Escherichia coli (iJO1366) Cell Envelope Operation
The cell's envelope includes five main structures:

- Phospholipids
- Murein Wall (Peptidoglycan)
- Lipoprotein
- Lipopolysaccharides
- Membrane Proteins

Cell Envelope Synthesis
Cell Envelope Subsystems

iJO1366 includes the pathways for the synthesis of the cell's envelope in nine subsystems

- Cell Envelope Biosynthesis Subsystem
- Membrane Lipid Metabolism Subsystem
- Glycerophospholipids Metabolism Subsystem
- Murein Biosynthesis Subsystem
- Murein Recycling Subsystem
- Lipopolysaccharide Biosynthesis - Recycling Subsystem
- Inorganic Ion Transport and Metabolism
- Transport, Inner Membrane Subsystem
- Transport, Outer Membrane Subsystem
- Transport, Outer Membrane Porin Subsystem

Lesson Outline

- iJO1366 Subsystems used in Cell Envelopes
  - Phospholipids
  - Murein Wall (Peptidoglycan)
  - Lipoprotein
  - Lipopolysaccharides
  - Membrane Transport & Secretion
iJO1366
Cell Envelope Biosynthesis Subsystem

• The Cell Envelope Biosynthesis Subsystem contains the following pathways.
  ✓ The synthesis of saturated fatty acids attached to an acyl carrier protein (ACP)
  ✓ The synthesis of unsaturated fatty acids attached to an acyl carrier protein (ACP)
  ✓ The synthesis of Murein (peptidoglycan) precursors
  ✓ The synthesis of Antigen precursors
iJO1366 Membrane Lipid Metabolism Subsystem

• The Membrane Lipid Metabolism Subsystem contains the pathway for fatty acid catabolism.

• This subsystem is not used in the synthesis of the cell envelope.

• When fatty acids are not used as a carbon source this pathway needs to be disabled of the optimization software will select it over the natural pathway included in the Cell Envelope Biosynthesis Subsystem.
iJO1366

Glycerophospholipids Metabolism Subsystem

- The Glycerophospholipids Metabolism Subsystem contains the saturated and unsaturated lipids that can be produced by E.coli, they include:
  - Phosphatidylethanolamine
  - Phosphatidylglycerol
  - Cardiolipin

- The length of the lipids spans from 12 to 18 carbons.

- The lipids actively included in simulations are determined by the biomass function.
Murein Biosynthesis Subsystem

- This subsystem models the production of the murein repeating units used to create the murein sacculus.
- The murein sacculus is modeled through the biomass function that determines the assumed murein molecules required for cell growth.
  - The Core biomass function ("BF_Core") only assumes one murein peptide.
  - The Wild Type biomass function ("BF_WT") assumes five different murein peptides,
**iJO1366 Murein Recycling Subsystem**

- *E. coli* contains up to 20 murein hydrolases.
- Murein hydrolases are enzymes that digest murein or murein fragments into small, soluble fragments.
- These hydrolases include autolysins and lytic transglycosylases.
- About 40-50% of the murein in the murein sacculus recycled per generation.
- About 90% of the recycled murein is reinserted back into the murein sacculus.
iJO1366
Lipopolysaccharide Biosynthesis - Recycling Subsystem

- The Lipopolysaccharide (LPS) Biosynthesis - Recycling Subsystem contains the following pathways.
  ✓ The synthesis of Lipid A
  ✓ The synthesis of KDO and the LPS core
  ✓ The synthesis of antigens including the enterobacterial common antigen, the O16 antigen, the lipid A diphosphate antigen, and the Ara4N antigen
  ✓ The biomass function is used to determine the antigen included in the model simulations.
Transport, Outer Membrane Porin Subsystem

The Transport, Outer Membrane Porin Subsystem includes the passive bidirectional diffusion of 270 metabolites.

- Diffusion of metabolites from the extracellular space to the periplasm.
- Uses the "tex" suffix for each reaction name.
Transport, Outer Membrane Subsystem

- The Transport, Outer Membrane Subsystem contains the following:
  - 46 reactions
  - Passive bidirectional diffusion of 10 metabolites,
  - Passive unidirectional diffusion of 9 metabolites using an undefined system,
  - Facilitated irreversible diffusion of 7 metabolites,
  - Active TonB system unidirectional transport of 11 metabolites,
  - Active proton antiport transport of 1 metabolites,
  - Active TolC system unidirectional secretion of 7 metabolites,
  - Active unidirectional secretion of 1 metabolites using an undefined system.
Transport, Inner Membrane Subsystem

- The Transport, Inner Membrane Subsystem contains the following.
  - 332 Reactions
  - Passive bidirectional and unidirectional diffusion
  - Facilitated irreversible diffusion
  - Active unidirectional transport using the ABC system
  - Active unidirectional transport using the PTS system
  - Active symporters and antiporters
  - Reductases
  - Permeases

![Diagram of Transport, Inner Membrane Subsystem]
The Inorganic Ion Transport and Metabolism contains the transport and secretion reactions for the following metabolites.

- Iron derivatives, including ferric 2,3-dihydroxybenzoylserine, $\text{Fe}^{(III)}$dicitrate, enterochelin, $\text{Fe}^{(III)}$hydroxamate, ferrichrome, ferroxamine, coprogen, aerobactin

- Ammonia, calcium, cadmium, chloride, cobalt, copper, sulfur dioxide, sulfate, sulfite, thiosulfate, tungstate, zinc, silver, mercury, potassium, magnesium, manganese, molybdate, nickel, nitrile oxide, nitrous oxide, nitrite, nitrate, oxygen, phosphate, selenite, selenite, sodium,
Lesson Outline

• iJO1366 Subsystems used in Cell Envelopes
  • Phospholipids
    ✓ Fatty Acids
    ✓ Glycerophospholipids
  • Murein Wall (Peptidoglycan)
  • Lipoprotein
  • Lipopolysaccharides
  • Membrane Transport & Secretion
Fatty Acids

• The physical properties of fatty acids are largely determined by the length and degree of unsaturation of their hydrocarbon chains.

• The hydrocarbon chain accounts for the poor solubility in water. A longer the chain and the fewer the double bonds reduces the water solubility.

• The carboxylic acid group is polar and accounts for the slight solubility of short-chain fatty acids in water.

• In fully saturated fatty acids free rotation around each carbon-carbon bond gives the chain great flexibility and allows the molecules to be tightly packed together.

• In unsaturated fats, a cis double bond forces a kink in the hydrocarbon chain that limits how tightly packed the molecules can be.

Unsaturations prevent close packing and lower the melting point (increase fluidity)
Bacterial Fatty Acids

- Fatty acids are chains of methylene carbons with a carboxyl group at one end.
- Fatty acids differ in the number of carbon atoms, in the number of double bonds, in where the double bonds are located in the molecule, and whether the molecule is branched.
- Fatty acids do not occur free, unattached, in bacteria but are covalently attached to other molecules.
- Fatty acids may be esterified to a carbohydrate. The Lipid A found in a lipopolysaccharide consists of a fatty acid esterified to glucosamine.
- Fatty acids can also be esterified to protein. The lipoprotein is covalently attached to the peptidoglycan and protrudes into the outer membrane.
- Unsaturated fatty acids and branched-chain fatty acids add additional fluidity to the membrane.

iJO1366
Cell Envelope Biosynthesis Subsystem

Saturated Fatty Acid Synthesis

Unsaturated Fatty Acid Synthesis

Cell Envelope Biosynthesis Subsystem.json
Fatty Acid Biosynthesis
(Cell Envelope Biosynthesis Subsystem)

Long-chain acyl-ACP
For Membrane
Phospholipids

Fatty Acid Biosynthesis (saturated)
Aerobic Fatty Acid (Saturated) Synthesis with Glucose  
(Escher_Catabolite_Repression_Aerobic_Core_Flux.csv)
Aerobic Fatty Acid (Unsaturated) Synthesis with Glucose
The Membrane Lipid Metabolism Subsystem contains the pathways for fatty acid catabolism.

Fatty Acid Degradation (Beta-Oxidation)

Note: When fatty acids are not used as a carbon source this pathway needs to be disabled of the optimization software will select it over the natural pathway included in the Cell Envelope Biosynthesis Subsystem.
Degradation of Hexadecenoyl-CoA

Constraint-based Metabolic Reconstructions & Analysis
H. Scott Hinton, 2018
Lesson Outline

• iJO1366 Subsystems used in Cell Envelopes
• Phospholipids
  ✓ Fatty Acids
  ➤ Glycerophospholipids
• Murein Wall (Peptidoglycan)
• Lipoprotein
• Lipopolysaccharides
• Membrane Transport & Secretion

• Glycerophospholipids or phosphoglycerides are glycerol-based phospholipids.
• Each glycerophospholipid molecule consists of a small polar head group and two long hydrophobic chains. In the cell membrane, the two layers of phospholipids are arranged as follows:
  ✓ the hydrophobic tails point to each other and form a fatty, hydrophobic center
  ✓ the ionic head groups are placed at the inner and outer surfaces of the cell membrane.
iJO1366
Glycerophospholipids
Metabolism Subsystem

• The Glycerophospholipids Metabolism Subsystem contains the saturated and unsaturated lipids that can be produced by *E.coli*, they include
  ✓ Phosphatidylethanolamine
  ✓ Phosphatidylglycerol
  ✓ Cardiolipin

✓ The length of the lipids spans from 12 to 18 carbons.
✓ The lipids actively included in simulations are determined by the biomass function.

