**Vesicular Trafficking:**

A eukaryotic cell may resemble a big city with several districts. Each of them is specialized in a particular role, such as energy production, manufacturing products, exportation, importation, communication with other cities, recycling, and so on. To work properly, a rich and complex communication system is needed between districts, which are carried out by carriers that go through multiple pathways.

Cellular districts are the intracellular compartments and many of them are membrane-bound compartments, i.e. organelles. Each organelle is specialized in one or several functions. For example, endoplasmic reticulum is in charge of synthesizing proteins for secretion and lipids for making membranes, Golgi apparatus produces carbohydrates to be attached to proteins and lipids, and is also a distribution center, lysosomes are the main digestion centers, and mitochondria and chloroplasts synthesize ATP.

Communication between some organelles is mediated by vesicles, which carry molecules both in the interior and as part of the membrane of the vesicle. Vesicular trafficking includes all the communication pathways mediated by vesicles, as well as the organelles that send or receive vesicles. There are two main roads in this trafficking road map. One, known as secretory pathway, starts in the endoplasmic reticulum that sends vesicles to the Golgi apparatus, which in turn sends vesicles targeted to the plasma membrane (exocytosis). This pathway releases molecules to the extracellular space, and also carries molecules to the plasma membrane. The other pathway is an importing pathway that begins at the plasma membrane where vesicles and other large compartments are originated by membrane invagination (endocytosis). These vesicles fuse with the endosomes, which end up becoming lysosomes. Lysosomes degrade the endocyted molecules, both those from the extracellular space and those forming the membrane of the vesicles. It is a degradation pathway. There are many other communication pathways mediated by vesicles so that it looks like that all the organelles are connected through vesicles between each other. However, there is this rule saying that the communication by vesicles between two organelles use to be bidirectional, i.e., the organelle A sends vesicles to the organelle B, and at the same time the organelle A receives vesicles from the organelle B. For example, the endoplasmic reticulum sends vesicles to the Golgi apparatus, which in turn sends vesicles back to the endoplasmic reticulum. The same happens between the plasma membrane and endosomes, and between Golgi apparatus and endosomes.

Figure 1. Main pathways of communication mediated by vesicles between organelles. Roads and compartments constitute the vesicular traffic. Communication is usually bidirectional. No all the communication paths are depicted.

There are organelles, such as mitochondria, chloroplasts and peroxisomes, which are not part of the vesicular traffic because they do not frequently send nor receive vesicles. However, these organelles communicate with other organelles by other mechanisms. For example, by physical contacts of their membranes. It is frequently observed the mitochondrial external membrane in close apposition to endoplasmic reticulum membranes. Some authors propose that there is a high transfer of molecules, mostly lipids, through these areas of membrane contacts. There are also transporters that exchange lipids between membrane of different compartments.

**Exocytosis:**

Exocytosis is the fusion of vesicles with the plasma membrane. Vesicles for exocytosis are mostly shipped from the trans domain of the Golgi apparatus, are moved to the cell periphery and fuse with the plasma membrane. Vesicles can be also shipped by other organelles like endosomes. There is a bidirectional trafficking between endosomes and plasma membranes.

**Types of exocytosis**

There are two types of exocytosis: constitutive and regulated

1. Constitutive exocytosis is present in almost every cell and carries molecules needed by the plasma membrane and extracellular matrix. It is the default exocytic pathway, a continuous trafficking where the amount of vesicles depends on the physiological state of the cell.

2. Regulated exocytosis is present in cells specialized in secretion, such as endocrine cells, neurons, intestine epithelial cells, glandular cells, and others. For example, through regulated exocytosis, molecules are released into the intestine lumen for digestion, or, like hormones, they are released to the extracellular matrix to modulate the physiology of other cells located either close or quite far in body traveling, where they arrives through blood vessels. Vesicles of regulated exocytosis fuse with the cell membrane after a signal, which is usually an increase in the cytoplasmic calcium concentration. So, they don not fuse spontaneously. Furthermore, ATP and GTP are needed as energy sources.

### Molecule selection

There are three ways for molecules to be endocytosed: unspecifically as a soluble form or pinocytosis specifically recognized and linked to a membrane receptor or receptor mediated endocytosis, and as part of the membrane of the endocytic vesicle or compartment.

#### Pinocytosis

Pinocytosis is the nonspecific incorporation of soluble molecules inside vesicles or larger compartments by endocytosis. It is clear that vesicles, or other type of membrane compartments, carry molecules in solution when detached from the plasma membrane. So, all endocytic processes carry molecules by pinocytosis. However, soluble material is particularly important in a type of endocytosis known as macropinocytosis, which, as its name suggest, is specialized on incorporating large amount of soluble extracellular molecules.

