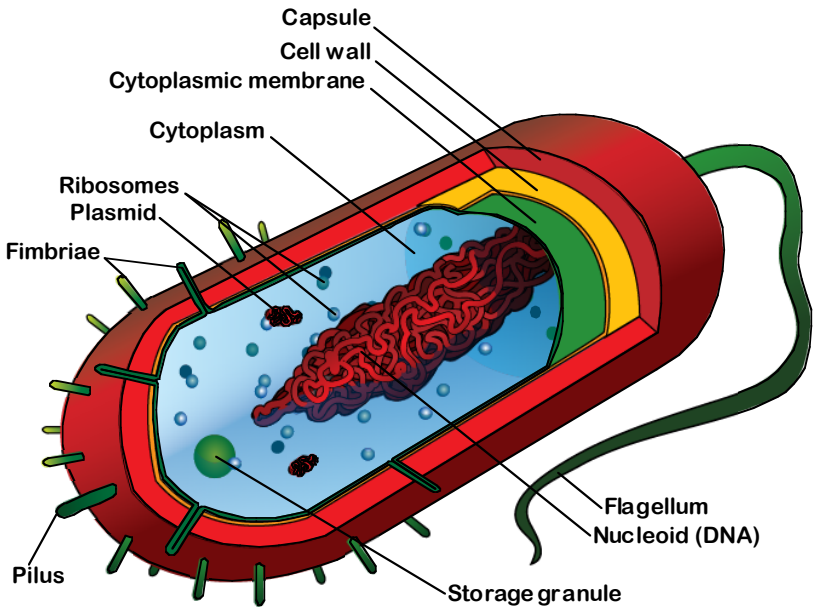
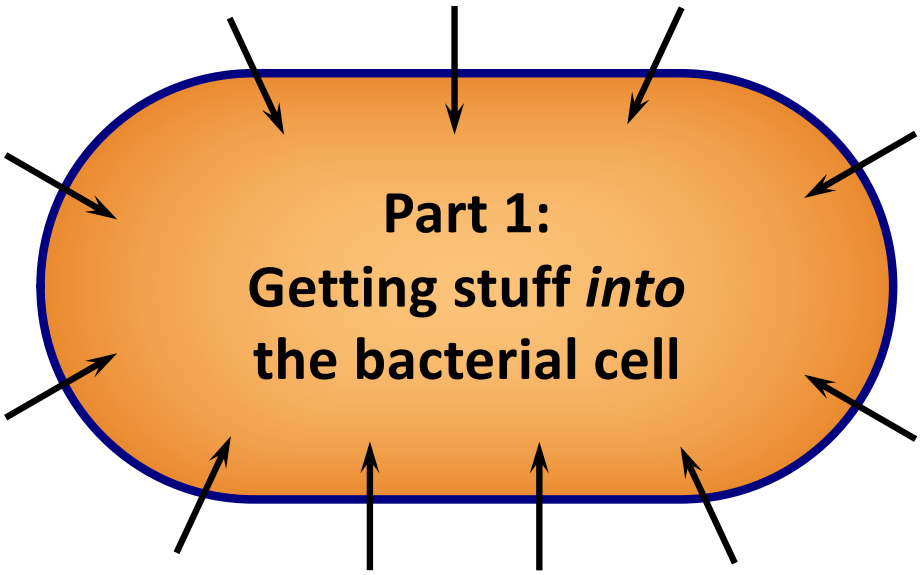
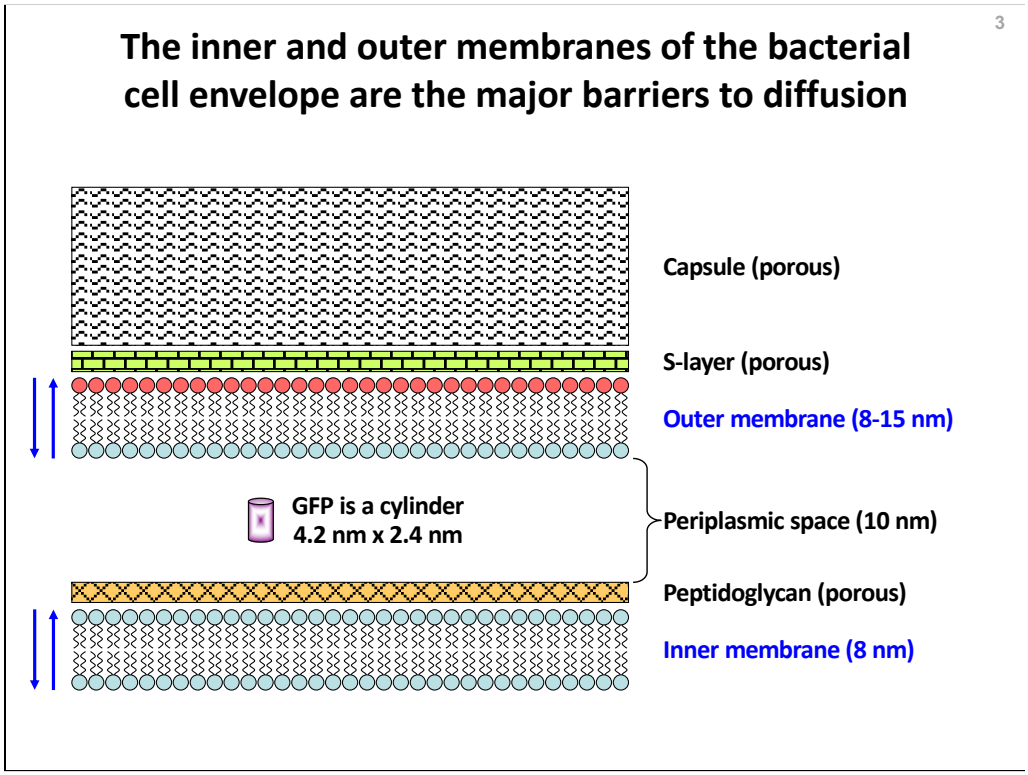
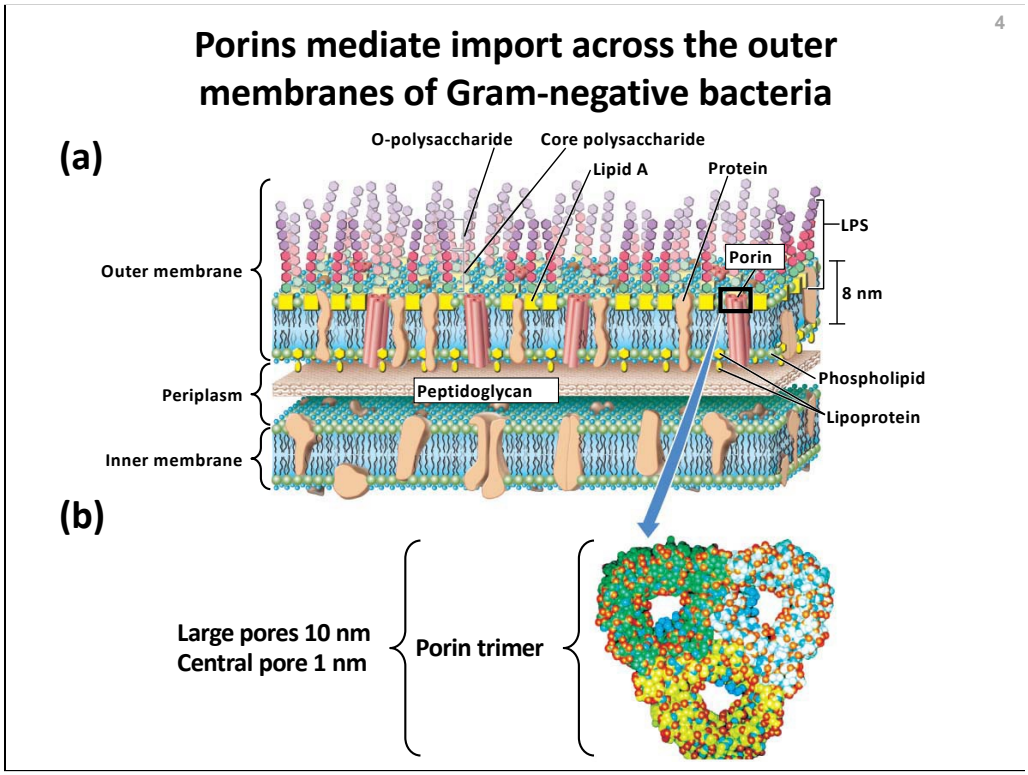
Transport across the bacterial cell envelope




Part 1:
Getting stuff *into*
the bacterial cell



As a familiar size reference ("molecular ruler"): green fluorescent protein (GFP) is a beta-barrel protein consisting of 238 amino acids with a molecular mass of 27 kDa and has the shape of a cylinder with a length of 4.2 nm and diameter of 2.4 nm.



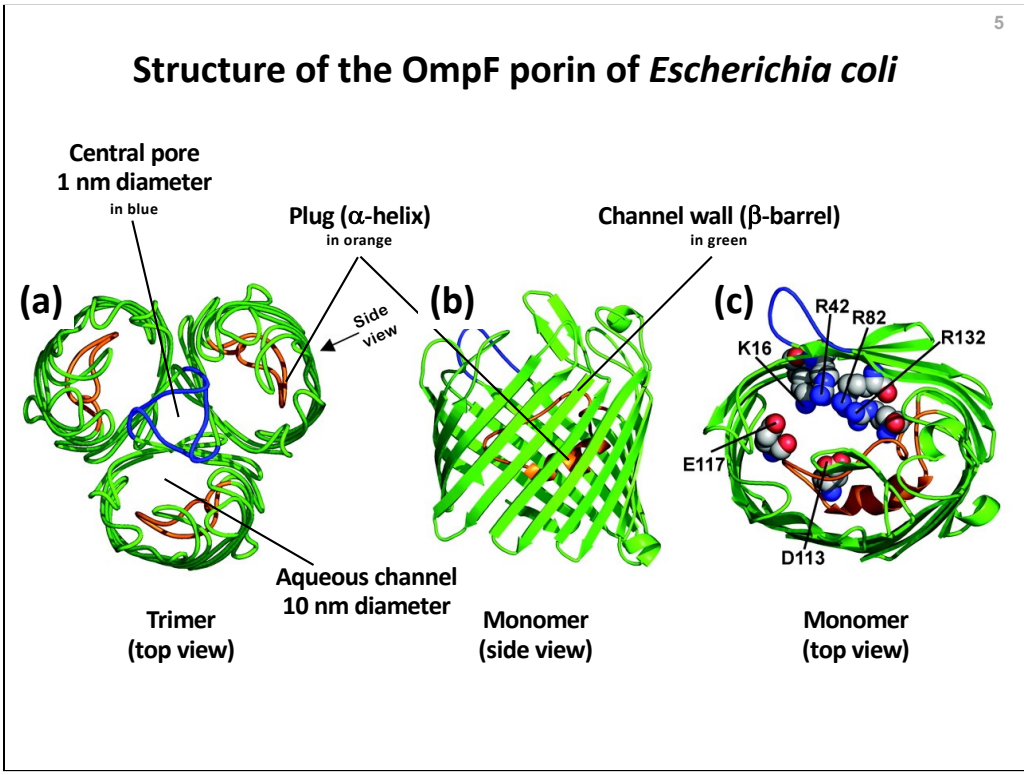
SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 3.20. The Gram-negative cell wall. Note that although the outer membrane is often called the "second lipid bilayer," the chemistry and architecture of this layer differ in many ways from that of the cytoplasmic membrane, as we saw in Week 2 of BIO-372.

(a) Arrangement of lipopolysaccharide (LPS), lipid A, phospholipid, porins, and lipoprotein in the outer membrane.

(b) Molecular model of porin proteins. Note the four pores present, one within each of the proteins forming a porin molecule and a smaller central pore between the porin proteins. The view is perpendicular to the plane of the membrane. Model based on X-ray diffraction studies of *Rhodobacter blasticus* porin.

Note that an average protein is about 2-6 nm in diameter. So proteins of this size could, in principle, pass through the large pores of porin (which are 10 nm in diameter). Prokaryotic ribosomes are large multi-protein complexes, about 20 nm in diameter. The amino acids that make up proteins range in size from the tiny glycine, with a molecular mass of roughly 75 Da, to the 204 Da mass of tryptophan, the largest of the naturally occurring amino acids. Their respective lengths vary from 0.4 nm to 1 nm, so all amino acids could, in principle, pass through the large pores of porin (10-nm diameter) or the small central pore of porin (1-nm diameter).



SOURCE: Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.* 67: 593-656 PMID:14665678.

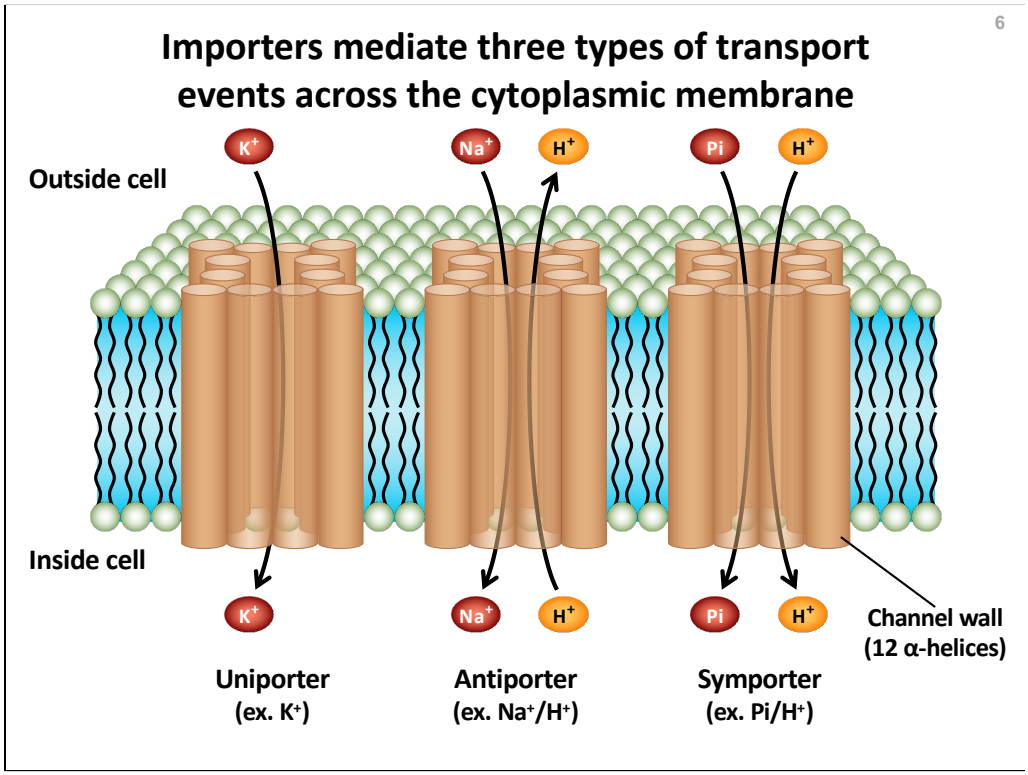
Figure 2. Structure of the OmpF porin of *E. coli*.