Glycerophospholipids Metabolism Subsystem.json
iJO1366 Produced Glycerophospholipids

Cytoplasmic Phosphatidylglycerol

Periplasmic Phosphatidylglycerol

Periplasmic Cardiolipin

Cytoplasmic Phosphatidylethanolamine

Periplasmic Phosphatidylethanolamine

Glycerophospholipids
Synthesis Pathway from Acetyl-CoA to Glycerophospholipid Phosphatidylethanolamine
Saturated Phospholipid Biosynthesis

Glycerophospholipids Metabolism Subsystem.json

Glycerol 3-phosphate

1,2-dihexadecanoyl-sn-glycerol 3-phosphate

Palmitoyl-ACP (n-C16:0ACP)

Phosphatidylethanolamine (dihexadecanoyl, n-C16:0)

All molecule drawings copied from https://biocyc.org
Unsaturated Phospholipid Biosynthesis

Glycerophospholipids Metabolism Subsystem.json

All molecule drawings copied from https://biocyc.org
Lesson: iJO1366 Cell Envelope Operation

Constraint-based Metabolic Reconstructions & Analysis

H. Scott Hinton, 2018

iJO1366 Model Production of Phospholipids (Biomass Function Driven)

Glycerophospholipids Metabolism Subsystem.json

Aerobic_Reaction_Flux.csv

Internal Membrane Phospholipids (Biomass Function)

Internal Membrane Phospholipids (Biomass Function)
iJO1366 Supported Lipids

The iJO1366 model’s support of lipids varies with the biomass function:
• The “Core” biomass function includes cytoplasmic and periplasmic flux for two lipid structures:
  ✓ Saturated
    ▪ pe160_c, pe160_p - phosphatidylethanolamine (dihexadecanoyl, n-C16:0)
  ✓ Unsaturated
    ▪ pe161_c, pe161_p - phosphatidylethanolamine (dihexadec-9enoyl, n-C16:1)
• The “Wild Type” biomass function the cytoplasmic and periplasmic flux for two lipid structures:
  ✓ Saturated
    ▪ pe160_c, pe160_p - phosphatidylethanolamine (dihexadecanoyl, n-C16:0)
    ▪ pg160_c, pg160_p - Phosphatidylglycerol (dihexadecanoyl, n-C16:0)
    ▪ clpn160_p - cardiolipin (tetrahexadecanoyl, n-C16:0)
  ✓ Unsaturated
    ▪ pe161_c, pe161_p - phosphatidylethanolamine (dihexadec-9enoyl, n-C16:1)
    ▪ pe181_c, pe181_p - phosphatidylethanolamine (dioctadec-11-enoyl, n-C18:1)
    ▪ pg161_c, pg161_p - Phosphatidylglycerol (dihexadec-9-enoyl, n-C16:1)
    ▪ pg181_c, pg181_p - Phosphatidylglycerol (dioctadec-11-enoyl, n-C18:1)
    ▪ clpn161_p - cardiolipin (tetrahexadec-9-enoyl, n-C16:1)
    ▪ clpn181_p - cardiolipin (tetraoctadec-11-enoyl, n-C18:1)

### Core Biomass Function
- pe160[c] 0.017868
- pe160[p] 0.045946
- pe161[c] 0.021060
- pe161[p] 0.054154

### Wild Type Biomass Function
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- clpn161[p] 0.002290
- clpn181[p] 0.001180
- clpn181[p] 0.0024732
- clpn181[p] 0.004957
- clpn181[p] 0.012747
- clpn181[p] 0.005707
- clpn181[p] 0.004892
- clpn181[p] 0.004439
- clpn181[p] 0.003805
- clpn181[p] 0.002288
- clpn181[p] 0.001961

Glycerophospholipid Production Using the Core Biomass Function

The iJO1366 Core biomass Functions includes the following glycerophospholipids:

✓ Saturated
  ▪ pe160_c, pe160_p - phosphatidylethanolamine (dihexadecanoyl, n-C16:0)
✓ Unsaturated
  ▪ pe161_c, pe161_p - phosphatidylethanolamine (dihexadec-9enoyl, n-C16:1)

Other glycerophospholipids can be created by changing the biomass function.

Glycerophospholipids Metabolism Subsystem.json

Escher_Catabolite_Repression_Aerobic_Core_Flux.csv
Glycerophospholipid Production Using the Wild Type Biomass Function

The iJO1366 can support the production of 15 Glycerophospholipids, including:

✓ Saturated
  - pe160_c, pe160_p – Phosphatidylethanolamine
  - pg160_c, pg160_p - Phosphatidylglycerol
  - clpn160_p - Cardiolipin

✓ Unsaturated
  - pe161_c, pe161_p - Phosphatidylethanolamine
  - pe181_c, pe181_p - Phosphatidylethanolamine
  - pg161_c, pg161_p - Phosphatidylglycerol
  - pg181_c, pg181_p - Phosphatidylglycerol
  - clpn161_p - Cardiolipin
  - clpn181_p - Cardiolipin
Lesson Outline

- iJO1366 Subsystems used in Cell Envelopes

- Phospholipids
  - Fatty Acids
  - Glycerophospholipids

- Murein Wall (Peptidoglycan)
  - Murein Overview
  - Murein Synthesis
  - Murein Recycling

- Lipoprotein

- Lipopolysaccharides

- Membrane Transport & Secretion
Biological Function of Murein

- Murein (peptidoglycan) is a continuous bag-shaped macromolecular structure encasing the cytoplasmic membrane.

- Its main function is to protect the cell structural integrity from internal osmotic pressure. It is also responsible for the maintenance of the defined cell shape and is a major component of the cell division process. Its absence from cells, in a hypotonic medium, will lead to swelling and to the rupture of the cytoplasmic membrane. Under certain conditions, cells lacking murein can be maintained as protoplasts, or spheroplasts, but they lose their shape and have a compromised cell division process.

- The main structural features of this giant macromolecule are linear glycan chains interlinked by short peptides.

- The glycan chains are composed of alternating linked units of N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc). The carboxyl group of each N-acetylmuramic acid residue is substituted by a peptide subunit, which is most often L-alanyl-γ-D-glutamyl-diaminopimelyl-D-alanyl-D-alanine in nascent peptidoglycan but will eventually lose one or both of the D-alanine residues in mature peptidoglycan. Neighboring glycan chains are interlinked either by a direct peptide linkage between a peptide subunits of one of the other chains or by a short peptide bridge between two peptide subunits.


Murein Physiology

Architecture of the murein layer. The drawing shows two murein layers and indicates (by dotted lines) how they can be stacked on one another to form a multilayered murein. Glycan strands are represented by solid bars. The peptide cross bridges are indicated by black lines (acceptor stem peptides) and black arrows (donor stem peptides).


Murein sacculus of *E. coli*. (a) Electron micrograph of an isolated murein sacculus. Bar, 0.5 µm. (b) Idealized schematic drawing of the architecture of the murein sacculus. The parallel lines represent a few of the vast number of glycan strands, and the arrows indicate the peptide bridges.
"In murein the glycan strands are made of the aminosugars N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), which are linked together by β-1,4 glycosidic bonds. The presence of the lactyl group of the muramic acid allows the covalent attachment of peptides, which can be cross-linked to form the characteristic net structure of murein. Whereas the chemistry of the glycan strands shows only few variations among different bacteria, such as O or N acetylation, the peptides vary a lot.

Importantly, a dibasic amino acid has to be present to enable the cross-linking peptide bond to be formed. In Escherichia coli, this is meso-diaminopimelic acid, an intermediate in the biosynthetic pathway leading to lysine. The stem peptides linked to the glycan strands are arranged helically along the strand, protruding in all directions and forming angles to one another of about 90°. Therefore, in a monolayered murein, only every second peptide can be cross-linked by turns to the right and left. Determination of the degree of cross-linkage indicates that under certain growth conditions all peptides in the plane of the glycan strands can be cross-linked."

Muropeptide Structures (II)

(A) Monomeric muropeptide structures identified from the murein of *E. coli*. The different peptide moieties (R) that can substitute the lactyl group of MurNAc are listed. (B) Chemistry of the major cross-linked dimers and trimers found in the murein of *E. coli*. A2pm, diaminopimelic acid.

D,D-(Ala-A2pm) crosslinked dimer

D,L-(A2pm-A2pm) crosslinked dimer

D,D-(Ala-A2pm) crosslinked trimer

Murein is a hetero-polymer made of linear glycan strands of alternating, β-1,4-linked N-acetylg glucosamine (GlcNAc) and N-acetyl muramic acid (MurNAc) residues, that are cross-linked by short peptides.

The terminal residues of the glycan strands are GlcNAc and 1,6-anhydroMurNAc, which is MurNAc with an intra-molecular ether-linkage from C-1 to C-6.

The peptides are attached by an amide linkage to the lactyl group of MurNAc and they are unusual because they contain rare D-amino acids.

The initial sequence of the newly synthesized pentapeptide is L-Ala-D-iGlu-m-A2pm-D-Ala-D-Ala, with the dibasic meso-diaminopimelic acid (m-A2pm) residing at position 3. The D-Glu residue is linked via its γ-carboxyl group to the L-center of m-A2pm.

Depending on the species and growth conditions, a fraction of the peptides contains Gly instead of D-Ala at position 4 or 5.

In murein sacculi isolated from E. coli, the fraction of pentapeptides is very low due to their rapid proteolytic degradation to tetrapeptides (L-Ala-D-iGlu-m-A2pm-D-Ala), tripeptides (L-Ala-D-iGlu-m-A2pm) and dipeptides (L-Ala-D-iGlu).

The murein lipoprotein is anchored to the outer membrane by its N-terminal lipid residues. Lpp is attached to murein by a peptide bond between the ε-amino group of its C-terminal Lys residue and the L-carboxyl group of the m-A2pm residue in the murein peptide.
The muropeptide composition of isolated *E. coli* sacculi shows some variation depending on the strain, the growth medium and temperature, and the growth phase.


<table>
<thead>
<tr>
<th>Muropeptide</th>
<th>Exponential growth phase</th>
<th>Stationary growth phase</th>
<th>Muropeptide</th>
<th>Exponential growth phase</th>
<th>Stationary growth phase</th>
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<tbody>
<tr>
<td>Di</td>
<td>1.22</td>
<td>1.97</td>
<td>Monomers</td>
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<td>Dimers</td>
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The Muropeptide Composition of *E. coli* KN 126 Grown in LB-medium
Particular Muropeptides

“Several aspects of the structure of murein seem to be of particular importance. The prevailing cross-linkage is via D,D-peptide bonds between m-A2pm and D-Ala, which are known to be formed by a penicillin-sensitive transpeptidation reaction. By contrast, the cross-linkages via L,D-peptide bonds between two m-A2pm residues are uncommon (about 2% of the linkages) in exponentially growing cells. However, the relative amount of L,D-cross-linkage can more than double during the stationary phase of growth. The murein is cross-linked not only by dimeric peptide bridges connecting two glycan strands but also to a significant extent (4.6%) by trimeric peptide bridges connecting three glycan strands. Tetrameric cross-links are extremely rare and represent only about 0.16% of the linkages. Structural considerations indicate that the three (four) strands cross-linked by a trimeric (tetrameric) peptide cannot be arranged in one plane. One (two) of the three (four) strands must be positioned either above or below the plane defined by the other two strands. This raises the question about the function of such a structural element in a monolayered murein.