#### Receptor mediated endocytosis

Receptor-mediated endocytosis captures specific extracellular molecules by means of receptors located in the plasma membrane. More than 25 receptor types have been found involved in this type of endocytosis. Molecules and small particles, which may be at low concentrations in the extracellular space, are efficiently included into vesicles by this mechanism. Molecules (also known as ligands) are recognized by these receptors, and the ligand-receptor complexes gather in small plasma membrane areas where vesicles are going to be formed. After pinching off from the plasma membrane, vesicles containing a high concentration of ligands are moved away from the plasma membrane.

### Types

Several types of endocytosis have been described depending on the vesicle size, the material to be incorporated, and the mechanism of vesicle formation. Here, we will deal with the following types of endocytosis: clathrin coated vesicles, caveolae, non-coated vesicles, and macropinocytosis. Phagocytosis is being included here too, although it is a particular mechanism capturing large particles such as bacteria, viruses, and cellular fragments, which are first recognized by receptors and then surrounded by membrane.

#### Clathrin coated vesicles

C**lathrin coated vesicles**. This endocytic pathway is the main mechanism for incorporating integral proteins and lipids. Clathrin coated vesicles are formed in plasma membrane areas where the cytoplasmic protein clathrin is present. In fibroblasts, these areas may be up to 2 % of the total surface of the plasma membrane. Clathrin shows a three arms molecular structure, and when these proteins gather together is able to assemble into a regular pentagonal net. This organization and the way they assemble help in the membrane invagination and final closure of the vesicle. The clathrin assembling produces vesicles of about 120 nm in diameter. Clathrin, however, is not in direct contact with the plasma membrane. Other proteins, known as adaptor proteins, are intermediaries between plasma membrane and clathrin, and are also needed for clathrin assembling. Furthermore, adaptor proteins are needed for the selection of proteins which are going to be incorporated into the vesicles: transmembrane proteins as well as receptor-ligand complexes. Adaptor proteins directly bind to the cytosolic domain of transmembrane proteins, including receptors. Once vesicle is closed and moves away from the plasma membrane, the clathrin scaffold is disorganized, individual clathrin proteins are released into the cytoplasm, and vesicle is now moved to the target compartment, usually early endosomes. Free clathrin molecules are thus able to start another endocytic process.

#### Caveolae

They are small invaginations (45-80 nm) of the plasma membrane that can be observed in most eukaryotic cells. It is supposed that most caveolae become vesicles. Caveolae are abundant in endothelial cells, muscle cells and adipocytes. Caveolae membrane contains the protein caveolin, as well as other glycosylphosphatidylinositol-linked proteins, and is enriched in sphingolipids (sphingomyelin and glycosphingolipids), and span cholesterol. The expression of caveolin in a cell is enough to form caveolae. There are around 100 to 200 caveolin molecules in one caveola and there are different types of caveolin in one caveola. Caveolae may be observed in the Golgi complex as well, so it has been suggested that vesicles formed from caveolae may work as transporter of certain molecules between plasma membrane and Golgi complex. However, most vesicles coming from the plasma membrane caveolae are fused with early endosomes. Some authors suggest that these endosomes are different from other early endosomes, and they suggest the name caveosome.

In some cell types, such as endothelial cells, muscle cells, and adipocytes, caveolae may have a more prominent role in endocytosis. Other functions may be modulating signal transduction by gathering receptors of the plasma membrane, such as tyrosine kinase receptors. They can also participate in the lipid traffic between plasma membrane and organelles. Choleric toxin, folic acid, and other molecules enter the cell by means of caveolae. They may be involved in fighting some tumors.

#### Non-coated vesicles

This type of endocytosis has been proposed because endocytic vesicles are still formed when both clathrin and caveolae endocytic pathways are inhibited. Furthermore, invaginations and vesicles show a slightly different morphology. Some toxins, such as cholera toxin, enter the cell in this type of vesicles. It is largely unknown how the mechanism of vesicle formation is and how transported molecules are selected, but lipid rafts may have some role here. It is also unknown whether this endocytic pathway is regulated or not.

#### Phagocytosis

Phagocytosis is a type of endocytosis that engulfs large particles like bacteria, cellular leftovers, and viruses. For instance, macrophages are responsible for removing pathogens like viruses and bacteria, and thousands of red blood cells every day. Protozoa, however, use phagocytosis for feeding. There are cells specialized in phagocytosis like macrophages, neutrophiles, and dendritic cells. Macrophages are resident cells in different tissues and sometimes receive particular names. For example, they are the Kuffer cells in the kidney and microglia in the brain. It is plausible that phagocytosis has evolved from a feeding mechanism in unicellular organisms to a defense role in multicellular animals.

