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Origin and Evolution of Epithelial Cell Types (chapter 5)

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1 Chapter 5 : Origin and Evolution of epithelial cell types

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14

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43 **5.1. Introduction**

44 **5.1.1. Epithelial-like structures are not a prerogative of animals**

45 Multicellularity emerged several times independently during the evolution of life (Abedin et
46 King 2010; Hinman et Cary 2017; King 2004; King, Hittinger, et Carroll 2003; Niklas 2014;
47 Parfrey et Lahr 2013), and so did epithelial-like tissues (Dickinson, Nelson, et Weis 2011, 2012;
48 Ganot et al. 2015; Maizel 2018; Miller et al. 2013). In cell biology (Lowe et Anderson 2015),
49 epithelia are defined generally as a sheet of cells tightly bound together and capable of
50 coordinated movements during morphogenesis. In the different lineages where they emerged,
51 they usually shape and line organs (when present), cavities and free external borders, they
52 control molecule and ion exchanges between the body and the environment and/or between
53 different compartments of the body involved in vital physiological processes.

54 Because multicellularity and epithelial-like structures emerged several time independently, the
55 molecules involved in their composition and patterning are different (Kania, Fendrych, et Friml
56 2014; Reynolds 2011). Nevertheless, very ancient proteins predating multicellularity can play
57 a key role in the establishment of epithelial features (Nagawa, Xu, et Yang 2010) such as
58 Rho/ROP GTPase for polarity because of their conserved involvement in controlling
59 cytoskeleton and vesicular trafficking, and other ancient proteins can have been co-opted
60 independently to perform a quite similar result, such as catenins in Ameobozoa and Metazoa
61 (see section 2).