(a) View of the porin trimer from the top, that is, in a direction perpendicular to the plane of the membrane. Loop 2, colored blue, plays a role in interaction of the monomer with its neighboring monomer subunits. The L3 loop, colored orange, narrows the channel.

(b) View of the monomeric unit from the side, roughly in the direction of the arrow in panel A. Loops 2 and 3 are colored as in panel A.

(c) View of the monomeric unit from the top, showing the "eyelet" or the constricted region of the channel. The eyelet is formed by Glu117 and Asp113 from the L3 loop, as well as four basic residues from the opposing barrel wall (Lys16, Arg42, Arg82, Arg132), all shown as spheres.

Opening and closing of the porin channel, by movement of the L3 loop, is voltage-gated in many porins, including OmpF.



SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 3.11. Structure of membrane-spanning transporters and types of transport events. In prokaryotes (bacteria and archaea), membrane-spanning transporters typically contain 12 alpha-helices (each shown here as a cylinder) that aggregate to form a channel through the membrane. Shown here are three transporters with differing types of transport events. For antiporters and symporters, the co-transported substrate is shown in orange. Antiporters transport the substrate and co-substrate in opposite directions. Symporters transport the substrate and co-substrate in the same direction. All of the examples shown are "simple transporters" (see Slide 8).

Abbreviations: Pi is inorganic phosphate (HPO_4^{2-}).

7

Imagine [A] is high outside the cell and low inside the cell, while [B] is low outside the cell and high inside the cell. Which type of transporter would be most useful to import B “uphill” against its concentration gradient?

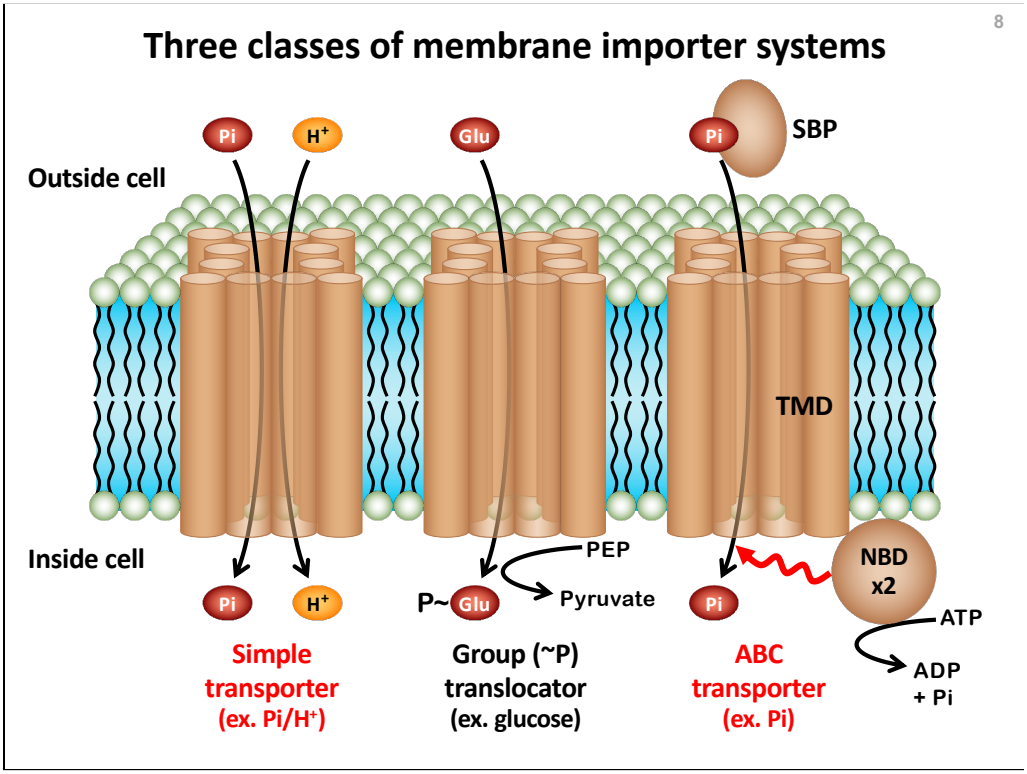
A. A uniporter that transports A.

B. A uniporter that transports B.

C. A symporter that transports A and B.

D. An antiporter that transports A and B.

Answer: (C)

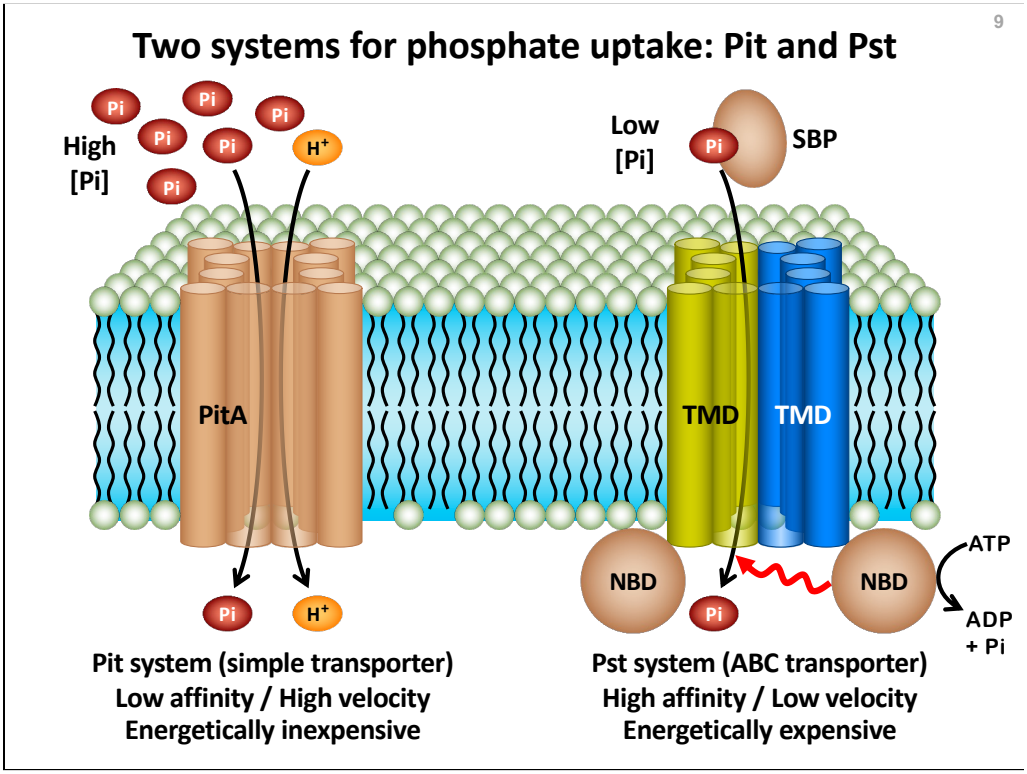


SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 3.10. The three classes of membrane transport systems: simple transporters; group translocators; ATP Binding Cassette (ABC) transporters. The three proteins of the ABC transporter system are: substrate-binding protein (SBP); transmembrane domain protein (TMD); nucleotide-binding domain protein (NBD). The ABC transporter complex contains two associated NBD proteins, only one of which is shown in the diagram.

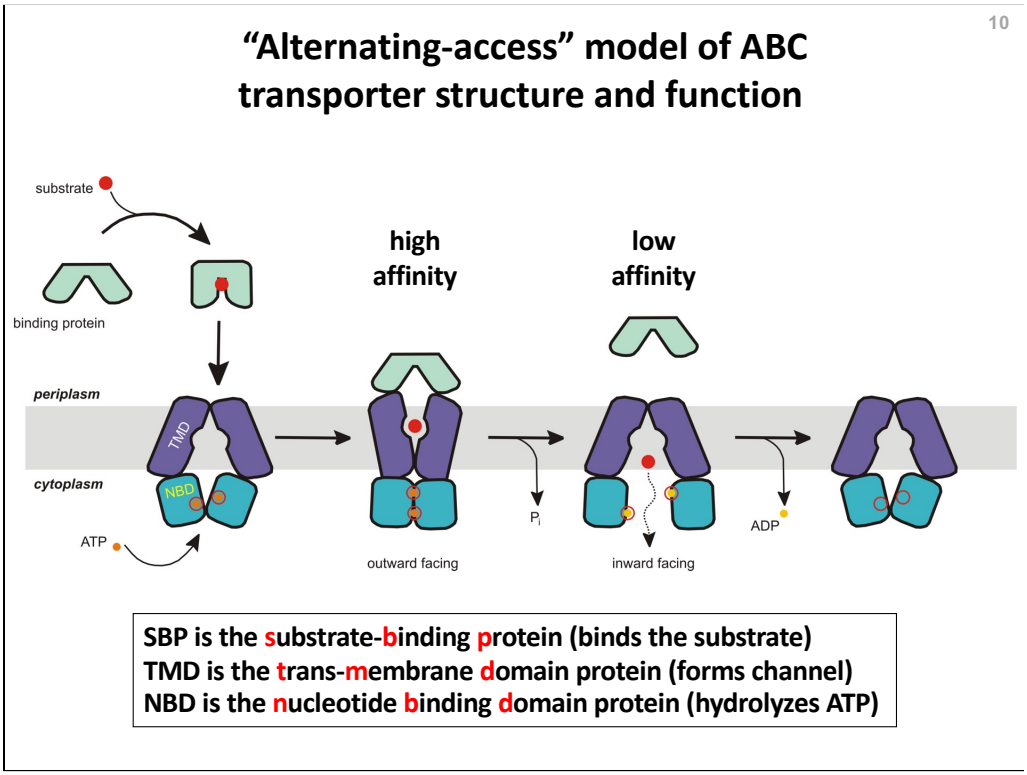
Note how simple transporters and ABC transporters move substances across the membrane without chemically modifying them, whereas group translocators result in the chemical modification (by phosphorylation) of the transported substance.

Abbreviations: Pi, inorganic phosphate (HPO_4^{2-}). SBP, substrate-binding protein. TMD, transmembrane domain protein. NBD, nucleotide-binding domain protein.



SOURCE: Lamarche MG, Wanner BL, Crépin S, Harel J (2008) The phosphate regulon and bacterial virulence: a regulatory network connecting phosphate homeostasis and pathogenesis. *FEMS Microbiol. Rev.* 32: 461-473. PMID: 18248418.

Prokaryotes often contain two or more importers for the same essential nutrients, such as inorganic phosphate (HPO_4^{2-}). At high substrate concentrations, "simple transporters" are used. The HPO_4^{2-}/H+ symporter (called “Pit” on the left) is an example. Simple transporters are high-velocity and energetically efficient because transport energy is provided by the proton motive force. The tradeoff is that simple transporters have low affinity for their substrates. Consequently, when the external concentration of substrate falls below a critical threshold, these transporters are no longer effective. Under low-substrate conditions, "ABC transporters" take over. The HPO_4^{2-} importer (called “Pst” on the right) is one example. ABC transporters display high affinity for their substrates, permitting them to scavenge nutrients even when the external substrate concentration is extremely low. The tradeoff is that ABC transporters are low-velocity and energetically costly because the transport energy is provided by ATP hydrolysis driving protein conformational switching (see Slide 10).



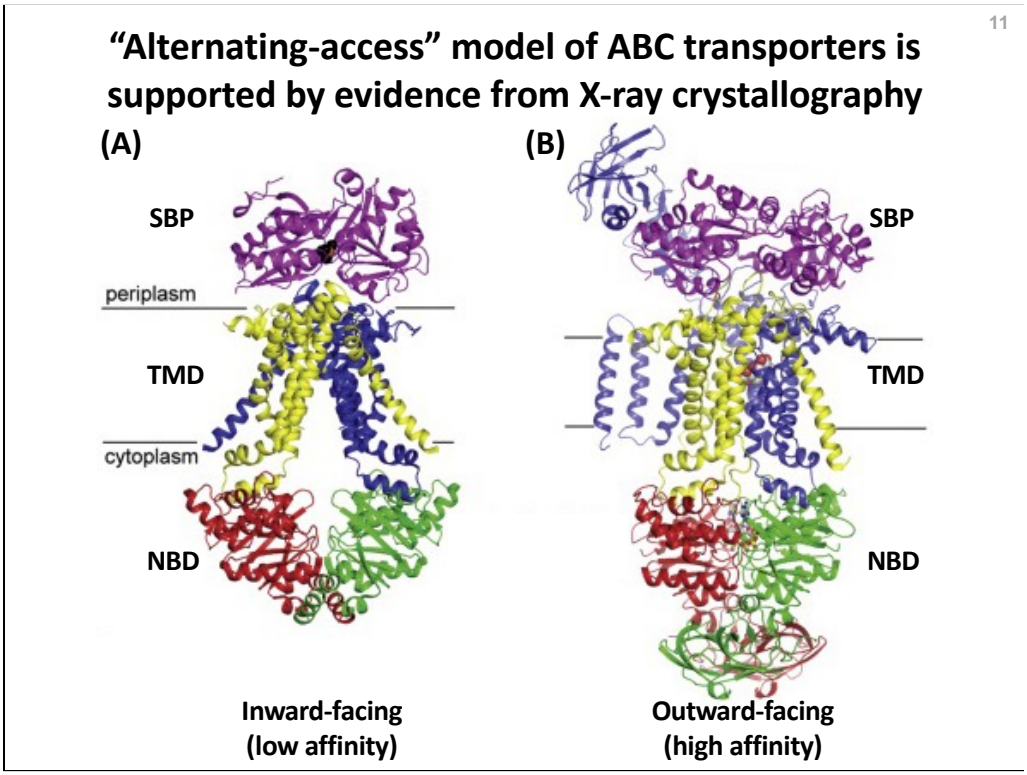
SOURCE: Oldham ML, Davidson AL, Vhen J (2008) Structural insights into ABC transporter mechanisms. *Curr. Opin. Struct. Biol.* 18: 726-733 PMID:18948194.

Figure 1. A model for "alternating-access" transport by ABC transporters, modified from: Oldham ML, Khare D, Quiocho FA, Davidson AL, Chen J (2007) Crystal structure of a catalytic intermediate of the maltose transporter. *Nature* 450(7169): 515-521 PMID: 18033289.

ABC transporters require energy in the form of adenosine triphosphate (ATP) to translocate substrates across cell membranes. These proteins harness the energy of ATP binding and hydrolysis to drive conformational changes in the trans-membrane domain (TMD) proteins. The "alternating-access model" describes the mechanism by which cyclical TMD conformational changes mediate transport of molecules across the membrane. In this model, the substrate-binding site alternates between outward-facing and inward-facing conformations. The relative binding affinities of the two conformations for the substrate determines the net direction of transport. For ABC importers, since translocation is from the periplasm to the cytoplasm, the outward-facing conformation has higher binding affinity for substrate than the inward-facing conformation.

The "ATP switch model" describes conformational changes in the nucleotide-binding domain (NBD) proteins as a result of ATP binding and hydrolysis. This model presents two principal conformations of the NBDs: formation of a closed dimer upon binding two ATP molecules and dissociation to an open dimer facilitated by ATP hydrolysis and release of inorganic phosphate (Pi) and adenosine diphosphate (ADP). Switching between the open and closed NBD dimer conformations induces conformational changes in the TMD, resulting in substrate translocation.

