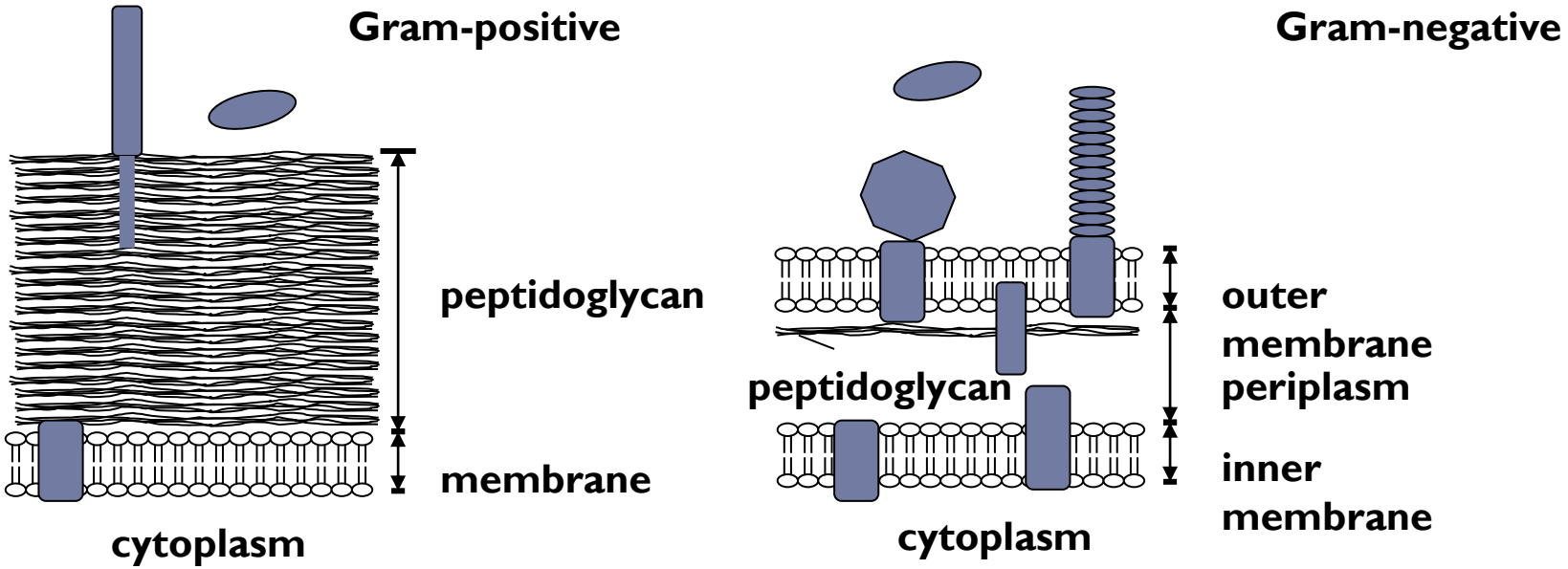
Chapter 2: Bacterial secretion

a.a. 2020-21

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.
- ≈ 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 : they transport adhesins, toxins, exoenzymes, proteases and other virulence factors.
Factors are released into the medium or delivered into host cells.

Bacterial secretion systems

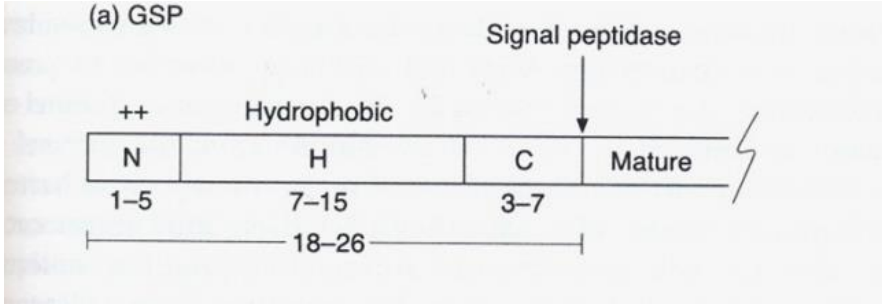
Secretion Apparatus	Secretion Signal	Steps in Secretion	Folded Substrates?	Number of Membranes	Gram (+) or Gram (−)
Sec	N-terminus	1	No	1	Both
Tat	N-terminus	1	Yes	1	Both
T1SS	C-terminus	1	No	2	Gram (−)
T2SS	N-terminus	2	Yes	1	Gram (−)
T3SS	N-terminus	1–2	No	2–3	Gram (−)
T4SS	C-terminus	1	No	2–3	Gram (−)
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 (+)

Sec Secretion Pathway

Sec secretion pathway or general secretory pathway (GSP): responsible for translocation of **most proteins** across the plasma membrane and for insertion of membrane proteins into the membrane. Sec translocates precursors of proteins **in an unfolded state**.

Very conserved transport machinery: homologous to that of eukaryotic endoplasmic reticulum, and archea system

The machinery of Sec pathway recognizes a specific hydrophobic **N-terminal leader sequence** on proteins destined for translocation.
The leader sequence is cleaved by a leader peptidase during the process of transport.

