

# A Historical and Evolutionary View of Tight Junctions



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**Abstract** The essential functions of a polarized epithelium are to separate the spaces between tissues and regulate the exchange of materials between them, functioning as an interface with the external environment. Tight junctions (TJ) are the anatomical structures responsible for creating this barrier. These cell junctions are regulated and selective, and vary depending on the tissue in which they are found.

However, for many years it was considered that TJ were at the border of cells and their function was limited to blocking the passage of substances between cells, so it is understandable that they received names as “terminal bar.” It was not until the arrival of electron microscopy that it was possible to resolve that these “terminal bars” are, in fact, a complex of cell junctions.

In this chapter, we will see the history and how the concept of tight junctions evolved. We will discuss the main functions of this type of cellular contacts and the experiments that allowed to study their structure and biology.

**Keywords** Transporting epithelium · Cell contacts · Tight junctions · Evolution · Microscopy

## Abbreviations

TJ      Tight junctions  
TER    Transepithelial electrical resistance  
TrEp   Transporting epithelium

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## A Single Cell in the Ocean

At the beginning of the twentieth century, life was considered a God-given gift to humanity, and biologists were reluctant to analyze it using a thermodynamic basis.

Nevertheless, Erwin Rudolf Josef Alexander Schrödinger, who was earlier awarded the Nobel Prize (1933) for his wave equation, wrote one of the most influential biological books ever named, *What Is Life?* [1], in which he proposed that living organisms are considered to be thermodynamically open systems. This idea would be easily understood by imagining a single cell in the sea exchanging substances with its environment, and this environment behaves as an infinite and constant reservoir that is not exhausted by the removal of nutrients nor spoiled by receiving catabolites and detritus excreted by the cells.

On the other side, if we think in a cell lodged in a recondite fold of the brain, the liver, or any other organ of a mammal, we can note that its surrounding is reduced to an extremely thin film, even though it behaves as an infinite and constant reservoir as if it was an immense ocean.

Consequently, this makes us wonder how evolution could generate metazoans whose cells can be meters away from the outer environment (think of a hepatocyte sunken in the liver of a whale). Evolution coped with this situation by developing transporting epithelia (TrEp), consisting of barriers of tightly packed cells, which generate a fluid compartment named “internal medium” that occupies some 20% of the human body.

Hence our cells do not exchange directly with the sea, but with this internal medium. In spite of being a comparatively small space, this internal medium may act as a constant reservoir because TrEp manage to regulate its composition through a complex mechanism called homeostasis, derived from Greek “homoios” (similar) and “stasis” (stability).

The epithelia acts as an interface with the external environment, withstands strong mechanical or chemical stress, and works as a diffusion barrier between compartments with different compositions, protecting the organism from toxins and microorganisms, extruding metabolic wastes, and taking up nutrients [2–4].

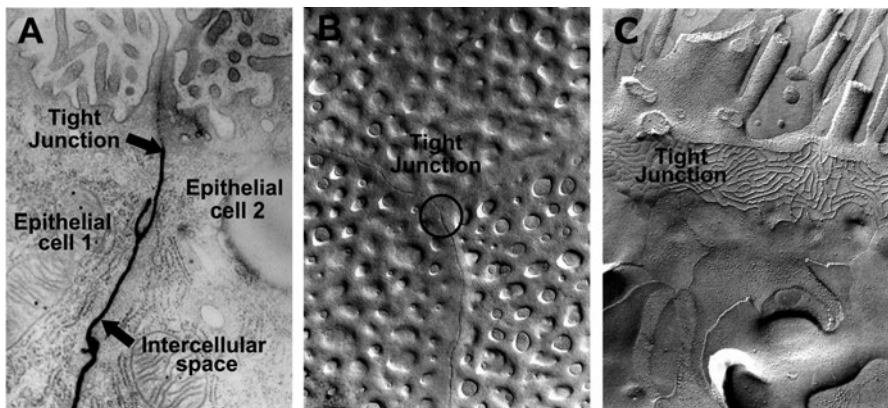
Exchange of substances through transporting epithelia proceeds through a transcellular and a paracellular route. It requires two fundamental features of TrEp: the first one consists of polarized cells that have an apical membrane domain which is structurally, molecularly, and physiologically different than the basolateral one. And the second one, the paracellular route, proceeds instead between the epithelial cells and is limited by cell-cell contacts called tight junctions (TJ) that partially seal the interspace between cells and transform them into selective permeability barriers restraining substances flow through the intercellular space [5, 6].

