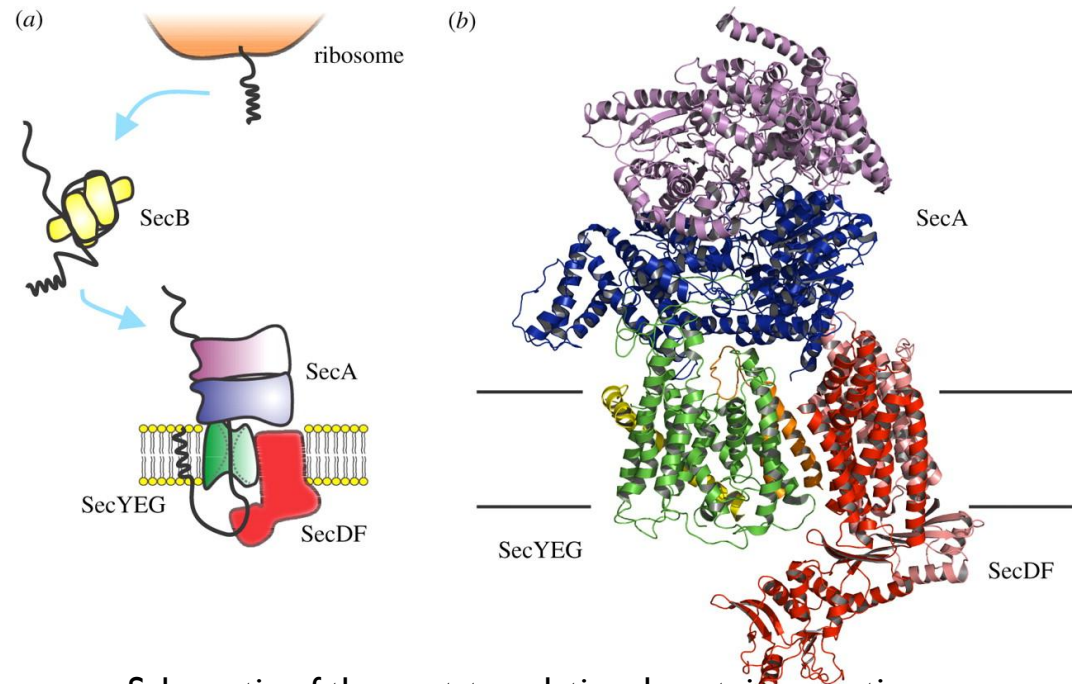
1. **Sec A/SecB route**, a post-translational pathway
2. **SRP (signal recognition particle) route**, a co-translational pathway

Protein translocation through the SecA/SecB route

Secreted proteins are recognized by the **SecB** chaperone after that translation has (mostly) been completed.

Several **SecB** proteins recognizes the leader sequence and binds as a tetramer and targets the unfolded protein to the translocase by binding to **SecA**.

Phil. Trans. R. Soc. B (2012) 367, 1016–1028



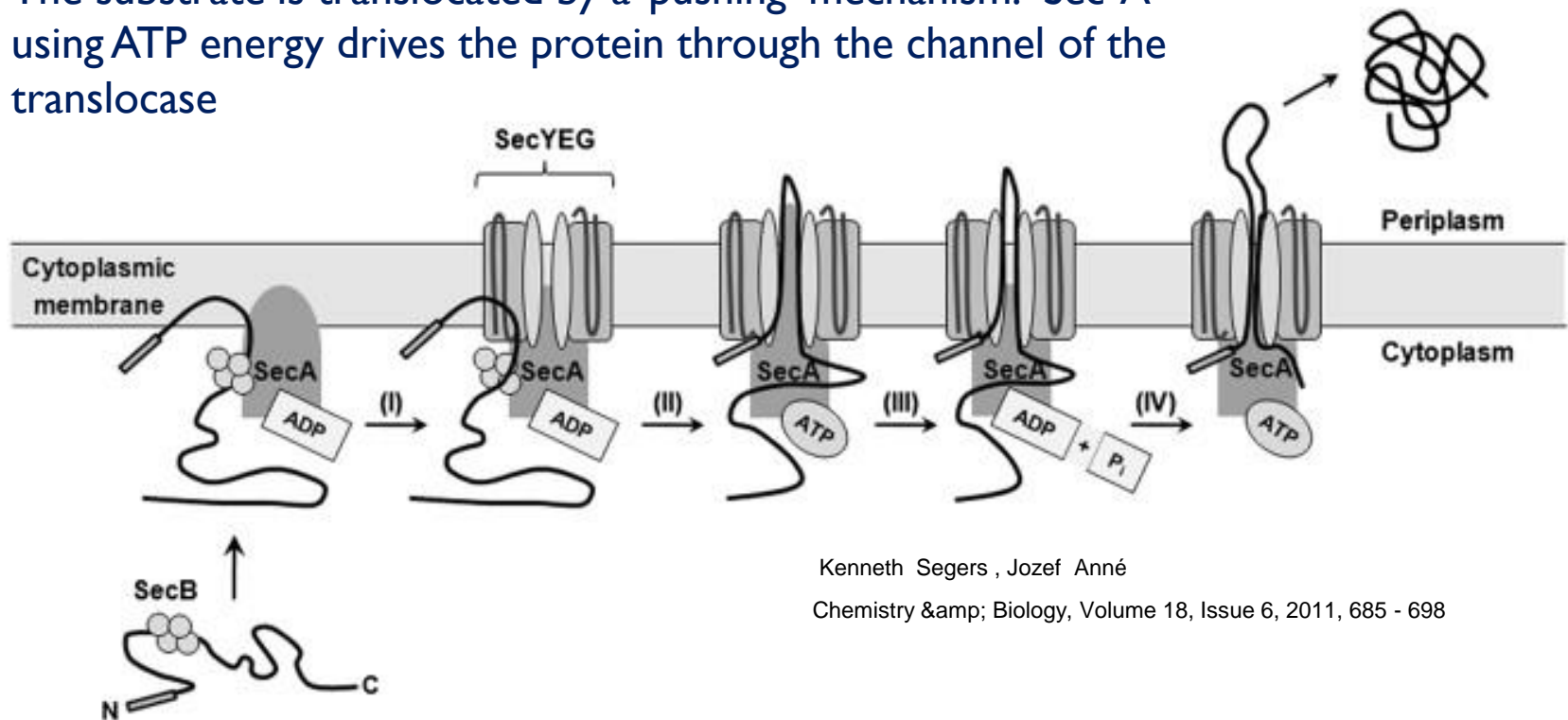
Schematic of the post-translational protein secretion pathway in *Escherichia coli*.