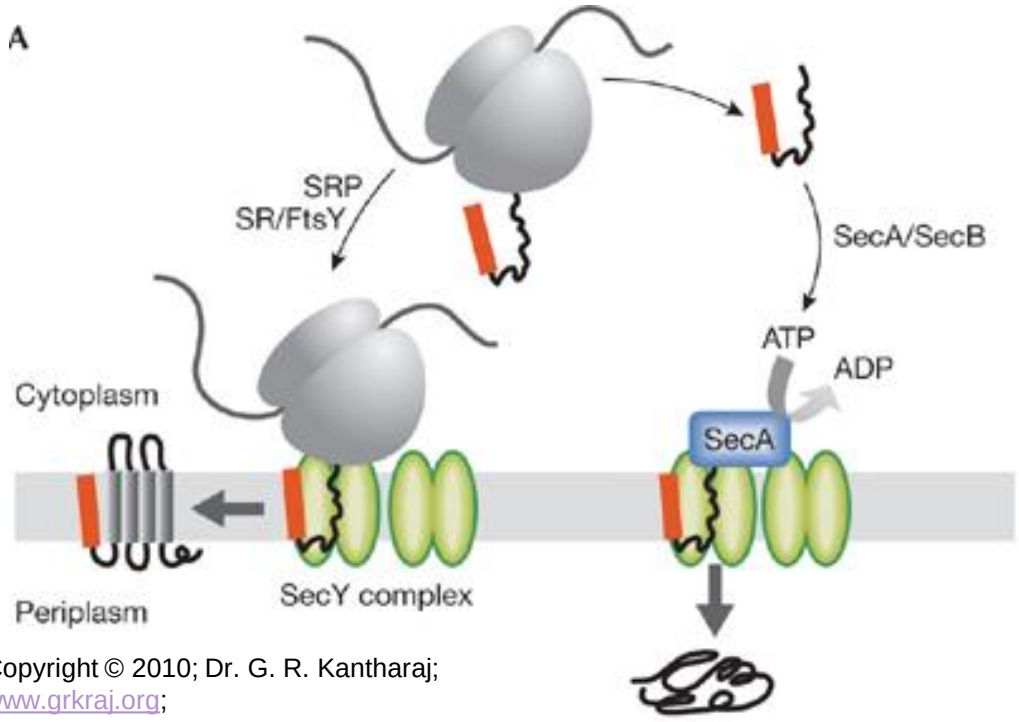


Signal sequence (leader sequence)

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 SecYEG (** 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 partners, there are 2 known routes in which the translocon SecYEG can function:

1. **Sec A/SecB route,** a post-translational pathway

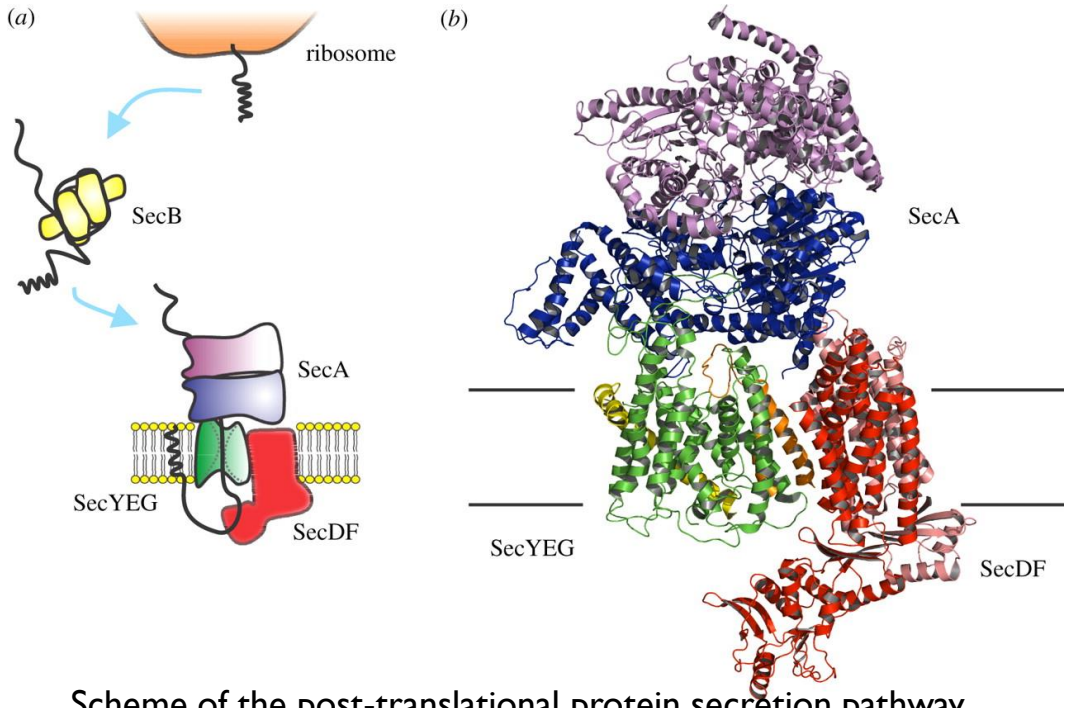
2. **SRP (signal recognition particle) route,** a co-translational pathway (homologous to ER)