62 After this short synopsis, this chapter will focus on the origin and evolution of animal epithelia
63 only.

64

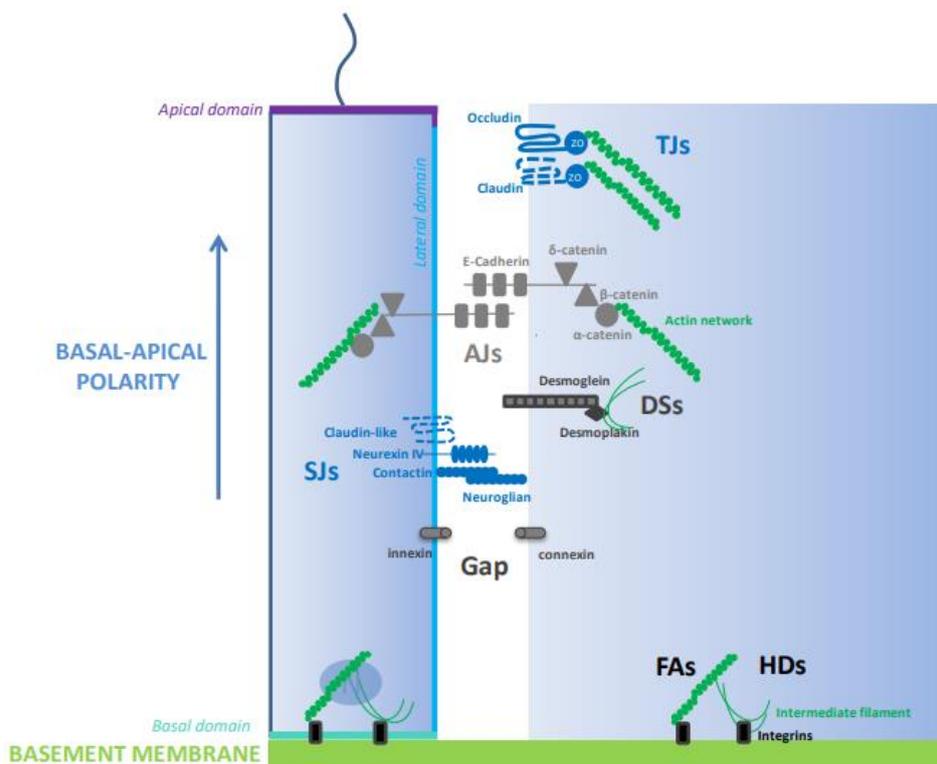
65

66 5.1.2. The definition of epithelium in Metazoa

67 Epithelia of animals are usually considered as one of the four fundamental tissue types along
68 with connective, nervous and muscular tissues (Lowe et Anderson 2015; Yathish et Grace
69 2018). Epithelial tissues cover all the surfaces of the body exposed to the external environment
70 and line organs and body cavities (named in this case: Covering and lining epithelia) hence
71 providing protection and compartmentalization of the body. Epithelia also form much of the
72 glandular tissue of an animal body (in this case they are named glandular epithelia). During
73 embryological development, epithelia are patterned early, after cleavage (Tyler 2003). Indeed,
74 the organization of cells within tissues is the first sign of cell differentiation during
75 embryogenesis and the epithelial blastoderm is the starting point for morphogenesis during the
76 development of a wide range of metazoans (Pozzi, Yurchenco, et Iozzo 2017). It is only from
77 epithelium that mesenchyme arises by epithelial-mesenchymal transition (EMT) during
78 gastrulation (Tyler 2003). In bilaterian adults, epithelial cells derive from all three major
79 embryonic layers (endo-, meso-, and ectoderm): for example in vertebrates, the skin, part of the
80 mouth and nose, and the anus develop from the ectoderm; cells lining the airways and most of
81 the digestive system originate from the endoderm while the epithelium lining vessels derive
82 from the mesoderm. Epithelia are fundamental structures controlling permeability and allowing
83 selective transfer of molecules between animal and its environment and between body
84 compartments. Epithelial tissues also provide protection from physical, chemical, and
85 biological agents and allow for coordinated tissue movements. Consequently, disruptions in
86 epithelial properties cause developmental defects and are responsible for diseases in adult
87 tissues (Miller et al. 2013; Royer et Lu 2011; Sekiguchi et Yamada 2018).

88 Currently, as for many other animal morpho-anatomical features, the histological
89 characteristics defining an epithelium were postulated according to what was found in model
90 animals, all pertaining to the bilaterian taxa, such as mammalian epithelial cells and *Drosophila*

91 epithelial tissues (for review see Tyler, 2003). From these studies, epithelia have been defined
 92 as layers of cells showing coordinated polarity, with differences in structure and function
 93 between the apical side facing the external medium (or internal cavities) and a basal side in
 94 contact with a basement membrane (BM) made of collagen IV (figure 5.1) (Rodriguez-Boulan
 95 et Nelson 1989). In addition, epithelial cells exhibit cell-cell junctions and cell-matrix contacts
 96 (figure 5.1) which maintain cohesion between cells during morphogenesis processes and allow
 97 the coordination of cell movements by providing a seal between cells (Jefferson, Leung, et Liem
 98 2004).



99

100 **Figure 5.1: Schematic drawing summarizing the three features defining animal epithelia** : 1) **Cell polarity** (basal-
 101 apical polarity : cell can harbor different features along this polarity axis, for instance cilia in some cells); 2) **Cell-
 102 cell and cell-matrix junctions** found in Bilaterians and their core molecular composition (here only structural
 103 components are noted rather than all the components needed for their trafficking and patterning). Left, junction
 104 types found in protostomes and non-chordates: Septate Junctions (SJs). Right, junction types found in vertebrates:
 105 Desmosomes (DSs), Tight Junctions (TJs). In between and on both sides are junction types found in all bilaterians:
 106 focal Adhesions (FAs), Hemi-Desmosomes (HDs), Gap junctions, Adherens Junctions (AJs); 3) **Basement
 107 membrane**, a dense sheet of extracellular matrix proteins providing support and increasing cohesiveness of cells.

108

109 **5.1.3. The classification of animal epithelial cell types**

110 Despite this general definition, epithelia can harbor different organizations and structures. In
111 addition to the distinction based on their general role (compartmentalizing *versus* glandular
112 epithelia), epithelial tissues are identified by both the number of layers (simple, stratified or
113 pseudostratified) and the shape of the cells (squamous, cuboidal or columnar) (summarized in
114 table 5.1 see Lowe and Anderson 2015 for more details).

