

# The origin of human epithelial tissue

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**Summary.** The histological structure of human epithelial tissue is complex, but all epithelia share three major features: cohesion, polarity and attachment. These functions are mainly achieved by the presence of specialized structures such as intercellular junctions, polarity protein complexes and basement membranes. In the present review, we have analyzed the presence of each of these structures in several groups of animals that are considered to be at the base of the animal evolution tree. Interestingly, these characters seem to have evolved independently, and a careful histological and structural analysis of the phylogenetic tree shows different groups of animals in which epithelia are absent and groups in which cells show only some of the specialized structures found in differentiated epithelia. These findings could contribute to understand how epithelial tissues evolved and determine their current protective functions.

**Key words:** Histology, Epithelium, Evolution

## Introduction

Deciphering the exact structure and composition of the human body has been one of the main goals of biomedical science for centuries. With the development of the first microscopes capable of amplifying human structures, histology was born as a scientific discipline (Mazzarini et al., 2021). However, it took two centuries of research before the cell theory was established by Schleiden and Schwann (Mazzarello, 1999), who demonstrated that our body is mainly composed by cells and extracellular products generated by these cells. With the generalization of this theory to the nervous tissue by Ramón y Cajal, the cell theory became universally applicable to all human structures, and it is currently accepted that the human body consists of a well-defined combination of tissues whose functions are strictly

coordinated (Mazzarello, 1999; Campos, 2015).

In general, it has been estimated that the human body contains around  $3.0 \times 10^{13}$  cells (Sender et al., 2016), although the exact number of cell types in the human body is still in debate, and novel cell types are described every year in the literature (Regev et al., 2017). Human cells are very well organized, forming numerous specific types of tissues that are considered to be variations of four types of basic tissues: epithelial, connective, muscle, and nervous tissue (Khan and Farhana, 2021; Neumann and Neumann, 2021). The definite function of each organ and each structure derives from the exact combination of tissues found in such structures.

From the beginning, histological studies were based on direct observations of human tissues using magnifying instruments, and evolutionary studies were not described until very recently. From the evolutionary point of view, the complex conformation of the human body is the result of millions of years of sequential changes driven by evolution (Gregory, 2008). From the simplest forms of life found in the fossil registry to the current evolutionarily advanced animals, evolution gave rise to numerous different body plans and animal types, but also to the different tissues of modern animals. According to their body symmetry, modern animals can be classified as non-symmetrical (poriferans and placozoans), non-bilaterian animals with radial symmetry (cnidarians and ctenophores) and bilaterian (evolutionarily advanced groups of animals showing bilateral symmetry) (Richter and King, 2013). In turn, the embryonic development of each of these groups of animals reveals important differences, with non-symmetrical animals showing only one germ layer, non-bilaterian animals normally showing two germ layers (diploblastic animals) and bilaterians having three germ layers at the earliest embryonic stages (triploblastic animals) (Hashimshony et al., 2015). A structural analysis of the basic tissues found in each group of modern animals might allow us to reconstruct the phylogenetical history of human tissues and shed light on the evolutive process of development of these tissues.

Apart from evolutive studies, the rapid development of genomic and proteomic analysis methods have significantly contributed to histological science, and

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recent reports based on these methods were able to describe important histological features in several types of human tissues (Kangas et al., 2022). Most likely, application of these techniques to other types of human tissues will contribute to improve current knowledge on the fine structure and function of human tissues.

In the present work, we have revised the current knowledge on the development of the epithelial tissue from an evolutive perspective and we have analyzed the different tissues related to the epithelium that can be found in the different currently-living modern animal groups in order to shed light on the origin of the epithelial tissue from an evolutive standpoint.

### A brief history of early animal evolution

Animal evolution is a complex process, and many questions are still unanswered. As the simplest forms of life, unicellular organisms were the first that appeared in the fossil registry. However, these organisms derived from previously-formed macromolecules that joined together to form the first living cell, and it is thought that RNA was the first macromolecule that appeared on our planet (Orgel, 2004). Assembling of different types of macromolecules was the key phenomenon that gave rise to the last universal cellular ancestor (LUCA), from which all living cells derived (Krupovic et al., 2020). Although the exact nature of the LUCA remains controversial, the first cell was probably similar to the currently-living prokaryote cells that include bacteria and archaea life forms. Another crucial leap in evolution was the onset of the first eukaryotic cell, which has been hypothesized to happen as a result of endosymbiosis between primitive prokaryotic cells around 1.5 billion years ago (Tellez, 2014).