The covalent linkage of a lipoprotein to the murein, which can be cleaved off by pronase treatment during the preparation of pure murein sacculi gives rise to muropeptides substituted at their A2pm residues by the two carboxyl-terminal amino acids of the lipoprotein, Lys and Arg. Therefore, a muropeptide analysis also contains some information about the amount and specific linkage of the murein lipoprotein to the sacculus. A peculiar structure of the murein of E. coli is a 1.6-anhydromuramic acid present at one end of the murein strands. All of the normally reducing ends seem to be blocked thereby, since free reducing ends have not been found. The 1,6-anhydro ring is formed by an endogenous lysozyme-like enzyme.

The 1,6-anhydromuramic acid that is a natural tag of one of the ends of the glycan strands can be used to calculate the average length of all glycan strands in the murein by determining the ratio of all muropeptides to all 1,6-anhydromuropeptides. Depending on the growth conditions, an average length of about 29 disaccharide units was found. A surprising result was obtained when analyzing the length distribution of the glycan strands by HPLC. It turned out that most of the strands are rather short, with lengths of around 5 to 10 disaccharide units, which equals about 1/300 of the total circumference of the cell. The distribution showed an amazingly broad variation from 1 to more than 100 disaccharide units. Whether the extremely long strands have a specific function and topological localization in the sacculus is not known yet.”

Lesson Outline

- iJO1366 Subsystems used in Cell Envelopes
- Phospholipids
  - Fatty Acids
  - Glycerophospholipids
- Murein Wall (Peptidoglycan)
  - Murein Overview
  - Murein Synthesis
  - Murein Recycling
- Lipoprotein
- Lipopolysaccharides
- Membrane Transport & Secretion
Murein (Peptidoglycan) Precursors

- A peptidoglycan monomer consists of two joined amino sugars, N-acetylglucosamine (NAG) or (UDP-N-acetyl-D-glucosamine or 'uacgam_c') in the iJO1366 model and N-acetylmuramic acid (NAM) UDP-N-acetylmuramate or 'uamr_c', with a pentapeptide coming off of the NAM.
- In *E. coli*, the pentapeptide consists of the amino acids L-alanine, D-glutamic acid, meso diaminopimelic acid (Meso-2,6-Diaminoheptanedioate in iJO1366 model), and two D-alanines.
- The synthesis of these murein precursors begins with D-fructose 6-phosphate.
- The murein precursors are synthesized in the cytoplasm.
Murein (Peptidoglycan) Synthesis

Murein is synthesized in four major stages

1. The precursors to peptidoglycan are UDP derivatives of amino acids made in the cytoplasm. They include N-acetylglucosamine (UDP-N-acetyl-D-glucosamine or ‘uagcma_c’) and N-acetylmuramic acid (UDP-N-acetylmuramate or ‘umr_c’).

2. The modified amino sugars are transferred to a lipid carrier (Undecaprenyl phosphate or ‘udcpp_c’) in the membrane which will carry the amino sugars across the membrane to the periplasm through the “Undecaprenylphosphate cycle.”

3. The peptidoglycan is then polymerized in the periplasm.

4. A transpeptidation reaction cross-links the peptidoglycan.

Peptidoglycan Precursors Synthesis
(Cell Envelope Biosynthesis Subsystem)
Lipid II Biosynthesis Flux

Glucose Carbon Source
Aerobic Condition
Minimal Media
Wild-Type Biomass Function
Undecaprenylphosphate Cycle
Undecaprenylphosphate Cycle

(Glucose, Aerobic, Wild-Type Biomass Functions)
Peptidoglycan Synthesis

Pauline Macheboeuf, Carlos Contreras-Martel, Viviana Job, Otto Dideberg, Andréa Dessen; Penicillin Binding Proteins: key players in bacterial cell cycle and drug resistance processes, FEMS Microbiology Reviews, Volume 30, Issue 5, 1 September 2006, Pages 673-691,
Structure of the Peptidoglycan of Escherichia coli

Basic murein disaccharide tetrapeptide subunit (monomer)

- N-acetylglucosamine (GlcNAc)
- N-acetylmuramic acid (MurNAc)
- L-Ala
- d-Glu
- meso-A2pm
- d-Ala

Cross-linking murein subunits

Waldemar Vollmer, Didier Blanot, Miguel A. De Pedro; Peptidoglycan structure and architecture, FEMS Microbiology Reviews, Volume 32, Issue 2, 1 March 2008, Pages 149-167,
Murein Biosynthesis Subsystem

- The murein sacculus is modeled through the biomass function that determines the required murein molecular flux required for cell growth.
- This subsystem models the production of the murein repeating units used to create the murein sacculus.
- The Core biomass function ("BF_Core") only assumes one murein peptide, murein5p5p_p.
- The Wild Type biomass function ("BF_WT") assumes five different murein peptides, murein4p4p_p, murein3p3p_p, murein4px4p_p, murein3px4p_p, murein4px4px4p_p.
- There are 15 murein metabolites that are included in the iJO1366 model that can be activated by altering the biomass function.
- Note that all reactions except MPTG and MPTG2 are in the periplasm.
<table>
<thead>
<tr>
<th>Murein Metabolite</th>
<th>Murein Metabolite (Muropeptides) Description</th>
<th>Biomass Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>murein3p3p_p</td>
<td>Two linked disacharide tripeptide murein units (uncrosslinked, middle of chain)</td>
<td>WT</td>
</tr>
<tr>
<td>murein3px3p_p</td>
<td>Two disacharide linked murein units, tripeptide crosslinked tripeptide (A2pm-&gt;A2pm) (middle of chain)</td>
<td>WT</td>
</tr>
<tr>
<td>murein3px4p_p</td>
<td>Two disacharide linked murein units, tripeptide crosslinked tetrapeptide (A2pm-&gt;D-ala) (middle of chain)</td>
<td>WT</td>
</tr>
<tr>
<td>murein4p3p_p</td>
<td>Two linked disacharide tetrapeptide and tripeptide murein units (uncrosslinked, middle of chain)</td>
<td>WT</td>
</tr>
<tr>
<td>murein4p4p_p</td>
<td>Two linked disacharide tetrapeptide murein units (uncrosslinked, middle of chain)</td>
<td>WT</td>
</tr>
<tr>
<td>murein4px4p_p</td>
<td>Two disacharide linked murein units, tetrapeptide crosslinked tetrapeptide (A2pm-&gt;D-ala) (middle of chain)</td>
<td>WT</td>
</tr>
<tr>
<td>murein4px4p4p_p</td>
<td>Three disacharide linked murein units (tetrapeptide crosslinked tetrapeptide (A2pm-&gt;D-ala), one uncrosslinked tetrapeptide) (middle of chain)</td>
<td>WT</td>
</tr>
<tr>
<td>murein5p3p_p</td>
<td>Two linked disacharide pentapeptide and tripeptide murein units (uncrosslinked, middle of chain)</td>
<td>WT</td>
</tr>
<tr>
<td>murein5p4p_p</td>
<td>Two linked disacharide pentapeptide and tetrapeptide murein units (uncrosslinked, middle of chain)</td>
<td>WT</td>
</tr>
<tr>
<td>murein5p5p_p</td>
<td>Two linked disacharide pentapeptide murein units (uncrosslinked, middle of chain)</td>
<td></td>
</tr>
<tr>
<td>murein5p5p5p_p</td>
<td>Three linked disacharide pentapeptide murein units (uncrosslinked, middle of chain)</td>
<td></td>
</tr>
<tr>
<td>murein5px3p_p</td>
<td>Two disacharide linked murein units, pentapeptide crosslinked tripeptide (A2pm-&gt;A2pm) (middle of chain)</td>
<td>Core</td>
</tr>
<tr>
<td>murein5px4p_p</td>
<td>Two disacharide linked murein units, pentapeptide crosslinked tetrapeptide (A2pm-&gt;D-ala) (middle of chain)</td>
<td></td>
</tr>
<tr>
<td>murein5px4px4p_p</td>
<td>Three disacharide linked murein units (pentapeptide crosslinked tetrapeptide (A2pm-&gt;D-ala) tetrapeptide crosslinked tetrapeptide (A2pm-&gt;D-ala)) (middle of chain)</td>
<td></td>
</tr>
</tbody>
</table>

Murein Peptides

Murein3p3p

Murein4p4p

Murein5p5p

D-Ala

D-Ala D-Ala

D-Ala

D-Ala

Molecular structures from biocyc.org
**Murein Composition in *E. coli* (iAF1260 & iJO1366)**

<table>
<thead>
<tr>
<th>Crosslinked Composition</th>
<th>Monomers</th>
<th>Dimers</th>
<th>Trimers</th>
<th>References</th>
<th>Overall Mol % (Metabolite)</th>
<th>Compound Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mol % in sacculus:</td>
<td>0.5</td>
<td>0.45</td>
<td>0.05</td>
<td>Glauner et al, Schiffer et al</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peptide Length Composition</th>
<th>Overall Mol % (Metabolite)</th>
<th>Compound Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncrosslinked 4p</td>
<td>0.4</td>
<td>murein4p4p</td>
</tr>
<tr>
<td>Uncrosslinked 3p</td>
<td>0.1</td>
<td>murein3p3p</td>
</tr>
<tr>
<td>4p-&gt;4p *</td>
<td>0.405</td>
<td>murein4px4p</td>
</tr>
<tr>
<td>3p-&gt;4p *</td>
<td>0.045</td>
<td>murein4px3p</td>
</tr>
<tr>
<td>4p-&gt;4p-&gt;4p *</td>
<td>0.05</td>
<td>murein4px4px4p</td>
</tr>
</tbody>
</table>

*All A2pm -> Ala crosslinked

4p = 4-peptide
3p = 3-peptide

Note: Under certain conditions an appreciable amount of the sacculus can be 3p -> 3p crosslinked


Murein Primary Pathway

Murein polymerizing transglycosylase
Murein crosslinking transpeptidase
Murein D, D-carboxypeptidase
Murein D, D-endopeptidase

Molecular structures from biocyc.org
Murein Biosynthesis: Core vs. Wild Type Biomass Function

- Core Biomass Function
- Wild Type Biomass Function

Murein Biosynthesis Subsystem.json
Escher_Catabolite_Repression_Aerobic_Core_Flux.csv
Murein Biosynthesis Subsystem.json
Escher_Catabolite_Repression_Aerobic_WT_Flux.csv
Murein Biosynthesis Subsystem Genes

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- Note that all reactions except MPTG and MPTG2 are in the periplasm.
Lesson Outline

- iJO1366 Subsystems used in Cell Envelopes

- Phospholipids
  - Fatty Acids
  - Glycerophospholipids

- Murein Wall (Peptidoglycan)
  - Murein Overview
  - Murein Synthesis
  - Murein Recycling

- Lipoprotein

- Lipopolysaccharides

- Membrane Transport & Secretion
Murein Turnover and Recycling

**Murein Turnover**

“The phenomenon of murein turnover in *E. coli* was detected rather late because turnover is coupled to murein recycling. The turnover material that is released at a rate of 40 to 50% per generation is quite efficiently recycled. About 90% of the turnover material is reinserted into the murein sacculus. The turnover products are 1,6-anhydro-MurNAc-β-1,4-GlcNAc-tetra- and tripeptides, indicating that they are released by the action of lytic transglycosylases. The absence of dimers or trimers suggests that turnover is due to the concerted action of both lytic transglycosylases and endopeptidases. The turnover products accumulate in the periplasm, from where they are reimported into the cytoplasm. Several recycling pathways exist.”