## Evolution of Knowledge on the Tight Junction Structure and Composition

For almost a century, the anatomical formation of TrEp was studied by dissecting a frog skin and gastric or intestinal mucosa and mounting them between two Lucite chambers with saline solutions on both sides. Fluxes through the intercellular spaces were practically negligible, because these spaces were sealed by molecular and anatomical structures, whose details could not be observed with optical microscopes.

This seal was also expected to be impermeable and located at the very limit of cells between the lumen and intercellular space. Therefore, it is understandable that the anatomical formation detected by light microscopy at the outermost edge of intercellular space received names such as *Schlussleisten*, “terminal bars,” *bandelettes de fermeture*, “hoops,” “occluding junctions,” “tight junctions,” “gaskets,” and “attachment belts” [7–12].

The introduction of electron microscopy from 1950 to 1955 permitted the observation of plasma membranes stained with osmium tetroxide ( $\text{OsO}_4$ ), and the plasma membrane appeared as a sequence of three layers, [dark]-[light]-[dark], which corresponded to the [cytoplasmic polar groups]-[hydrophobic chains]-[external polar groups] of biochemical models. The finer resolution of electron microscopy also revealed that the “terminal bar” is, in fact, a complex of different types of specialized intercellular junctions, which received the names TJ (*zonula occludens*), intermediate junction (*zonula* or *fascia adherens*), and desmosome (*macula adherens*) [6]. In fact, desmosomes were well known from earlier studies, and neighboring cells may also establish gap junctions. In Fig. 1a, the intercellular space shown



**Fig. 1** Structure of tight junctions. (a) Transmission electron microscopy of two adjacent cells from the epithelium. Lanthanum hydroxide applied from the lower solution; it cannot diffuse beyond the TJ. (b) Freeze fracture showing the meeting point of three epithelial cells, with the corresponding TJ. (c) A freeze fracture of the epithelium of the mouse's small intestine that passed exactly at the level of a TJ, showing the belt of junctional strands. In some of them, the cut passed through the membrane of one of the cells and in some other segments through the neighboring one

between two neighboring cells is full of lanthanum hydroxide, this marker was added at the basal side of the epithelium, and it diffused freely between the cells until the TJ stopped it [13–17].

A few years later, freeze-fracture studies of both epithelia and endothelia revealed that TJ consists of a distinctive reticular pattern or meshwork of fibrils embedded in the plane of the membrane. Figure 1b shows the intestinal epithelium as seen from the lumen. The apical domain appears as an archipelago in the upper part of the photo because the microvilli were cut in several transverse angles during the preparation of the specimen, giving the appearance of a cleared woodland. Three neighboring cells contact each other at the TJ, which looks like a piece of needlework. And in Fig. 1c, the TJ appears as if it was made of different strands that came together to form a continuous belt, which forms a seal all around the outermost edge of the intercellular space [18–21].

When studies of membrane permeability to water and solutes were extended from single cells to the epithelia, it seemed natural to assume that in these structures, permeation occurs across cell membranes and not through the intercellular space [12, 22–28].

The suggestion that the occluding junction essentially constitutes a tight seal was supported by a demonstration that the diffusion of macromolecules detectable by transmission electron microscopy, such as hemoglobin and ruthenium red, stops exactly at the locations of these junctions (Fig. 1a) [23, 26, 29].