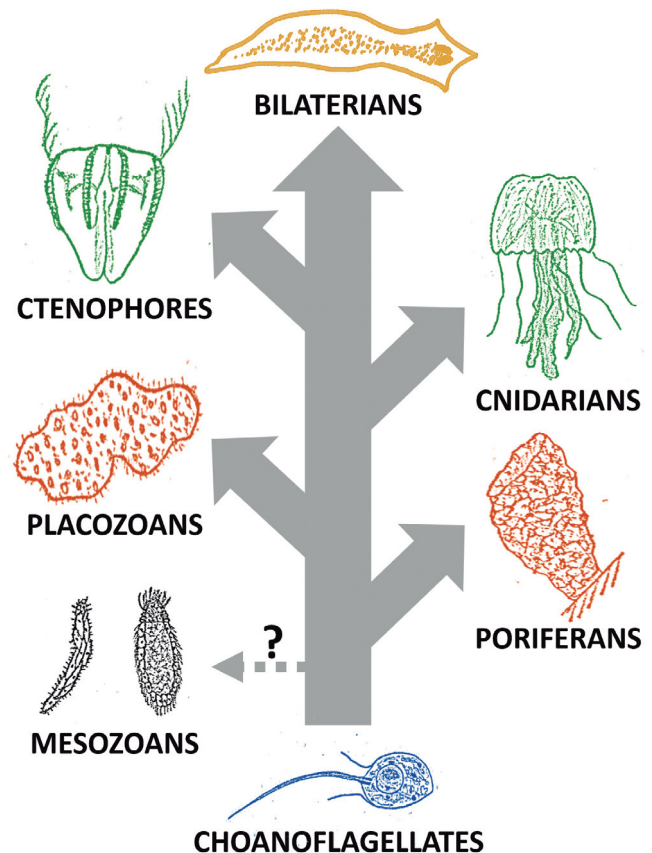
Then, the first evidence of multicellular life forms resembling a relatively complex structure was found in the early Ediacaran period (approx. 635 to 550 million years ago), when intriguing soft-bodied organisms with large size and body symmetry were found for the first time in history (Knoll et al., 2006). Although this was the first time that large species were found in the fossil registry, the real nature and fate of the Ediacaran fauna remain unknown (Darroch et al., 2018). Most likely, all these species extinguished and disappeared during the Ediacaran period, and these organisms could represent extinct groups without any modern representatives (Darroch et al., 2018). In fact, it has been hypothesized that the Ediacaran fauna was mostly composed by colonial, non-metazoan organisms (Couso, 2009).

Apart from the enigmatic Ediacaran fauna, the first clear evidence of metazoan organisms was found during the early Cambrian period, from about 539 to 520 million years ago, when morphologically complex and phylogenetically diverse animals arose (Erwin, 2020). Indeed, most modern metazoan phyla appeared at the same time during the so-called Cambrian explosion, and Cambrian fossils include several representatives of all types of modern metazoan phyla. Subsequently, it is

thought that the progressive evolution of these primitive body forms gave rise to all currently living animals. The reasons for this evolutive path of primitive animals remain unknown. However, it has been suggested that gene mutation and, especially, changes in the regulatory genome, are at the basis of evolution (Holló, 2015).

### The onset of the first metazoans

A morphological, molecular and genetic analysis of the simplest eukaryotic cells found in modern environments reveals the presence of several types of cells sharing several similarities with metazoan animal tissues. Unicellular eukaryotic organisms are currently classified in an independent kingdom called Protists (Dacks et al., 2008). However, this kingdom is very diverse and includes very different types of organisms. One of the most interesting protist cell types are choanoflagellates, which are considered to be related to metazoans (Nielsen, 2019). Choanoflagellates are abundant free-living protozoans that have been able to colonize most marine and fresh water niches and feed on bacteria. These organisms have a mobile flagellum that



**Fig. 1.** Basic classification tree of the most primitive modern animals according to their body plan configuration. Choanoflagellates are thought to be the origin of metazoans and are shown at the base of the tree.

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is surrounded by a collar consisting of numerous cilia forming a circular structure (Fig. 1) (Brunet and King, 2017). The role of the collar complex is related to nutrition, as it can induce water to flow and facilitate predation of bacteria that are phagocytized by the cell. Other groups of protists that have been suggested to be related to metazoans are filastereans and ichthyosporeans, although choanoflagellates are most closely linked with animals (Sebé-Pedrós et al., 2017).

The transition from a single-cell organism to a multicellular metazoan animal is one of the major evolutionary transitions in the history of life (Sebé-Pedrós et al., 2017; Niklas and Newman, 2020). However, the mechanisms associated with this transition are unknown, and the lack of fossils significantly hampers research in this area, and different hypotheses have been proposed. One of the hypotheses that reached higher acceptance is the idea that multicellularity evolved independently from unicellular ancestors several times during evolution, with several evolutive events in plants and fungi and one in animals, suggesting the existence of a first primitive common ancestor of all multicellular animals, which could form a monophyletic group (DeSalle and Schierwater, 2008; Niklas and Newman, 2013). In this milieu, observation of free-living modern protists offers some interesting clues. In most cases, protists live as independent single-cell organisms able to move from one place to another. However, several types of protists are capable of forming multicellular groups by cell aggregation (Sebé-Pedrós et al., 2017). In this regard, it has been found that clonal cell division allows some choanoflagellates to occasionally form colonies that are aggregated by small intercellular bridges (Dayel et al., 2011). Although these aggregate colonies cannot be considered as a metazoan animal, it could be considered as a first step towards tissue formation. Interestingly, analysis of the genomes of unicellular choanoflagellates demonstrated that these organisms contain some gene sequences showing certain homology with cadherins, although typical cadherins are not present in these organisms (Nichols et al., 2012).

