NBL 355-655 Module 3 Review Q&A

1. *What are cellular “organelles?”*

Cellular organelles are specialized structures in cells. The majority (but not all) cellular organelles are enclosed within a membrane and some have a double membrane. Cellular organelles carry out various and specific functions within a cell. For example, mitochondria, which are separated from the cytosol by two membranes (a double membrane), perform the TCA (Krebs) cycle, electron transport and oxidative phosphorylation to produce ATP. The nucleus, which is also separated from the cytosol by two membranes (a double membrane), is where the chromosomal DNA is located and replicated during cell proliferation, and where transcription (synthesis of RNA) occurs. In the cytoplasm, soluble proteins (which comprise about half of all proteins) are synthesized by free ribosomes, which often form together in long chains, each producing the same protein and are called polyribosomes or polysomes. The other half of proteins are integral membrane proteins, which are synthesized by ribosomes associated with the rough endoplasmic reticulum (RER) and are described more below.

1. *What are the components of biological membranes? Which cellular organelles are bounded by a biological membrane (or double membrane)? What are all the functions of biological membranes?*

Biological membranes are composed of lipid bilayers and integral membrane proteins. Lipid bilayers are composed of phospholipids, cholesterol and cholesterol esters, and sphingolipids. Biological membranes include the plasma membrane, membranes forming the smooth endoplasmic reticulum (SER) and rough ER (RER), Golgi apparatus, nuclear membranes (outer and inner), mitochondrial membranes (outer and inner), and membranes that form secretory vesicles, endosomes, synaptic vesicles and lysosomes. Thus the membrane bound organelles include the nucleus, mitochondria, RER and SER, endosomes, secretory vesicles and lysosomes. Cell biologists often refer to membrane bound organelles as compartments. The following organelles do not contain membranes: proteasomes and polyribosomes (polysomes). Some cell biologists consider the cytoskeleton filaments (microtubules, actin microfilaments and neurofilaments- Module 3) in the category of cellular organelles. Cytoskeletal filaments don’t contain membranes, but some can bind to membranes and are involved in transport of vesicles and/or regulation of membranes.

Functions of biological membranes: The plasma membrane contains and maintains the contents of cells, prevents necessary molecules from exiting, prevents unwanted toxic molecules from entering, and allows the formation of the membrane potential and change in the membrane potential. For intracellular organelles, membranes provide compartmentalization, in the nucleus it maintains and protects the chromosomes-genome, and for others it concentrates substrates and enzymes inside organelles, and prevents degradative enzymes in the cytoplasm from degrading molecules inside the organelle.

1. *Biological membranes are composed of lipid bilayers and membrane proteins. What are the four main types of lipid components that form the bilayer and what is the one main type of protein component of biological membranes?*

Lipids: phospholipids, cholesterol, cholesterol esters (CEs), and sphingolipids

Main protein type: Integral membrane proteins (IMPs). IMPs are proteins that are permanently inserted into or attached to a biological membrane. The great majority of IMPs are transmembrane proteins (TMPs), which are proteins that cross/span the membrane from one side to the other side because they have one or several membrane spanning domains. Cell biologists often use the terms IMPs and TMPs interchangeably. In most of the lecture I talk about TMPs. The other IMP (that are not TMPs) include is a small fraction of soluble proteins that can attach to membranes by different mechanisms, such as by forming a covalent bond with a lipid or when a region of the protein can bind to the lipid bilayer on one side.

1. *What are triglycerides? What do we obtain them from and what cells synthesize and store them? What are cooking oils? In what two main ways can fatty acids differ in their chemical structure?*

Chemically, triglycerides contain three long chain fatty acids esterified to a glycerol backbone. Triglycerides can be obtained by consuming vegetable or animal fat, and are also synthesized in the body and stored in lipid droplets, mainly by adipocytes.