Protein translocation through the SecA/SecB route

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

Several SecB polypeptides 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

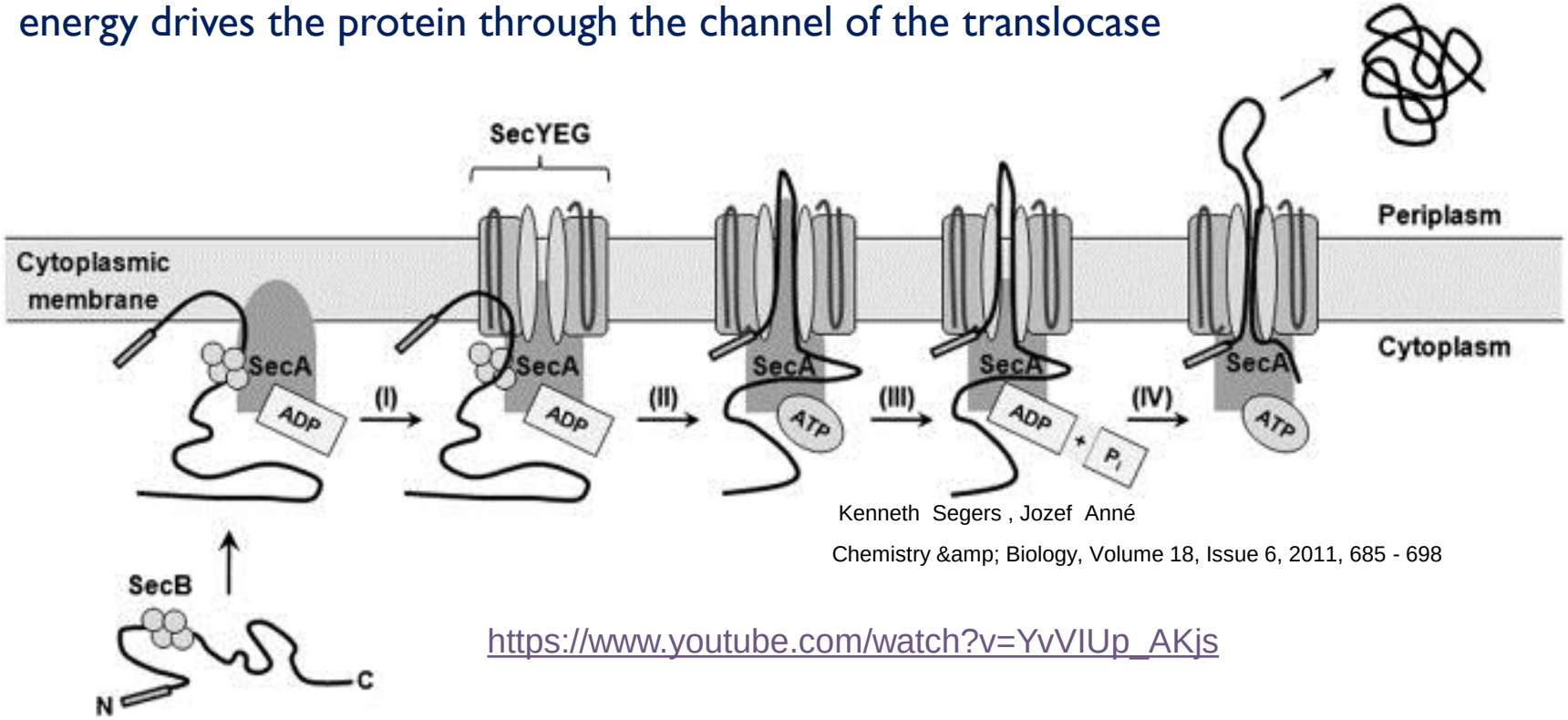


Scheme of the post-translational protein secretion pathway in Escherichia coli.

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

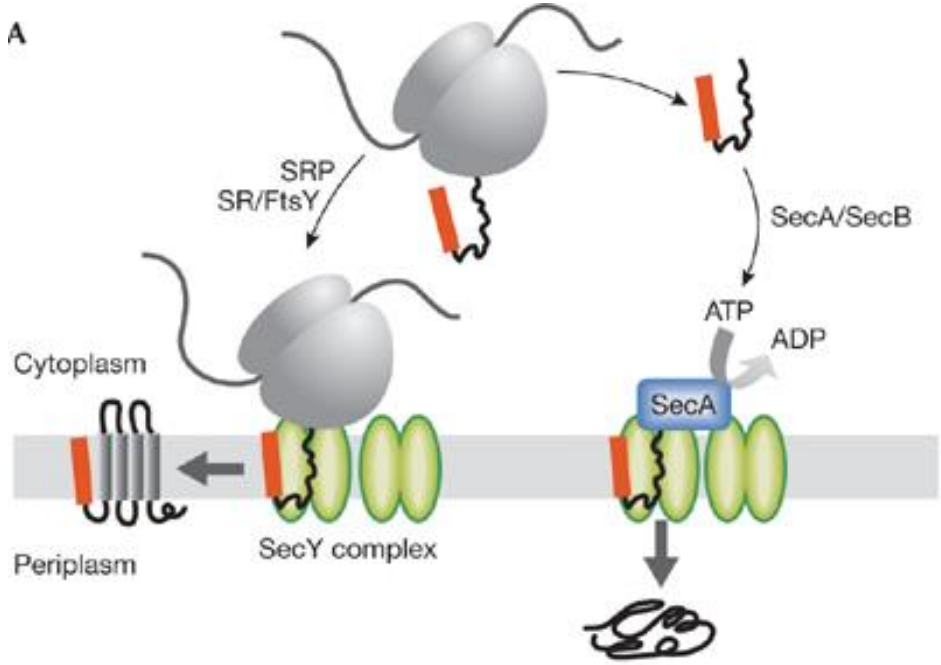
Chemistry & Biology, Volume 18, Issue 6, 2011, 685 - 698

https://www.youtube.com/watch?v=YvVIUp_AKjs

Secretory preproteins (thick black line); the N-terminal portion of the signal peptide is represented as a gray rectangle and it is 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. (System homologous to that of eukaryotic cells)



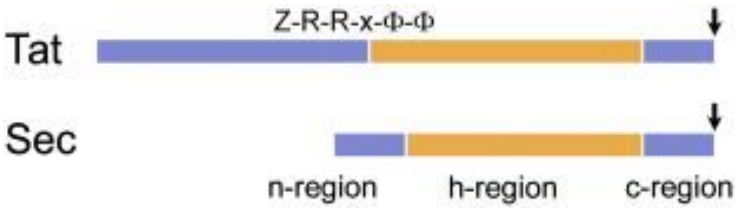
Signal sequence and N-terminal hydrophobic end is bound by the **signal recognition particle (SRP),** a ribonucleoprotein complex that comprises the protein **Ffh** and the **4.5S RNA**.
The complex is recognized by the specific **FtsY** receptor. And targeted to the **SecYEG complex.**

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-**a**rginine **t**ranslocation (TAT) pathway

TAT pathway: translocation of proteins that **are fully folded in the cytoplasm**. Substrates requirement: two consecutive **RR** residues, **twin-arginine,** within the signal sequence.