115

116 *Table 5.1 General classification of animal epithelia according to their organization*

		Cell shape			
		flat	cube	parallelepiped	irregular
Number of cell layers	1	Simple squamous	Simple cuboidal	Simple columnar	pseudostratified
	2 or more	Stratified squamous	Stratified cuboidal	Stratified columnar	

117

118

119 These different kinds of epithelial tissue generally perform different functions (absorption,
120 regulation, excretion, filtration, secretion, protection, detection) according to their body
121 position and their structural organization.

122

123 **5.1.4. The definition of junction types**

124 Junction types involved in the establishment and patterning of epithelia are usually classified
125 in two major categories: cell-cell junctions and cell-matrix junctions.

126 In bilaterians, cell-cell junctions include **sealing junctions** that link cells to form a regulated
127 barrier: these are tight junctions (TJs) in Chordata and septate junctions (SJs) in Protostomia.

128 Even though TJs and SJs have the same function they are quite different in morphology and
129 their molecular composition differ. TJs, the most apical junctions in vertebrates, are composed
130 of the transmembrane proteins occludin and claudins that are linked to the actin cytoskeleton

131 through zonula occludens (ZO) proteins (figure 5.1, for recent reviews see Garcia et al., 2018;
132 Yathish and Grace, 2018; Zihni et al., 2016). SJs are more basal (Tyler 2003) and contain
133 claudin-like proteins, contactin (Cont), neurexin IV (Nrx IV) and neuroglian (Nrg) among many
134 other components (figure 5.1) (for review see Hall and Ward, 2016).

135 A second type of cell-cell junction, **signaling gap junctions** allow direct cell-to-cell
136 communication (Skerrett et Williams 2017; Yathish et Grace 2018) and are composed in
137 vertebrate and in prostostome lineages of respectively connexin and innexin proteins, two
138 similar but non homologous proteins (figure 5.1). In other words, two distinct lineages found a
139 convergent solution to solve the same problem of intercellular communication (Alexopoulos et
140 al. 2004; Skerrett et Williams 2017).

141 The third type of cell-cell junctions are **anchoring junctions** linking cells between them
142 (adherens junctions (AJs), desmosomes (DSs)). Contrary to DSs (containing the desmosomal
143 transmembrane cadherin Desmogleins and cytosolic desmoplakins, (Johnson, Najor, et Green
144 2014; Magie et Martindale 2008; Yathish et Grace 2018) which are restricted to vertebrates and
145 which provide a link to intermediate filaments through desmoplakin, AJs are present in all
146 bilaterian taxa and comprise classical cadherins which interact to the actin cytoskeleton through
147 α -, β - and δ -catenins (figure 5.1) (Garcia, Nelson, et Chavez 2018; Miller et al. 2013; Yathish
148 et Grace 2018).

149 Finally concerning cell-matrix junctions, they enable the attachment of epithelial cells to the
150 underlying basement membrane. These are focal adhesion (FAs) and hemi-desmosomes (HDs).
151 As well as cell-cell junctions, cell-matrix junctions are needed to achieve collective migration
152 and coordination of various epithelial morphogenetic processes important during development,
153 tissue shaping and wound healing. Both FAs and HDs are highly specialized structures based
154 on interactions between integrins, fibrillar proteins of the extracellular matrix and the internal
155 intermediate filament or actin network (figure 5.1) (De Pascalis et Etienne-Manneville 2017;

156 Magie et Martindale 2008; Miller et al. 2018; Walko, Castañón, et Wiche 2015a). While FAs
157 are found in non-epithelial cells types (such as, for example, neurons or fibroblasts (Fischer et
158 al. 2019)), hemidesmosomes (HD) are specific to epithelia (Walko et al., 2015).