SecA is a molecular motor located at the inner surface of the translocon in the membrane.



A model for protein export by Sec system

The substrate is translocated by a 'pushing' mechanism. Sec-A using ATP energy drives the protein through the channel of the translocase



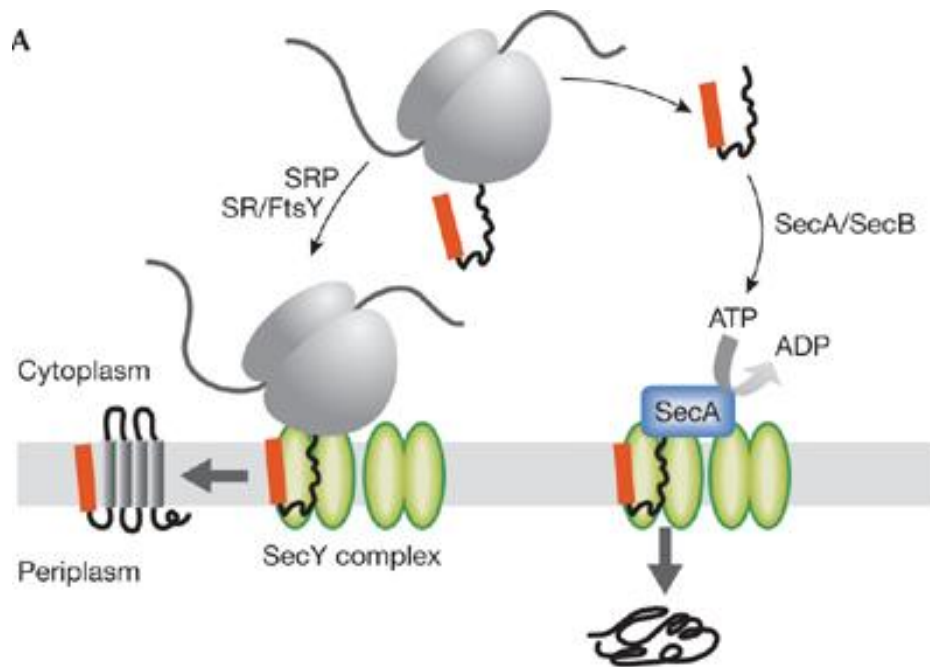
Kenneth Segers , Jozef Anné

Chemistry & Biology, Volume 18, Issue 6, 2011, 685 - 698

Sec-dependent protein translocation in Gram-Negative. Secretory preproteins (thick black line; the signal peptide is represented as a gray rectangle) are targeted to SecA by the molecular chaperone SecB.

Co-translational SRP mediated transport

Integral membrane proteins and proteins with **very hydrophobic signal sequences** are inserted into the cytoplasmic membrane co-translationally.