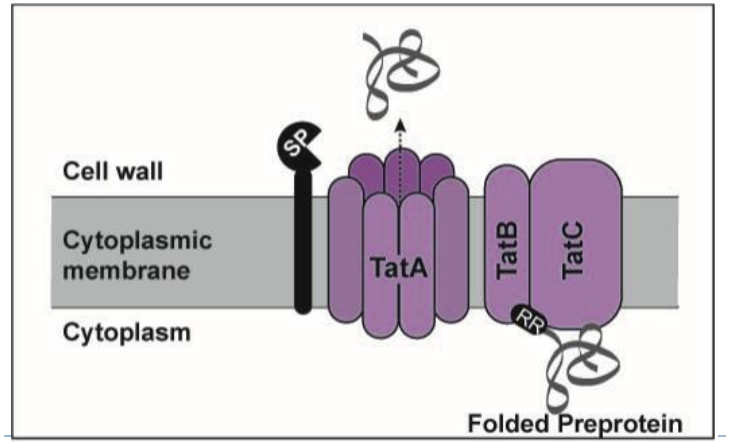
Present in Gram- and Gram+ bacteria, and archea. Described for targeting of proteins into plant chloroplasts and mitochondria. In *E. coli* substrates are complexes with co-factors, oligomeric complexes, respiratory chain complexes, etc.



Tat and sec signal peptide, from E. coli

The TAT pathway system 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 omoligomeric 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.