In contrast, cooking oils are fatty acids, and include, for example, olive oil, canola oil, corn oil, sunflower oil, peanut oil, palm oil and coconut oil. A fatty acid is a carboxylic acid with a long aliphatic (hydrocarbon) chain. Fatty acids differ in a) their carbon chain length (ranging from about 5-8 carbons in short chain FAs to 21-22 carbons in very long chain FAs, and b) their level of saturation (presence of double bonds); they can be either saturated, monounsaturated, or polyunsaturated.

1. *What is the general structure of a phospholipid? What does amphipathic/amphiphilic mean? What are the molecules that can be attached to the phosphate head group of a phospholipid?*

A phospholipid contains a glycerol backbone, two long chain fatty acids, and a phosphate head group that can also have another molecule covalently attached to it. Amphipathic or amphiphilic describes a molecule possessing both hydrophilic (polar or charged) and hydrophobic (nonpolar) properties. Phospholipids are amphipathic because they possess a water soluble head group (the phosphate group plus another polar or charged group covalently attached to it) and the two nonpolar/hydrophobic hydrocarbon tails esterified to the glycerol backbone. Phosphatidic acid (PA) is the simplest phospholipid. It is composed of a glycerol backbone, two long chain fatty acids and a phosphate head group. Attachment of choline to PA produces phosphatidylcholine (PC). Attachment of ethanolamine to PA produces phosphatidylethanolamine (PE). Attachment of serine to PA produces phosphatidylserine (PS). Attachment of inositol produces phosphatidylinositol (PI).

1. *What type of structure do oils spontaneously form in aqueous solution? What type of structure do phospholipids spontaneously form in aqueous solution? Why does this form instead of a micelle? What is a hydrophobic bond and what drives it?*

Oils or fatty acids spontaneously form micelles in aqueous solution, with all of the nonpolar/hydrophobic hydrocarbon regions facing the center of the micelle. In contrast, phospholipids spontaneously form lipid bilayers in solution because the phosphate head group region is very large, polar and charged, and depending on what else is attached, the polar and charged groups can attract or repel each other. More phospholipids can fit into a more compact structure in the bilayer structure than in the micelle.

A hydrophobic bond is a type of non-covalent bond. The hydrophobic bond is actually driven by the increase in hydrogen bonding between water molecules by excluding nonpolar/hydrophobic molecules. This maximizes hydrogen bonding between water molecules and minimizes the area of contact between water and nonpolar molecules. This is thermodynamically the more favorable state.

1. *What type of molecule is cholesterol and where is it found? How do we obtain and synthesize cholesterol? What effect does cholesterol have on biological membranes?*

Cholesterol is a sterol (a subgroup of steroids), and it is found in all membranes in the cell. Cholesterol is obtained from animal fat and is also synthesized by the liver. (Plant sterols are often called phytosterols.) At physiological temperatures, cholesterol decreases the membrane fluidity and decreases the membrane permeability, and thus stabilizes the membrane and makes it a better barrier (less permeable).

1. *Transmembrane proteins (TMPs) contain at least one membrane spanning domain (MSD). The majority of TMPs we’ll discuss in this course have multiple MSDs. What is the most common type of secondary structure that forms an MSD? What types of amino acids are found in a majority of the MSD sequence? Why does a MSD usually contain about 20 (17-22) amino acids? What does the hydropathy analysis predict? How few and how many MSDs can a protein have?*

The majority of MSDs are alpha helices (though some are beta barrels). The majority of amino acid (usually more than 75%) in a MSD are hydrophobic (nonpolar) amino acids. Given the structure of the protein alpha helix (there are about 3-4 amino acid residues in one turn of the helix) it would require about 20 amino acids (about 6 complete turns of alpha helix), to span the distance across the membrane lipid bilayer, which is about 4-5 nm. Transmembrane proteins (TMPs) have at least one MSD but can have multiple MSDs. Note that many TMPs are also anchored, through their cytoplasmic loops or domains, to cytosolic scaffolding or cytoskeleton proteins (through noncovalent protein-protein interactions) that tend to tether the TMPs to specific regions of the plasma membrane. Hydrophathy (hydrophobicity) analyses predict which regions of the protein will be MSDs. Many receptors, transporters and ion channels have many MSDs, ranging between about 5-14. The largest TMPs can have as many as 20-35 MSDs.