In the resting state of ABC transporters, the NBDs are in an open-dimer configuration with low affinity for ATP. This open conformation possesses a chamber accessible to the interior of the transporter. The transport cycle is initiated by binding of substrate to the high-affinity site on the TMDs, which induces conformational changes in the NBDs and enhances the binding of ATP. Two molecules of ATP bind cooperatively to form the closed dimer. The closed NBD dimer induces a conformational change in the TMDs such that the TMD opens, forming a chamber with an opening opposite to that of the initial state. The affinity of the substrate to the TMD is reduced and the substrate is released. Hydrolysis of ATP follows and then sequential release of P_i and then ADP restores the transporter to its resting configuration.

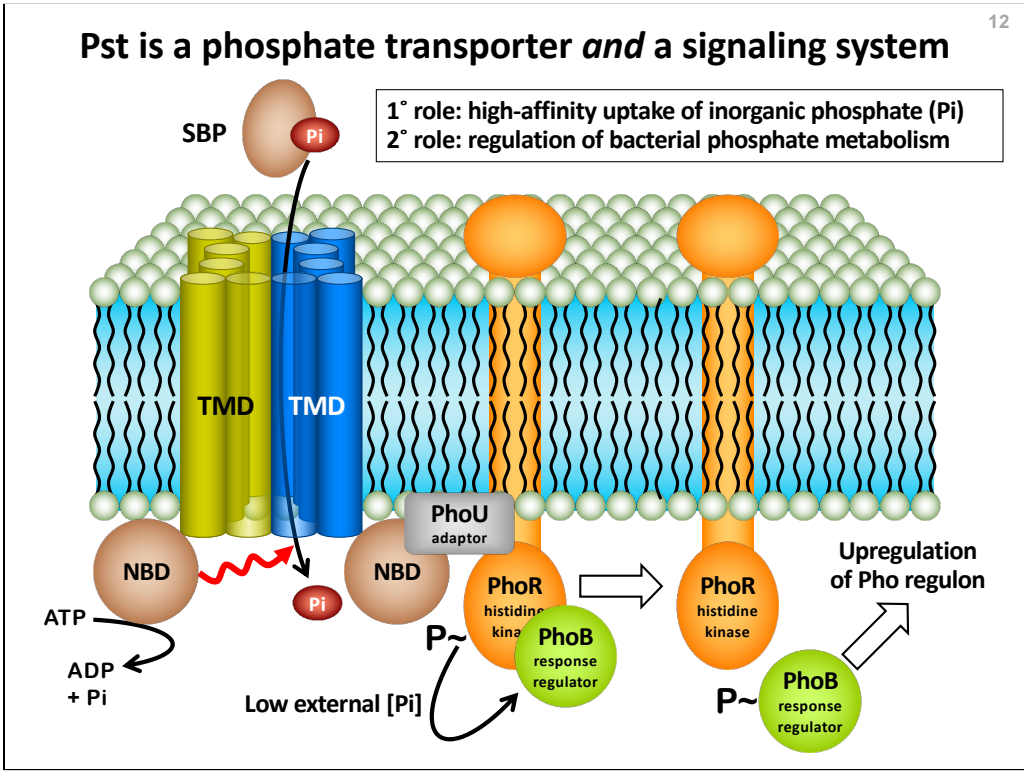


SOURCE: Oldham ML, Davidson AL, Vhen J (2008) Structural insights into ABC transporter mechanisms. *Curr. Opin. Struct. Biol.* 18: 726-733 PMID:18948194.

Figure 1. The "alternating-access" model of ABC transporter function is supported by studies of protein structure using X-ray crystallography. Structures of the inward-facing molybdate/tungstate transporter AfModBC-A **(A)** and the outward-facing maltose transporter MalFGK2-E **(B**) are shown. For each structure, the substrate-binding protein (SBP) is colored in purple, the transmembrane domain (TMD) subunits are colored in blue and yellow, and the nucleotide-binding domain (NBD) subunits are colored in red and green.

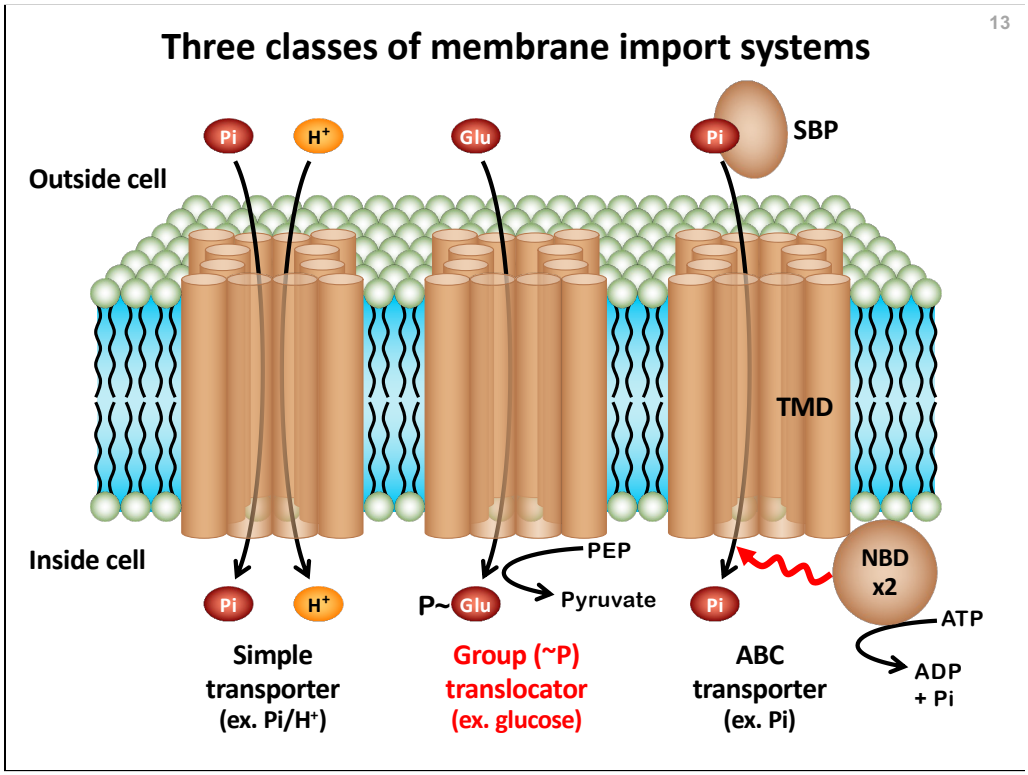
The crystal structure of the *Escherichia coli* maltose transporter **(B)** adopts an outward-facing conformation with a large occluded periplasm-facing transmembrane cavity formed by the TMDs and capped by the maltose binding protein. The substrate is found at the base of this cavity and the NBDs are dimerized around bound ATP.

Abbreviations: **SBP** is the substrate-binding protein (which binds the substrate with high affinity and specificity). **TMD** is the trans-membrane domain protein (which forms the substrate-translocation channel). **NBD** is the nucleotide binding domain protein (which hydrolyzes ATP).



SOURCE: Lamarche MG, Wanner BL, Crépin S, Harel J (2008) The phosphate regulon and bacterial virulence: a regulatory network connecting phosphate homeostasis and pathogenesis. *FEMS Microbiol Rev* 32: 461-473. PMID: 18248418.

ABC transporters often serve two functions: (1) high-affinity uptake of a substrate (the primary function), and (2) signal transduction to a two-component regulatory system (the secondary function). The **Pst** ABC transporter is an example. The Pst substrate binding protein (SBP) has an extremely high affinity for its substrate, inorganic phosphate (HPO_4^{2-}), which permits efficient uptake even when this nutrient is scarce in the environment. The Pst system interacts with the PhoR/PhoB "two-component system" via the adaptor protein PhoU. Two-component systems comprise a bifunctional histidine kinase-phosphatase (for example, PhoR) and a transcriptional response regulator (for example, PhoB). PhoR undergoes autophosphorylation by transferring the gamma-phosphate from ATP to a unique histidine residue in the PhoR "catalytic domain" (P~PhoR). Under **low phosphate** conditions, when the Pst system is essential for phosphate scavenging, **PhoR functions as a kinase** by transferring the phosphate from its catalytic domain histidine residue to a unique aspartate in the PhoB "receiver domain". The phosphorylated form of PhoB (P~PhoB) is active for DNA binding and increased transcription of the “Pho regulon” by RNA polymerase in the “induced” (high expression) state. The Pho regulon comprises a set of genes that are important for physiological adaptation to low-phosphate conditions. Under **high phosphate** conditions, when the Pit system is primarily responsible for phosphate scavenging, **PhoR functions as a phosphatase**, maintaining PhoB in the unphosphorylated (inactive) state and keeping expression of the Pho regulon in the “uninduced” (low expression) state.



SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

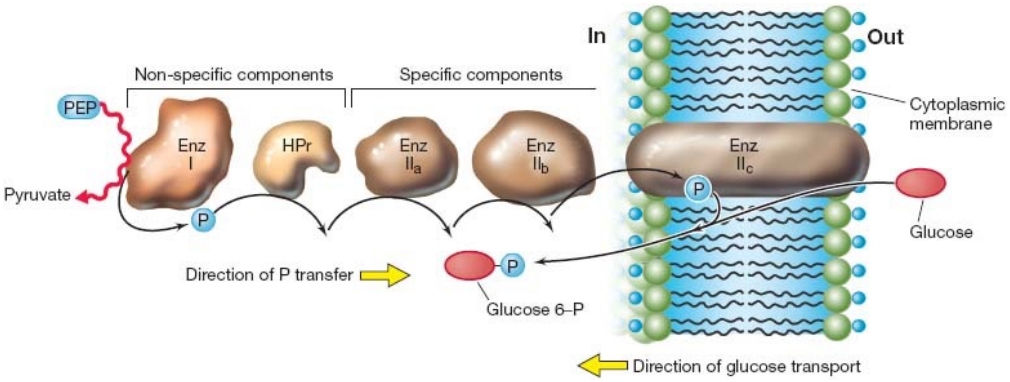
Figure 3.10. The three classes of membrane transport systems: simple transporters; group translocators; ATP Binding Cassette (ABC) transporters. The three proteins of the ABC transporter system are: substrate-binding protein (SBP); transmembrane domain protein (TMD); nucleotide-binding domain protein (NBD). The ABC transporter complex contains two associated NBD proteins, only one of which is shown in the diagram.

Note how simple transporters and ABC transporters move substances across the membrane without chemically modifying them, whereas group translocators result in the chemical modification (by phosphorylation) of the transported substance.

Abbreviations: Pi, inorganic phosphate (HPO_4^{2-}). SBP, substrate-binding protein. TMD, transmembrane domain protein. NBD, nucleotide-binding domain protein.

The PTS (phosphotransferase system) is a "group translocator" that mediates the uptake of sugars

1˚ role: high-affinity uptake of glucose and other sugars
2˚ role: regulation of bacterial carbon metabolism



Glucose + Pi → Glucose-6-Pi ΔG˚' = +3.3 kcal/mol
PEP → Pyruvate + Pi ΔG˚' = –14.8 kcal/mol

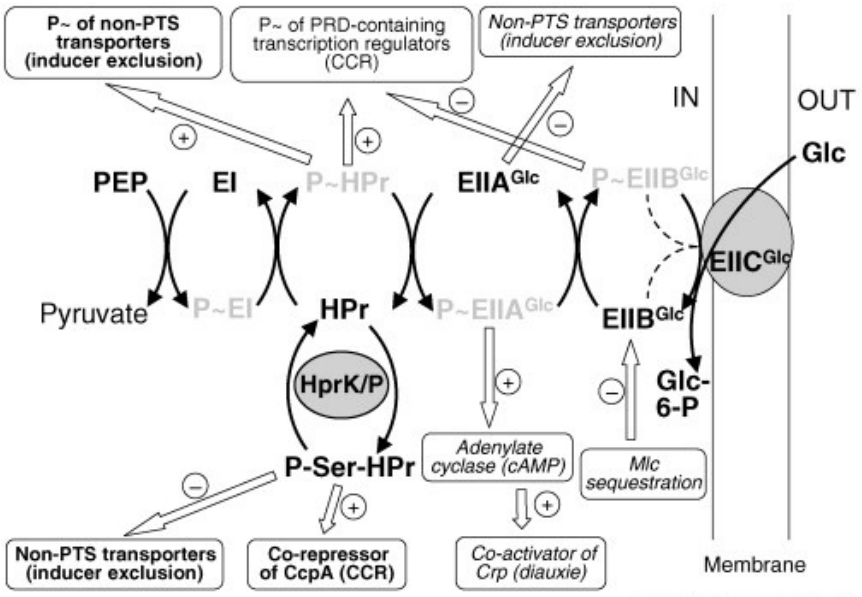
ATP + H_2O → ADP + Pi ΔG˚' = –7.3 kcal/mol

SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 3.13. Mechanism of the phosphotransferase system (PTS) of *Escherichia coli*. For uptake of glucose, the PTS system consists of five proteins: Enzyme I, Hpr, Enzyme II_a, Enzyme II_b, and Enzyme II_c. Sequential phosphate transfer occurs from phosphoenolpyruvate (PEP) to Enzyme I, then to HPr, then to Enzyme II_a, then to Enzyme II_b, then finally to Enzyme II_c, which actually transports and phosphorylates the sugar. Enzyme I and HPr are non-specific and they will transport anykind of monomeric sugar, whereas the Enzyme II components are specific for each particular monomeric sugar. Glucose-6-phosphate (Glucose-6-Pi) feeds directly into the glycolytic pathway, so it is constantly being "drained" from the system.

Abbreviations: Enz, Enzyme; PEP, phosphoenolpyruvate.

The PTS also regulates carbon metabolism (2º role)



SOURCE: Deutscher J (2008) The mechanisms of carbon catabolite repression in bacteria. *Curr Opin Microbiol* 11: 87-93. PMID:18359269.

Figure 1. The bacterial phosphotransferase system (PTS) and its regulatory functions. The PTS transports and phosphorylates numerous carbohydrates. Phosphoenolpyruvate (PEP) functions as phosphoryl donor for the phosphorylation cascade formed by the proteins Enzyme I (EI), HPr, Enzyme EII_a (EIIA), Enzyme EII_b (EIIB), and Enzyme EII_c (EIIC). PEP hydrolysis also provides the energy for the transport step catalyzed by the membrane-bound Enzyme EII_c (EIIC). In addition, several PTS proteins also carry out regulatory functions. The PTS components are present in the cell in both dephosphorylated and phosphorylated forms.

Uptake of the PTS substrate is fast when the extracellular concentration of the PTS substrate is high, resulting in a low ratio of PEP to pyruvate and a predominance of the **dephosphorylated** forms of the PTS proteins due to fast transfer of phosphate through the PTS system (black letters).

Uptake of the PTS substrate is slow when the extracellular concentration of the PTS substrate is low, resulting in a high ratio of PEP to pyruvate and a predominance of the **phosphorylated** forms of the PTS proteins due to slow transfer of phosphate through the PTS system (grey letters).

Depending on their phosphorylation state, the PTS proteins can carry out numerous regulatory functions by interacting with or phosphorylating their target proteins ('+' means stimulating and '–' means inhibiting the relevant effect on the target protein).

You do not need to memorize the information on this slide!!! The purpose of this slide is just to illustrate, once again, that transporters can also function in signal transduction pathways.