**Cell Adhesion:**

In animals, the organization of cell and tissue largely depends on the ability of cells to adhere to the extracellular matrix or between each other. Cell adhesion relies on transmembrane proteins, known as adhesion proteins, found in the plasma membrane. These proteins made possible the emergence of animals during evolution, all of them pluricellular organisms. Actually, adhesion proteins are very similar when comparing the different animal groups, including marine sponges. Adhesion is not just for anchoring and placing cells to form tridimensional structures, but also for communication between each other. In other words, the type of adhesion and to what a cell is adhered to be very useful information for the cells.

Cells move through tissues by way of adhesion. Cells don't swim, but crawl. For traveling, cells first need to be attached to some element of the environment, a cell or molecules of the extracellular matrix, and then drag the nucleus and the rest of the cytoplasm in the direction of moving. During embryo development, cells can move as coordinated groups. In this case, cells travel together by cell-cell adhesion.

Adhesion molecules are found in the plasma membrane. They diffuse laterally but get fixed when they get anchored to an extracellular molecule. One adhesion molecule does not make a strong bond, but cell adhesion is performed by many adhesion molecules that altogether make a strong linking, as if they were a molecular Velcro. Some adhesion molecules may interact laterally between each other and form molecular complexes that increase the adhesion strength in some local points of the cell surface. These are structures know as focal adhesions and adhesion junctions. Cells can regulate the intensity of adhesion and to what molecule they adhere to by way of different mechanisms. For example, cells can change the type and amount of adhesion molecules in the plasma membrane by synthesis, degradation, or hidden them temporarily in internal compartments by endocytosis and exocytosis. Another mechanism to control the strength and specificity of adhesion is by activating and inactivating the adhesion molecules in the plasma membrane.

There is adhesion molecules involved in the attachment of cells to the extracellular matrix and others in linking one cell to another.

**Cell-extracellular matrix adhesion**

Integrins are probably the most important proteins involved in the adhesion between cells and extracellular matrix, and comprise a large family of transmembrane proteins present in all animals. They are composed of two subunits (alpha and beta). In mammals, integrins families are formed by 18 alpha units and 3 beta units. By combination, they are able to form up to 24 different integrins, which are differentially expressed depending on the tissue and the physiological state of the cell. Integrins have 3 molecular domains. An intracellular domain that interacts with actin filaments of the cytoskeleton (sometimes with intermediate filaments), an extracellular domain that can bind collagen, fibronectins and laminins, and an intramembrane domain containing hydrophobic amino acid sequences inserted among lipid fatty acid chains. The ability of integrins to connect extracellular matrix and cytoskeleton makes it possible a structural continuity between the inner and the outer sides of the cell.

**Cell-cell adhesion.**

Some transmembrane molecules make direct adhesion contacts between cells. There are four types: cadherins, immunoglobulins, selectins and some types of integrins.  Cadherins are found in most animal cells and make homotypic contacts, i.e., they recognize and bind other cadherins located in neighboring cells. Cadherins may join laterally between each other to form groups for greater adhesion strength at certain points on the cell surface. There are more than 100 types of cadherins divided in classical and desmosomal cadherins. The name cadherin stands for calcium and adhesion, because they need calcium to make the adhesion contact. Some adhesion proteins belong to the immunoglobulin family and make homophilic contacts with other immunoglobulins located in neighboring cells, although they can also make heterophilic contacts. They are also a large and diverse family of proteins with selective tissue distribution. For example, N-CAM (neuronal cell adhesion molecule) is expressed in the nervous system. The binding strength of immunoglobulin proteins is weaker than cadherins, and it is thought to be suitable to fine tune the segregation of cells into groups inside tissues.

Selectins are another type of adhesion molecules involved in cell-cell adhesion by heterophilic contacts. They bind carbohydrates (sialic acid and fucose) located at the surface of adjoining cells. For example, they are needed during the exit of leukocytes from blood vessels toward the extracellular matrix of surrounding tissues. Integrins, which mainly participate in cell-extracellular matrix adhesion, are also involved in cell-cell adhesion. For example, some integrins can make heterophilic contacts with certain types of immunoglobulins of neighboring cells.

**Gap Junctions:**

Gap junctions, or intercellular junctions, are macromolecular complexes that form channels in the adjoining plasma membranes of neighbor cells. These channels make possible the direct cytoplasm-cytoplasm communication between the two cells. Virtually, all cells of solid animal tissues may establish communication with their neighbor cells by gap junctions. Although gap junctions are included in the textbook chapters dealing with cell adhesion, they actually should be studied together with the mechanisms of cell-cell communication, since this is their main function. Gap junctions were discovered in the 1960s by dye intracellular injections. A dye injected into one cell could be observed in adjacent cells a bit latter. This could be explained if there was a direct connection between the cytoplasms.