The next step in animal tissue evolution would be the formation of a multicellular animal with cells showing well-developed intercellular junctions. One the most widely accepted theories is the *gastrea* theory of Haeckel (Levit et al., 2022). This theory proposes that primitive flagellated cells joined together to form a ball-shaped multicellular organism called *blastea* in which all cells were identical and had direct contact with the external environment to allow individual access to nutrients. Most likely, these primitive flagellated cells were choanoflagellates or some other type of protists related to choanoflagellates, and therefore, the first multicellular organism could have been a choanoblastea (Nielsen, 2019). The result of this transition from unicellularity to multicellularity was the onset of the first multicellular animals and, thus, the first tissues or tissue-like structures in the history of life generated by differentiation of specific cell types.

Although the genetic and molecular factors involved in the evolution of multicellularity are not well understood, some key steps have been proposed (Niklas and Newman, 2013, 2020). Among others, it is probable that unicellular eukaryotic cells experienced, at least, the following steps in their transition to a multicellular organism: 1) a dramatic change in the expression of genes related to cell cycle and reproduction, 2) the development of the SAND domain in the genome of the cells, 3) modifications of the retinoblastoma cell cycle regulatory pathway, 4) genomic modifications related to cell-adhesion genes and gene diversity, including the development of intercellular adhesion systems allowing the multicellular organism to remain together, 5) evolution of genetic pathways related to cell-specific specialization and differentiation allowing the animal to acquire complex morphological configurations and functions, and 6) development of an efficient cell-cell communication system allowing cells to function in a coordinated manner.

### The origin of epithelial tissues

The epithelial tissue covers all body surfaces and is responsible for protecting the internal tissues from the external environment, thus providing the first barrier and line of defense (Mohapatra et al., 2021). To exert this function, epithelia are typically characterized by cohesion, polarity, and attachment. Intercellular cohesion is provided by the presence of abundant intercellular junctions whose function is to keep epithelial cells firmly attached in order to prevent water loss and to establish a firm protective barrier isolating the body from the external environment. These functions are exerted by the development of several types of adherent, gap and occluding (tight and septate) junctions at the membrane of epithelial cells (Brooke et al., 2012) and depends on several types of adhesive proteins, with two major protein superfamilies playing a major role: cadherins and immunoglobulins (Srivastava et al., 2010). Polarity is related to a specific spatial orientation in which an apical pole and a basal pole are found in the epithelium itself and in each individual cell found in the epithelium. Polarity is required for a correct development and morphogenesis of the epithelium and depends on the synthesis of specific polarity protein complexes (Assémat et al., 2008). Finally, epithelial attachment is achieved by means of a subjacent basement membrane whose function is to strongly attach the epithelium to the underlying body tissues to ensure that the epithelium will remain firmly adhered to these tissues and will support external mechanical forces. The presence of several types of collagens, especially type-IV collagen and adhesive proteins such as laminins, are the main constituent of epithelial basement membranes (Gatseva et al., 2019). In addition to protection, epithelial tissues play an important role in fabrication and secretion of multiple substances, absorption of molecules, sensory reception, and other related



functions.

Due to its crucial role and its allocation as the external barrier of every organism, it is very likely that the epithelial tissue was the first tissue that developed in the most primitive animals, and epithelia are currently found in most modern metazoans. However, an analysis of the most primitive modern metazoan animals (Fig. 1) reveals that evolution of epithelia did not occur in a single stage. Instead, it is the result of a complex set of evolutive hallmarks including several major steps (Nielsen, 2008). The analysis of modern animals reveals the existence of living representatives of intermediate steps of this process.

### The intriguing case of mesozoans as one of the simplest groups of living animals

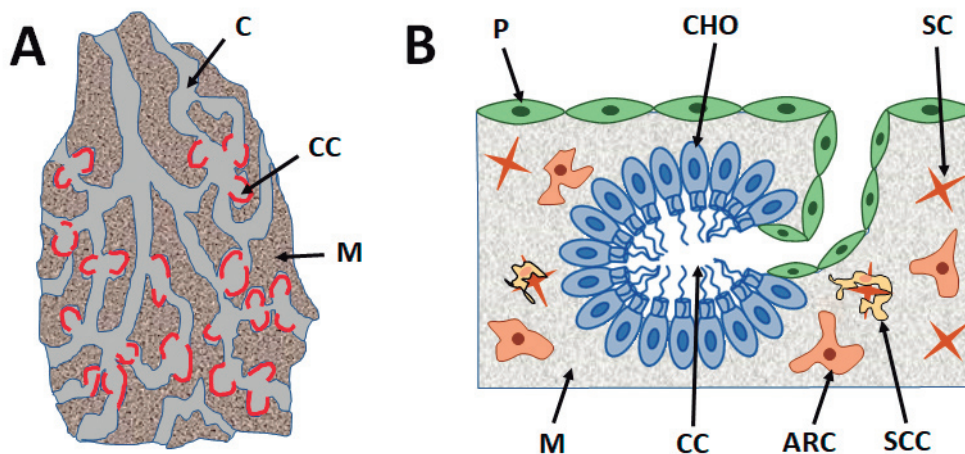
One of the simplest metazoan animals ever described were formerly included in the mesozoan group. Mesozoans are small parasites of cephalopods and other marine invertebrates and consist of a simple layer of cells covering a few internal cells that used to be reproductive cells (Schiffer et al., 2018). Due to their simplicity, mesozoans were traditionally considered as the simplest multicellular animals that could represent the link between protists and metazoans. However, recent genetic and molecular analyses demonstrated that mesozoans are derived from other groups of animals that are evolutionarily more advanced -probably, annelid worms- that experienced a dramatic body simplification and reduction, resulting in extremely simple animals as a result of their parasitic behavior (Zverkov et al., 2019). In fact, mesozoans are protected by a well-developed epithelial tissue showing intercellular junctions and the genome of these animals contains a series of genes that are typical of more evolved animals. In this regard, the cladistic analysis of the genes encoding for the 18S ribosomal RNA reveals important sequence similarities with triploblastic metazoans, suggesting that mesozoans could be simplified triploblastic bilaterian animals