1. *Where are transmembrane proteins (TMPs) synthesized? Insertion into the RER membrane is cotranslational for TMPs, why is this necessary?*

TMPs are synthesized by ribosomes associated with the rough endoplasmic reticulum (RER). Insertion of TMPs into the membrane occurs during translation (cotranslationally) because it would be thermodynamically unfavorable for a MSD (with all its hydrophobic R groups) to be exposed in aqueous solution, and the TMPs might aggregate through those domains, preventing their proper association with the membrane. It also ensures that the TMP is incorporated with the correct orientation (domains facing the ECF or cytoplasm) into the membrane.

1. *TMPs and secreted peptides and proteins (SPPs) are synthesized (translated) in the rough endoplasmic reticulum (RER). Insertion into the RER membrane is cotranslational for TMPs; why is this necessary? Insertion into the RER lumen is cotranslational for SPPs; why is this necessary?*

TMPs are integral membrane proteins because of their chemical structure (containing at least one MSD alpha helix that has a majority of hydrophobic amino acids), meaning that they are embedded in the lipid bilayer. Therefore they can’t diffuse into the aqueous cytoplasm and move in and out of the lipid bilayer. Since TMPs are stuck in the membrane, they require membrane trafficking to move them from their site of synthesis in the RER to their final destination, such as the plasma membrane. SPPs (secreted peptides and proteins) are secreted from the cell. The only way that peptides or proteins can be released by the cell is by exocytosis since hydrophilic molecules can’t move across the membrane on their own. Therefore SPPs require membrane trafficking pathways to move them from their site of synthesis in the RER to their final destination, outside the cell.

As described above, insertion of TMPs into the membrane occurs during translation (co-translationally) because it would be thermodynamically unfavorable for a hydrophobic membrane spanning domain to be exposed in aqueous solution, and the TMPs might aggregate through those hydrophobic domains, preventing their proper integration into the membrane. It also ensures that the TMP is incorporated with the correct orientation into the membrane so the domain facing the outside ECF and inside intracellular cytoplasm are correct. This becomes even more important as the number of transmembrane spanning domains increases. SPPs are inserted into the lumen of the RER co-translationally so that they can eventually be released/secreted from the cell by exocytosis.

1. *What are the functions of the secretory/biosynthetic pathway? Begin with synthesis in the RER and briefly describe how TMPs and SPPs “flow” in this pathway, including which organelles are involved, and what occurs at each step. For trafficking to the plasma membrane, there are two branches of the SBP: the regulated and constitutive secretory pathways. How are they different? Why are both necessary? What is exocytosis?*

The secretory/biosynthetic pathway is involved in the synthesis (translation), modification, sorting and trafficking of TMPs, SPP, intraorganellar proteins, and membrane lipids. The secretory/biosynthetic pathway involves the synthesis of TMPs in the RER and lipids in the smooth ER, modification of proteins in the Golgi, sorting of TMPs at the trans Golgi network (TGN) into specific vesicles, transport of the TMPs by transport vesicles or secretory vesicles, and fusion with the plasma membrane or early endosome. Lysosmes contain many degradative enzymes on the inside. An example of an intraorganellar protein is the acid hydrolyase exzyme located inside lysosomes.

For SPPs in the secretory/biosynthetic pathway, fusion of secretory vesicles with the plasma membrane allows the contents in the lumen (soluble peptides or proteins) to be released outside the cell. In neurons, the SBP is important for the delivery of lipids and TMPs to the plasma membrane, for the delivery of TMPs and intraorganelle proteins to the early endosome and lysosome, and for the release of neuropeptides, neurotrophic factors and extracellular matrix proteins outside of the cell.

In the constitutive secretory pathway, TMPs, lipids and SPPs are constantly trafficked and delivered into the plasma membrane or released out of the cell. In the regulated secretory pathway, transport or secretory vesicles do not fuse with the plasma membrane (by exocytosis) until a signal is received in the cell, which usually involves an increase in Ca2+ levels that then induces exocytosis. Exocytosis is the process by which a vesicle fuses with the plasma membrane. Following exocytosis, the vesicle lipids and the TMPs become incorporated into the plasma membrane itself, and the SPPs are released outside of the cell.

