Introduction
The normal functioning of the human body involves a vast diversity of communication events that are occurring simultaneously at the cell and tissue level in every organ of your body every millisecond of every day. As we will see in this lecture and those to follow, the disruption of any one of these signaling events can lead to a debilitating disease. After we summarize the types of intercellular signaling we’ll examine the role of the extracellular matrix in mediating these intercellular communications. Then we’ll look at a specialized structure called the gap junction that is essential to the functioning of a diversity of tissues and organs in the human body.

We can define three types of intercellular signaling in the human body:
- Endocrine - Cells in one part of the body send hormones via the bloodstream to influence other parts
- Paracrine - Cells secrete substances that influence other cells around them
- Autocrine - Cells secrete substances that influence themselves
Thus these types of intercellular communications are defined by the distance between the signaling cell and its target cell. Intercellular signaling can also be classified based upon the way in which the signaling molecules from one cell type impact the target cells. These are called modes of intercellular communication. In spite of the complexity of the human body and the diversity of intercellular communications that occur, the modes of cellular communication can be classified into four major groups:

- Communication via Diffusible Molecules (e.g., hormones, growth factors, neurotransmitters)
- Communication via Cellular Continuities (e.g., gap junctions)
- Communication via Cell Contact (e.g., adhesion molecule and its receptor)
- Communication mediated by the Extracellular Matrix (not covered in detail in this course).

**Gap Junctions**

Gap junctions are more accurately considered to be communicating junctions rather than cell adhesion junctions. But their structure likely results in both functions. Gap junctions are known to appear at specific times to mediate certain events. For example, gap junctions appear in the myometrium of the uterus during the later stages of pregnancy so that the uterine contractions can be precisely controlled during childbirth. During development, gap junctions appear in developing muscle cells (myoblasts) to co-ordinate their fusion into future muscle fibres (myoblasts). These topics are covered in the lecture on biomembrane fusion.

**Gap Junction Structure**

The following shows how gap junctions appear in the transmission and scanning electron microscopes. The third picture shows what purified gap junctional components look like.

- Gap junctions are made up of clusters of closely packed connexons
- Connexons consist of pairs of transmembrane channels
- The connexon hemichannel in one cell membrane docks with a connexon hemichannel in an adjacent cell
- The connexons are hexameric: they consist of arrays of 6 connexin protein subunits
- About 20 connexin subunit isoforms exist in mammals, chicks, amphibians and fish
- The connexon subunits of arthropods, where gap junctions were first identified, are called innexins (Phelan & Starich, 2001. BioEssays 23:388-396.)
- Recently, pannexins have been discovered in vertebrates; they are primarily localized to the CNS
- A connexon may be made of the same (homohexameric) or different (heterohexameric) subunits
Gap Junctions & Their Regulation
Gap junctions were introduced in an earlier lecture on cell junctions. In the 1966, Lowenstein proposed the existence of membrane junctions for the flow of small molecules between certain cells (Loewenstein, 1966. Ann. NY Acad. Sci. 137: 441-472). It was subsequently shown that many epithelial cells are physiologically- or electrically-coupled by the presence of a unique structure termed a gap junction. By injecting molecules into epithelial and other cells, it has been shown that small molecules including sugars, nucleotides, ions and signaling molecules can diffuse between cells through the connexons. The gap junctions of different cell types show different levels of permeability which are determined by various characteristics including the types of connexins in the connexons and the physiological state of the cell. Gap junctions have many important functions in cells. In the brain, gap junctions allow direct signaling between neurons, between glial cells and between neurons and glial cells.

- Penetrability determined by EM and microinjection studies (e.g., fluorescent dyes, labelled molecules, etc.)
- Molecules greater than 5000 MW cannot pass
- Molecules less than 5000 MW can pass
As will become clear when we discuss the roles of calcium ions, cyclic AMP and IP3, the ability of small molecules to transfer between cells via gap junctions has important implications to cell function.

