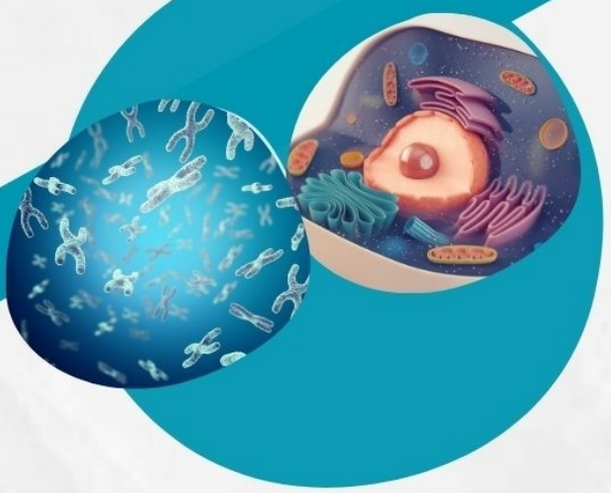
Sheets

Cell biology

Lecture 7,5



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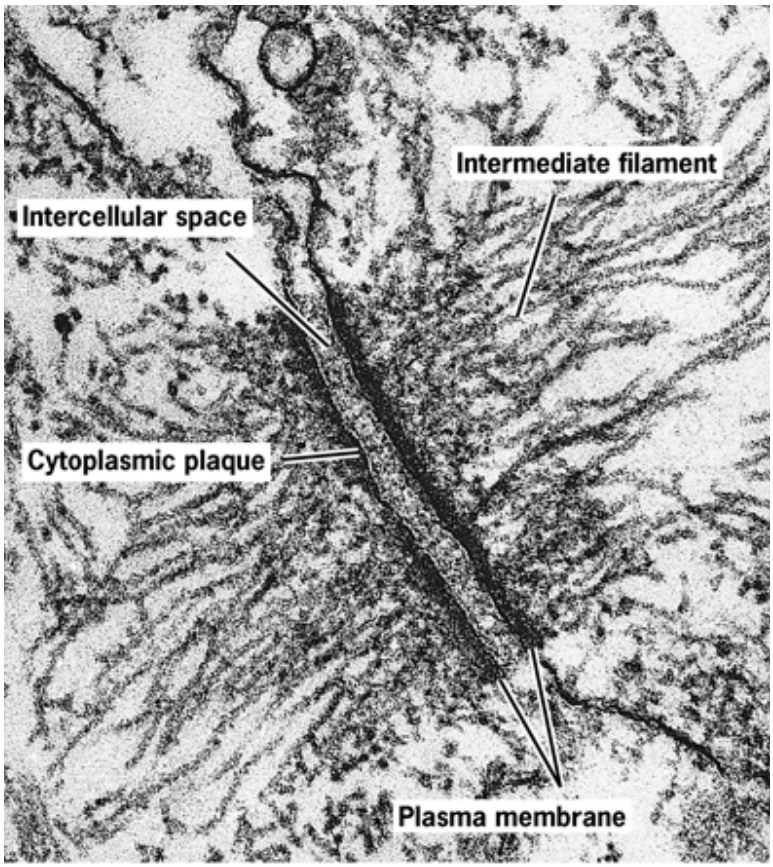

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FIGURE 7.27 Specialized cadherins, called desmoglein and desmocollin, bind to one another in the extracellular space. In the cytoplasm, the desmosomal cadherins bind indirectly to intermediate filaments. Electron micrograph of a desmosome from newt epidermis.

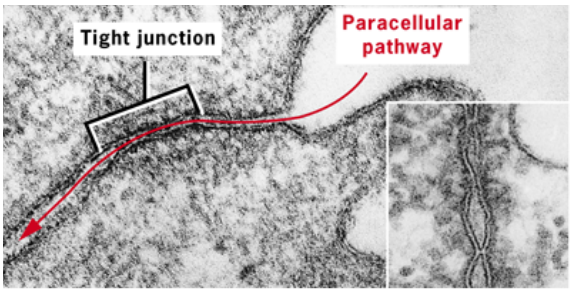
Review

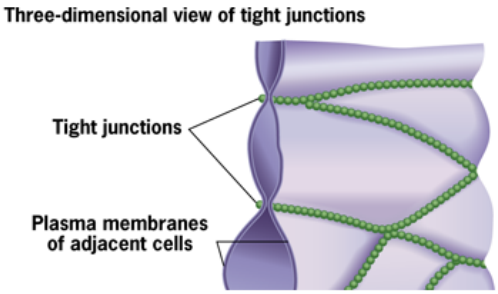
1. Which type(s) of cell junctions contain actin filaments? Which contain(s) intermediate filaments? Which contain(s) integrins? Which contain(s) cadherins?
2. How do cadherins, IgSF proteins, and selectins differ at the molecular level in the way they mediate cell–cell adhesion?
3. Distinguish between a hemidesmosome and a desmosome; between a desmosome and an adherens junction.