**Function**

Gap junction channels let simple carbohydrates, second messengers like cAMP or calcium, amino acid, and small RNAs swaping between the two connected cells. However, proteins, lipids, long RNAs, or large molecules in general, are not allowed to go through.

Gap junctions make possible both electric and metabolic synchronization of adjoining cells by allowing ions and molecules to be exchanged between them. For example, neurons can coupled be electrically synchronized (similar membrane potentials) and therefore synchronize their activity very efficiently by gap junctions. In this way, neurons can communicate between each other avoiding the release of neurotransmitters and without performing transduction mechanisms, which would slow the neuronal population synchronization. Gap junctions in neurons are known as electric synapses. Similarly, glial cells form a network of cells connected through gap junctions. The rhythmic contractions of the heart muscle, the uterus during birth, the intestine during peristaltic movements, and the accommodation of the iris muscle of the eye to different light intensities are mediated by cellular coupling through gap junctions. Non-excitable cells like hepatocytes and somatic cells of ovarian follicles are metabolically synchronized by gap junctions. Another example can be found during blood clotting when platelet cells attached to the blood vessel walls establish gap junctions between each other increasing the adhesion strength of the whole platelet aggregate.

## Apoptosis:

## Apoptosis is a molecular mechanism of eukaryote cells that ends up with cell death. It is a cellular suicide performed by an autodestruction molecular program triggered either by extracellular or intracellular stimuli. Apoptosis is also known as programmed cell death because the cell takes a precise and ordered chain of steps. It does not mean that cells are all going to die by apoptosis, that is, there is no apoptosis if there is no apoptotic stimuli.

In pluricellular organisms, apotosis is involved in many physiological and pathological processes. For example, morphogenesis during embryo development, tissular homeostasis and regeneration in adults, withstanding pathogens and cellular stress, and cancer. The amount of cells that die by apoptosis is huge, both during development and in adults. Blood and epithelial renewing in adults is intense, which means that an enormous amount of cells die by apoptosis.

### Molecular mechanism

The molecular mechanism for apoptosis has been conserved during evolution in most eukaryote cells. It is an ordered process and energy dependent, that needs to be initiated. A number of signals have been found to start apoptosis: extracellular signals that activate the so-called dead receptors, intracellular signals that activate organelles like mitochondria, and a third pathway that involves molecules like perforin and granzyme. These three pathways converge in degradative enzymes known as capasases (Figure 1.)

#### Dead receptors. Extracellular signals.

Extracellular signals can start apoptosis by activating transmembrane receptors known as death receptors. Death receptors are included in the TNF (tumor necrosis factors) receptor family.

#### Cellular stress. Internal signals.

This pathway involves stimuli that are not directly mediated by receptors. For example, lack of survival factors, high radiation, high temperature, toxic substances, and other insults triggers apoptosis by increasing cellular stress. These types of stimuli modify the cellular physiology that ends up affecting the permeability of the inner mitochondrial membrane, and some proapoptotic molecules are released from the mitochondrial matrix to the cytosol.

#### Pathogens

Cytotoxic T lymphocytes are able to kill cells that contain pathogens by activating receptors that start apoptosis. They can also introduce molecules into the infected cell to activate apoptosis.

### Cell process

During apoptosis, cells undergo several changes: cell retraction or shrinking, cytoplasm gets darker, organelles are more tightly packed, chromatin condenses, which is a change visible at light microscopy. Later, there are protrusions and folding of the plasma membrane so that the cell splits in several portions known as apoptotic bodies, which are always enclosed by membrane. Apoptotic bodies are phagocyted by macrophages. Since there is no release of intracellular molecules to the extracellular space through plasma membrane breakages, there is no inflammatory processes. In addition, macrophages do release any molecule after "eating" the apoptotic bodies. In this way, apoptosis is cell death without bothering the neighbor. However, opoptotic cells sometimes release molecules for cell proliferation, extracelular matrix remodeling and cytoskeleton reorganization.

Apoptotic bodies are quickly removed by macrophages, otherwise they can be broken, release their content and produce an inflammatory effect. Under macrophage activity inhibition, apoptosis leads to an inflammatory process. Macrophages recognize specific molecules on the external surface of the apoptotic bodies. Thus, the role of macrophages would be to be sure that cells that start apopotsis are going to die for real.

During aging, apoptosis is altered in different tissues. In some of them apoptosis is increases, but it is minimized in others. For example, the immune system, skeletal muscular tissue, cardiac muscle, and neurodegenerative pathologies show an increase in the apoptotic processes. Senescent cells, and cancer cells, are more resistant to apoptosis, that is why they are more easily observed during aging.