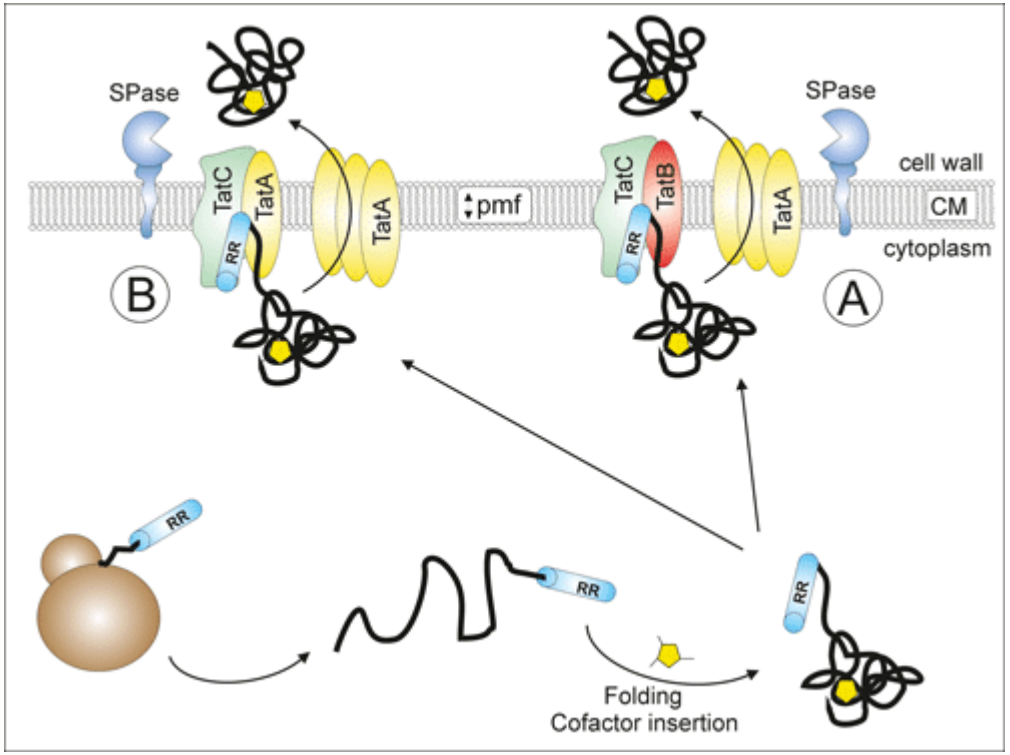


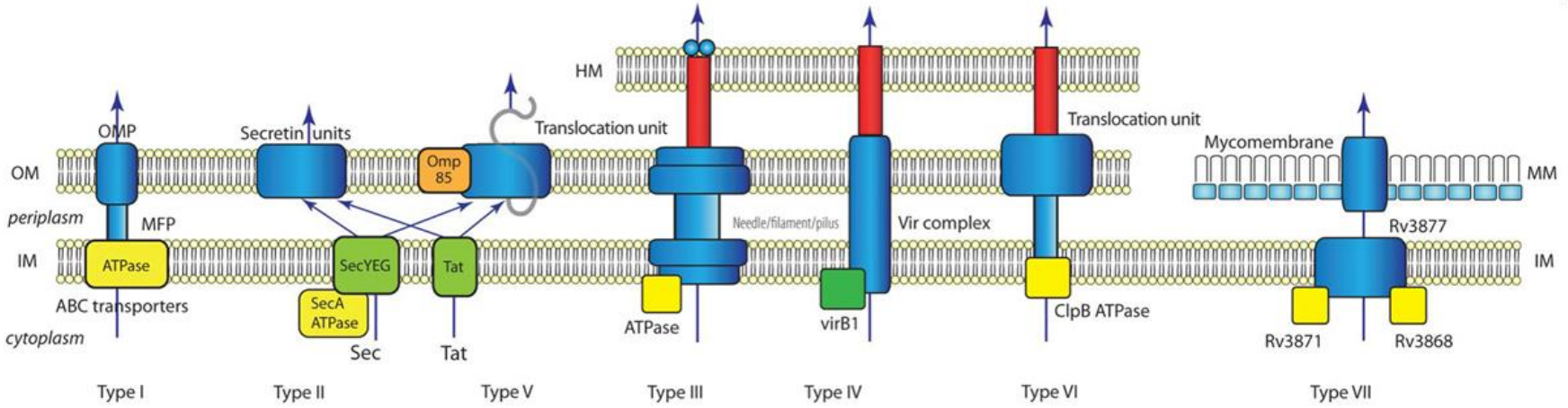
Fig B: Tat function in Gram + bacteria

http://www.fz-juelich.de/ibg/ibg-1/EN/Research/SystemicMicrobiology/protsec/Tat-dependent_protein_translocation.html

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.

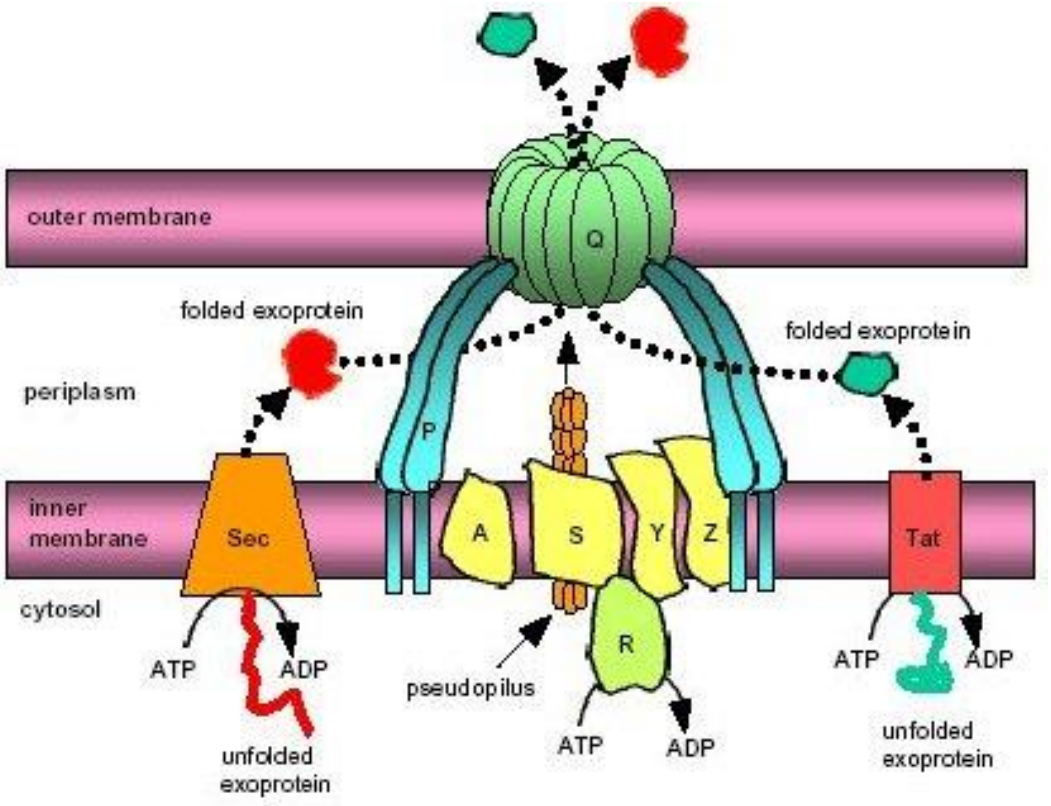


Tseng *et al. BMC Microbiology* 2009 **9**(Suppl 1):S2 Â

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)

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.
Homologous to type IV pilus.
Structural motifs on exoprotein surface determines substrate specificity.

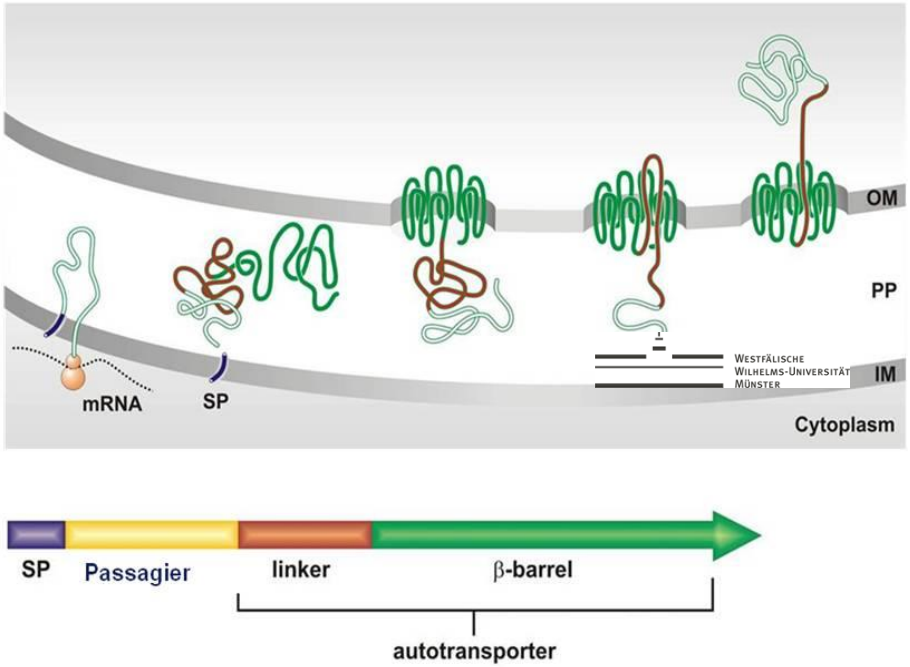
It is required pathogens to export toxins, exoenzymes etc Examples: *V. cholerae* (cholera toxin)

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. Two partner secretion).

