MCB Test 1 – Mueckler Review

9-30-17
Functions of Cellular Membranes

1. Plasma membrane acts as a selectively permeable barrier to the environment
   - Uptake of nutrients
   - Waste disposal
   - Maintains intracellular ionic milieu

2. Plasma membrane facilitates communication
   - With the environment
   - With other cells

3. Intracellular membranes allow compartmentalization and separation of different chemical reaction pathways
   - Increased efficiency through proximity
   - Prevent futile cycling through separation
   - Protein secretion
Fatty Acid Saturation

Palmitate
(ionized form of palmitic acid)

Cis

Trans

Oleate
(ionized form of oleic acid)
Bilayers are Thermodynamically Stable Structures Formed from Amphathic Lipids

Figure 10-11a  Molecular Biology of the Cell (© Garland Science 2008)
The Formation of Cell-Like Spherical Water-Filled Bilayers is Energetically Favorable
PhosphoLipid Movements within Bilayers

lateral diffusion (µM/sec)

flexion $(10^{12}-10^{13}/\text{sec})$
rotation $(10^8-10^9/\text{sec})$

flip-flop (rarely occurs)

Figure 10-11b Molecular Biology of the Cell (© Garland Science 2008)
A “Scramblase” Enzyme Catalyzes Symmetric Growth of Both Leaflets in the ER

Figure 12-58 Molecular Biology of the Cell (© Garland Science 2008)
A “Flippase” Enzyme promotes Lipid Asymmetry in the Plasma Membrane
Phospholipids are Involved in Signal Transduction

1. Activation of Lipid Kinases

extracellular signal

activated receptor protein

phosphorylated inositol phospholipid

PI-4,5P

PI-3,4,5P

activated PI 3-kinase

docked intracellular signaling protein

CYTOSOL

relay signal

Figure 10-17a  Molecular Biology of the Cell (© Garland Science 2008)
2. Phospholipases Produce Signaling Molecules via the Degradation of Phospholipids
Cholesterol Biosynthesis Occurs in the Cytosol and at the ER Membrane Through Isoprenoid Intermediates

Acetyl CoA + acetoacetyl CoA → HMG-CoA → HMG-CoA reductase → Mevalonate → Isopentenyl pyrophosphate (IPP) → Farnesyl pyrophosphate → Squalene → Cholesterol

(Rate-Limiting Step in ER)

Figure 10-26
Molecular Cell Biology, Sixth Edition
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3 Ways in which Lipids May be Transferred Between Different Intracellular Compartments

Vesicle Fusion

Direct Protein-Mediated Transfer

Soluble Lipid Binding Proteins

Figure 10-27
Molecular Cell Biology, Sixth Edition
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The 3 Basic Categories of Membrane Protein

**Integral**
- Single-Pass
- Transmembrane Helix
- Linker Domain

**Lipid-Anchored**
- Multi-pass
- β-Strands
- Fatty acyl anchor

**Peripheral**
- GPI Anchor
- (can also interact via PL headgroups)
Membrane Domains are “Inside-Out”

- **Polar side chains**
- **Nonpolar side chains**

**Right-Side Out Soluble Protein**
- Hydrophobic core region contains nonpolar side chains
- Polar side chains on the outside of the molecule can form hydrogen bonds to water
- Unfolded polypeptide
- Folded conformation in aqueous environment

*Figure 3-5 Molecular Biology of the Cell (© Garland Science 2008)*
Functional Characterization of Integral Membrane Proteins Requires Solubilization and Subsequent Reconstitution into a Lipid Bilayer

Figure 10-31 Molecular Biology of the Cell (© Garland Science 2008)
4 Ways that Protein Mobility is Restricted in Biological Membranes

- Intramembrane Protein-Protein Interactions
- Interaction with the cytoskeleton
- Interaction with the extracellular matrix
- Intercellular Protein-Protein Interactions
Ribosomal Subunits are Shared Between Free and Membrane-Bound Polysomes

Targeting information resides in the Nascent polypeptide chain
Signal-Mediated Targeting to the RER

Figure 13-6
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Properties of Secretory Signal Sequences

- Located at N-terminus
- 15-30 Residues in length
- Hydrophobic core of 8-12 residues
- Often basic residues at N-terminus (Arg, Lys)
- No sequence similarity
In Vitro Translation/Translocation System

- mRNA
- Rough microsomes
- Ribosomes
- tRNAs
- Soluble translation factors
- Low MW components
- Energy (ATP, creatine-P, creatine kinase)

Reticulocyte or wheat germ lysate
In Vitro Translation/Translocation System

mRNA + Translation Components + Amino acid* → Protein* → SDS PAGE
In Vitro Translation of Prolactin mRNA

Prolactin is a polypeptide hormone (MW ~ 22 kd) secreted by anterior pituitary.
Multipass Topologies are Generated by Multiple Internal Signal/Anchor Sequences

Type IVa

- stop-transfer sequence
- start-transfer sequence
- hydrophobic stop-transfer-peptide-binding site
- hydrophobic start-transfer-peptide-binding site
- translocator protein
- mature transmembrane protein in ER membrane

Figure 12-48
The Charge Difference Rule for Multispanning Membrane Proteins
Oligosaccharide Processing in the RER is Used for Quality Control
Disulfide Bridges are Formed in the RER by Protein Disulfide Isomerase (PDI)

Formation of a disulfide bond

![Diagram showing the process of disulfide bridge formation]

Figure 13-19a
Molecular Cell Biology, Sixth Edition
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Proteins are Incorporated Into Mitochondria Via Several Different Routes
Protein Import into the Matrix Requires ATP Hydrolysis and an Intact Proton Gradient Across the Inner Membrane
Targeting to the Inner Membrane Occurs Via 3 Distinct Routes

- **Stop-Transfer-Mediated**
  - Stop-transfer sequence
  - Preprotein
  - Cytosol

- **Oxa1-Mediated**
  - Oxa1-targeting sequence
  - Preprotein
  - Matrix-targeting sequence

- **Tom70/Tim22/54-Mediated**
  - Internal targeting sequences
  - Protein
  - ADP/ATP antiporter

**Single-Pass Proteins**
- Cytochrome oxidase subunit CoxVa

**Multi-Pass Proteins**
- ATP Synthase Subunit 9

**Figure 13-26**
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Targeting to the intermembranous space occurs via two distinct pathways:

1. **Direct Delivery**
   - Intermembrane-space-targeting sequence
   - Protein
   - COO⁻ → NH₃⁺
   - Cleaved matrix-targeting sequence
   - Tim23/17
   - Cytochrome c Heme Lyase

2. **IM Space Protease**
   - Intermembrane-space-targeting sequence
   - Matrix-targeting sequence
   - Preprotein
   - COO⁻ → NH₃⁺
   - Tom20, Tom22, Tom40
   - Cytochrome B2
Targeting to the Outer Membrane Via the SAM Protein Complex

Figure 12-27  Molecular Biology of the Cell (© Garland Science 2008)
Nuclear Transport

• Bidirectional
• Single Large Pore Complex
  Spans 2 lipid bilayers
• Nuclear Pores much larger
  than other translocons
Nuclear Import and Export Sequences are Recognized by Different Members of the Same Receptor Family (Keryopherinins)
Nuclear Import and Export Operate Via Reciprocal Use of the Ran-GDP/GTP Concentration Gradient

[Diagram showing the process of nuclear import and export involving Ran-GDP/GTP concentration gradient.]