7.5 Tight Junctions: Sealing the Extracellular Space إغلاق

A simple epithelium, like the lining of the intestine or lungs, is composed of a layer of cells that adhere tightly to one another to form a cellular sheet. Biologists have known for decades that when certain types of epithelia, such as frog skin or the wall of the urinary bladder, are mounted between two compartments containing different solute concentrations, very little diffusion of ions or solutes is observed across the wall of the epithelium from one

compartment to the other. Given the impermeability of plasma membranes, it is not surprising that solutes cannot diffuse freely through the cells of an epithelial layer. Why are they unable to pass between cells by way of the *paracellular pathway* (as in **Figure 7.28a**)? The reason became apparent during the 1960s with the discovery of specialized contacts, called **tight junctions** (or *zonulae occludens*), between neighboring epithelial cells. Tight junctions (TJs) are located at the most apical end of the junctional complex between adjacent epithelial cells (see Figure 7.25). An electron micrograph of a section through a TJ that has been cut to include the plasma membranes of the adjacent cells is shown in Figure 7.28*a*. A higher magnification view showing the interaction between the membranes of a TJ is shown in the inset of Figure 7.28*a*. It is evident that the adjoining membranes make contact at intermittent points, rather than being fused over a large surface area. As indicated in Figure 7.28*b*, the points of cell–cell contact are sites where integral proteins of two adjacent membranes meet within the extracellular space.





Source: (*a*) Courtesy of Daniel S. Friend, Harvard Medical School; Courtesy of Hiroyuki Sasaki and Shoichiro Tsukita; (*c*) ©1973 Philippa Claude, Daniel A. Goodenough. Originally published in *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.58.2.390; (*d*) Courtesy of D. Tarin.

FIGURE 7.28 Tight junctions. (a) Electron micrograph of a section through the apical region of adjoining epithelial cells showing where the plasma membranes of the two cells come together at intermittent points within the tight junction. Inset shows the tight junction structure at higher magnification. Tight junctions block the diffusion of solutes through the paracellular pathway between cells. (b) A model of a tight junction showing the intermittent points of contact between integral proteins from two apposing membranes. Each of these contact sites extends as a paired row of proteins within the membranes, forming a barrier that blocks solutes from penetrating the space between the cells. (c) Freeze-fracture replica showing the E face of the plasma membrane of one of the cells in a region of a tight junction. The grooves in the E face are left behind after the integral membrane proteins are pulled from this half of the membrane. (d) Scanning electron micrograph of the apical surface of an epithelium showing the encircling nature of the tight junctions.

Freeze fracture, which allows observation of the internal faces of a membrane (Figures 4.15 and 4.30), shows that the plasma membranes of a TJ contain interconnected strands (Figure 7.28*c*) that run mostly parallel to one another and to the apical surface of the epithelium. The strands (or grooves in the opposite face of a fractured membrane) correspond to paired rows of aligned integral membrane proteins that are illustrated in Figure 7.28*b*. The integral proteins of TJs form continuous fibrils that completely encircle the cell like a gasket and

make contact with neighboring cells on all sides (Figure 7.28*d*). As a result, TJs serve as a barrier to the free diffusion of water and solutes from the extracellular compartment on one side of an epithelial sheet to that on the other side. ② Tight junctions also serve as "fences" that help maintain the polarized character of epithelial cells (see Figure 4.30) by blocking the diffusion of integral proteins from the apical domain of the plasma membrane to its lateral and basal domains. Like other sites of cell adhesion, tight junctions are also involved in signaling pathways that regulate numerous cellular processes. ③ Selective permeability.

Not all TJs exhibit the same permeability properties. Part of the explanation can be seen under the electron microscope: TJs with several parallel strands (such as that in Figure 7.28*c*) tend to form better seals than junctions with a single or only a few strands. However, there is more to the story than numbers of strands. Some TJs are permeable to *specific* ions or solutes to which other TJs are impermeable. Studies over the past decade have shed considerable light on the molecular basis of TJ permeability.