**Murein Recycling**

“The major route for the uptake of the turnover products is via the transporter protein AmpG, a transmembrane protein proposed to act as a specific permease for intact muropeptides, which are mostly tripeptide-substituted muropeptides due to the presence of an L,D-carboxypeptidase in the periplasm. In the cytoplasm, the muropeptides have to be trimmed to the size that allows reutilization for the synthesis of murein precursors. A β-N-acetylmuramidase splits the disaccharide, and an amidase (AmpD) cleaves off the peptide. Interestingly, AmpD has a specificity for 1,6-anhydro muropeptides and therefore does not hydrolyze the murein precursor molecules such as UDPMurNAc-pentapeptide also present in the cytoplasm.

Besides intact muropeptides, degradation products of the turnover material are also taken up by the cell. The presence of an amidase (AmiA) in the periplasm causes degradation of the turnover products to their peptide and sugar parts. The peptides are taken up by the cell via two peptide transport systems, by a general oligopeptide permease (Opp), and by a specific murein peptide permease (Mpp). To avoid the need for a complete breakdown of the peptides to the amino acid level, a specific ligase (mpl) exists that catalyzes the attachment of the tripeptide to UDPMurNAc.”

Chemistry of the Murein of *E. coli*.

- On the right is shown how a new murein precursor linked to the undecaprenyl pyrophosphate group (represented by a spotted circle) is linked to the pre-existing murein by the formation of two bonds.
- Concomitant with cleavage of the D,D-peptide bond between the two D-Ala residues of the pentapeptide precursor, a transpeptidase forms a D,D-peptide bond between the carboxyl group of the penultimate D-Ala of the precursor and the epsilon amino group of a diaminopimelic acid residue present in a peptide moiety of the growing murein sacculus.
- A transglycosylase splits the pyrophosphate bond between the undecaprenyl group and the MurNAc of a nascent glycan strand in the sacculus and forms a glycosidic bond to the hydroxyl group at carbon 4 of the GlcNAc of the precursor molecule.
- The numbers point to the bonds cleaved by specific murein hydrolases present in *E. coli*:
  1. N-acetylglucosaminidase;
  2. lytic transglycosylase;
  3. N-acetylmuramyl-L-alanine amidase;
  4. D,D-endopeptidase;
  5. g-D-glutamyl-L-diaminopimelic acid endopeptidase;
  6. L,D-carboxypeptidase;
  7. D,D-carboxypeptidase; m-A2pm, meso-diaminopimelic acid.

Murein Hydrolases

Cleavage sites of the murein hydrolases.

Ami, N-Acetylmuramyl-L-alanine amidase; LTase, lytic transglycosylase; DD-EPase, DD-endopeptidase; LDEPase, LD-endopeptidase; DD-CPase, DD-carboxypeptidase; LD-CPase, LD-carboxypeptidase; R, peptide.

### Murein Hydrolases of *E. coli*

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specificity</th>
<th>MW [Da]</th>
<th>Gene</th>
<th>Intracellular localization</th>
<th>Function/remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slk70</td>
<td>LT</td>
<td>73,353</td>
<td>_<em>sleY</em></td>
<td>Periplasm</td>
<td>Lipoprotein</td>
</tr>
<tr>
<td>MlaA</td>
<td>LT</td>
<td>40,411</td>
<td>_<em>mlaA</em></td>
<td>Outer membrane</td>
<td>Lipoprotein, degradation to Slk35</td>
</tr>
<tr>
<td>MlaB</td>
<td>LT</td>
<td>40,256</td>
<td>_<em>mlaB</em></td>
<td>Outer membrane</td>
<td>Lipoprotein</td>
</tr>
<tr>
<td>MlaC</td>
<td>LT</td>
<td>40,113</td>
<td>_<em>mlaC</em></td>
<td>Outer membrane</td>
<td>Lipoprotein</td>
</tr>
<tr>
<td>MlaD</td>
<td>LT</td>
<td>49,417</td>
<td>_<em>mlaD</em></td>
<td>Outer membrane</td>
<td>Lipoprotein</td>
</tr>
<tr>
<td>EmtA</td>
<td>LT</td>
<td>26,575</td>
<td>__emtA (mlaE)</td>
<td>Outer membrane</td>
<td>Lipoprotein</td>
</tr>
<tr>
<td>AmiA</td>
<td>Ami</td>
<td>31,412</td>
<td>_<em>amiA</em></td>
<td>Periplasm</td>
<td>Cell separation</td>
</tr>
<tr>
<td>AmiB</td>
<td>Ami</td>
<td>47,985</td>
<td>_<em>amiB</em></td>
<td>Periplasm</td>
<td>Cell separation</td>
</tr>
<tr>
<td>AmiC</td>
<td>Ami</td>
<td>45,634</td>
<td>_<em>amiC</em></td>
<td>Periplasm, septation site</td>
<td>Cell separation</td>
</tr>
<tr>
<td>AmpD</td>
<td>1,6Ami</td>
<td>20,536</td>
<td>_<em>ampD</em></td>
<td>Cytoplasm</td>
<td>Recycling</td>
</tr>
<tr>
<td>PBP4</td>
<td>DD-EP/CP</td>
<td>51,798</td>
<td>_<em>dacB</em></td>
<td>Periplasm, membrane</td>
<td>Proteolytic cleavage to PBP8</td>
</tr>
<tr>
<td>PBP7</td>
<td>DD-EP</td>
<td>34,245</td>
<td>_<em>pdbG</em></td>
<td>Periplasm, membrane</td>
<td></td>
</tr>
<tr>
<td>MepA</td>
<td>DD/LD-EP</td>
<td>30,137</td>
<td>_<em>mepA</em></td>
<td>Periplasm</td>
<td>Recycling</td>
</tr>
<tr>
<td>NagZ</td>
<td>GA</td>
<td>37,595</td>
<td>__nagZ (ycfO)</td>
<td>Cytoplasm</td>
<td></td>
</tr>
<tr>
<td>PBP5</td>
<td>DD-CP</td>
<td>44,444</td>
<td>_<em>dacA</em></td>
<td>Inner membrane</td>
<td>Cell shape maintenance</td>
</tr>
<tr>
<td>PBP6</td>
<td>DD-CP</td>
<td>43,609</td>
<td>_<em>dacC</em></td>
<td>Inner membrane</td>
<td></td>
</tr>
<tr>
<td>PBP6B</td>
<td>DD-CP</td>
<td>43,346</td>
<td>_<em>dacD</em></td>
<td>Inner membrane</td>
<td></td>
</tr>
<tr>
<td>LdeA</td>
<td>LD-CP</td>
<td>33,567</td>
<td>__ldcA (ycgQ)</td>
<td>Cytoplasm</td>
<td>Recycling, essential in stationary phase</td>
</tr>
<tr>
<td>EnvC</td>
<td>Unknown</td>
<td>46,595</td>
<td>__envC (yibP)</td>
<td>Septation site</td>
<td>Cell separation</td>
</tr>
<tr>
<td>FlgJ</td>
<td>Unknown</td>
<td>34,475</td>
<td>_<em>flgJ</em></td>
<td>Flagellum</td>
<td>Flagellum synthesis</td>
</tr>
</tbody>
</table>

**Notes:**
- LT, lytic transglycosylase;
- Ami, N-acetylmuramyl-L-alanine amidase;
- 1,6Ami, 1,6-anhydro-N-acetylmuramyl-L-alanine amidase;
- GA, N-acetylglucosaminidase;
- DD-EP, DD-endopeptidase;
- LD-EP, LD-endopeptidase;
- DD-CP, DD-carboxypeptidase;
- LD–CP, LD-carboxypeptidase.

a Molecular weights are given for the unprocessed form.

b Endo-specific lytic transglycosylase.

c Membrane-associated.

d Hypothetical peptidase.

e Hypothetical muramidase.

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Lesson: iJO1366 Cell Envelope Operation

iJO1366
Murein Recycling Subsystem
Recycling Pathway of Murein Turnover Products in E. coli.

Constraint-based Metabolic Reconstructions & Analysis

Lesson: iJO1366 Cell Envelope Operation

H. Scott Hinton, 2018

Muropeptides

iJO1366
Murein Recycling Subsystem Genes

Periplasm

Cytoplasm

Muropeptides

Muropeptides
Murein Recycling Subsystem
Including All iJO1366 Murein Metabolites

Murein Recycling Subsystem-All Muropeptides.json
Aerobic Murein Recycle Flux with Glucose Carbon Source

- Note that the iJO1366 model in minimal media, under aerobic conditions, with glucose as the only carbon source, using the wild-type biomass function, only produces enough murein metabolites to meet the needs of the WT biomass function.
- Note that none of the murein is recycled.
- *E. coli* physiology suggests that 40-50% of the murein should be recycled.
Aerobic Murein Recycle Flux with Glucose Carbon Source after Doubling the Production of Lipid II

- Note that a flux value equivalent (0.027) to the additional flux required above the needs of the biomass function (0.027) is recycled back as a Lipid I precursor to help provide the additional Lipid II demand.
- The flux recycled through the "Alternate Carbon Metabolism Subsystem" is fed into the pathway for peptidoglycan production which also helps meet the additional Lipid II demand.
Lesson Outline

- iJO1366 Subsystems used in Cell Envelopes
- Phospholipids
  - Fatty Acids
  - Glycerophospholipids
- Murein Wall (Peptidoglycan)
  - Murein Overview
  - Murein Synthesis
  - Murein Recycling
- Lipoprotein
- Lipopolysaccharides
- Membrane Transport & Secretion
Lipoprotein Physiology

Lpp, the major lipoprotein, is considered to be the most abundant protein in *Escherichia coli*; estimates suggest that it is present at approximately 750,000 copies per cell, comprises 2% of the dry weight of the cell and accounts for more than 40% of the peptidoglycan on a weight basis. Lpp is necessary for the stabilization and integrity of the bacterial cell envelope and is thought to physically tether the outer membrane to the peptidoglycan layer.