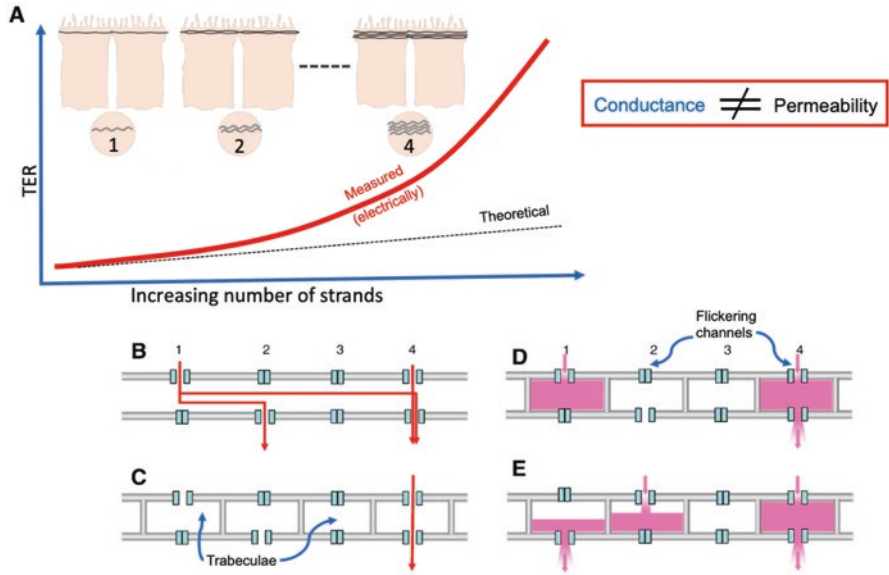
## Principal Functions of Tight Junction

The TJ have two principal functions in the TrEp; they act as gate and fence. The fence function of TJ refers to the maintenance of polarity as mentioned before, and TJ appears as a flat meshwork of anastomosing filaments in freeze-fracture surrounding the basolateral side at the outermost limit of the intercellular space and restricts the movement of the different components of the membrane from the apical to the basolateral domains maintaining the polarity of the plasma membrane, allowing the vectorial transit across TrEp [30].

The gate function refers to the capacity of TJ to regulate the passage of ions, molecules, and water through the paracellular pathway and can be detected by measuring the transepithelial electrical resistance (TER) of the tissue.

There are epithelia with high electrical resistance (i.e., the epithelium of the frog skin, above  $1500 \Omega \cdot \text{cm}^2$ ) and epithelia such as those of the small intestine, the gallbladder, and the proximal segment of a nephron with comparatively low resistance (approximately  $20\text{--}80 \Omega \cdot \text{cm}^2$ ). Obviously, the epithelia with high electrical resistance have a very small water flux through the paracellular route, while those with low electrical resistance have large fluxes through the paracellular route [31–34].

Decades ago, Phillippa Claude and Daniel Goodenough posed an obvious assumption, where each strand is an electrical resistor. If so, TER should increase linearly with the number of strands (Fig. 2a, segmented black line) [35]. However,



**Fig. 2** The role of trabeculae and flickering channels. (a) Segments of transporting epithelia with TJ having one, two, and four strands. The dotted black line below shows the theoretical correlation predicted. Redline instead depicts the actual TER found experimentally. (b) represents a TJ with two strands and randomly flickering ion channels. (c) is a TJ with two strands, randomly flickering channels but with trabeculae. Red lines represent electric currents applied to measure TER. (d) and (e) are the same TJ but explored through the diffusion of a colored marker. Red rectangle: electrical conductance is not equal to permeability

when the electrical resistance of different epithelia is plotted against the number of strands in their TJ, it is observed that the increase in resistance with each additional strand is not linear [2].