1. *What are the functions of the endosomal pathway? What is endocytosis? Begin with endocytosis at the plasma membrane and briefly describe how TMPs “flow” in the EP, including which organelles are involved, and what occurs at each step.*

The endosomal pathway is involved in the trafficking of transmembrane proteins and some nutrients from the plasma membrane to the early endosome (and then to the lysosome, if the protein is destined for degradation) or for recycling back to the plasma membrane. The endosomal pathway involves endocytosis from the plasma membrane, formation of endocytic vesicles, trafficking of endocytic vesicles to the early endosome, and sorting of TMPs and lipids at the early endosome. TMPs can be recycled by recycling endosomes back to the plasma membrane, or they can traffic to the late endosome/multivesicular body, and then go to the lysosome. Endocytosis is the infolding of a piece of the plasma membrane to form an endocytic vesicle. The endosomal pathway is important to maintaining the area of the plasma membrane (to balance the outflow process of the secretory/biosynthetic pathwat and exocytosis), for the delivery of nutrients like cholesterol and iron into the cell, to remove damaged TMPs by delivering them for degradation to the lysosome, and to control the number/density of TMPs on the plasma membrane (which is important in synaptic plasticity).

1. *The lysosome is part of the endosomal pathway. What is the function of the lysosome and how does it do it? Where are cytoplasmic soluble proteins degraded? Why do proteins need to be degraded?*

Lysosomes are involved in degradation of TMPs (and also other molecules). The proteasome is involved in degradation of cytosolic proteins. These organelles are involved in the removal of proteins that have become damaged by proteolysis, oxidation, or have become misfolded. The lysosome also contains many other hydrolytic enzymes that can degrade nucleic acids, carbohydrates, and lipids, and therefore lysosomes can also degrade damaged organelles like mitochondria. Those enzymes are inside the lysosome (they are intraorganellar protein), and they are delivered to the lysosome through the secretory/biosynthetic pathway. Proteasomes do not contain membranes since they are involved in degrading cytosolic proteins. Damaged proteins need to be degraded so they don’t aggregate, tie up normal proteins or disrupt normal processes, and following degradation, their amino acids can be recycled back into new proteins.

1. *What organelle is involved in generation of synaptic vesicles? What characteristics of neurons make their membrane trafficking in the SBP and EP more complicated than in other cells?*

Synaptic vesicles are generated by the synaptic endosome (also called the early endosome) in the presynaptic neuron. How is membrane trafficking more complicated in neurons? First, compared with other cells, neurons have a lot more plasma membrane because their axons can be very long, and they can have extensive dendritic branching. Thus during development, neurons must produce large amounts of lipid bilayers and TMPs that form the plasma membrane. Second, neurons are highly polarized cells with many different plasma membrane subdomains, for example the axonal membrane, the presynaptic axonal membrane, the postsynaptic dendritic membrane, the cell body, and the dendritic spine. Thus the neuronal SBP has to sort all the vesicles and deliver them to the different membrane microdomains. Third, the axon can be very long and the movement of organelles and vesicles to and from the presynaptic region requires fast axonal transport (FAT) that involves microtubules and microtubule-based motors. Fourth, neurons use the secretory and endosomal pathway not only for delivery and removal of TMPs, lipids and secreted factors for normal housekeeping functions, but also in synaptic signaling with the production of a specific type of vesicle called the synaptic vesicle, and the use of some secretory vesicles for neuropeptide signaling. Fifth, endosomal trafficking (and probably also the SBP) are involved in regulation of the number and types of postsynaptic receptors during synaptic plasticity, a process proposed to underlie learning and memory.