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

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

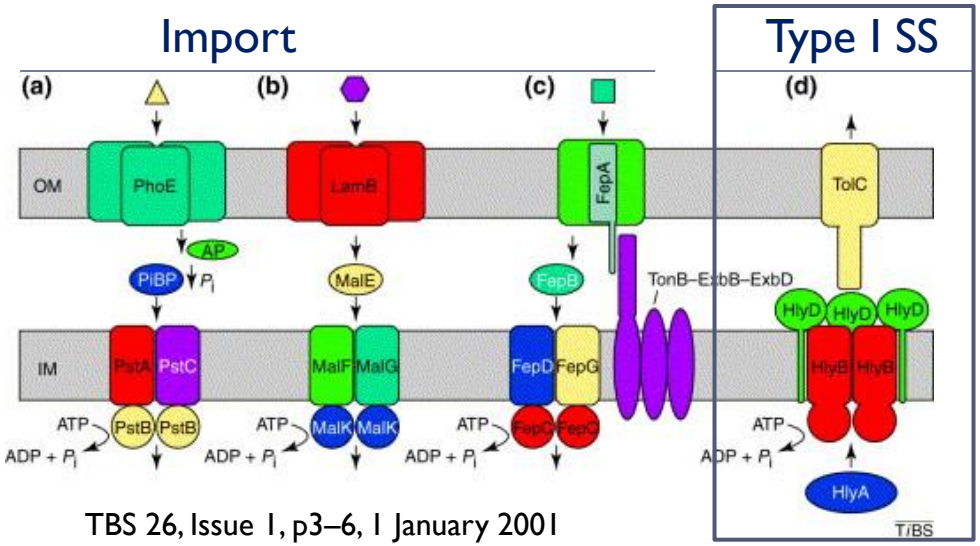


On the surface someT5SS proteins **undergo an autoproteolytic process**, while others remain anchored to the cell surface by the ß-domain). T5SS is important in bacterial pathogens in which autotransporters have a role in adhesion, and invasion with secretion of proteases and toxins.

T1SS and ATP-binding cassette

TISS 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)



TBS 26, Issue 1, p3–6, 1 January 2001

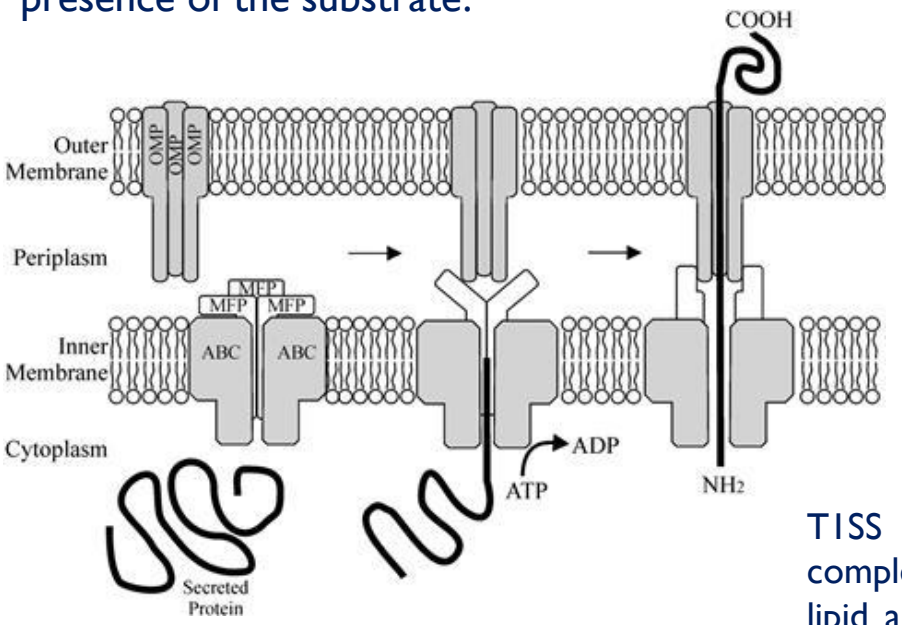
ATP-binding cassette (ABC): large family of transporters, operating in bacteria and eukaryotes which import nutrients or export small molecules such as antibiotics and toxins or proteins out of the cell.

It comprises four domains, two TMDs (transmembrane domains) and two NBDs (nucleotide binding domains), ATPases assembled in either one or several polypeptide chains.

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

Transport through T1SS

Each T1SS transporter is specific for one or a few substrates. Protein substrates contain a **C-terminal signal sequence** that is recognized by the ABC protein of the T1SS and remains uncleaved. ABC proteins form a stable complex in the inner membrane, and recruit TolC in the presence of the substrate.



