

Protein localization (Chapters 2 and 14).

- I. cytoplasm – not a problem
- II. cell (inner) membrane
- III. exported beyond cell (inner) membrane
- IV. secreted outside of cell (note different use than previous years)
- V. translocase systems for cell membrane (Sec and Tat)

VI. Sec system – General Export Pathway (GEP)

- A. SecY main component (SecE and SecG secondary)
- B. leader peptides at N terminus
- C. ATP driven

- D. posttranslational export (periplasmic, outer membrane, secreted)
 - 1. shorter leader
 - 2. SecB binds (prevents folding = chaperone)
 - 3. delivers to SecA and SecYEG system
 - 4. leader peptide cleaved by Lep releasing protein

- E. cotranslational (cell/inner membrane)
 - 1. longer leader (first transmembrane domain)
 - 2. Signal Recognition Particle (SRP) – ribonucleoprotein complex - conserved
 - a. binds to leader peptide
 - b. pauses translation
 - 3. docking protein FtsY at SecYEG
 - 4. SecA can be involved
 - 5. energy from translation
 - 6. YidC – helps protein thread into membrane

VII. disulfide bonds

- a. not formed in cytosol
- b. directed in periplasm by DsbA (disulfide oxidoreductase)

VIII. Export into/through outer membrane (Chapter 14, start page 620, Fig. 14.4)

A. outer membrane proteins likely are secreted into periplasm then insert into OM

B. Tat system (used in conjunction with export/secretion?)

1. leader has Twin Arginine Transport (Tat) = S-R-R-X motif
2. independent of Sec
3. can translocate folded proteins
4. three components (TatA,B,C)

C. Type 1 secretion

1. Not very common (Rtx toxins, *E. coli* hemolysin)
2. Sec and Tat-independent, no classical signal sequence
3. Secreted directly from cytoplasm to outside, bypassing periplasm
4. ABC (ATP binding cassette) transporter involved, along with outer membrane (TolC) and inner-outer membrane spanning protein
5. Targeting (signal) sequence is in C-terminus

D. Type 2 (also known as the General Secretion Pathway - GSP)

1. Widely used for many different proteins
2. Proteins secreted into periplasm by GEP, then transported outside by type 2 system
3. Transport system involves numerous proteins (>12) in both membranes
4. Homologous to Type 4 pili synthetic systems of gram-negative bacteria

E. Type 3

1. Injects proteins into host cells (plant or animal) - virulence factors
2. Complex multi-protein secretion apparatus in both membranes (>17)
3. Secreted proteins are GEP-independent, but secretion apparatus is GEP-dependent
4. Homologous to flagellum biosynthesis, especially hook and filament
5. injectosome (needle)
6. Targeting sequence in N-terminus, not cleaved
7. Secreted proteins have individual chaperones in the cytoplasm

F. Type 4

1. Pertussis toxin, Helicobacter CagA, Legionella, (originally DNA transfer - Agrobacterium, F plasmid conjugation)
2. Dedicated transport system exists (9 proteins)
3. Homologous to DNA transport systems of pili

G. Type 5

1. **Autotransporter** - Single protein that contains all necessary information to be secreted out of the cell.
2. **Two Partner Secretion** - First partner forms multimeric channel through outer membrane; second partner is transported through the channel out of cell.
3. In both systems the proteins are secreted into the periplasm by the GEP.
4. four domains
 - a. leader peptide
 - b. β -barrel through outer membrane
 - c. linker
 - d. passenger
 - e. also optional protease
5. chaperone-usheer –
 - a. some pili
 - b. usheer β -barrel through outer membrane
 - c. secreted pilin subunit in periplasm
 - d. chaperone guides pilin to usheer

H. Type 6 (I used to have Tat listed here, but it is considered a general export now)

1. Newly discovered (see commentary link on course web page)
2. Resembles phage tail
3. Virulence in several gram-negative bacteria

I. Gram-positive bacteria - sortase system (Fig. 14.7)

1. covalently binds secreted proteins to peptidoglycan or other protein
2. uses GEP (leader peptide)
3. C-terminal LPXTG sequence and hydrophobic domain
4. five sortase pathways so far