16

The energy for glucose uptake into the cell “uphill” (i.e., against its concentration gradient) ultimately comes from:

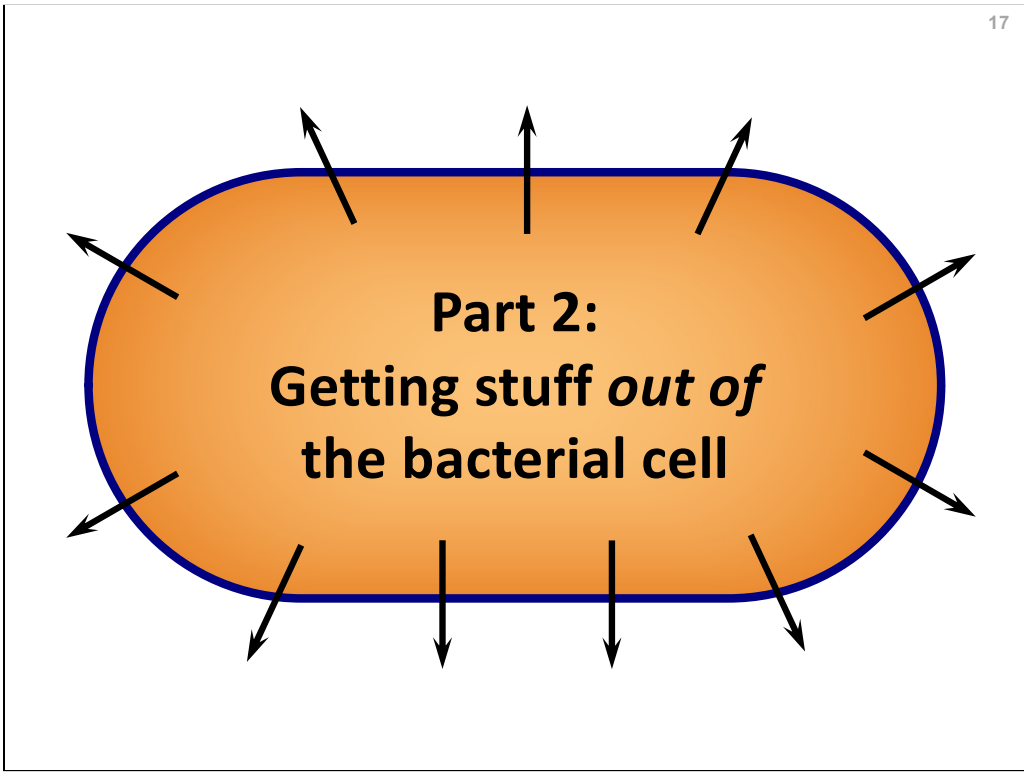
A. Concentration gradient of glucose across the membrane.

B. Coupled hydrolysis of phosphoenolpyruvate to pyruvate.

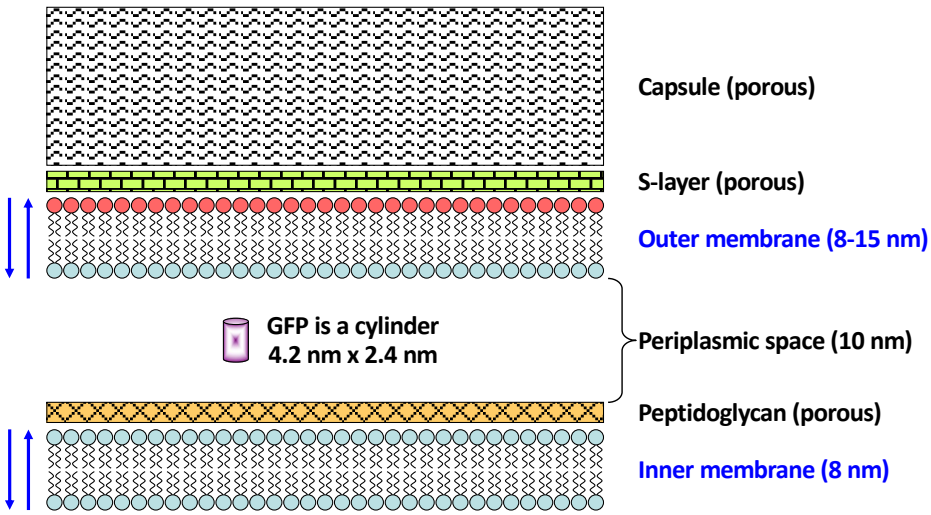
C. Coupled hydrolysis of ATP.

D. Conformational changes in the phosphotransferase system.

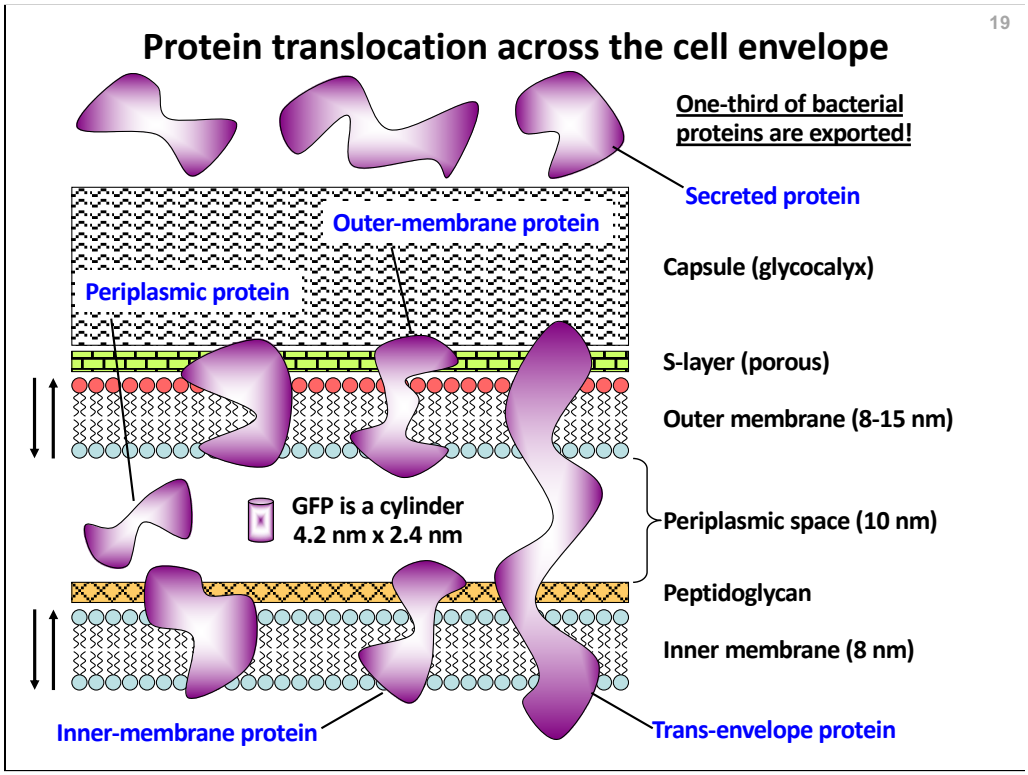
Answer: (B)


Part 2:
Getting stuff *out of*
the bacterial cell

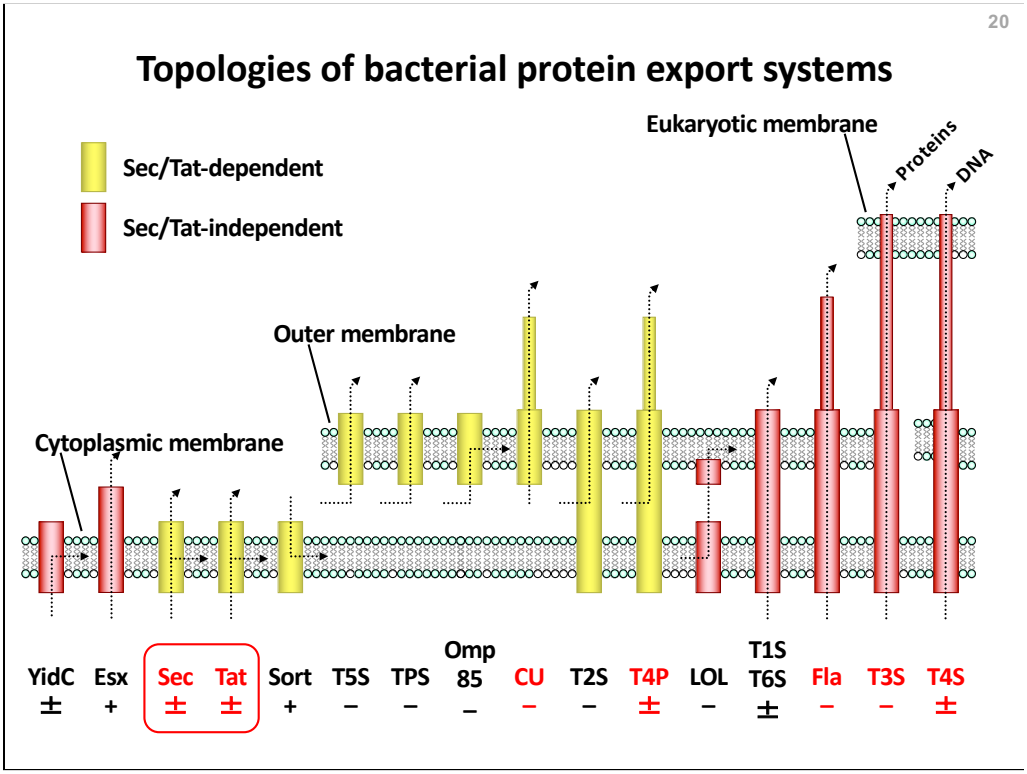
The inner and outer membranes of the bacterial cell envelope are the major barriers to diffusion



As a familiar size reference ("molecular ruler"): green fluorescent protein (GFP) is a beta-barrel protein consisting of 238 amino acids with a molecular mass of 27 kDa and has the shape of a cylinder with a length of 4.2 nm and diameter of 2.4 nm.



As a familiar size reference: GFP (green fluorescent protein) is a beta-barrel protein consisting of 238 amino acids with a molecular mass of 27 kDa and has the shape of a cylinder with a length of 4.2 nm and diameter of 2.4 nm.



SOURCE: Papanikou W, Karamanou S, Economou A (2007) Bacterial protein secretion through the translocase nanomachine. *Nat Rev Microbiol* 5: 839-851 PMID:17938627.

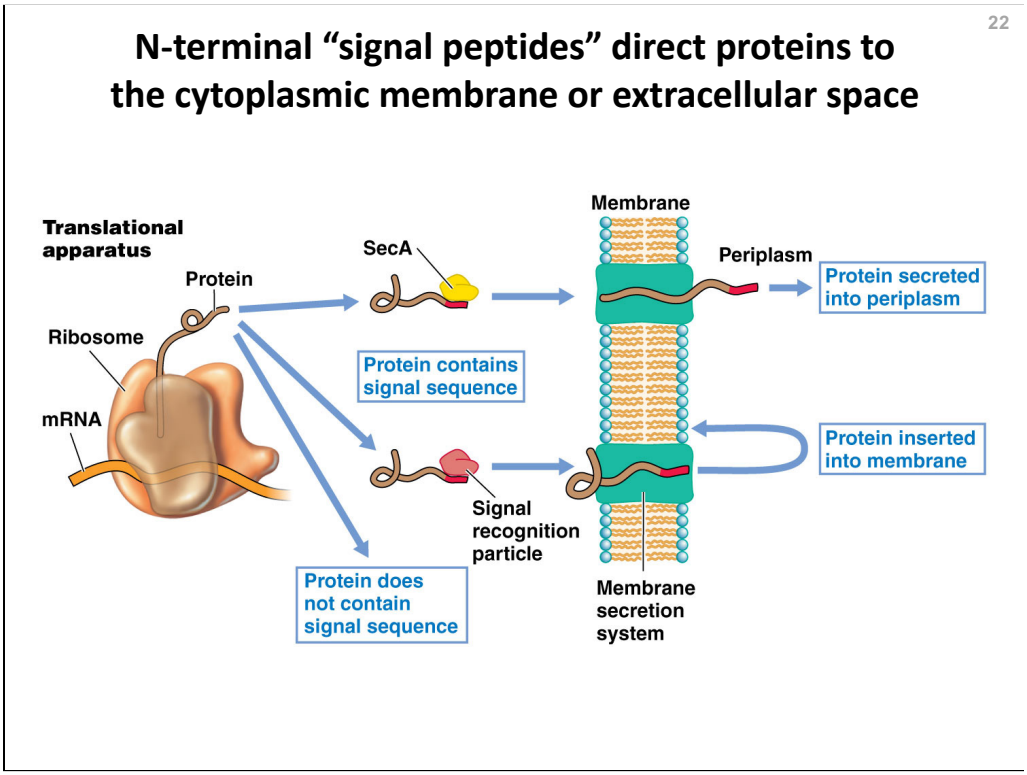
Box 1. The "zoo" of bacterial protein-export and protein-secretion systems. The figure shows a simplified schematic summary of the major protein-export, protein-secretion, and protein-membrane-integration systems in bacteria. The nomenclature of many systems follows the type-'n'-secretion or type-'n'-pilus convention. The arrows indicate the path that is taken by the exported protein. Arrows that initiate in the periplasm indicate that Sec-dependent or Tat-dependent translocation across the cytoplasmic membrane is a necessary first step for these systems.

Abbreviations: **Bam**, beta-barrel assembly machine; **CU**, chaperone-usher pathway; **Esx**, specialized secretion system that is found in Gram-positive bacteria; **Fla**, flagellum; **HM**, host cell membrane; **LOL**, lipoprotein outer-membrane localization; **Omp85**, outer membrane protein 85; **Sort**, sortase; **TPS**, two-partner secretion; **T2S**, type II secretion; **T3S**, type III secretion; **T4P**, type IV pili; **T4S**, type IV secretion; **T5S**, type V secretion (auto-transporter), **T6S**, type VI secretion; (**+**), present in Gram-positive bacteria only; (**–**), present in Gram-negative bacteria only; (±), present in both Gram-positive and Gram-negative bacteria.

You do not need to memorize all of the bacterial secretion systems shown here. The point of this slide is to illustrate that there is a tremendous variety of bacterial secretion systems with different topologies and functions. For the rest of this lecture, we will focus on the Sec and Tat "general secretion" systems. In future lectures we will take a look at some of the other secretion systems shown here.

The Sec protein export system

is a system to export proteins *before* they are folded



SOURCE: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 6.41. Export of proteins via the general secretory (Sec) system. The signal sequence is recognized either by SecA or by the signal recognition particle (SRP), which carries the protein to the membrane secretion system. SecA binds proteins that get secreted across the membrane, whereas the SRP binds proteins that get inserted into the membrane. In Gram-negative bacteria, SecA-secreted proteins will end up in the periplasmic space.

Günter Blobel won the Nobel Prize in Physiology or Medicine (1999) for his discovery of the role that N-terminal "signal peptides" play in directing proteins to their correct locations within the cell membrane or outside the cell membrane.