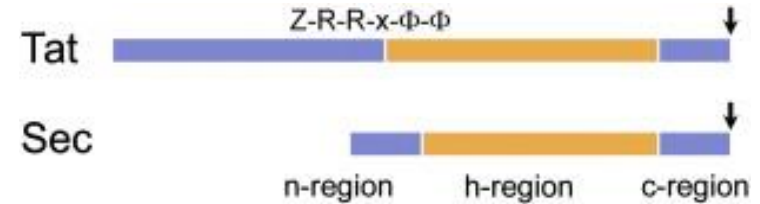
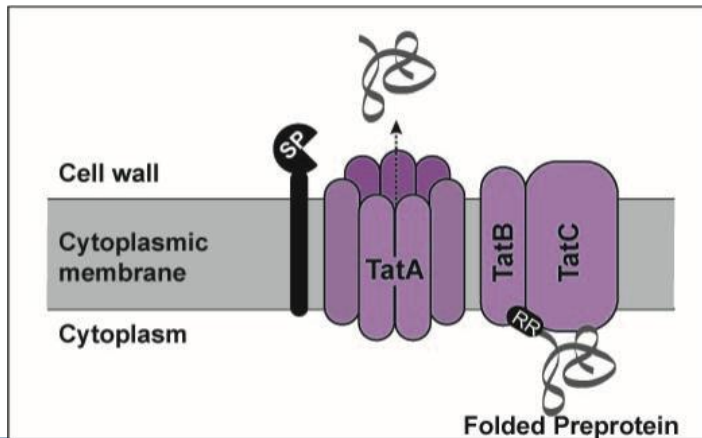
Signal sequence is bound by the **signal recognition particle (SRP)**, a ribonucleoprotein complex that comprises the protein **Ffh** and the 4.5S RNA. Ffh binds the SRP RNA, the signal peptide and with the FtsY receptor.

Co-translational membrane targeting of proteins by the bacterial signal-recognition particle (SRP) requires the specific interaction of the SRP-ribosome nascent chain complex with **FtsY**, the bacterial SRP receptor (SR).

Twin-arginine translocation (TAT) pathway

TAT is dedicated to translocation of proteins that **are fully folded in the cytoplasm**, such as complexes with co-factors, oligomeric complexes etc. Substrates requires a characteristic amino acid motif: two **consecutive RR residues** within the signal sequence (twin-arginine signal).

First described for targeting of proteins into plant thylakoids; conserved in Gram- and Gram+ bacteria.



Tat and sec signal peptide, from E. coli

The TAT pathway of protein secretion consists of 3 subunits: TatA, TatB, and TatC (TAT translocase).

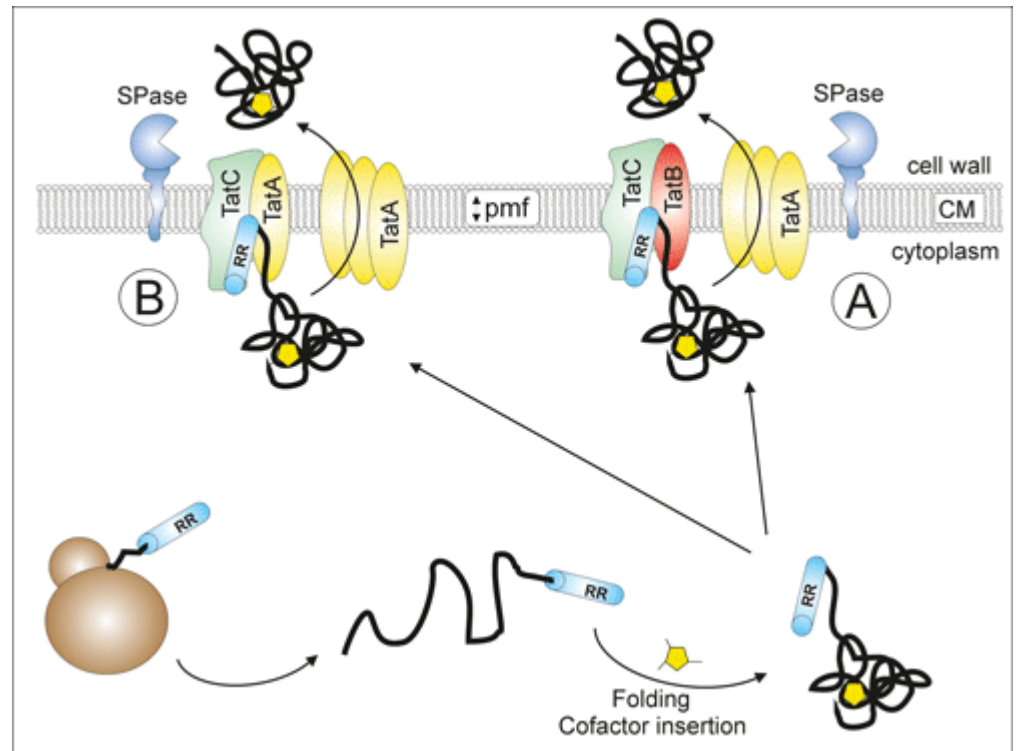
Proteins are recognized and targeted to the membrane-embedded TAT translocase **BC complex** by N-terminal twin-arginine signal.