It was originally thought that TJ strands were composed of a single protein, *occludin*. Then, it was found that cultured cells lacking a gene for occludin were still able to form TJ strands of normal structure and function. Subsequent studies by Shoichiro Tsukita and his colleagues at the University of Kyoto led to the discovery of a family of proteins called *claudins* that form the major structural component of TJ strands. The electron micrograph in **Figure 7.29** shows that occludin and claudin are present together within the linear fibrils of a TJ. At least 24 different claudins have been identified, and differences in the distribution of these proteins may explain selective differences in TJ permeability. For example, one small region of a human kidney tubule—a region known as the thick ascending limb (TAL)—has TJs that are permeable to magnesium (Mg^{2+}) ions. The loops of the claudin molecules that extend into the extracellular space are thought to form pores in the TAL that are selectively permeable to Mg^{2+} ions. Support for this concept has come from research on one specific member of the claudin family, claudin-16, which is expressed primarily in the TAL. The importance of claudin-16 in kidney function was revealed in studies of patients suffering from a rare disease characterized by abnormally low Mg^{2+} levels in their blood. These patients were found to have mutations in both copies of their claudin-16 gene. The Mg^{2+} levels are low because tight junctions containing the abnormal claudin are impermeable to Mg^{2+}. As a result, this important ion fails to be reabsorbed from the tubule and is simply excreted in the urine.

Summary for this paragraph

① ②

- Molecular composition: Claudins + occludins.
  - The first protein identified, but the cell can still form functional TJ strands without it.
  - Discovered later
  - at least 24 different types.

* The specific mix of claudins & occludins determines what can pass and what cannot

- In specific part of kidney (The TAL) TJs (Tight Junction) allow Mg^{+2} to be reabsorbed back into the blood by claudin-16.

↳ patients with mutation in the claudin-16 cannot reabsorb Mg^{+2} ⇒ it'll be lost in urine

Dangerously low Mg^{+2} in the blood

Feature	Description
Physical Form	Continuous strands/fibrils encircling the cell.
Main Proteins	Claudins (structural/ selective) and Occludins.
Barrier Role	Stops paracellular transport (between cells).
Fence Role	Maintains cell polarity (keeps proteins in their place).
Permeability	Varies; determined by the specific types of Claudins present.

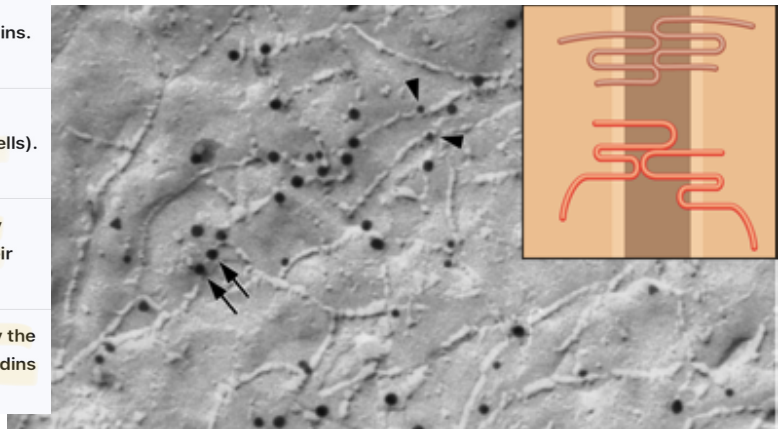


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FIGURE 7.29 The molecular composition of tight junction strands. Electron micrograph of a freeze-fracture replica of cells that had been joined to one another by tight junctions. The fracture faces were incubated with two types of gold-labeled antibodies. The smaller gold particles (arrowheads) reveal the presence of claudin molecules, whereas the larger gold particles (arrows) indicate the presence of occludin. These experiments demonstrate that both proteins are present in the same tight junction strands. Bar equals 0.15 µm. The inset shows a possible arrangement of the two integral membrane proteins as they make contact in the intercellular space. Both the claudins (red)ww and occludin (brown) span the membrane four times.

Another important function of tight junctions came to light in 2002. It was thought for decades that the impermeability of mammalian skin to water was solely a property of the outer, cornified layer of the skin (see Figure 7.1), which contains tightly packed protein filaments and associated lipids. It was discovered, however, that mice lacking a gene for claudin-1 died shortly after birth as a result of dehydration. Further investigation revealed that the cells in one of the outer layers of *normal* epidermis are connected to one another by tight junctions. Animals lacking the gene for claudin-1 were unable to assemble watertight epidermal tight junctions and, as a result, suffered from uncontrolled water loss.