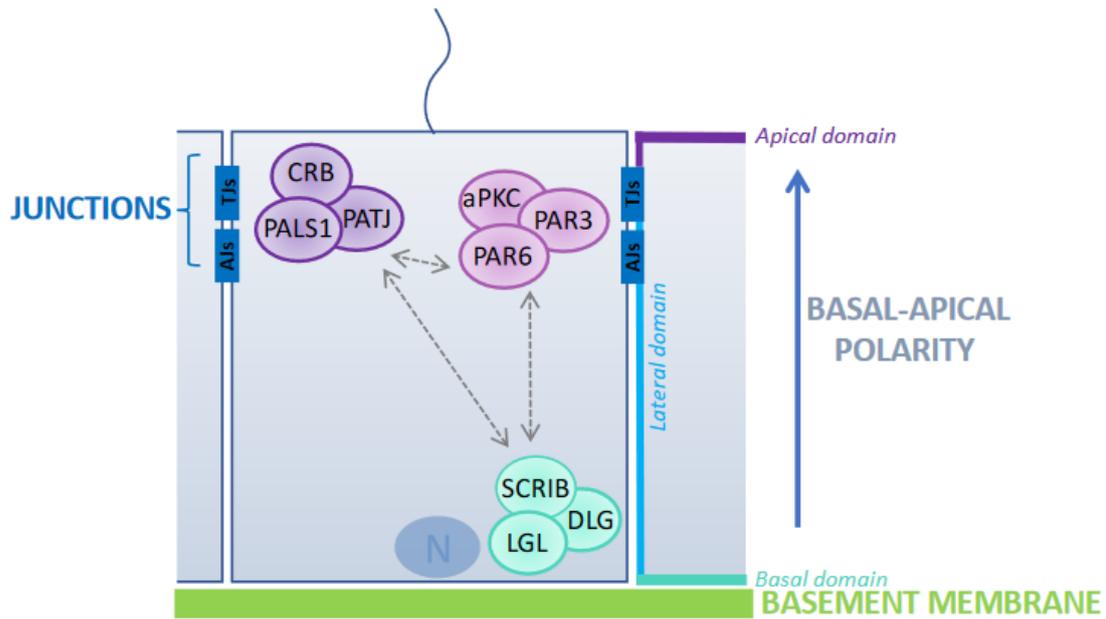
159 It is usually accepted that variations of types and of organization of junctions have allowed the
160 development and differentiation of tissue types and have played a key role in the evolution of
161 animal body plans (Abedin et King 2010; Magie et Martindale 2008).

162

163 **5.1.5. Cell polarity complexes and cell domains**

164 The precise location of the previously defined junctions is one of the numerous demonstrations
165 of cell polarity. This polarity is also obvious by the localization and orientation of other cell
166 features such as the nucleus, cilia or other cell extensions, the cytoskeleton and vesicular
167 trafficking. The cell is generally divided into three domains: the basal domain facing the
168 basement membrane, the apical domain facing the external medium or cavity lumens, and, in
169 between, the lateral domain which is more or less extended depending on the shape of cells
170 (table 5.1, figures 5.1 and 5.2). This polarized structural and ultrastructural organization of an
171 epithelial cell is patterned by at least three protein complexes named polarity complexes, each
172 composed of three interacting proteins (figure 5.2) (Assémat et al. 2008; Le Bivic 2013):

- 173 - The apical PAR complex is composed of atypical Protein Kinase C (aPKC), the Partition
174 defective 3 (PAR3) and Partition defective 6 (PAR6);
- 175 - The apical CRUMBS complex made of CRUMBS (CRB), stardust (Sdt, or MPP5
176 (Membrane Palmitoylated Protein 5) in mammals also known as PALS1 (protein
177 associated with Lin-7 1) and PALS1-associated tight junction protein (PATJ);
- 178 - The lateral SCRIBBLE complex is made with SCRIBBLE (SCRIB), lethal giant larvae
179 (LGL) and Disc large (DLG).



180

181 **Figure 5.2: Localization and protein composition of the three polarity complexes needed to pattern cell polarity**
 182 **in epithelial cells: PAR, CRUMBS and SCRIBBLE according to what was described in Le Bivic (2013).**

183

184 These polarity complexes interact and regulate each other and are themselves regulated by
 185 signaling pathways in a complex manner (Assémat et al. 2008; Le Bivic 2013).