TAT translocase

TatB and TatC bind the signal peptide of Tat-secreted proteins (figure A) and then homooligomeric complexes of TatA are recruited to the substrate-loaded receptor complex in a proton motive force (pmf)-dependent manner, followed by the translocation of the substrate across the cytoplasmic membrane (CM) in a step that still is very poorly understood.

A large pore formed in the membrane must be large (6-7 nm in diameter) and at the same time prevent movement of ions across the membrane.

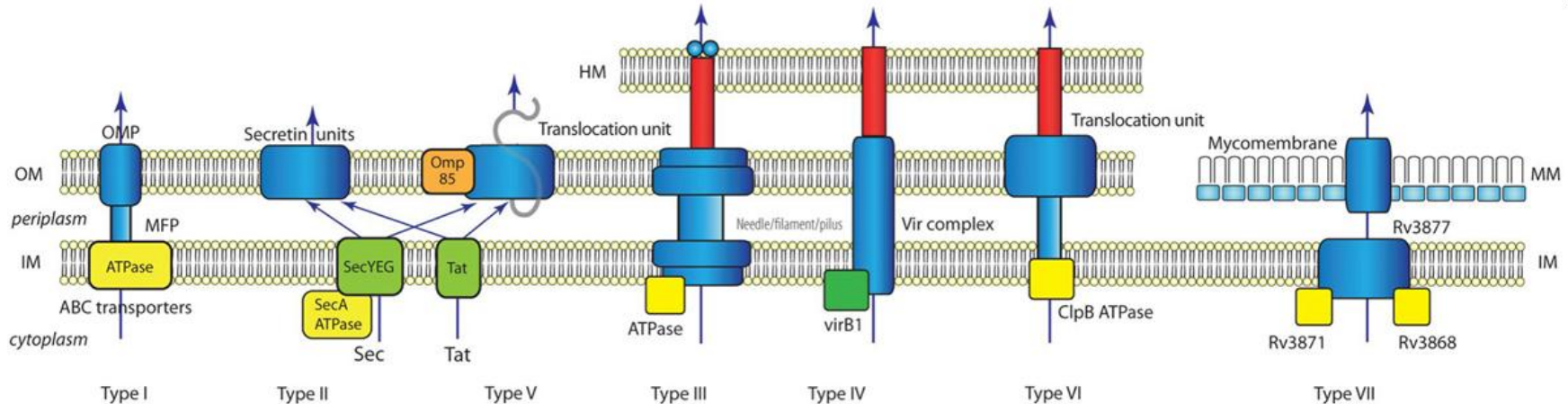
Fig B: Tat function in Gram + bacteria



Overview of different secretion systems

Gram-negative bacteria: six protein secretion systems are known, each of which shows considerable diversity.

Most proteins are exported into the periplasmic space via the **Sec pathway** or **Tat pathway**. In Gram – bacteria some proteins are then translocated across the outer membrane via the type II, and type V pathways.



Tseng et al. *BMC Microbiology* 2009 9(Suppl 1):S2 Å

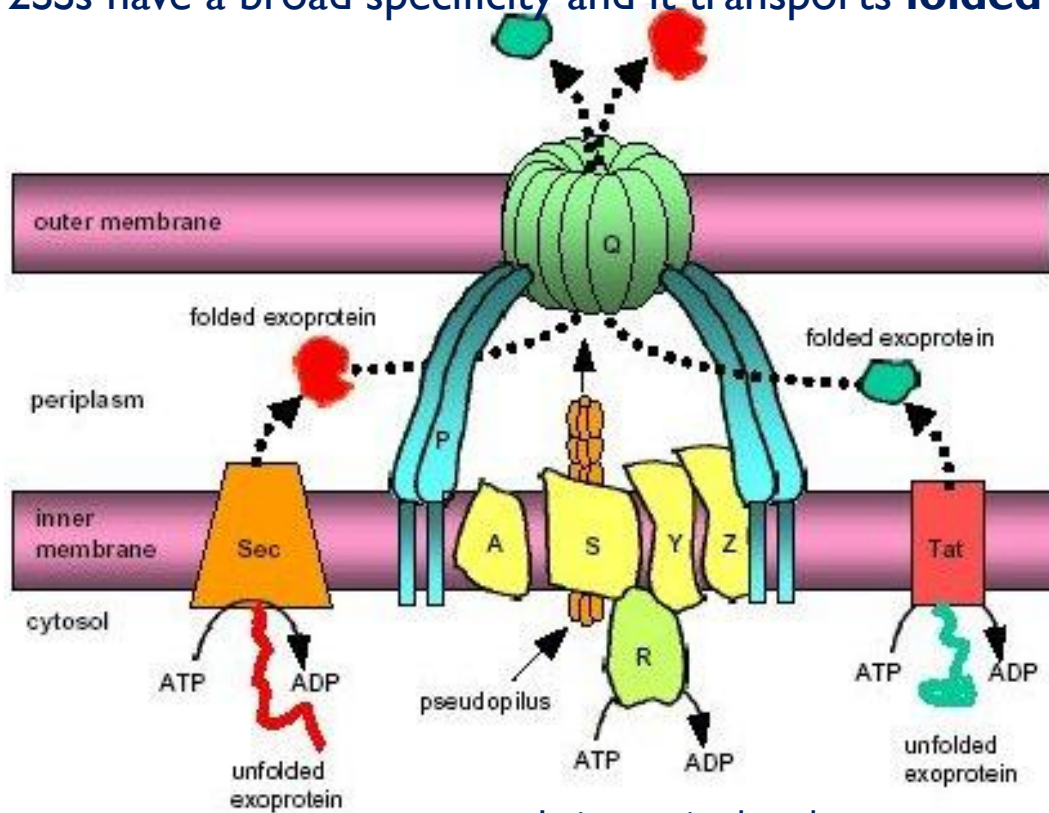
Some secreted proteins are transported across the inner and outer membranes **in a single step** via the type I, type III, type IV and type VI pathways. A specialized type VII secretion system translocates proteins across both the membrane of mycobacteria.