Lpp exists in two forms, historically known as free Lpp and bound Lpp but more recently referred to as transmembrane Lpp and periplasmic Lpp. Periplasmic (bound) Lpp is covalently linked to the peptidoglycan layer by its carboxy terminal lysine whereas transmembrane (free) Lpp spans the outer membrane and is surface exposed. The ratio of free Lpp:bound Lpp is estimated to be 2:1. Tracer experiments with labelled arginine suggest that free Lpp is synthesized first and then converted to bound Lpp and there is a dynamic equilibrium between the two forms within the cell. Transmembrane Lpp can be labelled with an outer membrane impermeable label whereas periplasmic Lpp cannot. Periplasmic Lpp can be degraded by proteases introduced into the periplasm whereas the proteases do not effect the transmembrane Lpp.

Cells lacking Lpp or with mutations affecting the attachment of Lpp to the murein (peptidoglycan) layer exhibit outer membrane blebs, are hypersensitive to toxic compounds, and release periplasmic proteins to the extracellular medium.

Lpp is expressed as a prolipoprotein, having a 20 amino acid signal sequence extending from the amino terminus. Lpp is then exported by the Sec complex. During translocation across the cytoplasmic membrane, Lpp undergoes N-acylation by the Lnt protein, and the mature lipoprotein is then translocated to the outer membrane by the LolABCDE system where it is covalently bound to the peptidoglycan layer. Studies using inhibitors of the proton motive force (pmf) and ATP-depleted cells indicated that both the pmf and ATP are required for translocation of an OmpF-Lpp chimeric protein. Immunoelectron microscopy revealed that free lipoprotein is inserted equally over the entire cell wall, that lipoprotein synthesis increases with cell length, and that cell shape depends on total lipoprotein content of the cell in that low total lipoprotein corresponds to a spherical shape and a higher lipoprotein content corresponds with a rod shape.

https://biocyc.org/gene?orgid=ECOLI&id=EG10544-MONOMER
iJO1366 Lipoproteins

• There is no pathway in iJO1366 for the synthesis of lipoproteins.
• The pathway loop for the synthesis of lipoproteins is shown to the right.
• The synthesis of lipoproteins is NOT supported in the iJO1366 model.
• The lipoproteins are included in the average protein determined for the biomass function.
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Lipopolysaccharides Physiology

Lipopolysaccharides (LPS), also known as lipoglycans and endotoxins, are large molecules consisting of a lipid and a polysaccharide composed of O-antigen, outer core and inner core joined by a covalent bond; they are found in the outer membrane of Gram-negative bacteria, and elicit strong immune responses in animals.

O-antigen - A repetitive glycan polymer contained within an LPS is referred to as the O antigen, O polysaccharide, or O side-chain of the bacteria. The O antigen is attached to the core oligosaccharide, and comprises the outermost domain of the LPS molecule. The composition of the O chain varies from strain to strain. For example, there are over 160 different O antigen structures produced by different E. coli strains. O antigen is exposed on the very outer surface of the bacterial cell, and, as a consequence, is a target for recognition by host antibodies.

Core - The Core domain always contains an oligosaccharide component that attaches directly to lipid A and commonly contains sugars such as heptose and 3-Deoxy-D-manno-oct-2-ulosonic acid (also known as KDO, keto-deoxyoctulosonate).

Lipid A - Lipid A is, in normal circumstances, a phosphorylated glucosamine disaccharide decorated with multiple fatty acids. These hydrophobic fatty acid chains anchor the LPS into the bacterial membrane, and the rest of the LPS projects from the cell surface. The lipid A domain is responsible for much of the toxicity of Gram-negative bacteria. When bacterial cells are lysed by the immune system, fragments of membrane containing lipid A are released into the circulation, causing fever, diarrhea, and possible fatal endotoxic shock (also called septic shock).

https://en.wikipedia.org/wiki/Lipopolysaccharide
Schematic Structure of the *E. coli K-12* Cell Envelope

Lipopolysaccharide Biosynthesis - Recycling Subsystem
Constitutive pathway for biosynthesis of the Kdo2-lipid A portion of LPS in *E. coli* K-12

Export of Nascent core-lipid A and O-antigen Precursors, and the Assembly of lipopolysaccharide (LPS) in *E. coli* K-12.

Biosynthesis of the L-Ara4N Unit and its Attachment to core-lipid A
Lipid A Biosynthesis

Molecular Images from KEGG Database - Lipopolysaccharide Biosynthesis - Recycling Subsystem.json
**Lipopolysaccharide Core Biosynthesis**

**Alpha-D-Ribose 5-phosphate**
(Pentose Phosphate Pathway)

**Lipid A**

**MOAT**

**MOAT2**

**Periplasmic Space**

**Core**

**Precursor**
Sedoheptulose 7-phosphate

**Constraint-based Metabolic Reconstructions & Analysis**

-80-

H. Scott Hinton, 2018
Enterobacterial common antigen (ECA) is a family-specific surface antigen shared by all members of the Enterobacteriaceae and is restricted to this family. It is found in freshly isolated wild-type strains as well as in laboratory strains like Escherichia coli K-12.

- ECA is not included in either the core or WT iJO1366 Biomass Functions.
The O-antigen usually consists of 10–25 repeating units containing two to seven sugar residues. Thus, the molecular mass of the LPS present in smooth strains will be up to ~25 kDa.

The present scheme of *E. coli* O-antigens comprises O1 to O181.

The complete pathway of O16-antigen not implemented in iJO1366

O16-antigen is not included in either the core or WT iJO1366

**Biomass Functions**

Core Lipopolysaccharide Biosynthesis (Limited LPS Core and No Antigen)
Wild Type Lipopolysaccharide Biosynthesis (No Antigen)
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  - Murein Recycling
- Lipoprotein
- Lipopolysaccharides
- Membrane Transport & Secretion
Primary Solute Transport in *E. coli*

**Diffusion**
- H₂O, O₂, CO₂

**Facilitated Diffusion**
- Glycerol

**Ion-Coupled Transport**
- Lactose

**ATP-binding Cassette Transporters**
- Galactose

**Group Translocation**
- Glucose

**Extracellular**
- Outer Membrane Porins

**Periplasm**
- Transporter Protein

**Cytoplasm**
- H₂O, O₂, CO₂

After Figure 6.10 in M. Schaechter et al, "Microbe," ASM Press, 2006
Outer Membrane Porin Transport

- The major proteins in the outer membrane are called porins.
- Porins form a small non-specific hydrophilic channels allowing the concentration gradient diffusion of both neutral and charged low molecular weight (<600 Da) solutes such as sugars and ions.
- Hydrophobic compounds cannot pass through the porins.
- Porins allow the passage of water and hydrophilic solutes to diffuse with concentration gradients both into and out of the cell.
- Hydrophobic and larger hydrophilic molecules require special structures (e.g. TonB) to provide selective transport through the outer membrane.
- The four major porins of *E. coli* are OmpC, OmpF, OmpN, and PhoE.
  - OmpC is 7% smaller than OmpF. OmpN is not highly expressed but has similar properties to OmpC.
  - PhoE is a channel for phosphate and other anions that is produced under conditions of inorganic phosphate limitations.
- Other outer membrane proteins for large molecules include LamB (maltose and maltodextrins), BtuB (vitamin B12), and Tsx (nucleosides).
- The protein from the outer membrane porins is included in the aggregate protein total in the biomass function.

- There is no differentiation between the major outer membrane proteins in the iJO1366 model. The COBRA reaction suffix “tex” is an Boolean “or” of the four major outer membrane proteins.
  - (b0241 or b0929 or b1377 or b2215)
  - (PhoE or OmpF or OmpN or OmpC)
- The LamB (b4036) porin is associated with the transport of glucose (GLCtex_copy2), maltohexaose (MALTHXtexi), maltopentaose (MALPTTtexi), maltotriose (MALTTTRtexi), maltotetraose (MALTTTRtexi), maltose (MALTTexi), and 1,4-alpha-D-glucan (14GLUCANtexi)
- The BtuB (b3966) porin (TonB system) is associated with the transport of adenosylcobalimin (ADOCBLtonex), cobinamide (CBItex), and vitamin b12 or cob(1)alamin (CBL1tonex).
- The Tsx (b0411) porin is associated with the transport of adenosine (ADOCBLtonex), deoxyadenosine (DADNtex), deoxycytidine (DCYTtex), deoxyuridine (DURItex), guanine (GUAtex), inosine (INStex), and uridine (URItex)

Primary Solute Transport in *E. coli*

**Diffusion**
- H₂O, O₂, CO₂

**Facilitated Diffusion**
- Glycerol

**Ion-Coupled Transport**
- Lactose

**ATP-binding Cassette Transporters**
- Galactose

**Group Translocation**
- Glucose

**Extracellular**
- Outer Membrane Porins

**Periplasm**
- Transporter Protein
- Sympporter
- H⁺

**Cytoplasm**
- H₂O, O₂, CO₂

**OM**
- Enzyme II
- Glycerophospholipids

**IM**
- Enzyme I

**Cytoplasm**
- Glycerol
- Lactose
- Glucose
- H⁺

**Murein**
- Lipoproteins

**Lipid A**
- Antigen Core

After Figure 6.10 in M. Schaechter et al, "Microbe," ASM Press, 2006
Passive Transport Through the Inner Membrane

- **Passive transport** uses no energy but relies on diffusion. It only operates when the solute concentration is higher outside than inside the cell.

- **Simple Diffusion** provides the entry for a few nutrients including dissolved oxygen, carbon dioxide and water. Simple diffusion does not provide either speed or selectivity.