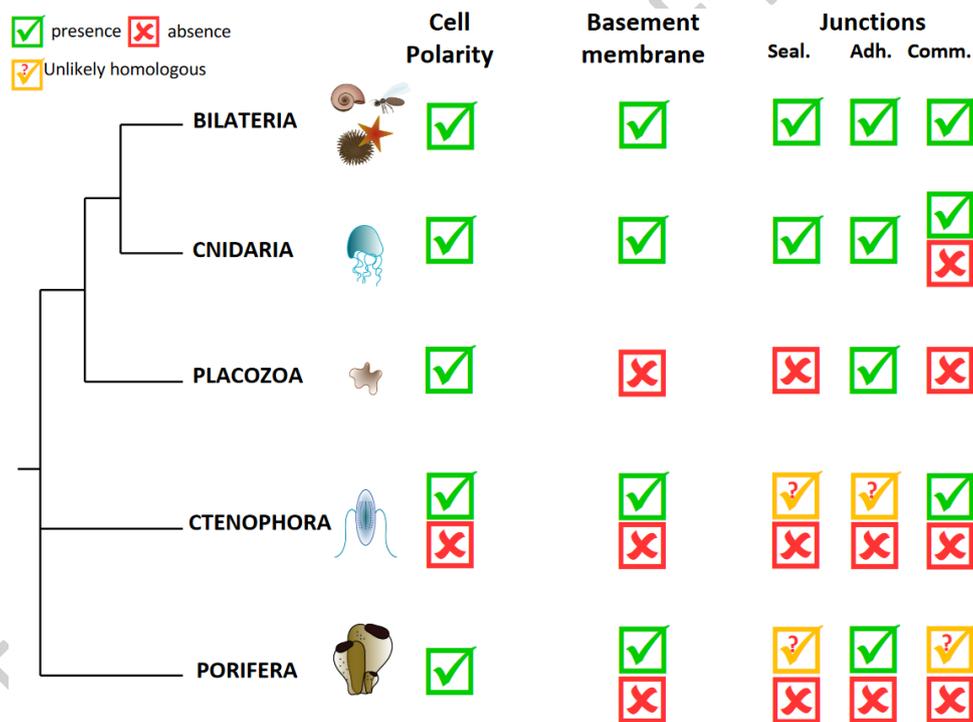
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187 **5.1.6. Do all metazoans have (the same) epithelia?**

188 There is no doubt that the last common ancestor of all extant animals was multicellular (King
 189 et Rokas 2017). And, whatever the variability in timing, relative importance and order of cell
 190 mechanisms involved between phyla (and sometimes among species of a same phylum (A.
 191 Ereskovsky 2010), in all metazoans, the patterning of an epithelial or epithelial-like cell sheet
 192 is one of the earliest important event occurring during embryogenesis after the acquisition of
 193 multicellularity (more often at the blastula stage). The acquisition of such an epithelial level of
 194 organization is intimately connected with the acquisition of cellular adhesion and cell-

195 communication toolkits. One of the main questions raised is: did these key cell properties
 196 emerge once or several times? In other words, are all animal epithelia and epithelial-like sheets
 197 homologous among metazoans? And is it possible to trace back the origin and evolution of all
 198 animal epithelial cell types?

199 To try to answer this question, the first step is to examine the presence/absence in non-bilaterian
 200 taxa of the previously cited three histological criteria defining an animal epithelium (figure 5.3)
 201 in order to: 1) decipher whether the term and definition of “epithelium” refers to equivalent
 202 structures throughout Metazoa, 2) determine, if epithelia are an ancestral character, which of
 203 epithelial features were ancestrally present.



204

205 **Figure 5.3: According to histological observations** (Fidler et al. 2017; Ganot et al. 2015; Ledger 1975; Sally P. Leys
 206 et Riesgo 2012; Magie et Martindale 2008; Moroz et Kohn 2016; Satterlie et Case 1978; Smith et Reese 2016) **the**
 207 **joint presence of the three criteria supposed to define an epithelium in animals is far from being systematically**
 208 **conserved in non-bilaterian phyla.** For junctions: “seal.” stands for sealing, “adh.” stands for adhesive and
 209 “comm.” For communicating. In this schema, if two symbols are indicated for the same character in one phylum,
 210 it means that the observation can be different depending on the species considered. The status “unlikely
 211 homologous” means that a type of junction harboring histological features compatible with either sealing,
 212 adhesive or communicating properties were described, but that their characteristics suggest that they are from a
 213 different nature than what is found typically in other animals (for sponges: (E. D. M. Adams, Goss, et Leys 2010b;
 214 Sally P. Leys et Hill 2012b; Sally P. Leys et Riesgo 2012; S. P. Leys, Mackie, et Reiswig 2007; S. P. Leys, Nichols, et
 215 Adams 2009); for ctenophores: (Hernandez-Nicaise, Nicaise, et Reese 1989; S. L. Tamm et Tamm 1991; Sidney L.