Type 2 secretion system (T2SS)

T2SS is an ubiquitous secretion pathway that specifically supports the transport across the **outer membranes** of most Gram- Sec- or TAT dependent proteins.

T2SSs have a broad specificity and it transports **folded proteins**.

Conserved components of the T2SS are located in both the inner and outer membranes where they assemble into a **supramolecular complex** spanning the bacterial envelope. (transenvelope machine).



It is required pathogens to export toxins, Examples: *V. cholerae* (cholera toxin)

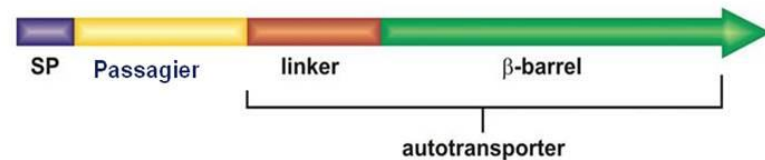
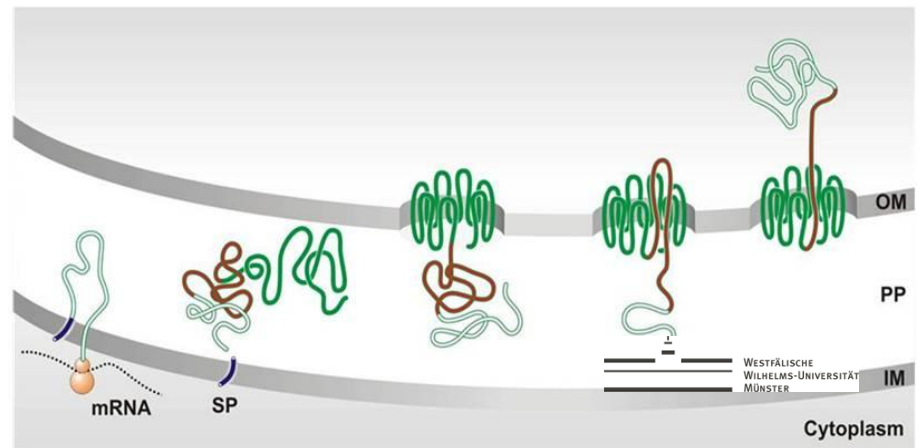
Type 5 secretion system (T5SS)

Proteins secreted via T5SS contain all the functional elements required to **promote their own secretion** – no other proteins dedicated to the transport.

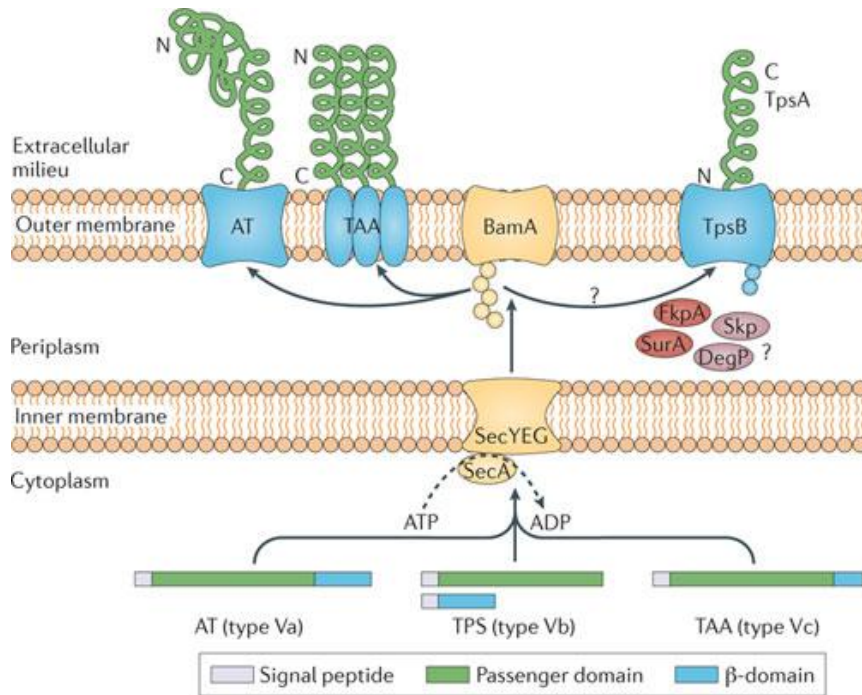
Functional domains: the **translocation unit (β -domain)** forming the autotransporter, and the **passenger domain** corresponding to the transported domain other than the Sec signal sequence.