(Zrzavý et al., 1998).

One of the most intriguing species within the mesozoan taxon is *Salinella salve*, a mysterious animal found only once at the end of the XIX century that seems to have disappeared since then (Tihelka and Cai, 2021). According to the only available reports on this species, *Salinella* is a very simple animal consisting of a single layer of ciliated cells and was initially classified as a monoblastozoan, i.e., animals formed from a single cell layer during embryonic development. As in the case of the rest of mesozoans, it is probable that *Salinella* evolved as a result of simplification and regression, although the lack of specimens available for study makes it impossible to demonstrate its real nature and origin. In consequence, evidence suggests that mesozoans are not the basis of evolution of modern animals and rather constitute secondarily reduced animals.

### The first proof of multicellularity is found in poriferans

Among the currently living animals, poriferans - sponges- are considered to be the most primitive metazoans. Sponges lack real tissues or organs, and their structure and physiology are very simple compared to evolved animals. For these reasons, poriferans show specific features of single-cell protists and complex metazoan animals (Adamska, 2016). Although the poriferan group is highly diverse important morphological and structural differences exist between specific groups, most sponges consist of a series of chambers that are interconnected by canals through which water containing nutrients is forced to circulate (Fig. 2) (Leys et al., 2005). Histologically, chambers are covered by choanocytes, the main cell type of poriferans, which contain a flagellum surrounded by a collar consisting of multiple microvilli. This structure is very similar to that found in choanoflagellates, and is responsible for inducing water circulation inside the animal and for capturing food particles (Adamska,



**Fig. 2.** Illustrative representation of the structure of poriferans. **A.** Gross section of the body of a typical sponge. **B.** Histological structure of a poriferan. C, canals; CC, choanocyte chamber; M, mesohyl; P, pinacocytes; CHO, choanocytes; SC, skeletal components; ARC, archeocytes; SCC, sclerocytes.

2016). In turn, the surface of the animal and some of the large canals are covered by flat cells called pinacocytes, whose function is protective and do not absorb food particles. Between the network of chambers and canals and the external layer of the animal covered by pinacocytes, a very primitive structure called the mesohyl is found. The mesohyl contains primitive skeletal components (calcified spicules, silicon dioxide skeletal elements or spongin fibers), sclerocytes (cells in charge of synthesizing the skeletal components) and archeocytes (totipotent undifferentiated cells) (Fig. 2). One interesting feature of modern sponges is totipotentiality, as it has been demonstrated that each poriferan cell can differentiate to all the other cell types of the animal (Müller, 2006).

Numerous studies have demonstrated a high homology between choanocytes and choanoflagellates, whose morphology, structure and ultrastructure are very similar (Brunet and King, 2017). Both types of cells have the typical collar complex with almost the same components and structure, although some specific adaptations to life in a chamber were found in choanocytes as compared to free-living choanoflagellates (Mah et al., 2014). In both cases, the role of these cells is capturing food particles, mainly bacteria, after attracting them to the collar by generating specific water flows, and digestion is intracellular in each specific cell, without the existence of a digestive system (Godefroy et al., 2019). All these similarities support the porifera-first hypothesis (Nielsen, 2019), in which poriferans are the first step in the evolution of animal multicellularity and sponges would be directly related to single-cell protists. Recent phylogenomic analyses are in agreement with this hypothesis (Simion et al., 2017).

However, sponges are believed not to have real tissues and have been classified for decades as non-epitheliozoan animals lacking true epithelial tissues, and it has been suggested that water can move and spread among all the sponge cells (Adams et al., 2010). The cells that make up the different histological layers of poriferans are devoid of the most important structures and features found in well-differentiated tissues of other types of animals (Adamska, 2016). As for the choanocytes and pinacocytes, these cells are attached to each other, but the typical intercellular junctions and the basement membrane found in all epithelial tissues are not found or appear only as immature, rudimentary precursors of these structures (Niklas and Newman, 2013). Therefore, poriferans have been traditionally considered as animals with spatially juxtaposed cells rather than real epithelial tissues. Although recent genetic and molecular analyses found that certain species of sponges may have a primitive epithelial-like tissue or a pseudo-epithelium able to express some of the main constituents of animal epithelia, this structure is structurally less differentiated and less developed than typical epithelia (Jonusaite et al., 2016).