Accordingly, we put forward a theoretical model explaining the relationship because a TJ is by no means a simple series of strands, but they have trabeculae and flickering channels that explain satisfactorily the values of TER found experimentally [5, 36]. Since each strand acts as a resistor, a TJ composed of two strands should in principle have a TER value twice that of a TJ with only one strand, while one with five strands would have a TER value five times higher and so on. Contrary to this theoretical expectation, the actual relationship between the TER and strand number found experimentally is not linear, but rather corresponds to the line shown in Fig. 2a. To account for this peculiarity, Phillipa Claude suggested that the strands possess channels that can be open or closed. TER should be the inverse of conductance (G). In turn, the conductance G and permeability should be directly related, i.e., conductance is the electrical manifestation of ion permeability. However, working with María Susana Balda and Karl Matter [36, 37], we discovered that in some situations in which we experimentally modified TJ, conductance (measured through the passage of current) and diffusion (measured through the flux of radioactive tracers) varied independently.

We produced a different model depicted in Fig. 2b. It represents a segment of a TJ that contains only two horizontal strands with ion channels. An electrical current (red lines) crossing the first strand can immediately cross the second through any of the ion channels that happen to be open at that precise moment (i.e., simultaneously). However, if the TJ has trabeculae (segments of strands going vertically from one strand to the next) (Fig. 2c), a current crossing the first strand through channel 1 can proceed only if the corresponding channel in the lower strand is also in the open configuration, i.e., through the fourth arrangement of channels in Fig. 2c. Figure 2d and e illustrates a situation in which permeability is studied by adding a tracer to the upper compartment. The tracer penetrates the TJ through any of the channels that happen to be open (channels 1 and 4 in the example). While the tracer that has penetrated through channel 1 is not able to keep flowing because the channel in the lower strand is closed, the tracer that penetrated through channel 4 can keep diffusing through the lower strand because this channel is also open. A moment later (Fig. 2e), the channel in the lower strand opens, and the tracer can pursue diffusion. Because the strands in the TJ contain trabeculae, the TJ is “compartmentalized”; thus, the overall increase in the TER is more pronounced with the addition of further strands (Fig. 2a, red curve).

In this description, we refer to a static arrangement. However, it must be taken into account that the structure and degree of tightness depend on the actin cytoskeleton and vary in response to intracellular signals mediated by a large number of protein species, including PKC, PLC, adenylate cyclase, calmodulin, nonreceptor tyrosine kinases, and G protein receptors. Thus, the junctional belt around the cells “is highly dynamic.” The TJ may even reversibly disassemble to allow the passage of leukocytes. It may also change as a cell ages or be present only during specific stages of development.

TJ do not communicate with neighboring cells. Cell-cell communication is due to communicating gap junctions [15]. The confusion comes from the fact that gap junctions are often established between the strands of TJ [13, 15–17]; hence one erroneously attributes communication to junctional strands.

## The Study of Tight Junction’s Assemble

Since the multitude of molecules that constitute the TJ and other cell-cell and cell-substrate junctions, many membrane molecules with a polarized distribution and the highly complex mechanisms responsible for junctions and polarization were perfected along ages of evolution. Thus, it is evident that this large number and variety of molecular species that required millions of years of evolution might not have coincided within minutes in the same multicellular organism.

Of course, in those years, epitheliologists took samples of mature epithelia to study the generation of TJ, but the TrEp was useless because TJ and polarity are synthesized, assembled, and functionally expressed in mature epithelia. To avoid this difficulty, it occurred to us to devise an artificial epithelium by seeding MDCK

cells derived from a dog kidney and cultured them at confluency on an artificial and translucent nylon net, covered with 1 cm in diameter of collagen, and left them overnight. A priori, the probability that the monolayer of the cell would attach to the collagen/Nitex support and have only one layer because no cells would attach to the apical border of the already attached cells was so remote that we almost discarded our plans. However, it is very hard to throw away a cherished idea: we did try to make an artificial transporting epithelium. Our enthusiasm grew as we started to constate that it worked! Twenty hours later, the discovery was delightful due to cells had established TJ, and they had polarized.