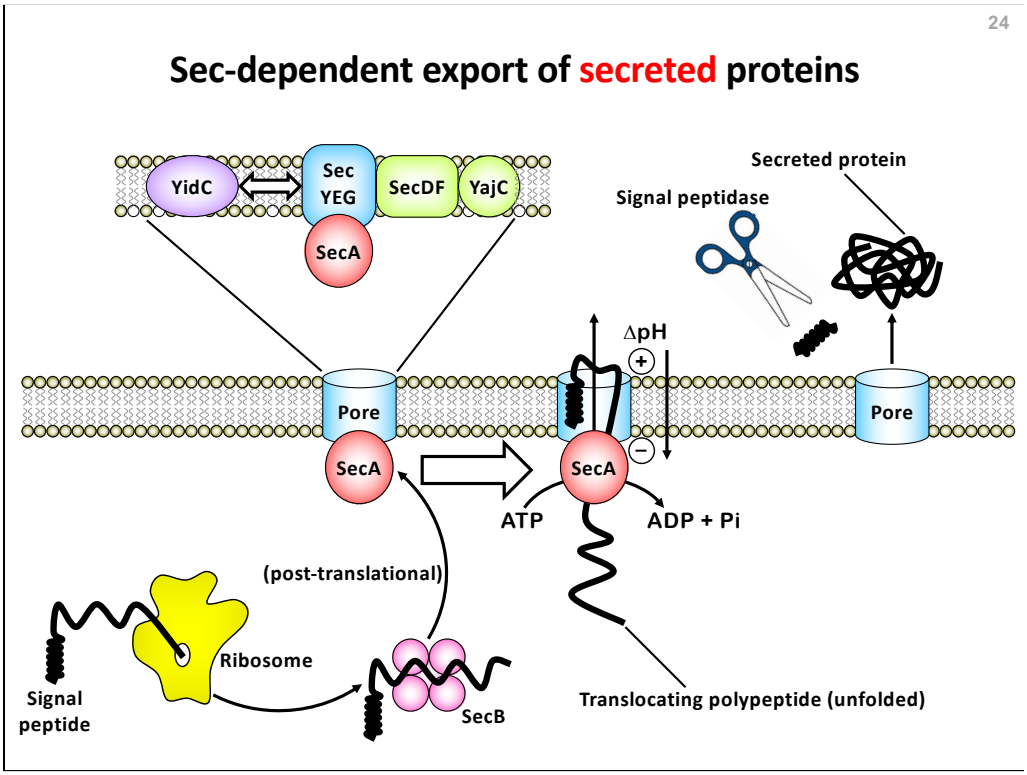
23

Signal peptides of the Sec protein export system

Sec signal peptide sequences are about 24 amino acids in length

Example: *Escherichia coli* outer membrane lipoprotein

You do not need to memorize the information on this slide! The purpose of this slide is just to illustrate the general nature and role of signal peptides in protein localization.



Source: Papanikou W, Karamanou S, Economou A (2007) Bacterial protein secretion through the translocase nanomachine. *Nat Rev Microbiol* 5: 839-851 PMID:17938627.

Source: Valent QA, Scotti PA, Hiugh S, de Gier JW, von Heijne G, Lentzen G, Wintermeyer W, Oudega B, Luirink J (1998) The *Escherichia coli* SRP and SecB targeting pathways converge at the translocon. *EMBO J* 17: 2504-2512 PMID:9564033.

Figure 1. Export of bacterial **secreted proteins** through the translocase nanomachine. Translocation is **post-translational**. The components are not drawn to scale. The bacterial pre-protein translocase for **secreted proteins** consists of the **SecYEG** pore complex (blue) and **SecA** (red), which functions as a docking protein and as an ATPase motor protein. SecYEG can also associate with the auxiliary proteins **YidC**, **SecDF**, **YajC** but we won't discuss these auxiliary proteins in BIO-372.

Stage 1: Secretory pre-proteins (thick black lines) are synthesized with amino-terminal **signal peptides** that are shorter and more hydrophilic than the signal peptides of trans-membrane proteins. As soon as the signal peptide emerges from the ribosome it is bound by the **SecB** chaperone (pink) and translation continues until the entire polypeptide has been synthesized and released from the ribosome.

Stage 2: The **SecB-pre-protein** complex docks with the **SecA** protein bound to the **SecYEG** pore complex and translocation across the cytoplasmic membrane begins. This is **post-translational** protein export, which occurs mainly with secreted proteins.

Stage 3: The pre-protein is translocated through the **SecYEG** pore until the entire pre-protein has crossed to the *trans* (external) side of the cytoplasmic membrane. Energy for export of secreted pre-proteins is provided by binding and hydrolysis of ATP by the **SecA** motor protein as well as the proton-motive force (H^+).

Stage 4: Once the pre-protein has finished crossing the cytoplasmic membrane, the N-terminal signal peptide is cleaved by **signal peptidase** and the mature protein folds into its final three-dimensional shape.

25

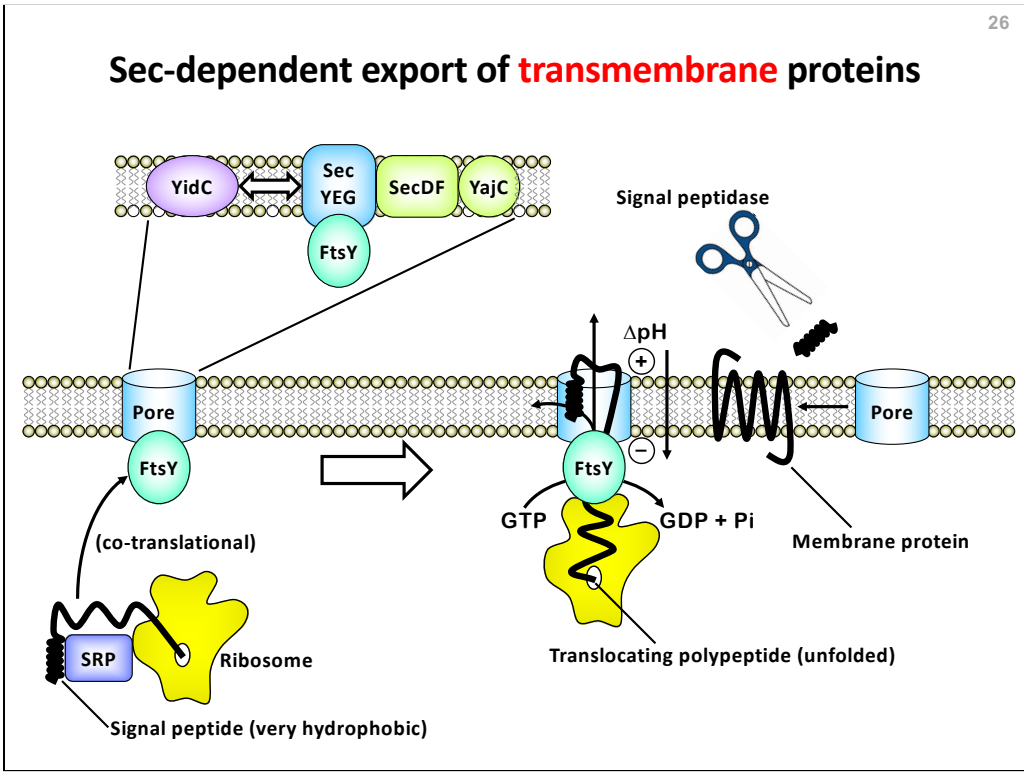
The energy for export of **secreted** proteins (proteins that end up outside the cytoplasmic membrane) ultimately comes from:

A. ATP hydrolysis by SecA.

B. GTP hydrolysis by the ribosome.

C. The proton motive force.

Answer: (A) and (C).



Source: Papanikou W, Karamanou S, Economou A (2007) Bacterial protein secretion through the translocase nanomachine. *Nat Rev Microbiol* 5: 839-851 PMID:17938627.

Source: Valent QA, Scotti PA, Hiugh S, de Gier JW, von Heijne G, Lentzen G, Wintermeyer W, Oudega B, Luirink J (1998) The *Escherichia coli* SRP and SecB targeting pathways converge at the translocon. *EMBO J* 17: 2504-2512 PMID:9564033.

Figure 1. Export of bacterial **trans-membrane proteins** through the translocase nanomachine. Translocation is **co-translational**. The components are not drawn to scale. The bacterial pre-protein translocase for **trans-membrane proteins** consists of the **SecYEG** pore complex (blue) and **FtsY**(turquoise), which functions as a docking protein. SecYEG can also associate with the auxiliary proteins **YidC**, **SecDF**, **YajC** but we won't discuss these auxiliary proteins in BIO-372.

Stage 1: Trans-membrane pre-proteins (thick black lines) are synthesized with amino-terminal **signal peptides** that are longer and more hydrophobic than the signal peptides of secreted proteins. As soon as the signal peptide emerges from the ribosome it is bound by the **SRP** "signal recognition particle" (blue) and translation pauses.

Stage 2: The **ribosome-SRP-pre-protein** complex docks with the **FtsY** protein bound to the **SecYEG** pore complex. Translation of the pre-protein resumes, accompanied by translocation of the pre-protein across the cytoplasmic membrane. This is **co-translational** protein export, which occurs mainly with trans-membrane proteins.

Stage 3: As the pre-protein is translocated through the SecYEG pore, some protein segments cross to the *trans* (external) side of the cytoplasmic membrane while other protein segments inert into the membrane. This process continues until the entire pre-protein has been translocated across and into the membrane. Energy for export of trans-membrane pre-proteins is provided by binding and hydrolysis of GTP by the **ribosome** as well as the proton-motive force (H^+).

Stage 4: Once the pre-protein has finished translocating across and into the cytoplasmic membrane, the N-terminal signal peptide is cleaved by **signal peptidase** and the mature protein folds into its final three-dimensional shape.

27

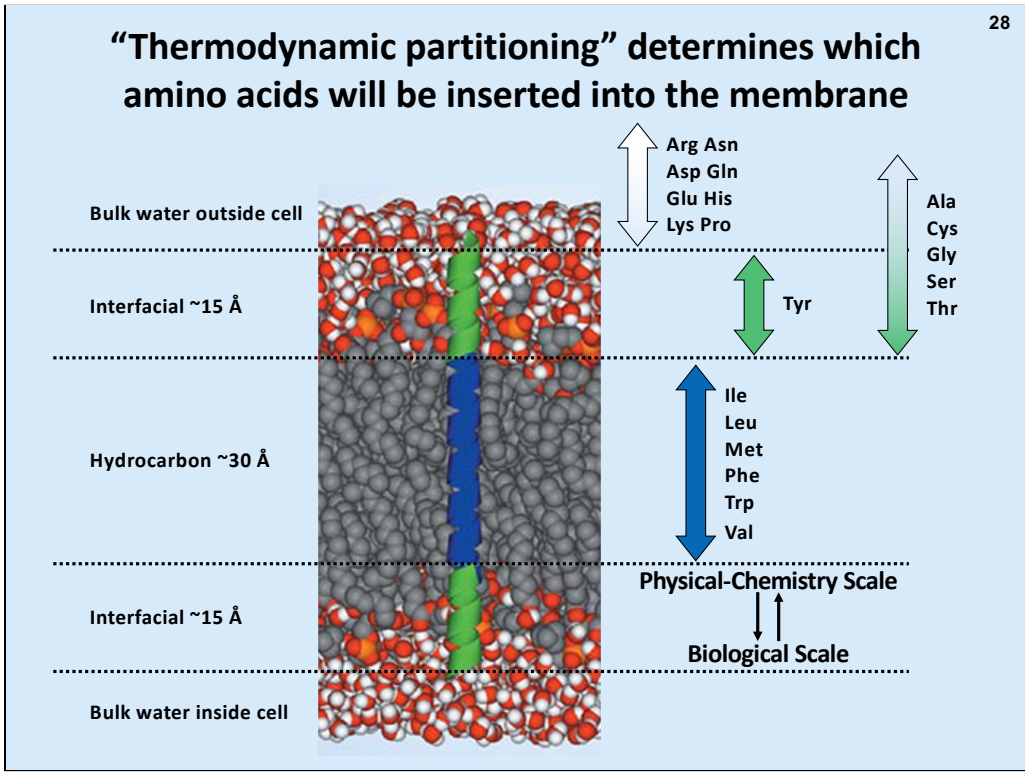
The energy for export of **transmembrane** proteins (proteins that are inserted into the cytoplasmic membrane) ultimately comes from:

A. ATP hydrolysis by SecA.

B. GTP hydrolysis by the ribosome.

C. The proton motive force.

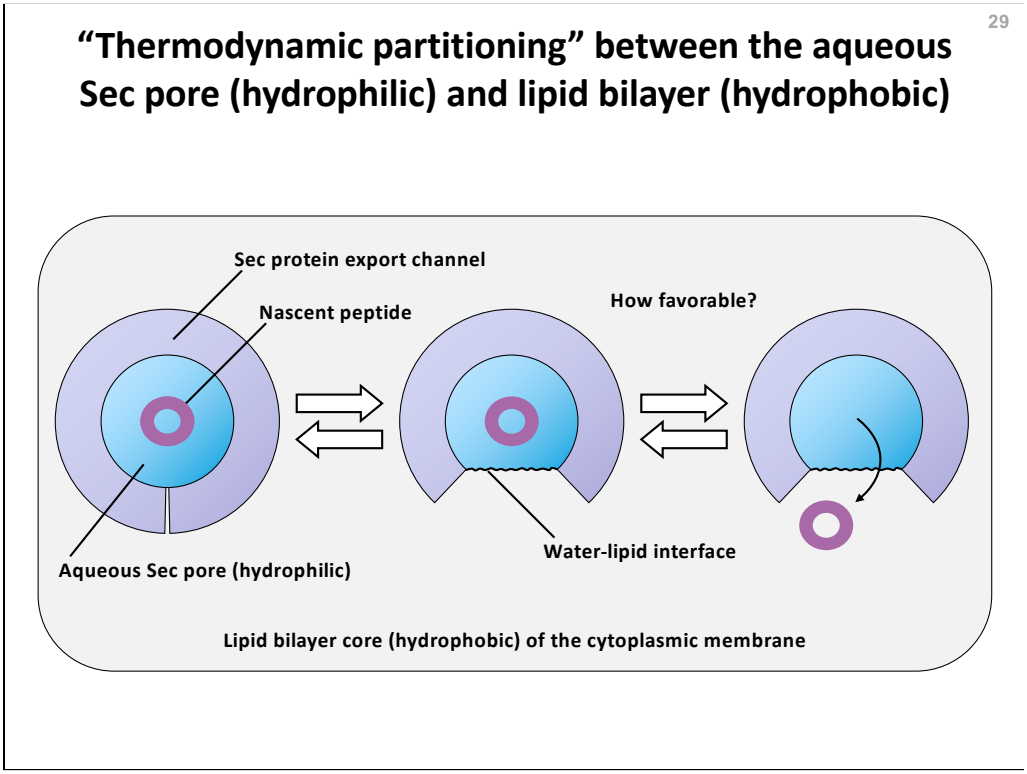
Answer: (B) and (C).



SOURCE: Bowie JU (2005) Border crossing. *Nature* 433: 367-369 PMID:15674274.

SOURCE: Hessa T, Kim H, Bihlmaier K, Lundin C, Boekel J, Andersson H, Nilsson I, White SH, von Heijne G (2005) Recognition of transmembrane helices by the endoplasmic reticulum translocon. *Nature* 433: 377-381 PMID:15674282.