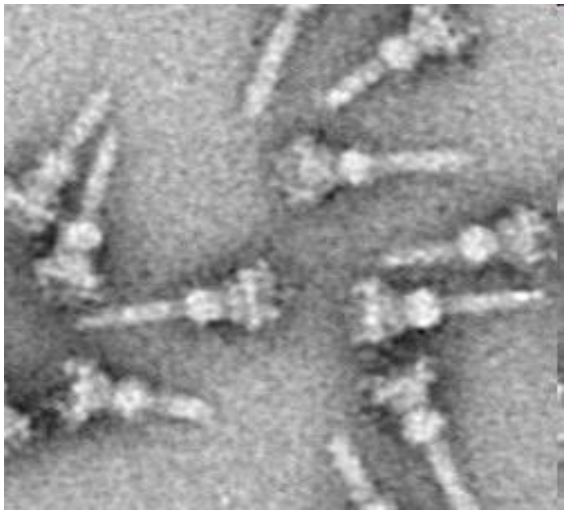
Timothy J. Wells and Ian R. Henderson,
University of Birmingham, Birmingham, UK

The complex forms a tunnel across the periplasm, which guarantees the continuous translocation of unfolded HlyA without any periplasmic intermediate.
Hydrolysis of ATP by the ABC protein provides the energy for the secretion of the substrate

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 tather than ATP

T3SS – the injectisome

T3SSs or "injectisomes" or "needle and syringe-like apparatus" because of their structure. T3SS secrete a wide variety of proteins (effectors) across both the IM and OM membranes and **transport substrates into a target eukaryotic cell membrane** in the same step.



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

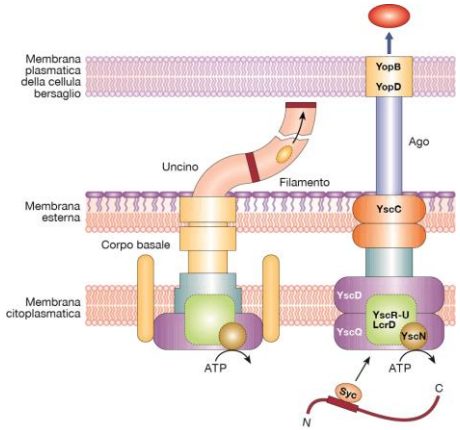
Secretion occurs without the formation of periplasmic intermediates, but through a pilus with **needle-like** structure with a tip. T3SS effectors are never extracellular, they remain invisible to the immune system:

Within the host, these effectors modulate or subvert specific host cell functions, thereby promoting bacterial invasion and inhibiting phagocytosis, preventing apoptosis, and cytokine release.

T3SS is present in a wide variety of gram-negative organisms (*Yersinia, Salmonella, Shigella, Pseudomonas* spp.) , with a **primary role in the virulence.**

The components of a T3SS

T3SS consists of ≈20-25 proteins, main parts:

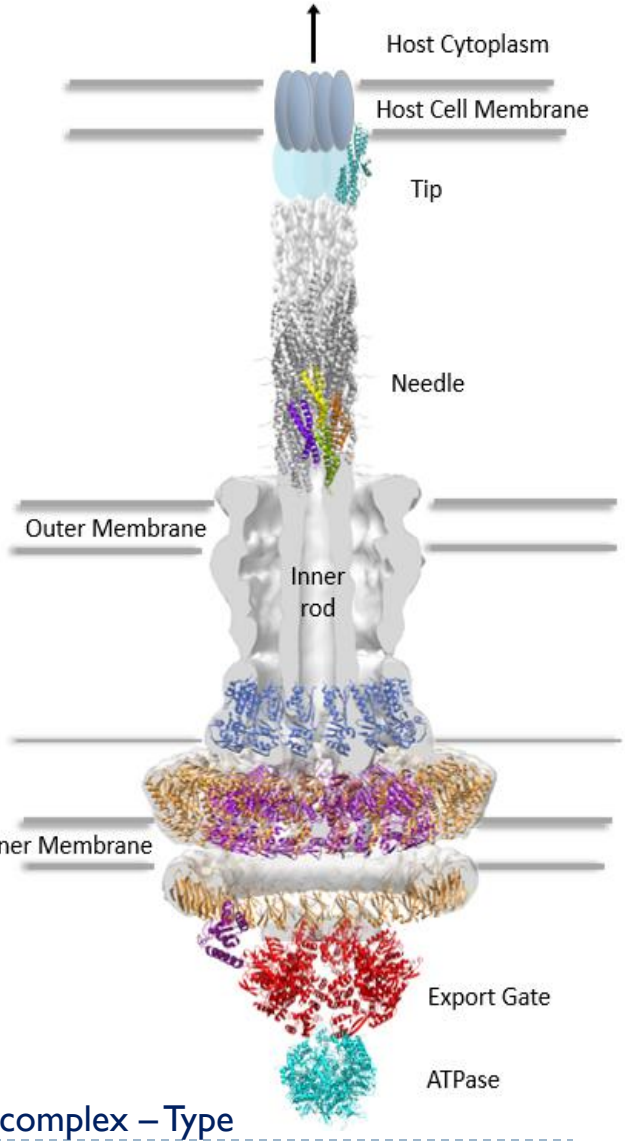


A basal body spans the IM and OM consisting of several rings with a center rod (homologous the basal body of flagellum, with ATPase and export gate).

A filament (needle): a polymeric protein assemble to form a hollow 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** secreted through the T3SS and inserted into the host membrane, forming a pore. It enables the translocation of **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