- **Facilitated Diffusion** is selective but requires a concentration gradient. Channel proteins form selective channels that facilitate the diffusion of specific molecules.

- **Active transport** is responsible for the entry of nearly all nutrients. They all require energy to pump molecules into the cell at high rates, often against a concentration gradient.
  - **Ion-coupled transport (secondary active transport)** is driven by an electrochemical gradient (proton or sodium motive force) that has been established across the cell membrane. This includes symport, antiport and uniport transporters.
  - **ABC transporter** requires ATP to pump solutes into the cell. Binding proteins, located in the periplasm, carry their specific ligand to a protein complex on the membrane. Hydrolysis of ATP is used to open the membrane pore and allow the unidirectional transport of the substrate into the cell.

- **Phosphotransferase system (PTS)** accomplished the transport by chemically modifying the solute. It will arrive in the cell as a different molecule.

  "M. Schaechter et al, “Microbe”, ASM Press, 2006"
ATP-binding Cassette (ABC) Transporters

- **Step 1:** The solute enters the periplasm through an outer membrane porin.

- **Step 2:** The solute binds to a specific periplasmic binding protein to form a solute:binding protein complex. The binding protein undergoes a conformational change that allows it to bind to the membrane transporter.

- **Step 3:** The liganded binding protein binds to the ABC transporter in the cell membrane and delivers the solute to the transporter. The binding process also stimulates the ATP hydrolysis in the ATPase.

- **Step 4:** The transporter complex translocates the solute across the cell membrane through a channel that opens transiently.

- **Step 5:** The transporter complex returns to its unstimulated state.

TonB System Operation

- Transport large metabolites that cannot enter through the outer membrane porin system.
- TonB is a specific outer membrane transport system.
- Has specific receptors for ferric siderophores, vitamin b$_{12}$ (cobalamin), group B colicins, phages T1, φ80 and T5.
- Couples energy from the proton motive force in the inner membrane to the outer membrane receptor.
- TonB causes a conformational change in the outer membrane receptor that results in the translocation of the solute through the receptor channel into the periplasm.
- ExbB and ExbD are accessory proteins required by TonB.
- An ABC transporter moves the solute from the periplasm to the cytoplasm.
## TonB Transport System in iJO1366

<table>
<thead>
<tr>
<th>Metabolite Name</th>
<th>Metabolite Symbol</th>
<th>Reaction</th>
<th>Reaction Formula</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosylcobalamin</td>
<td>adocbl_x</td>
<td>ADOCBLTonex</td>
<td>adocbl_e + h_p -&gt; h_c + adocbl_p</td>
<td>btuB and (TonB and exbD and exbB)</td>
</tr>
<tr>
<td>Aerobactin</td>
<td>arbtn_fe3_x</td>
<td>ARBTNtonex</td>
<td>arbtn_fe3_e + h_p -&gt; h_c + arbtn_fe3_p</td>
<td>None</td>
</tr>
<tr>
<td>Cobinamide</td>
<td>cbi_x</td>
<td>CBITonex</td>
<td>cbi_e + h_p -&gt; h_c + cbi_p</td>
<td>btuB and (TonB and exbD and exbB)</td>
</tr>
<tr>
<td>Cob(1)alamin</td>
<td>cbl1_x</td>
<td>CBL1tonex</td>
<td>cbl1_e + h_p -&gt; h_c + cbl1_p</td>
<td>btuB and (TonB and exbD and exbB)</td>
</tr>
<tr>
<td>Coprogen</td>
<td>cpgn_x</td>
<td>CPGNtonex</td>
<td>cpgn_e + h_p -&gt; h_c + cpgn_p</td>
<td>fhuE and (TonB and exbD and exbB)</td>
</tr>
<tr>
<td>Ferric-dicitrate</td>
<td>fe3dcit_x</td>
<td>FE3DCITTonex</td>
<td>fe3dcit_e + h_p -&gt; h_c + fe3dcit_p</td>
<td>fecA and (TonB and exbD and exbB)</td>
</tr>
<tr>
<td>Ferric 2,3-dihydroxybenzoylserine</td>
<td>fe3dhbzs_x</td>
<td>FE3DHBZ2stonex</td>
<td>fe3dhbzs_e + h_p -&gt; h_c + fe3dhbzs_p</td>
<td>(YbiL and (TonB and exbD and exbB)) or (FeuA and (TonB and exbD and exbB))</td>
</tr>
<tr>
<td>Ferrichrome</td>
<td>fecrm_x</td>
<td>FECRMtonex</td>
<td>fecrm_e + h_p -&gt; h_c + fecrm_p</td>
<td>fhuA and (TonB and exbD and exbB)</td>
</tr>
<tr>
<td>Fe-enterobactin</td>
<td>feenter_x</td>
<td>FEENTERtonex</td>
<td>feenter_e + h_p -&gt; h_c + feenter_p</td>
<td>fepA and (TonB and exbD and exbB)</td>
</tr>
<tr>
<td>Ferroxamine</td>
<td>feoxam_x</td>
<td>FEOXAMtonex</td>
<td>feoxam_e + h_p -&gt; h_c + feoxam_p</td>
<td>fhuA and (TonB and exbD and exbB)</td>
</tr>
</tbody>
</table>
Group Translocation

• PEP group translocation, also known as the phosphotransferase system (PTS).

• PEP transfers its phosphoryl to a histidine residue on Enzyme E I (EI). EI in turn transfers the phosphate to a Histidine Protein (HPr). From HPr the phosphoryl is transferred to Enzyme E II (EIIA). EIIA is specific for glucose and it further transfers the phosphoryl group to a juxtamembrane Enzyme II B (EIIB). Finally, EIIB phosphorylates glucose as it crosses the plasma membrane through the transmembrane Enzyme II C (EIIC), forming glucose-6-phosphate.

• The benefit of transforming glucose into glucose-6-phosphate is that it will not leak out of the cell, therefore providing a unidirectional pathway of glucose.

https://en.wikipedia.org/wiki/PEP_group_translocation

- The iJO1366 Representation of the glucose PTS system

GLCptspp: pep_c + glc__D_p -> g6p_c + pyr_c

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Description</th>
<th>Reaction Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACGAptspp</td>
<td>N-Acetyl-D-glucosamine transport via PEP:Pyr PTS</td>
<td>pep(_c) + acgam(_p) → acgam6p(_c) + pyr(_c)</td>
</tr>
<tr>
<td>ACMANAptspp</td>
<td>N-acetyl-D-mannosamine transport via PTS</td>
<td>pep(_c) + acmana(_p) → acmanap(_c) + pyr(_c)</td>
</tr>
<tr>
<td>ACMUMptspp</td>
<td>N-acetylmuramate transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + acmum(_p) → acmum6p(_c) + pyr(_c)</td>
</tr>
<tr>
<td>ARBTptspp</td>
<td>Arbutin transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + arbt(_p) → arbt6p(_c) + pyr(_c)</td>
</tr>
<tr>
<td>ASCBptspp</td>
<td>L-ascorbate transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + ascb(<em>L)</em>(_p) → ascb6p(_c) + pyr(_c)</td>
</tr>
<tr>
<td>CHTBSptspp</td>
<td>Chitobiose transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + chtbs(_p) → chtbs6p(_c) + pyr(_c)</td>
</tr>
<tr>
<td>FRUptspp</td>
<td>D-fructose transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + fru(_p) → f1p(_c) + pyr(_c)</td>
</tr>
<tr>
<td>GALTptspp</td>
<td>Galactitol transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + galt(_p) → galt1p(_c) + pyr(_c)</td>
</tr>
<tr>
<td>GAMptspp</td>
<td>D-glucosamine transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + gam(_p) → gam6p(_c) + pyr(_c)</td>
</tr>
<tr>
<td>GLCptspp</td>
<td>D-glucose transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + glc(<em>D)</em>(_p) → g6p(_c) + pyr(_c)</td>
</tr>
<tr>
<td>MALTptspp</td>
<td>Maltose transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + malt(_p) → malt6p(_c) + pyr(_c)</td>
</tr>
<tr>
<td>MANGLYCptspp</td>
<td>2-O-alpha-mannosyl-D-glycerate transport via PEP:Pyr PTS</td>
<td>pep(_c) + manglyc(_p) → man6pglyc(_c) + pyr(_c)</td>
</tr>
<tr>
<td>MANptspp</td>
<td>D-mannose transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + man(_p) → man6p(_c) + pyr(_c)</td>
</tr>
<tr>
<td>MLNptspp</td>
<td>Mannitol transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + mln(_p) → mln1p(_c) + pyr(_c)</td>
</tr>
<tr>
<td>SBTptspp</td>
<td>D-sorbitol transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + sbt(<em>D)</em>(_p) → pyr(_c) + sbt6p(_c)</td>
</tr>
<tr>
<td>SUCptspp</td>
<td>Sucrose transport via PEP:Pyr (periplasm)</td>
<td>pep(_c) + sucr(_p) → pyr(_c) + suc6p(_c)</td>
</tr>
<tr>
<td>TREptspp</td>
<td>Trehalose transport via PEP:Pyr PTS (periplasm)</td>
<td>pep(_c) + tre(_p) → pyr(_c) + tre6p(_c)</td>
</tr>
</tbody>
</table>

**iJO1366 Group Translocation Examples**
Transport Membrane Proteins in the iJO1366 Model

The iJO1366 *E. coli* model has four subsystems that are associated with membrane proteins.

- **Transport, Inner Membrane Subsystem** (332 Reactions)
  - Passive bidirectional and unidirectional diffusion,
  - Facilitated irreversible diffusion,
  - Active unidirectional transport using the ABC system,
  - Active unidirectional transport using the PTS system,
  - Active symporters and antiporters.

- **Inorganic Ion Transport and Metabolism**
  - Passive bidirectional and unidirectional diffusion,
  - Active unidirectional transport using the ABC system,
  - Active symporters and antiporters,
  - Active secretion transporters,
  - Outer membrane secretion proteins (3).

- **Transport, Outer Membrane Porin Subsystem**
  - This subsystem includes the passive bidirectional diffusion of 270 metabolites between the extracellular space and the periplasm.