However, we realized that those cells, obtained by harvesting with EGTA (a calcium chelator) and trypsin (an enzyme that hydrolyzes peptides into their amino acidic building blocks), were seriously damaged and spent most of the overnight hours repairing their structure and cell membrane. In this case, the cells were seed at confluency; after 30 min, the cells were transferred to a  $\text{Ca}^{2+}$  and cell-free medium. The next day, the confluent monolayer of cells did not have either TJ or polarity. Nevertheless, upon switching them to media-containing  $\text{Ca}^{2+}$ , the cells developed TJ and polarized in less than 2 h. We name this technique “calcium switch” and used to investigate how transporting epithelia develops TJ and cell polarity [38–40].

It must be taken into account that this synthesis of junctions and polarity takes place in cells that already have all the mechanisms and molecules involved or can synthesize them de novo if these were destroyed by trypsin. However, it is taken for granted that processes would mimic normal synthesis and assembly of TJ. The assumption is justified by studies on natural preparations, such as synthesis of TJ in the villi of the intestinal mucosa and other instances where cells migrate from the depth of a crypt to the apex of villum, or observed in steps from morula to embryo [41–44].

## Tight Junctions Under Special Situations

TJ's proper regulation in transporting epithelia allows the permeation of enormous macrophages while entirely blocking the passage of small molecules of toxins produced by an infection with bacteria [45]. On the other hand, the epithelia that form a nephron are capable of producing TJ with a TER precisely needed to withstand the osmotic gradient between plasma and the filtrated liquid circulating in the lumen of a particular segment. It's not surprising that failures or the absence of TJ expose the organism to grave risks [46].

The relationship between failures of TJ and terrible pathological processes, most of them autoimmune, is when TJ allow the passage of molecules that should not reach the extravascular space, contact the immune system, and trigger the synthesis of antibodies. These risks are mainly prevented by the property of TJ to be established in the mixtures of epithelial cells derived from any organ and even derived from different animal species. This property explains why multiple transitions from

one type of the epithelium to a different one along the digestive tract are perfectly sealed.

TJ are not only found in epithelia but also between endothelial cells of capillary vessels. As in the case of epithelia, the comparison of the relatively low permeability of the plasma membrane with the relatively high one of the capillary wall suggested long ago that most of the transendothelial flux of water and small solutes occurs in the intercellular space. This pathway is also limited by the tight junction, except for the endothelium of microvessels in hemopoietic tissues. The tightness of endothelial TJ may be very low, as in the spleen and endocrine glands, or very high, as in the brain and the retina. The number and arrangement of the strands in endothelia also vary from arteries and veins to small vessels [27, 47].

Additionally, under certain circumstances, TJ can be traversed by whole germ cells. They may also be traversed by leukocytes migrating toward the side of infection. This process seems to be quite delicate, as the seal is reestablished after the leukocyte reaches the opposite side.

TJ may even be found between cells that are neither epithelial nor endothelial, such as those of the glia [48], muscle fibers [49–51], and fibroblast [52], and may even be present between two regions of the same cell.

## Remarks

It has been more than a century after TJ have attracted the attention of light microscopists. Furthermore, the TJ is no longer considered a static, almost inert seal, whose only role is a mechanical barrier to the passage of substances. Today the “lip” of the TJ observed by transmission electron microscopy appears to be the tip of an iceberg, where the cytoskeleton, cell-cell contact molecules, scaffolding proteins, calmodulin, protein kinase C, phospholipase C, adenylate cyclase, and G-proteins coordinate to afford a weak sealing of just  $10 \Omega \text{ cm}^2$ , as in the proximal tube of the kidney, or a strong blockade of several thousands of ohms as in the urinary bladder.

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