1. *What is the blood-brain barrier (BBB) and why is it so important? What cells form and regulate the BBB? What cellular junction type is critical for the BBB? What could happen if the BBB is damaged? How does the BBB impact CNS drug design?*

The BBB is a barrier that separates the circulating blood from the extracellular fluid (ECF) that surrounds the neurons and most glial cells. (Neuroscientists often refer to the neural cells in the brain, including neurons and many glia, as the brain parenchyma.) The BBB protects the brain. It prevents the entry of pathogenic cells including viruses, bacteria, parasites, and fungi and thus prevents infection of brain cells. It also prevents entry of the great majority of normal immune cells and hence prevents damage from immune cells and an inflammatory response. It also prevents the entry of harmful substances such as toxins and poisons from the blood into the brain and causing damage. Furthermore, it prevents unwanted hydrophilic (water soluble) molecules (such as amino acid neurotransmitters, blood proteins, antibodies and protein hormones) from entering the CNS and disrupting neuronal and glial functions.

The cells that form the BBB are the vascular endothelial cells (VECs), which form the blood capillary itself, and the glial cells called pericytes that surround the VEC/capillary. The brain VECs are different from the rest of the body’s VECs in that they form specialized tight junctions that prevent/block the diffusion of molecules and movement of cells from the blood directly into the brain parenchyma. The pericytes (a type of glial cell) cover the VECs and release factors that stabilize and maintain those tight junctions. Tight junctions are a subtype of cellular junction formed by multiple transmembrane proteins that bind to each other and form a barrier to movement of molecules from one side to the other. Astrocytes also form end feet on the VECs and transport specific molecules (such as glucose, essential amino acids and water soluble vitamins) that cells in the CNS require, to the ECF. Astrocytes can regulate the capillaries by releasing factors that are detected by pericytes and affect vasodilation/vasocontriction. Regulation of blood vessel size is called functional hyperemia and the ability of neurons to affect this is called neurovascular coupling.

(In some models/figures, the astrocytic end feet are shown as part of the BBB. They are located next to the VECs, but whether they affect the tight junction of the VECs is still debated. However, they are necessary for the transport of nutrients, and they release factors that induce pericytes to regulate vasoconstriction or vasodilation.)

Damage to the BBB could allow unwanted molecules and cells to enter directly into the brain tissue. This would mean that toxins and pathogens could enter and cause infection or damage to the brain cells. Also, the body’s immune cells and immune mediators could enter the brain and cause inflammation, which could affect neural cells. Because of the BBB, an important consideration in CNS drug design involves the chemical nature of the drug. Most drugs used in the CNS have to have hydrophobic/nonpolar chemical nature, because hydrophobic/nonpolar molecules can diffuse directly across the membranes; hydrophilic/polar molecules cannot. Natural drugs like morphine, THC or CBD in marijuana, nicotine, and alcohol, because they are hydrophobic, can cross the BBB and activate receptors in the brain.

1. *What is the blood-CSF barrier (B-CSF-B), where is it located, and why is it important? What cells form the B-CSF-B? What cellular junction is critical for the B-CSF-B. T cells have been shown to be present in low levels in normal CSF? How could T cells get into the CSF. What could be the function of these T cells?*

The blood-CSF barrier is the barrier between the blood in the choroid capillaries (in the choroid plexus) and the CSF in the brain ventricles. The B-CSF-B ensures that hydrophilic molecules, poisons, toxins and toxic cells, and most immune cells in the blood can’t enter into the CSF in the ventricles (and subarachnoid space). Unlike other parts of the brain, the VECs in the choroid plexus do not form tight junctions. However, the choroid ependymal cells that surround the choroid capillaries do form tight junctions and therefore form a barrier to the entry of most cells and hydrophilic molecules from the blood into the CSF. All of that said, one type of immune cell called a T lymphocyte (T cell) is present in low levels in normal CSF, and therefore some T cells appear to be able to cross the blood-CSF barrier and enter and exit the CSF in the ventricles. These T cells are thought to function as immune surveillance cells that detect rogue pathogenic agents such as bacteria, viruses, parasites and fungi, and recruit and activate other T cells and other immune cells to remove the pathogens/infections.