The β -domain inserts into the outer membrane in a biophysically favored β -barrel structure. After formation of the β -barrel, the **passenger domain** inserts into the pore and is translocated to the bacterial cell surface.

On the surface some T5SS proteins undergo an autoproteolytic process, while others remain anchored to the cell surface by the β -domain).



Subgroups of autotransporter proteins



Different types of autotransporter proteins:
– monomeric (AT), two-partner system (TPS)– trimeric (TAA).

Schematic overview of the type 5SS

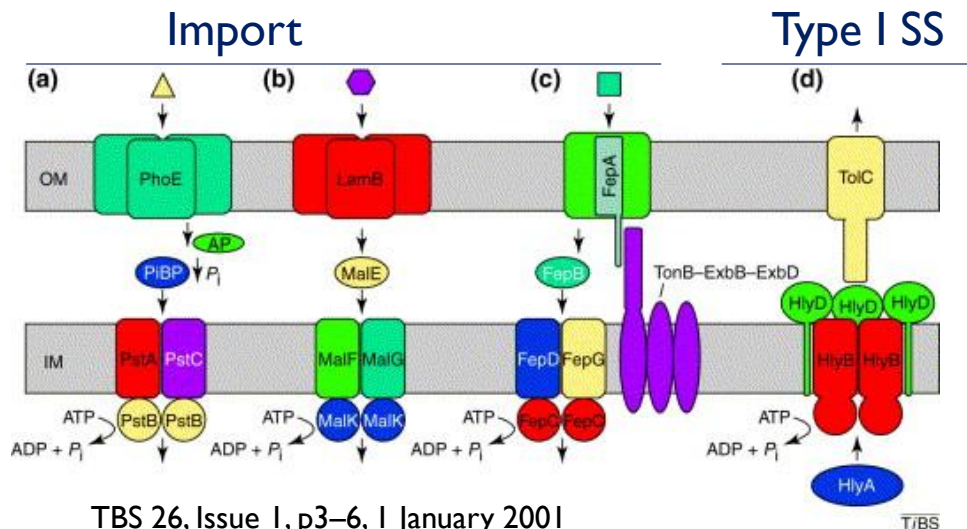
T5SS is important in bacterial pathogens in which autotransporters have a role in adhesion, and invasion. Secreted passengers include enzymes such as Ig proteases and toxins.

<http://www.helsinki.fi/goldman/Pages/research3.html>

T1SS and ATP-binding cassette

T1SS catalyze the **one-step translocation** of polypeptides across both, the inner and outer membranes. It is an ubiquitous system, which consists of only three protein subunits that span the cell envelope:

1. The IM spanning ABC (**ATP-binding cassette**) transporter:
2. A periplasmic connection protein (membrane fusion protein MFP)
3. A connected OM – spanning pore protein (OMP)



ATP-binding cassette (ABC): large family of transporters, operating in bacteria and eukaryotes which import nutrients or export small molecules such as antibiotics and toxins out of the cell. It comprises four domains, two TMDs (transmembrane domains) and two NBDs (nucleotide binding domains) assembled in either one or several polypeptide chains.