**Connexin Proteins Spontaneously Form Connexons**

As shown in the following experiment (Cx43 Experiment 1), pure phospholipid vesicles are impermeable to the fluorescent dye Lucifer Yellow. When the purified gap junction protein Cx43 was added, the Lucifer Yellow was able to enter into the phospholipid vesicles. Thus it is concluded the Crx43 can spontaneously form connexons allowing the dye to enter.

It was later shown that the flow of the dye through the connexon channels could be altered by phosphorylation of Cx43. When Mitogen Activated Protein Kinase, MAPK, an important protein kinase involved in many signaling events (see future lectures on signal transduction) is added to phospholipid vesicles containing Cx43, the Lucifer Yellow dye molecules are unable to enter the phospholipid vesicles. This suggests the MAPK phosphorylated the Cx43 making it unable to form permeable channels. When the MAPK was removed, the Cx43 was apparently unphosphorylated permitting the dye to pass through the functioning channels. While much work needs to be done on this area, it indicates that connexin proteins can spontaneously form connexons and that the functioning of those connexons can be regulated by the phosphorylation of the connexin protein.
Gap Junctions and Heart Function
Cardiac muscle is different from skeletal muscle because the contractile cells comprising it are connected electrically and not stimulated by nerves as is the case for skeletal muscle. Because cardiac muscle undergoes such strong regular contractions it has intercalated disks (id) that are strong adhesion regions that hold adjacent cardiac cells together. The contractile elements are seen within each myocyte. Adhesion components covered in this course (specialized adherens junctions, desmosomes) are also present.

Cardiac muscle is comprised of a multitude of electrically insulated cells that can only communicate via gap junctions. Thus the number, size and localization of these gap junctions are critical to normal heart function. In keeping with this disruption of cardiac gap junctions can lead to arrhythmias and other heart conditions. At least five connexins (Cx43, Cx40, Cx45, Cx31.9 and Cx37) are expressed in the heart which is comprised of cardiac myocytes, vascular and interstitial cells, and other cell types (e.g., adipocytes; mesothelium). Different regions of the heart express different amounts and combinations of these connexions. Atrial myocytes express abundant amounts of Cx43 and Cx40 but only a very limited amount of Cx45. Connexin
Cx43 is the primary ventricular gap junctional protein with only minor amounts of Cx45 and no detectable Cx40.

Ventricular myocytes possess gap junctions that are among the largest of any mammalian tissue. In addition to the differences in connexin expression, heart gap junctions also exist in different numbers and patterns in different regions. Much remains to be learned about the functions of these different gap junctions. One approach has been to study gap junctions and heart function after ischemia. Ischemia, which can occur naturally (e.g., heart attack) or experimentally, is a decrease in the blood supply to an organ or tissue caused by the blockage or constriction of blood vessels. In this case, the blood flow to the heart is stopped leading to a lack of oxygen in the affected tissue. When this is done in rat, as seen in the following figure, the level of phosphorylation of Cx43 drops rapidly suggesting the regulation of gap junctions becomes altered after ischemia.

**Gap Junctions in Breast Development**

Above we discussed the role of ECM in secretory tissue development using salivary glands as an example. Breast tissue is a secretory tissue designed for the storage and release of milk protein. Different types of connexin proteins are present in different regions of the developing breast indicating variations in the types of gap junctions that function in those regions.
Mammary gland duct and alveolus microanatomy and connexin expression in mouse and human (Figure 1 from Elizabeth McLachlin et al, 2007. J. Membrane Biol. 218: 107-121).

Focusing in on human breast duct in the above figure, note that Cx26 is expressed primarily in the duct luminal cells and is involved in milk production while Cx43 is localized in gap junctions in the contractile myoepithelial cells that regulate the release of the milk from the alveolus. Both of these gap junction proteins undergo developmental changes. If Cx26 gene expression is knocked down in mice at puberty alveolar development is impaired and lactation is prevented. If the Cx43 gene is knocked out the mice die at birth. A conditional knockout for Cx43 has not been generated. There is also evidence that in normal breast tissue both Cx26 and Cx43 have tumour suppressive roles.