- **Transport, Outer Membrane Subsystem** (46 reactions)
  - Passive bidirectional diffusion of 10 metabolites,
  - Passive unidirectional diffusion of 9 metabolites using an undefined system,
  - Facilitated irreversible diffusion of 7 metabolites,
  - Active TonB system unidirectional transport of 11 metabolites,
  - Active TolC system unidirectional transport and secretion of 7 metabolites,
  - Active proton antiport transport of 1 metabolites,
  - Active unidirectional transport of 1 metabolites using an undefined system.
Solute Transport Examples in iJO1366

- Diffusion
  - \( \text{O2tx}: \text{o2}_e \leftrightarrow \text{o2}_p \)
  - \( \text{O2tpp}: \text{o2}_p \leftrightarrow \text{o2}_c \)

- Facilitated Diffusion (Same as diffusion)
  - \( \text{GLYCtex}: \text{glyc}_e \leftrightarrow \text{glyc}_p \)
  - \( \text{GLYCtpp}: \text{glyc}_c \leftrightarrow \text{glyc}_p \)

- Ion-coupled Transport
  - Symporters
    - \( \text{LCTStex}: \text{lcts}_e \leftrightarrow \text{lcts}_p \)
    - \( \text{LCTStpp}: \text{h}_p + \text{lcts}_p \leftrightarrow \text{h}_c + \text{lcts}_c \)
  - Antiporters
    - \( \text{LCTSt3ipp}: \text{lcts}_c + \text{h}_p \rightarrow \text{h}_c + \text{lcts}_p \)
  - Uniporters
    - None, Potassium modeled as symporter

- ATP-binding Cassette Transport (ABC System)
  - \( \text{GALtex}: \text{gal}_e \leftrightarrow \text{gal}_p \)
  - \( \text{GALabcpp}: \text{atp}_c + \text{h2o}_c + \text{gal}_p \rightarrow \text{adp}_c + \text{gal}_c + \text{h}_c + \text{pi}_c \)

- Group Translocation
  - \( \text{GLCtex_copy1}: \text{glc\_D}_e \leftrightarrow \text{glc\_D}_p \)
  - \( \text{GLCptspp}: \text{pep}_c + \text{glc\_D}_p \rightarrow \text{g6p}_c + \text{pyr}_c \)

- TonB System
  - \( \text{FECRMtonex}: \text{fecrm}_e + \text{h}_p \rightarrow \text{h}_c + \text{fecrm}_p \)
  - \( \text{FECRMabcpp}: \text{atp}_c + \text{h2o}_c + \text{fecrm}_p \rightarrow \text{adp}_c + \text{fecrm}_c + \text{h}_c + \text{pi}_c \)
Gram-Negative Bacterial Secretion Pathways

- **Type I** – Protein enter periplasm via ATP-binding cassette, passes through a membrane fusion protein (MFP) and then through an outer membrane protein (OMP).
- **Type II** – A two step process started by entering the periplasm through a translocase and then transported across the outer membrane through a secreton.
- **Type III** – A complex protein structure that spans both the inner and outer membranes and includes an injectisome.
- **Type IV** – A complex protein structure that spans both the inner and outer membranes and can transfer DNA, toxins or monomeric proteins to a host cell or the extracellular media.
- **Type V** – Proteins are translocated across the outer membrane via a self-encoded transmembrane pore.

Assembly of TolC-dependent Type I Hemolysin Export Machinery
(This TolC system is not supported by iJO1366)

A model indicating reversible interaction of outer membrane protein, TolC, with substrate-specific inner membrane complexes (translocases) containing an adaptor protein and an energy-providing traffic ATPase in protein export.

Transport occurs across the inner membrane (IM), outer membrane (OM), and the intervening periplasmic space.

A model indicating reversible interaction of outer membrane TolC with substrate-specific inner membrane complexes (translocases) containing an energy-providing protein, typically an antiporter in drug efflux (RND pump).

Transport occurs across the inner membrane (IM), outer membrane (OM), and the intervening periplasmic space.

## Type I TolC System Secretion Reactions in iJO1366

<table>
<thead>
<tr>
<th>Metabolite Name</th>
<th>Metabolite Symbol</th>
<th>Reaction</th>
<th>Reaction Formula</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMtpp</td>
<td>cm_x</td>
<td>Chloramphenicol transport via TolC system</td>
<td>cm_p + h_p -&gt; h_c + cm_e</td>
<td>(AcrA and AcrB and TolC) or (AcrA and AcrD and TolC)</td>
</tr>
<tr>
<td>DOXRBCNtpp</td>
<td>doxrbcn_x</td>
<td>Doxorubicin transport via TolC system</td>
<td>doxrbcn_p + h_p -&gt; h_c + doxrbcn_e</td>
<td>(AcrA and AcrB and TolC) or (AcrA and AcrD and TolC)</td>
</tr>
<tr>
<td>FUSAtpp</td>
<td>fusa_x</td>
<td>Fusidic acid transport via TolC system</td>
<td>fusa_p + h_p -&gt; h_c + fusa_e</td>
<td>(AcrA and AcrB and TolC) or (AcrA and AcrD and TolC)</td>
</tr>
<tr>
<td>MINCYCtpp</td>
<td>mincyc_x</td>
<td>Minocycline transport via TolC system</td>
<td>h_p + mincyc_p -&gt; h_c + mincyc_e</td>
<td>(AcrA and AcrB and TolC) or (AcrA and AcrD and TolC)</td>
</tr>
<tr>
<td>NOVBCNtpp</td>
<td>novbcn_x</td>
<td>Novobiocin transport via TolC system</td>
<td>h_p + novbcn_p -&gt; h_c + novbcn_e</td>
<td>(AcrA and AcrB and TolC) or (AcrA and AcrD and TolC)</td>
</tr>
<tr>
<td>RFAMPtpp</td>
<td>rfamp_x</td>
<td>Rifampin transport via TolC system</td>
<td>h_p + rfamp_p -&gt; h_c + rfamp_e</td>
<td>(AcrA and AcrB and TolC) or (AcrA and AcrD and TolC)</td>
</tr>
<tr>
<td>TTRCYCtpp</td>
<td>ttrcyc_x</td>
<td>Tetracycline transport via TolC system</td>
<td>h_p + ttrcyc_p -&gt; h_c + ttrcyc_e</td>
<td>(AcrA and AcrB and TolC) or (AcrA and AcrD and TolC)</td>
</tr>
</tbody>
</table>

These are the only metabolites supported in the iJO1366 model. They are allowed to diffuse into the periplasm and then are pumped out by the TolC system. These metabolites are not supported in cytoplasm.
The preprotein is represented by a black line, with the gray region showing the signal sequence.

**Steps 1–3: Targeting.** A signal sequence and its immediate carboxy-terminal region comprise an initiation domain that is recognized by the Sec machinery. SecB, the Sec-system-specific chaperone, channels the preprotein to the Sec translocation pathway and, additionally, actively targets the bound precursor to the translocase by its ability to bind SecA. The preprotein-bearing SecA then binds to the membrane, at a high-affinity SecA-binding site. SecY, SecE and SecG form a hetero-trimeric complex, SecYEG, which constitutes a pathway ('channel') for polypeptide movement.

**Steps 4 and 5: Initiation.** The initiation step requires ATP but not its hydrolysis.

**Step 6: Continuation.** Continued translocation requires cycles of ATP hydrolysis and/or proton-motive force across the membrane. Translocation is thought to occur in a step-wise fashion with a step of 20–30 amino acid residues.

**Step 7: Completion.** As yet, little is known about the completion process, which occurs on the periplasmic side, leading to the release and/or folding of the substrate protein into the periplasmic space.

Tat Pathway – Folded Protein Inner Membrane Translocation
(Type II Secretion Pathways)

• Under resting (nontranslocating) conditions, TatA and TatBC form separate high-molecular-mass complexes within the membrane.

• (a) A depiction of the E. coli inner membrane is shown with the relative positions of the periplasm and cell cytoplasm shown.

• (b) The cycle is initiated when the twin-arginine signal peptide of a Tat substrate protein (blue) binds the TatBC complex in the membrane. The twin arginine motif is recognized directly by TatC.

• (c) In a proton-motive force (∆p)-dependent manner, the TatA complex then associates with the substrate bound TatBC module.

• (d) The TatABC complex is now fully assembled, and the substrate protein is translocated across the membrane through a channel formed by multiple TatA protomers. Protein transport is probably driven by the transport of protons across the membrane.

• Following transport, the TatA and TatBC complexes dissociate and return to the resting state (a).