Figure 1. Sub-compartments of the cytoplasmic membrane and the preferred locations of different amino acids. A snapshot of a lipid bilayer membrane and its three major sub-compartments: hydrocarbon core region, interfacial regions, bulk water regions. Grey, carbon atoms; red, oxygen atoms; white, hydrogen atoms bound to oxygen atoms; orange, phosphorus atoms. In an alpha-helix, 20 amino acids (blue) can span the hydrocarbon core, and 10 amino acids (green) can span the interfacial region. Arrows indicate where each amino acid (denoted by its three-letter symbol) would preferentially localize at thermal equilibrium based on transfer free-energy measurements.

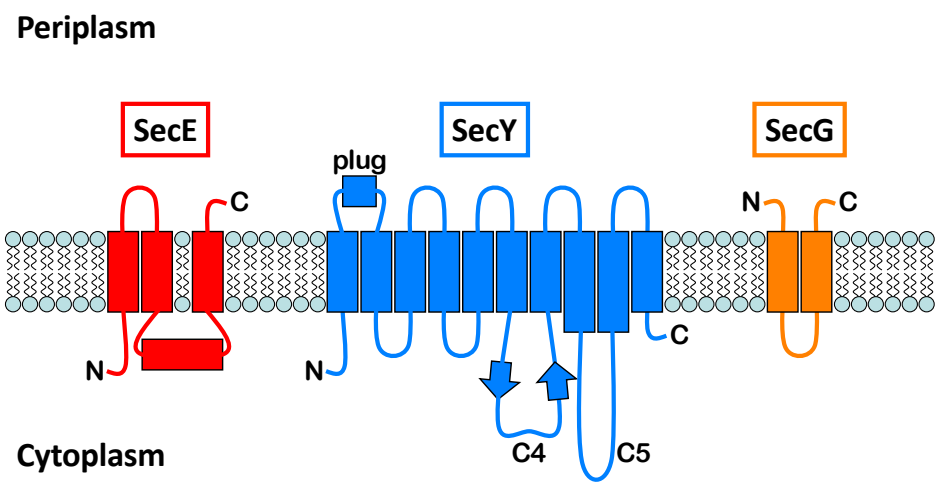


SOURCE: Bowie JU (2005) Border crossing. *Nature* 433: 367-369 PMID:15674274.

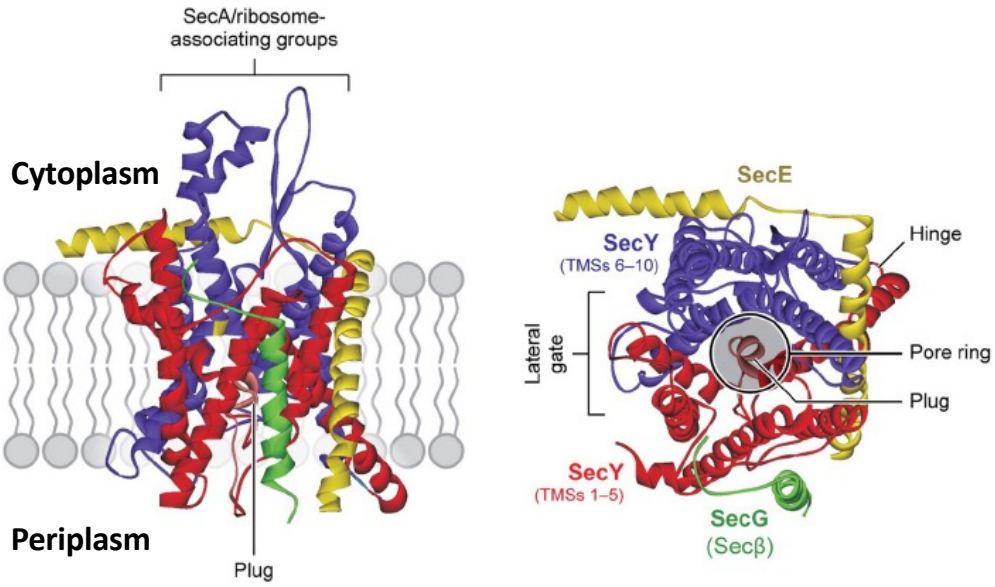
SOURCE: Hessa T, Kim H, Bihlmaier K, Lundin C, Boekel J, Andersson H, Nilsson I, White SH, von Heijne G (2005) Recognition of transmembrane helices by the endoplasmic reticulum translocon. *Nature* 433: 377-381 PMID:15674282.

Figure 2. A model of the membrane-insertion "decision". A top view of the membrane and the translocon pore that crosses it. Inside the pore is a peptide helix surrounded by water. The pore opens sideways into the membrane, allowing the helix to interact with the membrane lipids. If the peptide is more compatible with lipid than with water, it will transfer into the membrane; otherwise, it will continue to be moved through the pore. The figure is only intended to convey the basic principle and omits many mechanistic and structural issues.

Topology of the SecYEG protein export channel



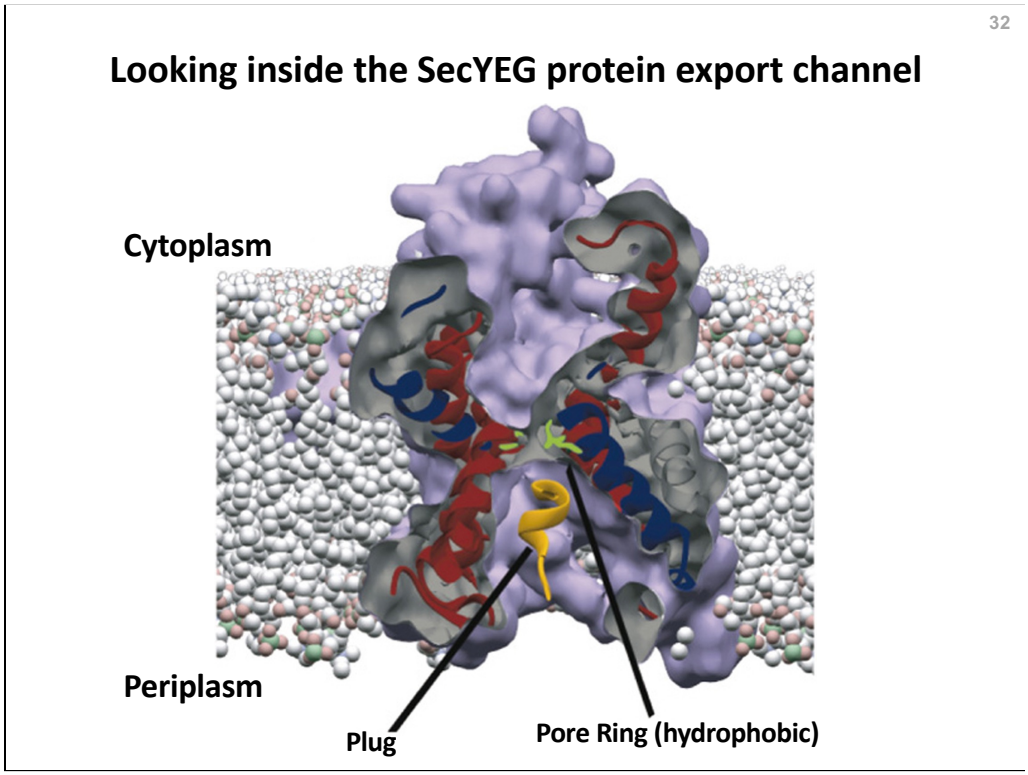
Structure of the SecYEG protein export channel



SOURCE: Driessen AJ, Nouwen N (2008) Protein translocation across the bacterial cytoplasmic membrane. *Annu Rev Biochem* 77: 643-67 PMID:18078384.

Figure 4. Structure of the **SecYEG** pore complex, the protein-conducting channel of the Sec translocase. **(A, left)** Membrane cross-section and **(B, right)** a cytosolic view of the structure of SecYEG. The protein-conducting channel consists of three subunits: **SecY**, which is embraced by **SecE** and the peripheral-bound **SecG**. The channel forms an hourglass-like structure with a pore ring of hydrophobic amino acid residues at its constriction. The pore is closed at the periplasmic side by a plug formed by a short alpha-helix of a periplasmic loop that folds back into the funnel. The two halves of the clamshell-like structure of SecY are indicated as TMS1-5 and TMS6-10 and are connected by a hinge region in the back. The clamshell opening in the front forms a lateral gate into the hydrophobic core of the lipid bilayer. Insertion of the signal peptide into the lateral gate is thought to widen the central pore opening and destabilize the plug, resulting in the opening of a vectorial water-filled channel.

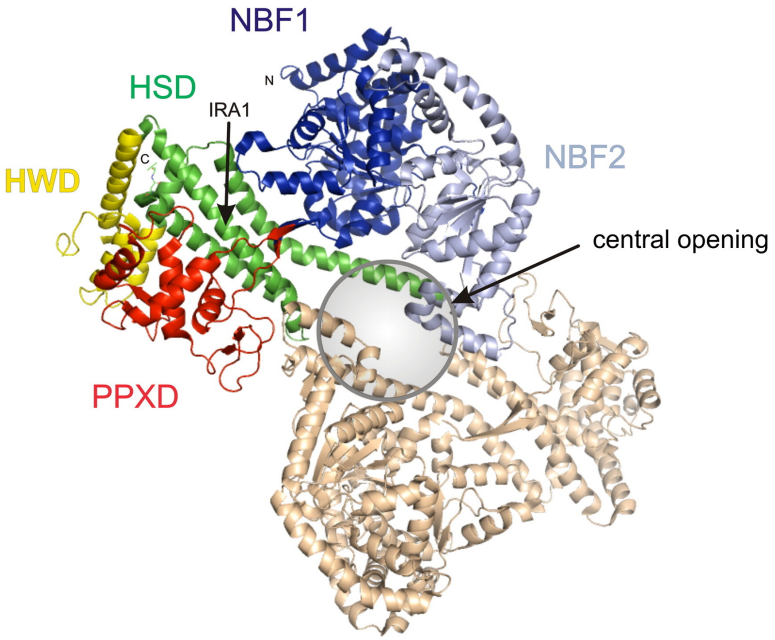
TMS, trans-membrane segment.



SOURCE: Rapoport TA (2007) Protein translocation across the eukaryotic endplasmic reticulum and bacterial plasma membranes. *Nature* 450: 663-669 PMID:18046402.

Figure 1. The SecYEG protein translocation channel. Cross-sectional view of the channel from the side.

Structure of the SecA protein export motor (dimer)

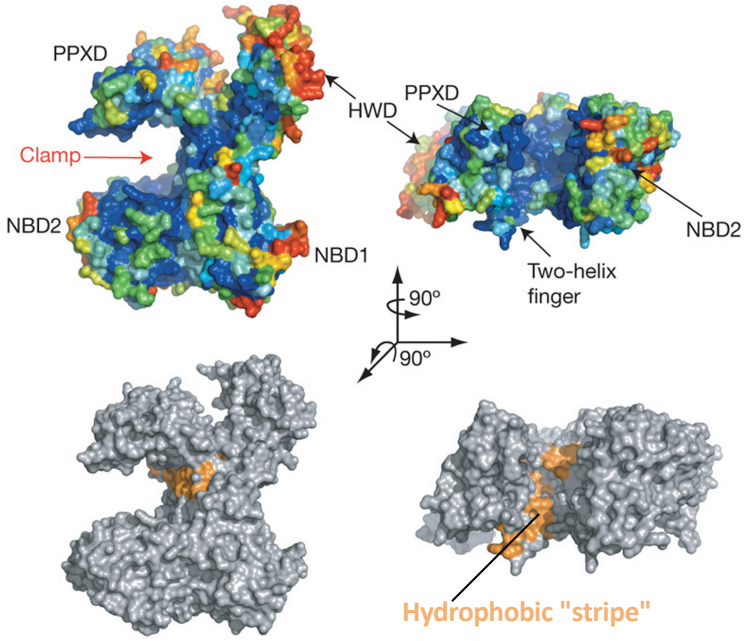


SOURCE: Driessen AJ, Nouwen N (2008) Protein translocation across the bacterial cytoplasmic membrane. *Annu. Rev. Biochem.* 77: 643-67 PMID:18078384.

SOURCE: du Plessis DJ, Nouwen N, Driessen AJ (2011) The Sec translocase *Biochim Biophys Acta* 1808: 851-865 PMID:20801097.

Figure 3. Structure of **SecA**, the motor subunit of the Sec translocase. The structure of SecA shows the different subdomains in color: **NBF1** (dark blue) and **NBF2** (light blue), nucleotide-binding folds 1 and 2; **PPXD** (red), preprotein cross-linking domain; **HSD** (green), alpha-helical scaffold domain; **HWD** (yellow), alpha-helical wing domain. The second protomer of the SecA dimer is represented as a grey ribbon. The intramolecular region of ATP hydrolysis 1 (**IRA1**), which controls hydrolysis of ATP at NBF1, localizes to the HSD (as indicated) and to a central opening at the SecA dimer interface.

Structure of the SecA protein export motor (monomer)



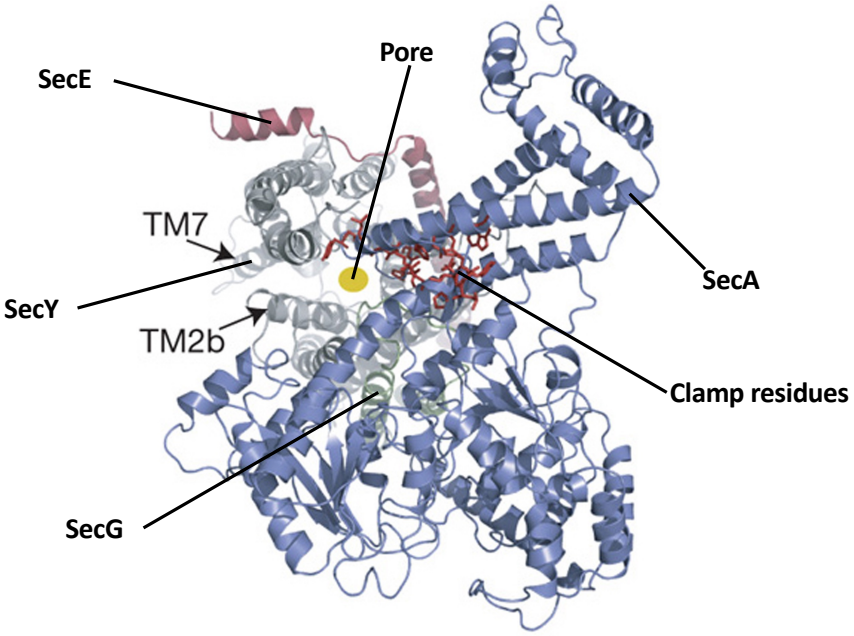
SOURCE: Zimmer J, Nam Y, Rapoport TA (2008) Structure of a complex of the ATPase SecA and the protein-translocation channel. *Nature* 455: 936-943 PMID:18923516.

SOURCE: Erlandson KJ, Miller SB, Nam ., Osborne AR, Zimmer J, Rapoport TA (2008) A role for the two-helix finger of the SecA ATPase in protein translocation. *Nature* 455: 984-987 PMID:18923526.