In general, the structure of canonical adherent, gap and occluding junctions (Brooke et al., 2012) was never

found in poriferans cells. However, primitive forms of intercellular attachment specializations were found, including an accumulation of adherent-like proteins in the membrane of adjacent pinacocytes (Lethias et al., 1983), parallel membrane structures showing a specific three-dimensional configuration of the plasma membranes of adjacent cells and certain types of septate junctions (Adams et al., 2010). The presence of these cell-cell junction structures would explain the fact that some types of fresh-water sponges, which need to face a hyposmotic environment, are able to form a cellular barrier showing transepithelial electrical resistance (Adams et al., 2010) and why free-living sponge embryos can swim without detaching. Whether fresh-water sponges developed these unique features late during evolution is a question that remains unanswered. At the genetic level, it has been demonstrated that gene sequences with partial homology to several cadherins and immunoglobulins are represented in the genome of several poriferans (Srivastava et al., 2010), although significant differences with the genes of highly evolved animals were found. Other genes related to claudin, Scrib and Dlg were also found in certain species of poriferans (Jonusaite et al., 2016). These findings reveal that poriferans constitute a very diverse group of animals in which species with different characteristics and different degrees of evolution coexist in the same animal taxon, with all of them belonging to a primitive evolutive stage as compared to other groups of animals. The fact that poriferan cells are considered to be very poorly differentiated and show interchangeability among cell types and even totipotentiality, confirms the primitive status of poriferans (Müller, 2006).

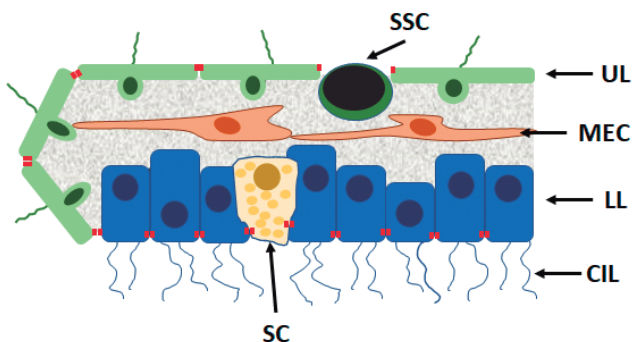
These findings are in agreement with the analysis of embryonic development in poriferans. It is well known that poriferans do not have several embryonic layers, and all body constituents derive from a single layer of cells that is able to adopt increasingly more complex three-dimensional configurations but do not experience gastrulation (Ereskovsky and Dondua, 2006). During embryogenesis, poriferan embryos experience a process of simple invagination that cannot be considered as gastrulation and probably occurred as a process of adaptative convergence with typical metazoans (Richter and King, 2013). Although differences among groups of sponges may exist, the absence of gastrulation and intestinal epithelium making digestion exclusively intracellular confirms that poriferans show only one germ layer.

### Placozoans: the first evidence of cell cohesion

Analysis of currently existing animals gave rise to the discovering of a very interesting group of metazoans called placozoans, represented by a single species, *Trichoplax adhaerens*. These animals are very small flattened benthic organisms that grow attached to the substrate (Schierwater et al., 2009). In many senses, placozoans are considered to be among the most

primitive and simplest animals in nature. Histologically, placozoans are formed by an upper layer of flat ciliated cells whose function is protective, with scattered rounded cells named shiny spherical cells, and a lower ciliated cell layer in charge of feeding (Srivastava et al., 2008; Smith and Reese, 2016). The lower layer cells constitute 72% of the total cell population and are organized at different levels, resembling a pseudostratified epithelium, with scattered secretory cells (Smith et al., 2014). Multi-nucleate elongated cells and interstitial liquid reside between both epithelia, but placozoa do not have extracellular matrix (ECM) or basement membranes (Schierwater et al., 2009) (Fig. 3).

Even with the lack of basement membranes, placozoans upper and lower cell layers resemble epithelial tissues in several aspects, especially regarding intercellular cohesion. Unlike poriferans, placozoans are the first group of metazoans showing true, well-differentiated intercellular junctions able to form a protective barrier to the external environment. Although gap and tight junctions have not been described in placozoans, cells in both external cell layers are kept joined by abundant adherent junctions connected to apical actin fibers resembling the situation of true epithelia (Smith and Reese, 2016). Moreover, genetic analyses showed that genes related to the cadherin/ $\beta$ -catenin/ $\alpha$ -catenin complex, which are necessary for the establishment of adherent junctions are present in the placozoan genome (Belahbib et al., 2018). Although these are not true epithelia due to the lack of a basal membrane and other types of intercellular junctions, both cell layers are commonly known as upper epithelium and lower epithelium and these animals are often considered as the first epitheliozoans. The morphological findings suggesting that placozoans are evolutionarily more advanced than poriferans were also confirmed at the phylogenetic level. In this regard, whole-genome phylogenetic analyses classify placozoans at the basis of a group including more complex animals, and exclude poriferans from this group



**Fig. 3.** Illustrative representation of the histological structure of a placozoan. UL, upper cell layer; MEC, multi-nucleate elongated cells; LL, lower cell layer; CIL, cilia; SC, secretory cell; SSC, shiny spherical cell. Intercellular junctions joining two adjacent cells are represented in red.