Tight junctions are also present between the endothelial cells that line the walls of capillaries. These junctions are particularly evident in the brain where they help form the *blood–brain barrier*, which prevents substances from passing from the bloodstream into the brain. Although small ions and even water molecules may not be able to penetrate the blood–brain barrier, cells of the immune system are able to pass across the endothelium through these junctions. These cells are thought to send a signal that opens the junction, allowing the cells to pass. While protecting the brain from unwanted solutes, the blood–brain barrier also prevents access of many potentially therapeutic drugs to the central nervous system. Consequently, a major goal of the pharmaceutical industry is to develop drugs that temporarily open the tight junctions of the brain to allow passage of therapeutic compounds.

Review

1. What does freeze-fracture analysis tell you about the structure of a junction that cannot be learned from examination of stained tissue sections?
2. How does the structure of a tight junction contribute to its function?

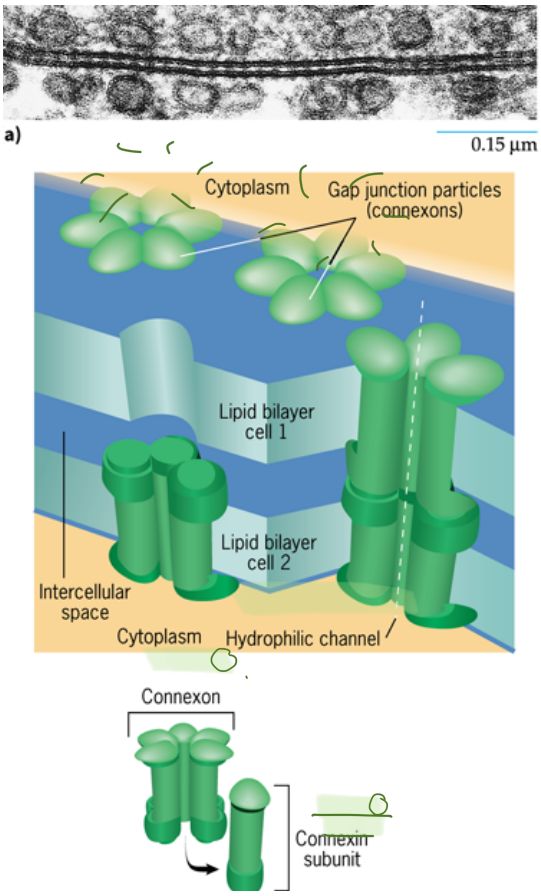
7.6 Intercellular Communication

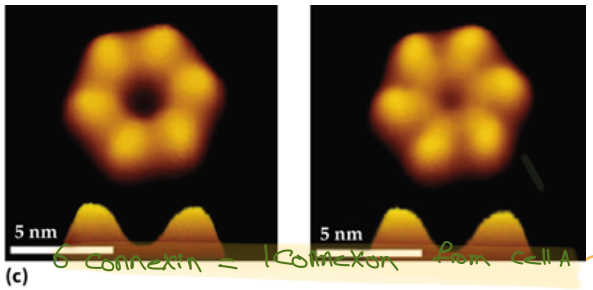
Gap junctions and plasmodesmata are specialized sites of communication between adjoining cells in animals and plants, respectively. The plasma membranes of a gap junction contain channels that connect the cytoplasm of one cell with the cytoplasm of the adjoining cell. Cells appear to be able to communicate with one another through a variety of means. Short-range communication can be achieved through specialized sites that join cells directly (gap junctions in animals and plasmodesmata in plants). Mid-range communication can be carried out through long tubules that stretch between cells. To carry out long-range communication with other cells, cells can send extracellular vesicles.

Gap Junctions

Gap junctions are sites between animal cells that are specialized for intercellular communication. Electron micrographs reveal gap junctions to be sites where the plasma membranes of adjacent cells come very close to one another (within about 3 nm) but do not make direct contact. Instead, the cleft between the cells is spanned by very fine strands (**Figure 7.30a**) that are actually molecular "pipelines" passing through the adjoining plasma membranes and opening into the cytoplasm of the adjoining cells (Figure 7.30*b*).

Range	Mechanism	Structure	Best For...
Short	Specialized Sites	Gap Junctions / Plasmodesmata	Fast, synchronized electrical/chemical signals. (small cargo)
Mid	Long Tubules	Tunneling Nanotubes	Targeted delivery of large cargo between neighbors.
Long	Extracellular Vesicles	Exosomes / Microvesicles	Broad signaling across tissues or the entire organism.