SOURCE: Tsukazaki T, Mori H, Fukai S, Ishitani R, Mori T, Dohmae N, Perederina A, Sugita Y, Vassylyev DG, Ito K, Nureki O (2008) Conformational transition of Sec machinery inferred from bacterial SecYE structures. *Nature* 455: 988-991 PMID:18923527.

Figure 4. The polypeptide clamp of SecA. **(A, upper)** Surface representation of a SecA monomer with conserved amino acid residues color coded (blue denotes the highest conservation and red denotes the lowest conservation). **(B, lower)** As in **(A)** except that the stripe of conserved hydrophobic residues along the proposed polypeptide clamp of SecA is shown in orange.

Structure of the SecA-SecYEG protein export complex

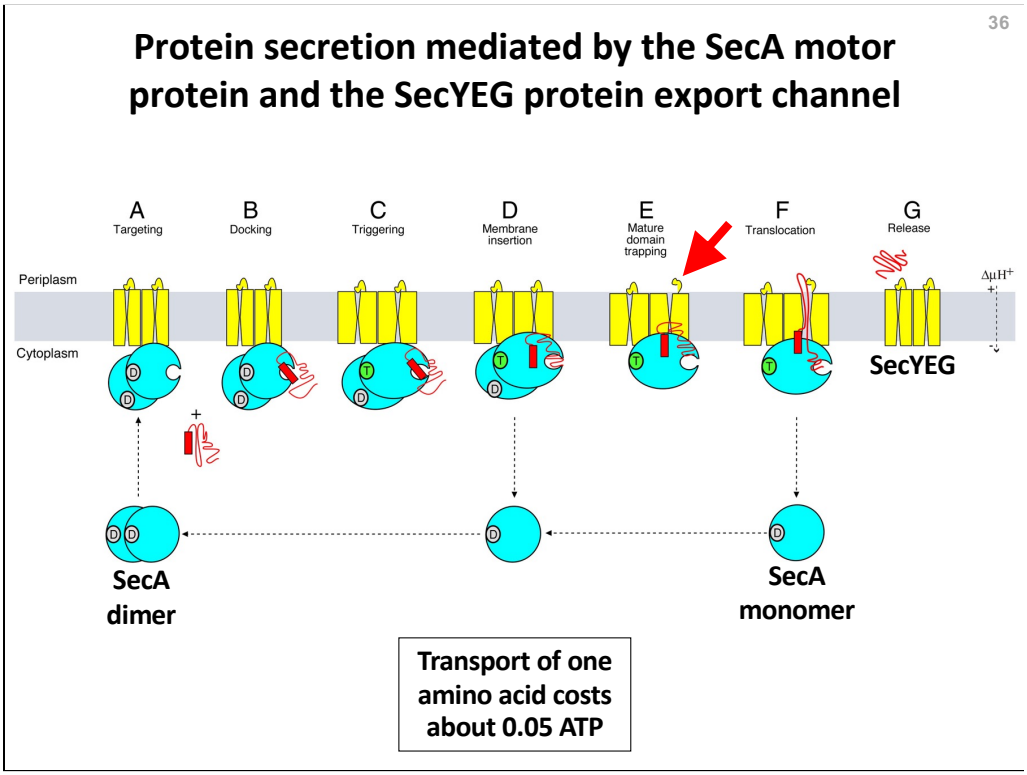


SOURCE: Zimmer J, Nam Y, Rapoport TA (2008) Structure of a complex of the ATPase SecA and the protein-translocation channel. *Nature* 455: 936-943 PMID:18923516.

SOURCE: Erlandson KJ, Miller SB, Nam ., Osborne AR, Zimmer J, Rapoport TA (2008) A role for the two-helix finger of the SecA ATPase in protein translocation. *Nature* 455: 984-987 PMID:18923526.

SOURCE: Tsukazaki T, Mori H, Fukai S, Ishitani R, Mori T, Dohmae N, Perederina A, Sugita Y, Vassylyev DG, Ito K, Nureki O (2008) Conformational transition of Sec machinery inferred from bacterial SecYE structures. *Nature* 455: 988-991 PMID:18923527.

Figure 4. The polypeptide clamp of SecA. **(C)** The SecA-SecYEG complex with SecA (blue), SecY (grey), SecE (red), and SecG (green). The PPX domain was removed for clarity. The hydrophobic stripe residues that were labeled orange in **(B)** are shown as red sticks. The yellow circle indicates the translocation pore in SecYEG.



Source: Chatzi KE, Sardis MF, Economou A, Karamanou S (2014) SecA-mediated targeting and translocation of secretory proteins. *Biochim. Biophys. Acta* 1843(8): 1466-1474 PMID: 24583121.

Figure 4. Schematic representation of preprotein translocation steps. The model represents discrete steps that have been experimentally demonstrated. SecYEG dimers (yellow) and SecA (cyan) monomer to dimer transitions (arrows) are shown. Signal sequence (red rectangle), preprotein mature domain (red line), ADP (D) and ATP (T) are also indicated. Changes in the orientation of SecA protomers and the shape of SecA and SecYEG indicate conformational rearrangements that lead to the activation of the holoenzyme.

(A) ADP-SecA exists as a dimer in the cytoplasm. It docks on the SecYEG channel with a 2:2 stoichiometry. A 50:50 distribution between membrane-bound and cytoplasmic SecA is thought to govern this equilibrium.

(B) A preprotein diffusing in the cytoplasm either alone or bound to chaperones like SecB, binds to SecA. The signal sequence and the mature domain dock independently on their respective SecA docking sites. Unlike the soluble SecA dimer that can accommodate two preprotein molecules, only one is bound on the SecA–SecYEG translocase with high affinity.

(C) The binding of the signal sequence lowers the activation energy of the holoenzyme (SecA-SecYEG) in a step known as "triggering". The conformation of the translocase undergoes an allosteric change.

(D) Triggering stabilizes the SecA membrane-inserted state. The mature domains of secretory chains are not yet fully engaged in the channel.

(E) The preprotein becomes "trapped", presumably by forming additional contacts through its mature domain with the SecA triggered dimer. SecA monomerizes at this step and catalyzes multiple rounds of ATP hydrolysis (indicated by red arrow).

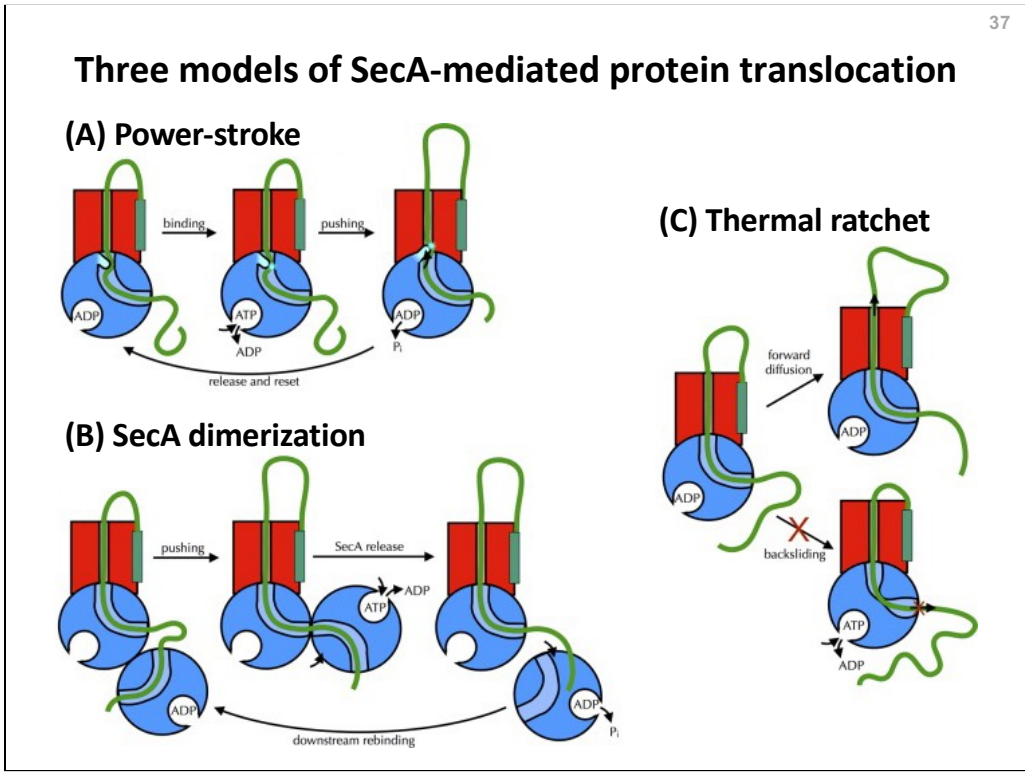
(E) ATP hydrolysis by SecA is coupled to mechanical work and mediates transfer of the polypeptide chain. In every catalytic cycle of ATP hydrolysis 20-30 amino acyl residues of the preprotein are translocated.

(E) To stimulate ATP hydrolysis, binding of the mature domain onto the PBD is allosterically transmitted to the ATPase motor. The base of the motor opens, and the IRA2 domain becomes disordered and detaches from the NBD. These events loosen up the NBD-IRA2 interface and the previously bound ADP can now diffuse out and is easily exchanged for ATP.

(E) PMF affects different stages of the translocation process. It facilitates the insertion and possibly the orientation of the signal sequences as translocation initiates. SecA de-insertion from the membrane is accelerated by PMF either by promoting ADP release from SecA or by conformational changes in SecY.

(F) Association of the signal sequence with the lateral gate of SecY causes the periplasmic plug to flip out. Next the signal sequence can diffuse into the bilayer, where the signal peptidase I (signal peptidase II for lipoproteins) whose catalytic domain resides in the trans side of the membrane, can cleave it off.

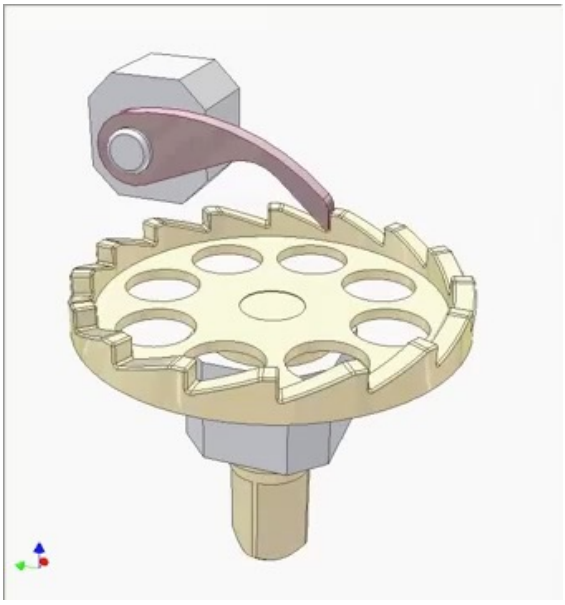
(G) The mature domain is released in the periplasm.



SOURCE: Corey RA, Allen WJ, Collinson I (2016) Protein translocation: what's the problem? *Biochem Soc Trans* 44(3):753-759 PMID: 27284038.

Figure 3. Proposed models for protein translocation by the Sec complex. Many models for how **SecA** drives protein translocation have been proposed, designed to accommodate the results of structural and functional studies. So far, however, such models make various assumptions, e.g., they postulate the existence of conformational changes that lack direct experimental evidence. In general, these models can be divided into three types: models involving a power stroke within SecA, those that invoke quaternary interactions between multiple SecA molecules, or those that act through biased (ratcheted) diffusion. **(A) Power-stroke model**. An example of a power-stroke mechanism, whereby conformational changes within SecA during the ATPase cycle physically push polypeptides through the channel. In the example shown on the slide, a so-called "2-helix finger (2HF) domain" of SecA binds to the pre-protein substrate, pushes it into the channel, then releases it and returns to its resting position. **(B) SecA dimerization model.** The observation that SecA can exist both as a monomer and in several different dimer forms has led to the proposal of multiple models in which SecA-SecA quaternary interactions drive transport. In the example shown on the slide, one SecA protomer holds the pre-protein substrate in the channel whereas the other binds to downstream regions. ATP binding alters the SecA dimer interface, pushing the substrate through the channel, and ATP hydrolysis releases SecA, allowing it to rebind downstream. **(C) Thermal ratchet model.** Rather than physically pushing the substrate through the channel, directional movement can be achieved by selectively allowing diffusion in one direction, while preventing it in the other. Such a "thermal ratchet" (a.k.a. "Brownian ratchet") would act by using ATP to prevent backsliding (somehow...via an unknown mechanism…). In the example shown on the slide, SecA senses that it is backsliding (somehow… via an unknown mechanism…) and constricts to halt movement; however this is entirely speculative, as an illustration of the core concept.

Ratchets restrict movement to one direction



See: Movie_Slide38.mov posted on Moodle.

39

Which of the following statements is true:

A. The Sec channel translocates proteins *before* they are folded (i.e., proteins in the still-unfolded, linear state).

B. The Sec channel translocates proteins *after* they are folded (i.e., proteins in the already-folded, three-dimensional state).

C. The Sec channel folds proteins *during* translocation.

Answer: (A)

The Tat protein export system

is a system to export proteins *after* they are folded

Tat, twin-arginine translocation system.

41

The Sec system exports proteins *before* that are folded (proteins in the unfolded, linear state) so it can't export:

(1) Proteins that can't fold in the oxidizing periplasm

Example: green fluorescent protein (GFP)

(2) Proteins that require insertion of cofactors

Example: redox enzymes (FAD, heme, etc.)