(Srivastava et al., 2008). However, most researchers consider that placozoans should not be considered as eumetazoans due to their structural and genetic simplicity, although it is clear that their position in the evolutive tree would be above poriferans (Richter and King, 2013).

As in the case of the sponges, it is thought that placozoa embryonic development is very simple, and only one germ layer is formed. The fact that digestion is exclusively intracellular, and no specific digestive epithelium is formed during development, supports this assumption (Ereskovsky and Dondua, 2006). Although a true epithelium is not found in placozoans, the development for the first time of a tight protective cell barrier containing functional intercellular junctions is a major breakthrough in tissue evolution. In fact, it has been proposed that cell interconnection through intercellular junctions allows the efficient interchange of nutrients among cells in the epithelium, thus allowing nutrition of all cells, including those that are not specialized in feeding (Nielsen, 2019). Therefore, this process is at the basis of cell differentiation and specialization, as a subpopulation of cells can now specialize in specific functions by not having to actively seek nutrients.

#### The onset of a true epithelial tissue in cnidarians and ctenophores

Cnidarians and ctenophores are aquatic animals showing radial symmetry. Although still primitive, cnidarians and ctenophores show the first evidence of true epithelial tissues, suggesting that both types of animals could have evolved from more primitive groups lacking true epithelia and cnidarians and ctenophores might have given rise to bilaterian animals (Li et al., 2021). In fact, several researchers suggest that evolutionarily advanced animals might derive from ctenophores rather than poriferans, and that simplicity in poriferans could have arisen secondarily. This hypothesis, called the ctenophore-sister hypothesis, would imply that non-symmetrical animals would not be the origin of bilaterian animal groups (Simion et al., 2017). Due to the presence of a true epithelium, cnidarians and ctenophores are considered as the first eumetazoan animals in the animal kingdom (Richter and King, 2013).

Structurally, cnidarians and ctenophores consist of an external single-cell epithelial layer derived from an embryonic ectoderm called epidermis, an internal simple epithelial layer derived from the endoderm called gastrodermis, and a thick intermediate layer often known as mesoglea. The mesoglea is not considered as a true tissue, and is formed by a mixture of several types of collagens and basement membrane components immersed in an abundant amount of water with very few or no cells (Fidler et al., 2014). Although the mesoglea is flexible, its mechanical stiffness is very poor (Sarras and Deutzmann, 2001). The external epithelium contains

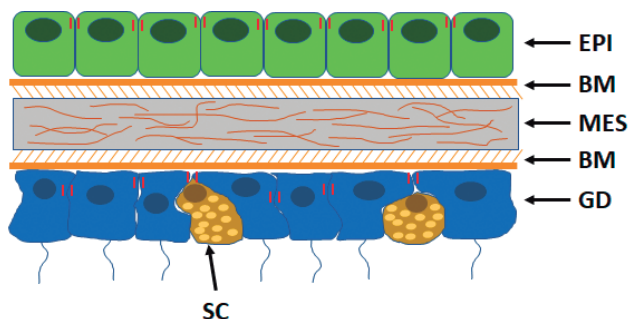


## Origin of epithelia

differentiated cells playing an important role in protecting the body contents, whereas the internal epithelium is specialized in absorbing food particles. Analysis of both epithelial layers reveals the typical properties of true epithelia, including cohesion, attachment and polarity (Fig. 4) (Vernale et al., 2021).

Regarding cohesion, it has been demonstrated that external and internal epithelia of cnidarians and ctenophores are joined together by abundant septate occluding junctions controlling the protein transit between adjacent cells (Smith and Reese, 2016). These junctions are organized as belt structures surrounding the apical membrane of epithelial cells, although differences among specific groups of animals have been described (Ganot et al., 2015). In addition, cnidaria possess belt desmosomes and gap junctions contributing to epithelial cell-cell cohesion, like the rest of epithelozoan animals (Ganot et al., 2015; Skerrett and Williams, 2017). The combination of several types of intercellular junctions provides these animals with strong cohesive properties and establishes a tight protective barrier to the external environment.

Epithelia are tightly attached to the subjacent tissues by well-defined attachment structures organized for the first time in the tree of life as a basement membrane. Basement membranes are specialized structures found at the interface between the epithelium and the connective tissue (Gatseva et al., 2019). The basement membrane allows the interchange of substances between the epithelium and the connective tissues and is able to regulate cell function, differentiation and development (Fidler et al., 2014). In the case of cnidarians, it has been demonstrated that the basement membrane, as well as the mesoglea, contain all the typical molecules found in the human basement membrane such as type IV collagen and other types of collagens, proteoglycans, laminins and fibronectin (Zhang et al., 2002; Parisi et al., 2021). In addition, it has been reported that cnidarians express the sulfilimine bonds that are required for an efficient physiology of collagen IV in basement membranes (Fidler et al., 2014), suggesting that the basement membrane found in these animals could be functional.



**Fig. 4.** Illustrative representation of the histological structure of cnidaria and ctenophores. EPI, epidermis; GD, gastrodermis; MES, mesoglea; BM, basement membrane; SC, secretory cell. Intercellular junctions joining two adjacent cells are represented in red.