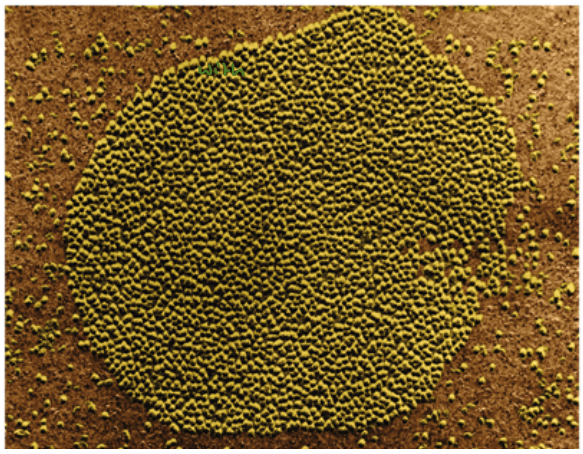


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FIGURE 7.30 Gap junctions. (a) Electron micrograph of a section through a gap junction perpendicular to the plane of the two adjacent membranes. The "pipelines" between the two cells are seen as electron-dense beads on the apposed plasma membranes. (b) Schematic model of a gap junction showing the arrangement of six connexin subunits to form a connexon, which contains half of the channel that connects the cytoplasm of the two adjoining cells. Each connexin subunit is an integral protein with four transmembrane domains. (c) High-resolution images derived from atomic force microscopy of the extracellular surface of a single connexon in the open (left) and closed (right) conformations. Closure of the connexon was induced by exposure to elevated Ca^{2+} ion concentration. (d) Freeze-fracture replica of a gap junction plaque showing the large numbers of highly concentrated connexons. (The crystal structure of a gap junction can be found in *Nature* 458: 597, 2009.)

Gap junctions have a simple molecular composition; they are composed entirely of an integral membrane protein called *connexin*. Connexins are organized into multisubunit complexes, called **connexons**, that completely span the membrane (Figure 7.30*b*). Each connexon is composed of six connexin subunits arranged in a ring around a central opening, or *annulus*, that is approximately 1.5 nm in diameter at its extracellular surface (Figure 7.30*c*, left).

During the formation of gap junctions, the connexons in the plasma membranes of apposing cells become tightly linked to one another through extensive noncovalent interactions of the extracellular domains of the connexin subunits. Once aligned, connexons in apposing plasma membranes form complete intercellular channels connecting the cytoplasm of one cell with the cytoplasm of its neighbor (Figure 7.30*b*). Large numbers of connexons become clustered in specific regions of the membrane, forming gap-junction plaques that can be visualized when the membrane is split down the middle by freeze fracture (see Figure 7.30*d*.)

As discussed in the Experimental Pathways feature, gap junctions are sites of communication between the cytoplasms of adjacent cells. The existence of gap-junction intercellular communication (GJIC) is revealed through the passage of either ionic currents or low-molecular weight dyes, such as fluorescein, from one cell to its neighbors (**Figure 7.31**). Mammalian gap junctions allow the diffusion of molecules having a molecular mass below approximately 1000 daltons. In contrast to the highly selective ion channels that connect a cell to the external medium (Section 4.6), gap junction channels are relatively nonselective. Just as ion channels can be open or closed, gap-junction channels are also gated. Channel closure can be triggered by a number of stimuli, including phosphorylation of connexin subunits, and changes in voltage across the junction (Figure 7.30*c*, right).

Property	Description
Cargo Type	Ions and low-molecular weight molecules.
Size Cutoff	Approximately 1000 Daltons.
Selectivity	Relatively nonselective (if it fits, it sits).
Regulation	Gated (can be opened or closed by the cell).
Triggers	Phosphorylation, Voltage, and chemical changes.

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FIGURE 7.31 Results of an experiment demonstrating the passage of low-molecular-weight solutes through gap junctions. Micrograph showing the passage of fluorescein from one cell into which it was injected (X) to the surrounding cells.