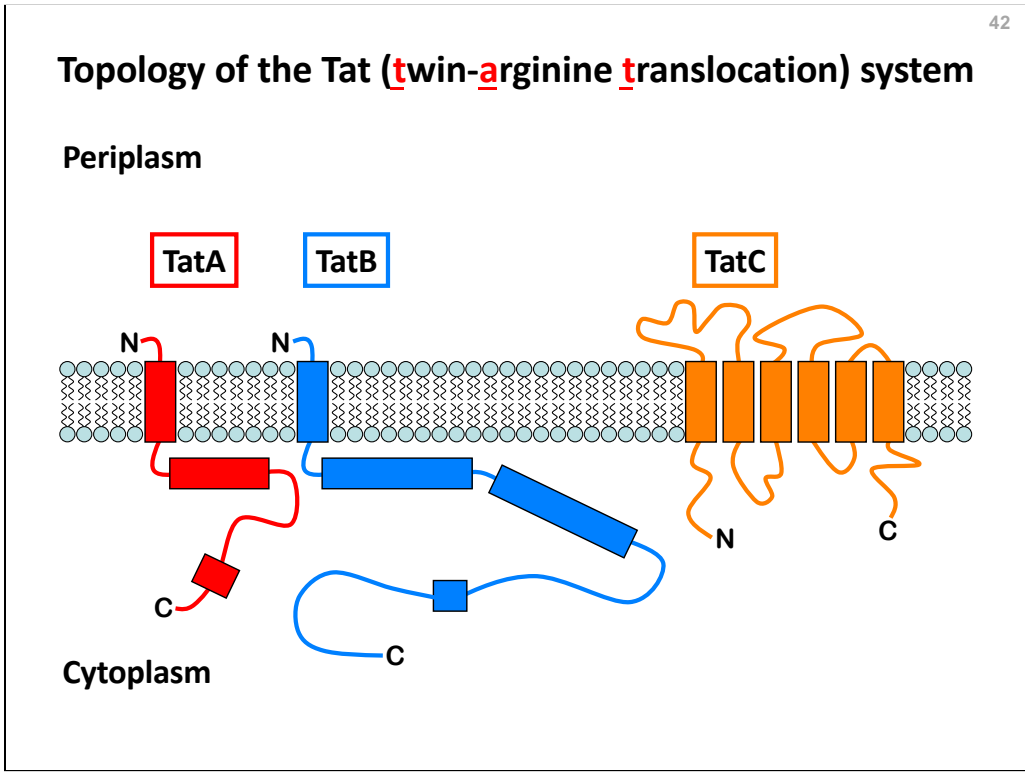
(3) Proteins that form multi-protein complexes

Example: protein complexes in the electron transport chain

(4) Some (but not all) integral membrane proteins

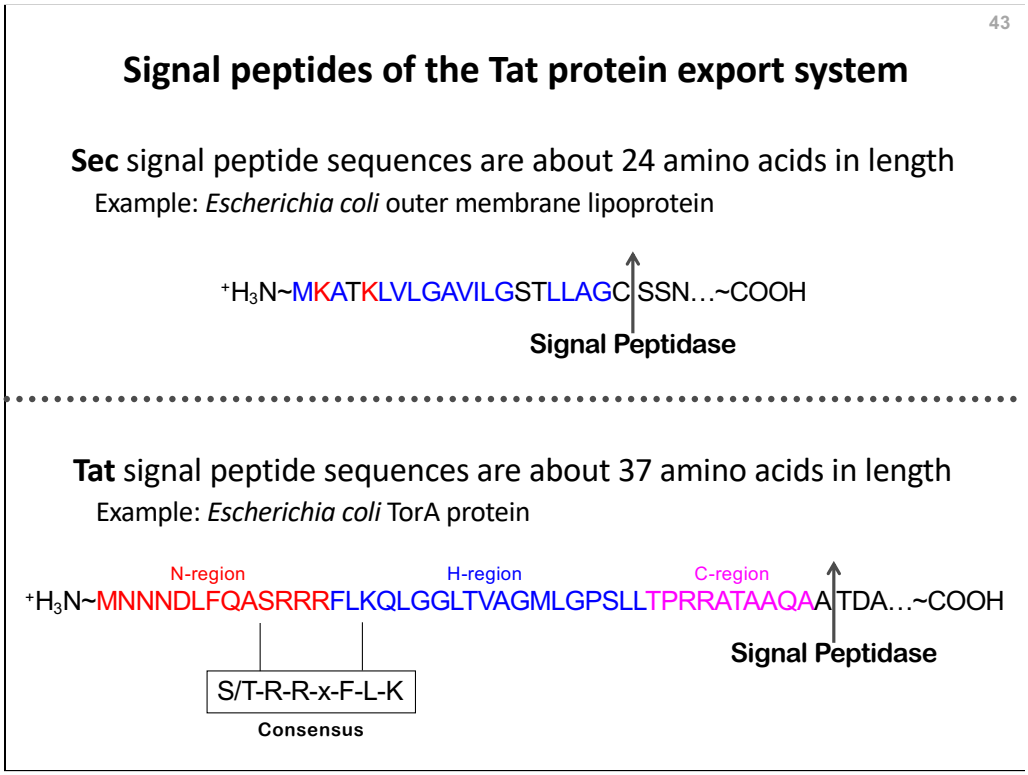
Example: cytochrome complexes

SOURCE: Lee PA, Tullman-Ercek D, Georgiou G (2006) The bacterial twin-arginine translocation pathway. *Annu Rev Microbiol* 60: 373-395 PMID:16756481.



SOURCE: Lee PA, Tullman-Ercek D, Georgiou G (2006) The bacterial twin-arginine translocation pathway. *Annu Rev Microbiol* 60: 373-395 PMID:16756481.

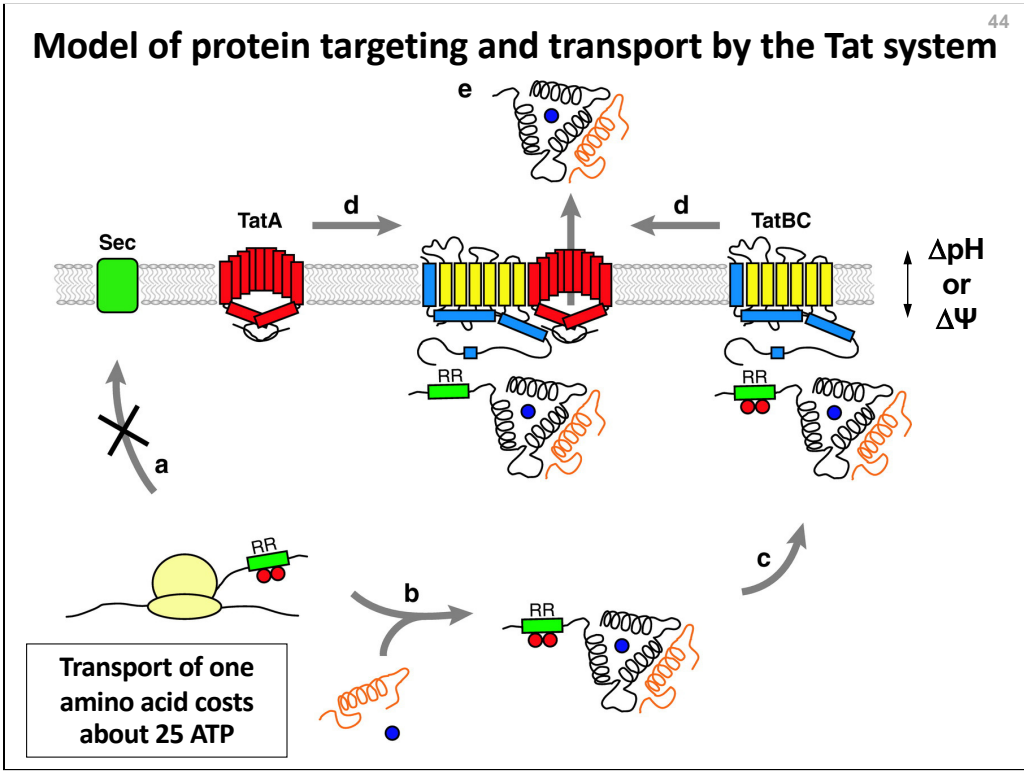
Figure 2. The predicted structure and topology of the bacterial Tat components. Predicted alpha-helical regions are shown as boxes.



SOURCE: Lee PA, Tullman-Ercek D, Georgiou G (2006) The bacterial twin-arginine translocation pathway. *Annu Rev Microbiol* 60: 373-395 PMID:16756481.

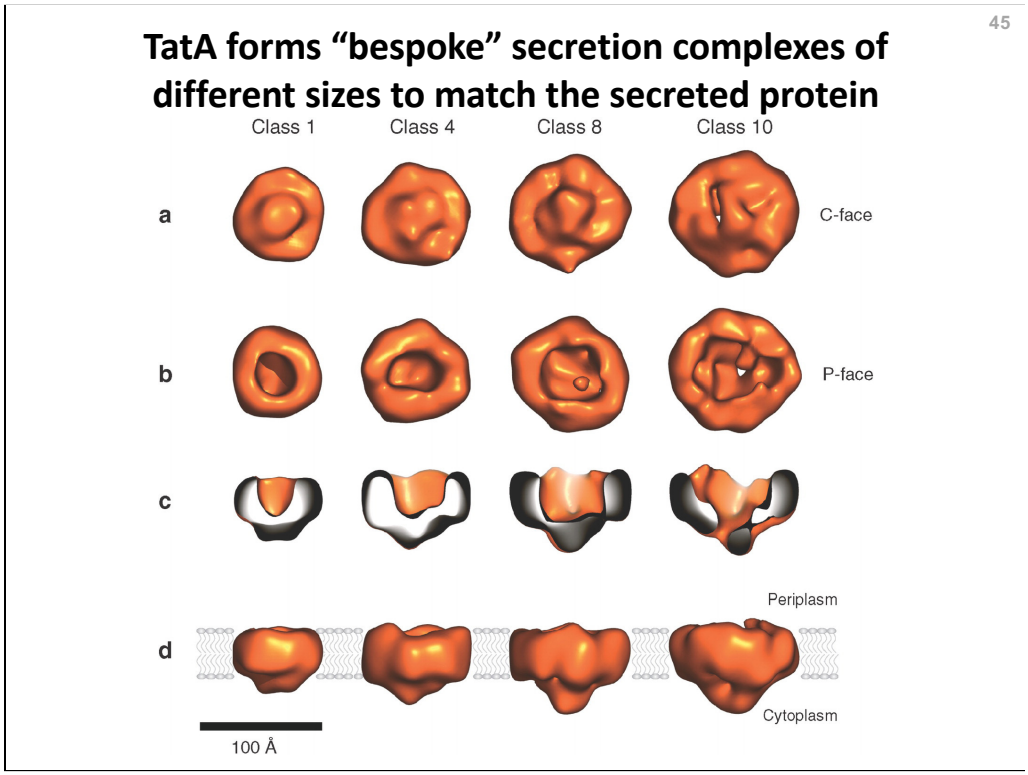
Figure 1. Features of a typical Tat signal peptide (ssTorA, from *Escherichia coli*). The N-terminal **n-region** is shown in red, the hydrophobic **h-region** is in blue, and the conserved **c-region** is in violet. The consensus Tat motif is boxed. The vertical dashed line indicates the site of cleavage by signal peptidase I.

You do not need to memorize the information on this slide! The point of this slide is just to illustrate the nature and role of signal peptides in protein localization.



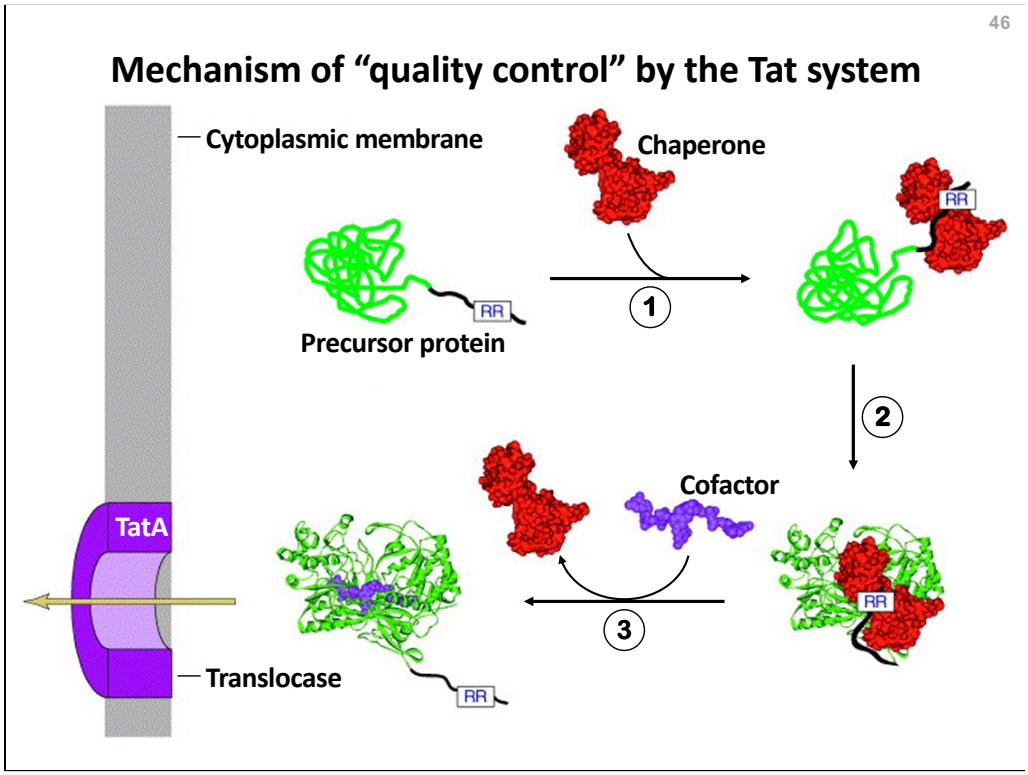
SOURCE: Lee PA, Tullman-Ercek D, Georgiou G (2006) The bacterial twin-arginine translocation pathway. *Annu Rev Microbiol* 60: 373-395 PMID:16756481.

Figure 3. Model of Tat targeting and transport. Sequence of events: Tat signal peptide docks with TatC or TatC/TatB complex → handoff to TatB → handoff to TatA → export. Energy is provided by the proton motive force; ATP is not required. Energy requirement: 79,000 protons (equivalent to ~ 10,000 ATP) per protein transported. In the membrane TatA is depicted in red, TatB in blue, TatC in yellow. **(A)** Upon emerging from the ribosome the preprotein must avoid targeting to other pathways such as Sec, which is aided by the characteristics of the signal peptide and mature protein and/or the binding of Tat-specific chaperones (red circles). **(B)** After folding, any cofactors and/or additional subunits are added prior to targeting to the TatBC receptor complex **(C)**. **(D)** The proton motive force drives the formation of an active translocase and the substrate is transported through a pore consisting mainly of TatA. **(E)** Upon removal of the signal peptide the mature protein is released on the periplasmic side of the membrane.



SOURCE: Lee PA, Tullman-Ercek D, Georgiou G (2006) The bacterial twin-arginine translocation pathway. *Annu Rev Microbiol* 60: 373-395 PMID:16756481.

Figure 4. Three-dimensional density maps of TatA complexes, solved by random canonical tilt electron microscopy. **(A)** TatA complexes viewed from the closed end of the channel on the cytoplasmic side of the membrane (C-face). **(B)** TatA complexes viewed from the open end of the channel on the periplasmic side of the membrane (P-face). **(C)** Side views of TatA. The front half of each molecule has been cut away to reveal internal features. **(D)** Views of TatA parallel to the membrane plane. The proposed position of the lipid bilayer is indicated in gray. Scale bar, 10 nm.



SOURCE: Berks BC, Palmer T, Sargent F (2005) Protein targeting by the bacterial twin-arginine translocation (Tat) pathway. *Curr Opin Microbiol* 8: 174-181 PMID:15802249.

Figure 2. A model for the mechanism of quality control in the biogenesis of an extracellular protein (TMAO reductase, in the example shown here) that is exported by the Tat translocon. **(1)** The substrate-specific chaperone (TorD, in the example shown here) binds to the Tat signal peptide of the precursor protein (TMAO reductase). This prevents the precursor protein from interacting with the TatA translocase that is located in the cytoplasmic membrane. **(2)** The chaperone also binds to the cofactorless mature region of the precursor protein, maintaining it in a partly folded state that is competent for cofactor insertion. **(3)** Cofactor insertion leads to release of the chaperone from the enzyme. The precursor protein's signal peptide is now free to direct export of the precursor protein via the TatA translocase. The TMAO reductase cofactor is *bis*(molybdopterin guanine dinucleotide)molybdenum.

47

Which of the following statements is true:

A. The Tat channel translocates proteins *before* they are folded (i.e., proteins in the still-unfolded, linear state).

B. The Tat channel translocates proteins *after* they are folded (i.e., proteins in the already-folded, three-dimensional state).

C. The Tat channel folds proteins *during* translocation.

Answer: (B)