Polarity is a crucial feature of true epithelia and is a consequence of cell differentiation and specialization (Nielsen, 2019; Wang, 2021). Acquisition of epithelial polarity allows the epithelium to adapt to specific environmental situations by adopting a particular mechanical behavior adapted to these situations. Polarity requires a series of complex genetic and molecular mechanisms related to cell function and differentiation, such as cytoskeleton contractility, migration, folding, invagination, deformation and other specializations related to environmental adaptation (Wang, 2021). These mechanisms are mainly controlled by three polarity complexes: Crumbs, Scribble and Par and by a high number of genes and gene pathways that need to work coordinately (Vernale et al., 2021).

A very interesting consequence of the development of true epithelia able to establish a tight barrier to the external environment is the possibility of cell specialization and differentiation. In the case of cnidarians, we found that two different layers are formed by cell differentiation during embryonic development: the ectoderm and endoderm, implying that cnidarians are the first animals that can be considered as diploblastic organisms (Nielsen, 2008). Once the endodermal layer is formed as a properly sealed epithelium, cnidarian embryos display a typical invagination to create a gut cavity that will allow extracellular digestion to occur in a closed space surrounded by endodermal cells (Ereskovsky and Dondua, 2006). Cell specialization and differentiation derived from this phenomenon opened the door to the complex differentiation processes found in bilaterian animals. On the other hand, ctenophores seem to have gone one step further than cnidaria regarding embryonic development. Although the general structure of both cnidarians and ctenophores is very similar, and both types of animals show the same types of epithelia, ctenophore embryos have developed the mesoderm layer and can be considered as the first triploblastic organisms, along with bilaterians (Nielsen, 2008).

## Evolution and specialization of epithelial tissues in bilaterians

Most animal groups, including mammals can be classified as bilaterians. Once the true epithelial tissue was developed in cnidaria and ctenophores, more evolved groups of animals kept this feature and added new molecular components to their epithelia to achieve improved tissue functions. From the most primitive acoelomate animals devoid of a general cavity of the body (platyhelminths and nemertines) to the evolutionarily advanced coelomates (mollusks, annelids, echinoderms and chordates, among other groups), all these animals possess well-defined epithelia (Fig. 5) and three embryonic layers and are therefore defined as eumetazoa (Richter and King, 2013). In fact, all bilaterians have properly-structured basement membranes containing type IV collagen, several types of intercellular junctions and other features that are typical

of well-differentiated epithelia, such as stratification and differentiation in basal and supra-basal cell layers (Nielsen, 2019).

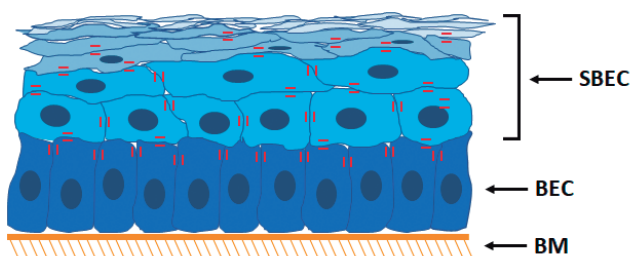
Transition from a diploblastic to a triploblastic body plan implies the formation of specific tissues and organs, most of which consist of a combination of several types of basic tissues, including epithelial tissue. Therefore, epithelial tissues found in bilaterians show specific differentiation patterns according to their location and function, and it has been demonstrated that different cell types allow epithelia to exert their functions at the tissue level to maintain homeostasis (Montoro et al., 2020). In general, epithelia found in bilaterian animals can reach increasing levels of complexity during differentiation, and the following types of epithelia have been described according to their histological structure (Bragulla and Homberger, 2009): 1) simple epithelia, characterized by the presence of a single layer of epithelial cells, 2) pseudostratified epithelia, showing a single layer of cells reaching different height, with all cells keeping contact with the basement membrane, 3) stratified epithelia showing several cell layers including a basal layer and one or more suprabasal cell layers, and can be cornified or non-cornified, and 4) transitional epithelia, which is a modification of stratified epithelia in which cell morphology can change in response to stretching. Each configuration depends on the activation of specific gene pathways and the synthesis of biomolecules related to epithelial cell differentiation. Among the most important biomolecules driving differentiation, cytoskeleton components stand out because of their role in controlling cell morphology and response to biomechanical stress forces, including microfilaments, microtubules and intermediate filaments (Bragulla and Homberger, 2009) that can be more or less developed depending of the specific type of epithelia. One of the main components related to epithelia differentiation are the cytokeratins, which appear very late in evolution and are typical of vertebrate epithelia (Fuchs, 1983; Bragulla and Homberger, 2009). Their function is very relevant for epithelial physiology, and each type of epithelium and each epithelial cell have been suggested to express its own subset of keratins. As an example of this, basal cells of vertebrate epithelia show abundant amounts of

keratins with a molecular weight of 46, 50, 56, and 58 KD, whereas differentiating epidermal cells express larger keratins (60-70 KD) (Fuchs, 1983). Development of genes encoding for each kind of keratin and for regulating their gene expression is at the basis of the evolution of the different types of animals classified within the bilaterian group (Holló, 2015).