<table>
<thead>
<tr>
<th>Gene</th>
<th>B-number</th>
<th>Reactions</th>
<th>Reaction Name</th>
<th>Gene Reaction Rule</th>
</tr>
</thead>
<tbody>
<tr>
<td>hybO</td>
<td>b2997</td>
<td>HYD1pp</td>
<td>Hydrogenase (ubiquinone-8: 2 protons) (periplasm)</td>
<td>(b0972 and b0973 and b0974) or (b2994 and b2995 and b2996 and b2997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HYD2pp</td>
<td>Hydrogenase (menaquinone8: 2 protons) (periplasm)</td>
<td>(b0972 and b0973 and b0974) or (b2994 and b2995 and b2996 and b2997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HYD3pp</td>
<td>Hydrogenase (Demethylmenaquinone-8: 2 protons) (periplasm)</td>
<td>(b0972 and b0973 and b0974) or (b2994 and b2995 and b2996 and b2997)</td>
</tr>
<tr>
<td>napG</td>
<td>b2205</td>
<td>NO3R1bpp</td>
<td>Nitrate reductase (Ubiquinol-8)</td>
<td>(b2203 and b2206) or (b2202 and b2205 and b2204)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NTRI3pp</td>
<td>Nitrite Reductase (Ubiquinone-8, periplasm)</td>
<td>b4070 and b4071 and b4072 and b4073</td>
</tr>
<tr>
<td>torZ</td>
<td>b1872</td>
<td>DMSOR1pp</td>
<td>Dimethyl sulfoxide reductase (Menaquinol 8) (periplasm)</td>
<td>b1872 and b1873</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMSOR2pp</td>
<td>Dimethyl sulfoxide reductase (Demethylmenaquinol 8) (periplasm)</td>
<td>b1872 and b1873</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TMAOR1pp</td>
<td>Trimethylamine N-oxide reductase (menaquinol 8) (periplasm)</td>
<td>(b0996 and b0997) or (b1872 and b1873)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TMAOR2pp</td>
<td>Trimethylamine N-oxide reductase (demethylmenaquinol 8) (periplasm)</td>
<td>(b0996 and b0997) or (b1872 and b1873)</td>
</tr>
<tr>
<td>dmsA</td>
<td>b0894</td>
<td>DMSOR1</td>
<td>Dimethyl sulfoxide reductase (Menaquinol 8)</td>
<td>(b1587 and b1588 and b1589 and b1590) or (b0894 and b0895 and b0896)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DMSOR2</td>
<td>Dimethyl sulfoxide reductase (Demethylmenaquinol 8)</td>
<td>b0894 and b0895 and b0896</td>
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<td>TMAOR1</td>
<td>Trimethylamine N-oxide reductase (menaquinol 8)</td>
<td>(b0894 and b0895 and b0896) or (b1587 and b1588 and b1590)</td>
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<td>TMAOR2</td>
<td>Trimethylamine N-oxide reductase (demethylmenaquinol 8)</td>
<td>b0894 and b0895 and b0896</td>
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<td>ynfE</td>
<td>b1587</td>
<td>SELR</td>
<td>Selenate reductase</td>
<td>b1587 and b1588 and b1589 and b1590</td>
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<td></td>
<td>TMAOR1</td>
<td>Trimethylamine N-oxide reductase (menaquinol 8)</td>
<td>(b0894 and b0895 and b0896) or (b1587 and b1588 and b1590)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SELR</td>
<td>Selenate reductase</td>
<td>b1587 and b1588 and b1589 and b1590</td>
</tr>
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<td>ynfF</td>
<td>b1588</td>
<td>DMSOR1</td>
<td>Dimethyl sulfoxide reductase (Menaquinol 8)</td>
<td>(b1587 and b1588 and b1589 and b1590) or (b0894 and b0895 and b0896)</td>
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<td>fdoG</td>
<td>b3894</td>
<td>FDH4pp</td>
<td>Formate dehydrogenase (quinone-8) (periplasm)</td>
<td>(b3892 and b3893 and b3894) or (b1474 and b1475 and b1476)</td>
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<td>amiA</td>
<td>b2435</td>
<td>AGM3App</td>
<td>N-Acetyl-D-glucosamine(anhydrous)N-Acetylmuramyl-tripeptide amidase (periplasm)</td>
<td>b2435 or b2817 or b4169</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AGM4App</td>
<td>N-Acetyl-D-glucosamine(anhydrous)N-Acetylmuramyl-tetrapeptide amidase (periplasm)</td>
<td>b2435 or b2817 or b4169</td>
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Tat Reactions in iJO1366
General Secretory Pathways (Gsp) dependent exoproteins are shown as grey circles, have initially been exported across the IM via the Sec or Tat machinery (not shown).

The exoproteins are subsequently recognised by the Gsp machinery and transported across the OM via the secretin, GspDQ. The secretin GspDQ is shown as a homomultimeric ring forming a channel with a large central opening. GspM₂ is proposed to direct the location of GspLᵥ at a specific site into the cell envelope. GspLᵥ is anchoring the GspEₚ NTPase (traffic ATPase) to the inner face of the cytoplasmic membrane. The GspEₚ NTPase is represented as a homomultimer (hexameric ring).

The opening and closure of the GspEₚ central ring is linked to cycles of ATP hydrolysis and promotes (as shown by the arrow) the helical assembly of the pseudopilins. Pseudopilins are thus using the central cavity of the GspEₚ hexamer to be incorporated into a pseudopilus structure that pushes exoproteins through the secretin channel. The Gspₚ component is interacting with the periplasmic N-terminal domain of the GspDQ secretin (closed). In the absence of such interaction, as shown on the right with the dotted Gspₚ form, the central cavity of the GspDQ secretin is made accessible to exoproteins (open). Preceding assembly into a pseudopilus, the GspGₚ⁻Kₓ pseudopilins are processed (removal of the leader peptide, shown as a T bar) by the prepilin peptidase, GspOₚ. The pseudopilus is arbitrarily represented as a succession of different pseudopilins. It should be noted that the major pseudopilin GspGₚ is indicated in orange, whereas minor pseudopilins are indicated in green. The atypical pseudopilin GspKₓ is represented in dark green and is supposed to arrest pseudopilus elongation.

Type III Secretion
(Not included in the iJO1366 Model)

• The type III secretion system is composed of a complex protein structure spanning both the inner and the outer membranes.

• One of the main features of this transport system is that it can allow the direct injection of protein into a host eukaryotic cell. By acting as a molecular syringe. This apparatus has also been called the injectisome.

• The type III system is similar to the flagella and Hpr pilus export apparatus.

• It is a strictly Sec-independent secretion pathway.


Type IV Secretion
(Not included in the iJO1366 Model)

- The type IV secretion system is related to the systems that are involved in conjugative transfer of DNA and can be exemplified by the secretion of the pertussis toxin in Bordetella pertussis.
- Depending on the bacterial species or the protein secreted, Sec-dependent and Sec-independent secretion of effector molecules have been observed.
- It allows the secretion of
  (i) nucleoprotein DNA conjugation intermediates;
  (ii) multi-subunit toxins, such as the pertussis toxin (PT); or
  (iii) monomeric protein, such as RecA.
- Recently, this pathway has been divided into two subclasses:
  (i) type IVa corresponds to machinery assembly containing VirB homologues of Agrobacterium tumefaciens, and
  (ii) type IVb corresponds to functional secretion systems assembled from Tra homologues of the IncI ColIb-P9 plasmid of Shigella flexneri.
- Proteins using this pathway can be secreted either into the extracellular milieu or directly into a host cell.

Type V Secretion
(Not included in the iJO1366 Model)

- Proteins using type V secretion systems (T5SS) are translocated across the inner membrane via the Sec system.
- Type 5aSS inserts as a β-barrel into the outer membrane assisted by Bam, forming a pore for the translocation.
- Type 5bSS act as a split variant of type 5aSS systems: the passenger domain and translocation domain are two separate protein chains but expressed from the same operon structure. Called two-partner secretion (Tps) systems.
- Type 5cSS are obligate trimers, with most functioning as bacterial adhesins. Often called trimeric autotransporter adhesins (TAAs).
- Type 5dSS similar to type 5aSS systems except their domains are connected via an additional periplasmic domain.
- Type 5eSS are inverse autotransporters, based on the fact that the domain order is reversed.

The autotransporter polypeptide is threaded through the inner membrane (IM) by the Sec machinery (in magenta). Many autotransporters, including the one pictured, have an extended signal peptide (in yellow) which remains attached to the Sec translocon and tethers the autotransporter to the translocon. In the periplasm, chaperone proteins such as Skp (orange), FkpA (red) and SurA (in blue) and DegP (in blue) bind to the autotransporter and keep it unfolded. The chaperone/protease DegP is also involved in quality control of autotransport. The signal peptide is eventually cleaved by signal peptidase (not shown) releasing the autotransporter into the periplasm. The C-terminal membrane anchor (in brown) is recognized by the POTRA (P) domains of BamA (in purple); the Bam complex aids in inserting the b-barrel membrane anchor into the outer membrane (OM). The linker region (light green) then forms a hairpin inside the pore of the barrel. The passenger domain (dark green) is pulled through the pore. The energy for this presumably derives from the sequential folding of the passenger domain on the outside of the cell. Once the passenger domain has been secreted, the linker assumes an a-helical conformation and plugs the pore.

The translocator TpsB protein (brown) and the passenger TpsA protein (green) are synthesized as separate polypeptide chains, which are then transported across the IM by the Sec machinery (magenta). The TpsB protein folds into a b-barrel structure in the OM, with two periplasmic POTRA domains (P). TpsA proteins such as FHA and HMW1 contain extended signal peptides (shown in yellow). Periplasmic transit of both proteins is presumably facilitated by chaperones (orange, with asterisk). In the case of FHA, DegP acts as a chaperone. The N-terminal TPS domain (light green) targets the TpsA protein to its cognate TpsB partner. The TpsA protein is then translocated across the OM into the extracellular space. The final topologies of HMW1 and FHA differ, and the alternative conformations strongly speak for a hairpin as the transport intermediate: mature HMW1 (second structure from the left) has its C-terminus locked in the periplasm by a disulphide bond (orange) that prevents its passage through the pore of HMW1B, its TpsB partner. The N-terminal TPS domain of HMW1 is cleaved, probably in the periplasm, to produce the mature passenger (dark green). FHA remains associated with FhaC through its N-terminal TPS domain, with the C-terminus distal from the cell surface, and is cleaved or partially degraded. The C-terminus of FHA is processed at the cell surface by the autotransporter protease SphB1.

Trimeric autotransport presumably follows a largely similar sequence of events to classical autotransport, the major difference being the presence of three polypeptide chains rather than just one. Many trimeric autotransporters also contain extended signal peptides, shown in yellow. As yet unidentified chaperones (orange, marked with asterisks) keep the polypeptides in a translocation-competent, unfolded state. The Bam complex (purple) is required for trimeric autotransporter biogenesis and recognizes the C-terminal membrane anchor (in brown, the three membrane anchor monomers are coloured in different shades). The Bam complex assists in trimerization of the b-barrel and membrane insertion. The linker regions (different shades of light green) form hairpins within the pore, and this leads to translocation of the polypeptides encoding the passenger domain (in different shades of dark green). The periplasmic chaperone/protease DegP is involved in quality control of trimeric autotransport. The passenger domain trimerizes after secretion and remains covalently attached to the membrane anchor.

**Type VI Secretion**  
(Not included in the iJO1366 Model)

- The most recently identified of the Gram-negative protein secretion systems is the Type VI secretion system (T6SS), a complex, single-step secretion system.

- The 13 core components (TssA-M) are believed to form a large molecular machine extending from the bacterial cytoplasm, across the inner membrane, periplasm and outer membrane, and ultimately into a target cell.

- Secretion of effector proteins is in a 'one-step' manner, independent of the Sec or Tat export machineries.

- No secretion signals (substrate-defining motifs directly recognized by the T6SS machinery) have yet been identified.

Lesson Outline

- iJO1366 Subsystems used in Cell Envelopes
- Phospholipids
  - Fatty Acids
  - Glycerophospholipids
- Murein Wall (Peptidoglycan)
  - Murein Overview
  - Murein Synthesis
  - Murein Recycling
- Lipoprotein
- Lipopolysaccharides
- Membrane Transport & Secretion