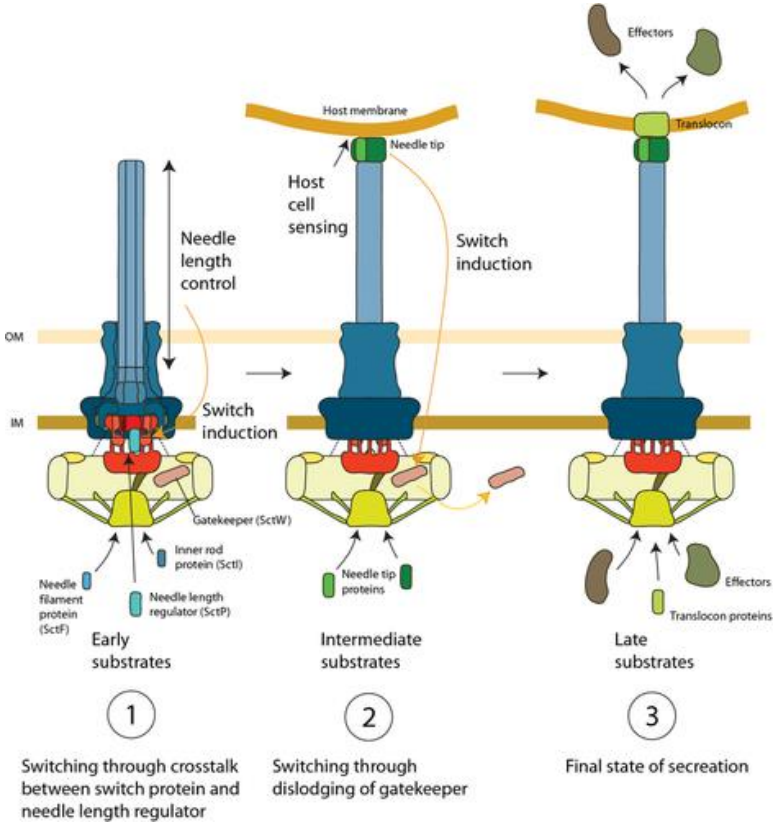
T3SS is usually assembled as uncompleted system and protein effectors are not translocated until the bacterium makes contact with the host cell.

These are states of secretion leading to substrate specificity switching:

1) Secretion of early substrates: assembly of the filament. Length is controlled.

2) Intermediate substrates: needle tip proteins which sense contacts with host cell membrane.

3) After contact the removal of gatekeeper protein occurs and starts translocation of the translocon proteins and effector proteins through the secretion channel opened.



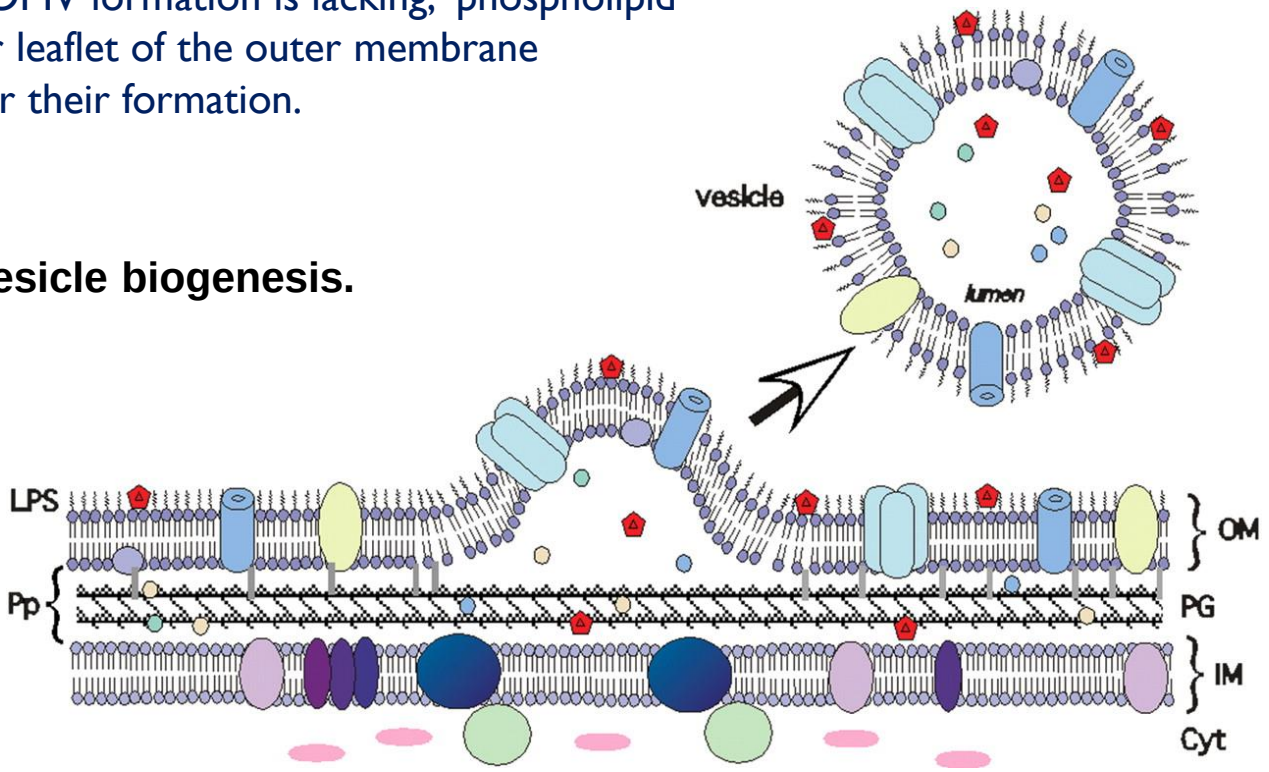
Diepold A, FEMS Microbiol Rev 38 (2014) 802–822

https://elifesciences.org/articles/39514

Bacterial outer membrane vescicles (OMV)

Vesicles released from the envelope of growing bacteria serve as secretory vehicles for proteins, DNA and lipids of Gram-negative bacteria.
OMVs are ascribed the functionality to provide a manner to communicate among themselves, with other microorganisms in their environment and with the host.
A general mechanism of OMV formation is lacking, phospholipid accumulation in the outer leaflet of the outer membrane seems to be important for their formation.

Model of vesicle biogenesis.



Meta J. Kuehn, and Nicole C. Kesty Genes Dev. 2005;19:2645-2655