Although bilaterians is a very heterogeneous group, a common characteristic of bilaterian animals is the presence of a set of genes of the HOX family controlling embryogenesis and determining the body plan along the head-to-tail axis of bilaterian animals (Mallo, 2018). HOX genes are involved in the determination of segmental identity and body configuration, and their expression can guide specific cell populations during embryonic morphogenesis (Mallo and Alonso, 2013). Regulation of HOX genes expression is very complex and is interconnected with other signaling pathways such as the WNT, FGF and GDF pathways, and shows differences among animal groups (Alexander et al., 2009). However, the basic mechanisms of HOX function are evolutionarily conserved and can be used to trace phylogenetic relationships between the different groups of bilaterians. The different morphogenetic patterns found in each kind of animal allowed evolution to develop the multiple types of bilaterians currently found in nature, as morphogenesis enabled animal form diversification via development (Richter and King, 2013).

## Conclusions

As the most external protective layer of the organism, the epithelial tissue plays a very important role in maintaining the homeostasis of most living organisms (Mohapatra et al., 2021). This function is strictly dependent on the histological structure of each type of epithelium, which need to provide the organism with an adequate barrier to the external medium that relies on the presence of intercellular junctions, basement membrane and a proper differentiation of each type of cell in the epithelium. In addition, the epithelium should be able to strictly control the passage of molecules throughout the epithelial barrier (Castro Dias et al., 2019). All these functions and structures found in the human epithelium are the result of millions of years of animal evolution, and most of the crucial steps towards the evolution of functional epithelia are represented in currently-living animals. First, it is likely that the first metazoans arose after an intriguing process of cell confluence from unicellular eukaryotic cells. This would contribute to explain why poriferans lack true epithelia and are rather based on the presence of adjacent cells that are not joined by real intercellular junctions. As a result of evolution, it is also likely that poriferans gave rise to the onset of placozoans, the first animals showing true intercellular junctions that, although primitive, established the first functional barrier to the external environment, which was then perfected by more



**Fig. 5.** Illustrative representation of the histological structure of a well-developed stratified epithelium found in bilaterians. SBEC, supra-basal epithelial cells; BEC, basal epithelial cells; BM, basement membrane. Intercellular junctions joining two adjacent cells are represented in red.



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evolved animals by the development of complex cell-cell junctions of different types, including the highly-efficient tight junctions (Otani and Furuse, 2020). The lack of an intermediate group of animals makes it very difficult to establish the connection between placozoans and animals with true epithelia displaying complex intercellular junctions and basement membrane. However, the analysis of modern animals suggests that the next evolutive step towards the generation of a true epithelium can be found in cnidaria, which show the first epithelial tissues fulfilling the criteria for real epithelia - cohesion, polarity, and attachment-, although ctenophores could also be good candidates. The progressive acquisition of more complex functions by developing animals could have driven the evolution of these primitive epithelia into the complex epithelial tissues currently found in the human body. These complex tissues would have evolved by cell specialization, with a sequential improvement in the structures associated to cohesion, polarity and attachment.

Analysis of tissues found in nature allowed us to establish a possible link between the most primitive pseudoepithelial tissues and the most differentiated and evolved epithelia found in the human body. Although the present work contributes to understanding how the most primitive epithelial tissues evolved, a very important unanswered question is how these tissues further evolved and differentiated to give rise to the multiple types of epithelia currently found in humans, including glandular epithelia. Another open question is related to the possible future evolution of epithelial tissues. According to the above-described evolutive process, we may hypothesize that human tissues will continue with the progressive development of more efficient and functional intercellular junctions and basement membranes, the synthesis of novel types of cytokeratins with increased functional properties and the specialization of the different cell layers of stratified epithelia to achieve advanced functions.

In addition, a very important leap in the evolution of human tissues is the development of novel human tissues by tissue engineering (Langer and Vacanti, 1993). Described almost three decades ago, tissue engineering methods allow the generation of bioengineered tissues in the laboratory from living cells and biomaterials (Chaudhuri et al., 2017). For the first time in the history of evolution, researchers can fabricate novel tissues with improved properties in the laboratory that are not driven by evolution, but are generated *de novo* for a specific directed purpose. Initially, the first bioartificial tissues generated by tissue engineering had the ultimate goal of biomimicry to resemble native tissues, such as the human skin (Carriel et al., 2012; Dearman et al., 2021), oral mucosa (Alaminos et al., 2007), nerve (Carriel et al., 2014) or cornea (Alaminos et al., 2006), among others, with some of these tissues reaching the clinical setting with promising results (Egea-Guerrero et al., 2019; Rico-Sánchez et al., 2019). However, the rapid advance

of methods related to biomaterial functionalization (Shadjou et al., 2018), nanotechnology (Chato-Astrain et al., 2020) and cell differentiation (Garzón et al., 2020) allows the design and fabrication of functionalized human tissues able to provide the bioartificial product with improved functions and properties. Given that the human being is considered to be the ultimate product of evolution, the novel tissues generated by tissue engineering could also be considered as evolutive steps towards the generation of tissues with improved features, although these tissues were generated in the laboratory and not as a result of natural evolution. The clinical application of the novel improved tissues generated by tissue engineering will determine the real usefulness of these novel types of tissues.

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