Experimental Pathway

The Role of Gap Junctions in Intercellular Communication

Based on the information presented in Chapter 4, you might presume that synaptic transmission always occurs by movement of neurotransmitter molecules from the presynaptic neuron to the postsynaptic cell. This was the prevailing view until the 1950s, when Edwin Furshpan and David Potter of University College in London found a notable exception. Furshpan and Potter were studying synaptic transmission between giant neurons in the nerve cord of the crayfish. They noted that a small, *subthreshold* depolarization induced in the presynaptic nerve cell produced a very rapid (0.1 msec) depolarization in the postsynaptic cell.[1,2] If the nerve cells had been connected by a chemical synapse, a subthreshold change in membrane potential should not have been propagated to the postsynaptic cell, because it would not be sufficient to stimulate the release of neurotransmitter molecules. Even if neurotransmitter molecules were released, they could not possibly induce such a rapid change in the postsynaptic cell. Furshpan and Potter concluded that the two nerve cells were connected by a different type of synapse, an *electrotonic synapse*, in which ionic currents in the presynaptic cell could flow directly into the postsynaptic cell on the other side of the synapse. It was presumed that this type of cell–cell connection, which allows for the flow of ions between cells, was peculiar to excitable cells, such as neurons, which are specialized for cell–cell communication.

During the early 1960s, Yoshinobu Kanno and Werner Loewenstein of Columbia University were studying the permeability properties of the nuclear envelope, the membranous complex that bounds the nucleus. To determine whether ions were capable of flowing across the nuclear envelope, they had turned to the very large cells that make up epithelial tissues of the larval fruit fly (cells that contain the giant chromosomes that had proved so useful to geneticists). These cells were large enough to allow penetration of microelectrodes capable of inducing and recording ionic currents (**Figure 1**). To their surprise, Kanno and Loewenstein found that when ions were injected into the nucleus of one cell, not only did the ion flux (measured as an electrical current) spread into the cytoplasm of that cell, but it flowed directly into the cytoplasm of an adjacent cell. In fact, the potential recorded in the adjacent cell was almost as great as that in the cell in which the current was originally induced. Kanno and Loewenstein concluded that the epithelial cells that make up the salivary gland are *electrically coupled* to one another, meaning that ions are able to flow freely from cell to cell through low-resistance cell junctions.[3] If small inorganic ions could pass through these junctions between neighboring cells, what about larger substances? When a small volume of the fluorescention fluorescein (molecular weight of 376 daltons) was injected with a micropipette into the cytoplasm of one cell, the fluorescence rapidly spread into adjacent cells until the entire epithelial layer glowed from the presence of the tracer (as in Figure 7.32). In contrast, none of the fluorescent dye leaked out of the cells into the external medium, indicating that fluorescein molecules were diffusing directly from the cytoplasm of one cell into the cytoplasm of adjacent cells by means of permeable cell–cell contacts.[4] Similar observations were soon made about a variety of different types of epithelial and mesenchymal cells, including those of various mammals, indicating that

= Reading + understanding the idea."

1. The Discovery: Chemical vs. Electrical Synapses

• The Old View: Before the 1950s, scientists thought all nerve signals moved via chemical neurotransmitters.

• The Exception (Furshpan & Potter, 1950s): While studying crayfish nerve cords, they found a signal that moved too fast (0.1 msec) to be chemical.

• The Conclusion: They discovered the electrotonic synapse (electrical synapse). Instead of releasing chemicals, ionic currents flow directly from one neuron to the next.

2. Electrical Coupling in Non-Nerve Cells

• Kanno & Loewenstein (Early 1960s): They studied epithelial cells from fruit fly salivary glands.

• The Experiment: They injected ions into the nucleus of one cell using microelectrodes.

• The Result: The ions didn't just stay in that cell; they flowed directly into the cytoplasm of the adjacent cell.

• The Conclusion: These cells are electrically coupled, meaning ions flow freely through low-resistance junctions between neighbors.

3. Proving the Pathway with Dyes

• The Tracer Experiment: Scientists injected a fluorescent dye called fluorescein (weight: 376 daltons) into one cell.

• Key Observation: The dye spread rapidly until the entire epithelial layer glowed.

• Why it matters: Because the dye did not leak into the outside medium, it proved the molecules moved directly from cytoplasm to cytoplasm through permeable cell contacts (Gap Junctions).

4. Key Scientific Terms to Know

• Subthreshold Depolarization: A small change in electrical charge that isn't strong enough to trigger a full "action potential" but can still pass through a gap junction.

• Electrically Coupled: When two cells act as one electrical unit because ions can move freely between them.

• Low-Resistance Junctions: Another name for gap junctions, highlighting that they offer very little "resistance" to the flow of ions.

Summary of Evidence for Gap Junctions:

1. **Speed: Signals move faster than chemical neurotransmitters.**
2. **Ionic Flow: Electrical currents pass directly between adjacent cells.**
3. **Dye Transfer: Small molecules (under 1000 daltons) can move between cells without touching the extracellular space.**