TBS 26, Issue 1, p3-6, 1 January 2001

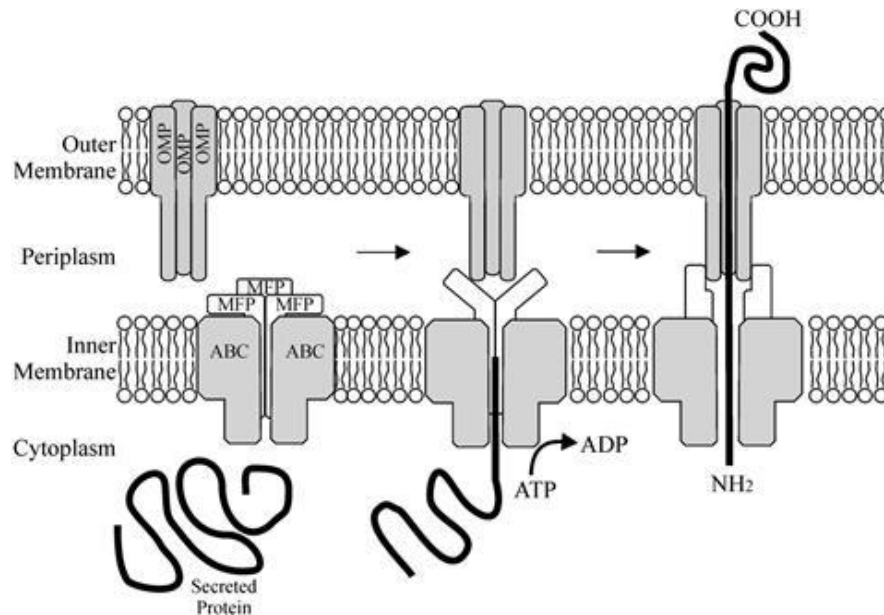
Escherichia coli hemolysin (HlyA) is transported by a complex as well as S-layer proteins, Iron siderophores, toxins).

Transport through T1SS

Type I secretion system transports various molecules, drugs, and proteins of various sizes. Each transporter is specific for one or a few substrates.

T1SS protein substrates contain a **C-terminal signal sequence** that is recognized by the T1SS and remains uncleaved.

Hydrolysis of ATP by the ABC protein provides the energy for the secretion of the substrate

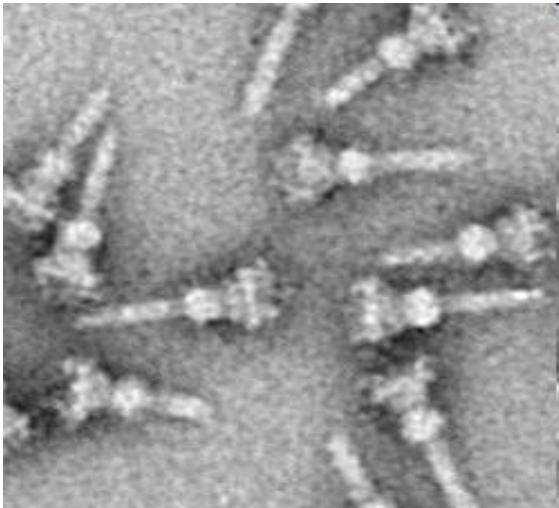


Timothy J. Wells and Ian R. Henderson, University of Birmingham, Birmingham, UK

T1SS is similar to the **multidrug efflux system**, a complex, consisting of an IM transporter (not ABC) and a lipid anchored periplasmic protein, couples to TolC or a family member. Energy derive from A proton-substrate antiport

T3SS – the injectisome

T3SSs have been described as “injectisomes” and “needle and syringe”-like apparatuses because of their structure. They secrete a wide variety of proteins across both the IM and OM membranes and also transport substrates into a target eukaryotic cell membrane in the same step.



A TEM image of isolated **T3SS** **needle** complexes from *Salmonella typhimurium*

Secretion occurs without the formation of periplasmic intermediates, but through a pilus with **needle-like** structure with a tip.

T3SS is present in a wide variety of gram-negative organisms (*Yersinia*, *Salmonella*, *Shigella*, *Pseudomonas* spp.) , with a **primary role in the virulence**. Within the host, these effectors modulate or subvert specific host cell functions, thereby promoting bacterial invasion.



The components of a T3SS

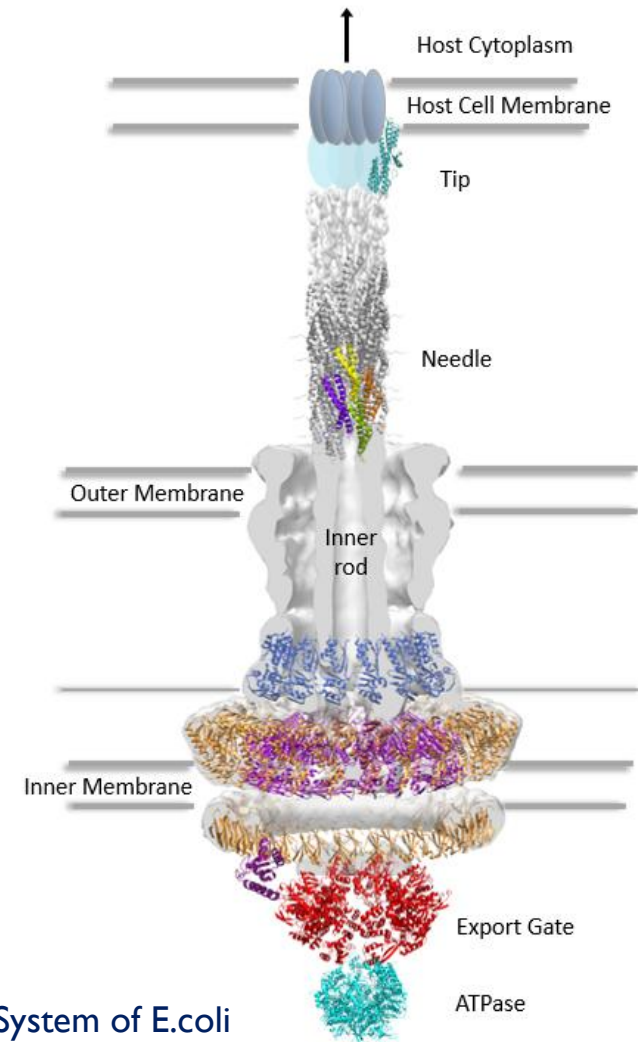
T3SS consists of $\approx 20-25$ proteins, main parts:

A basal body spans the bacterial inner and outer membranes consisting of several rings with a center rod (homologous the basal body of flagellum, with ATPase and export gate).

A filament (needle) : a polymeric protein assemble to form a hollow extracellular appendage extending 60 nm from the OM (wide enough for unfolded effectors)

A translocon: a needle **tip** protein assembled at the apex of the needle and two **hydrophobic translocator proteins** which are secreted through the T3SS and inserted into the host membrane, forming a pore.

The pore in the host membrane enabling the translocation of different **effector proteins** into the cells. Regulatory proteins, act as **channel plug** preventing release of effectors in the absence of a target cell.



Syringe and needle complex – Type III Secretion System of E.coli

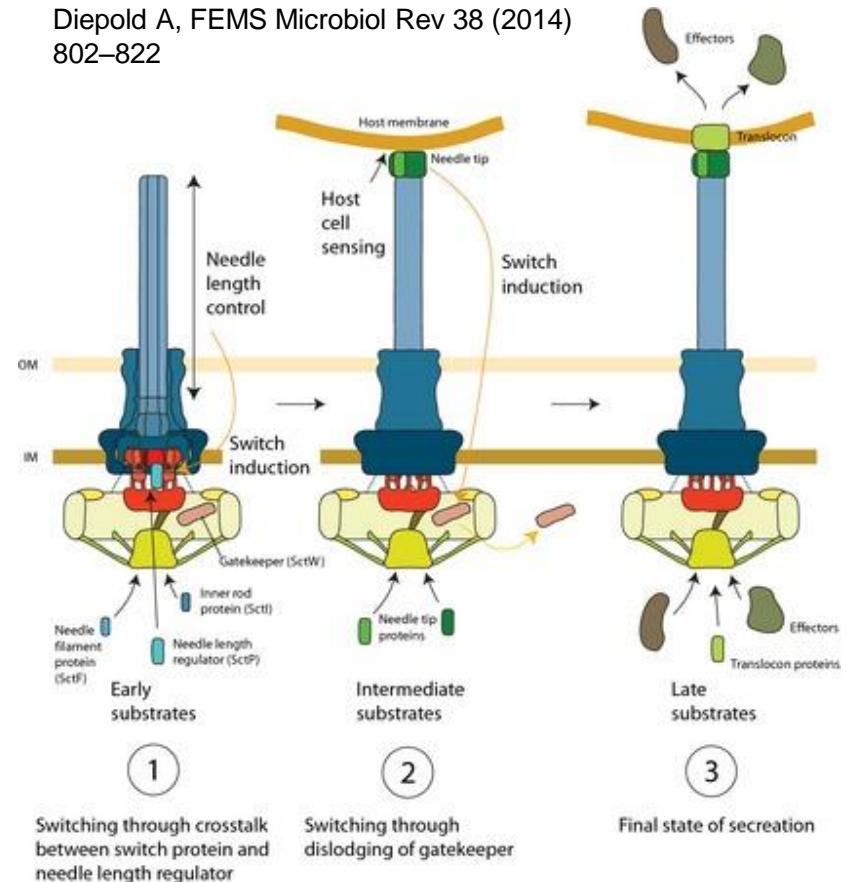
T3SS is a contact-dependent secretion system

T3SS is often a contact-dependent SS. It is assembled as an uncompleted system and protein effectors are not translocated until the bacterium makes contact with the host cell.

These are states of secretion leading to substrate specificity switching.:

- 1) Secretion of early substrates: assembly of the filament
- 2) Intermediate substrates: needle tip proteins
- 3) After contact with host cell membrane: removal of gatekeeper protein and translocation of the effectors through the secretion channel opened.

Diepold A, FEMS Microbiol Rev 38 (2014) 802–822



T3ss effectors are never extracellular, they remain invisible to the immune system: they interfere with cytoskeleton and induce cytokine release and apoptosis.