216 Tamm et Tamm 2002)). This schema shows that either many non-bilaterian species do not fit the definition of an
217 epithelium or that the definition postulated decades ago does not depict and fit the diversity really found across
218 animal epithelia.

219

220 The first finding is that the organization of epithelial or epithelial-like sheets found in non-
221 bilaterian phyla is much more variable – even in a single phylum – than it is in bilaterians. The
222 second obvious finding is that, among non-bilaterians, only cnidarian epithelia meets the three
223 criteria of polarity, basement membrane and junctions (whatever their type) at the same time
224 (Ganot et al. 2015; Magie et Martindale 2008). In contrast, Placozoa do not meet the accepted
225 definition of an epithelium (according to the very partial data available so far in this phylum)
226 because of the absence of a basement membrane (Fidler et al., 2017; Ruthmann et al., 1986).
227 As far as Ctenophora and Porifera are concerned only some of the species have tissues that fully
228 meet the definition of epithelium: this is the case for the Homoscleromorpha class in Porifera
229 (Belahbib et al. 2018; Boute et al. 1996; A. V. Ereskovsky et al. 2009; Sally P. Leys et Hill
230 2012a; Sally P. Leys et Riesgo 2012), and the genera *Beroe* and *Pleurobrachia* in Ctenophora
231 (according to the partial data available so far in this phylum) (Fidler et al. 2017). It is therefore
232 very surprising that the presence of a *bona fide* epithelium was questioned only for sponges.
233 Indeed, while Porifera were classically excluded from the “Eumetazoa” clade partly because of
234 the absence of “true” epithelia with a basement membrane, and even several years after Boute
235 et al. (1996) showed a basement membrane existed in homoscleromorph sponges (A. V.
236 Ereskovsky et al. 2009), in contrast the term “epithelium” is currently used for Placozoa (Armon
237 et al. 2018; Smith et Reese 2016) and Ctenophora (Sidney L. Tamm et Tamm 2002). Given
238 such a terminological inconsistency, there are two solutions: 1) either the present definition of
239 epithelium with 3 criteria is kept as it is, meaning that Placozoa, some Ctenophora and most
240 Porifera have to be considered devoid of epithelia; 2) or consider that the epithelium is a
241 synapomorphy of metazoans whatever the presence or absence of basement membrane. This

242 second solution has the positive consequence of taking into account the obvious conservation
243 of physiological properties (mechanical resistance and occlusion to small molecules) that is
244 found in epithelia of a demosponge (a group lacking basement membranes), a property that
245 appears to be shared with bilaterian epithelia that have occluding junctions (E. D. M. Adams,
246 Goss, et Leys 2010a; Dunn, Leys, et Haddock 2015; Sally P. Leys et Riesgo 2012).

247 Today, according to most authors, there is no longer any doubt on the presence of functional
248 epithelia in the last common ancestor of animals that harbor cell polarity, adhesion properties
249 and basement membrane support (Fidler et al. 2017; King et Rokas 2017; Sally P. Leys et Hill
250 2012a; Sally P. Leys et Riesgo 2012; Medwig et Matus 2017). We now have to explore how
251 and when such an epithelium originated and evolved: this is the item of the following section.

252 Tracing back the evolution of epithelial cell types is not only related to the questionings
253 concerning germ layer homology, we have also to consider that the similarity of cell features
254 (here presence of junctions, polarity and basement membrane, as previously mentioned) can
255 result from convergent evolution by co-option of similar molecular actors or that shared
256 proteins can have undergone neofunctionalization. In order to try to tackle this issue, the recent
257 development of comparative whole organism single cell transcriptomic approaches (sc-
258 RNAseq) should enable to define cell type specific core regulatory complex (CoRC) (Arendt
259 2008; Arendt et al. 2016; Marioni et Arendt 2017). Thanks to the conservation of regulatory
260 mechanisms, a homologous cell type is expected to remain recognizable across species (Arendt
261 2005, 2008). We will also discuss this point in section 2.

262 **5.2. The Origin of animal epithelia**

263 In order to understand the origin of this metazoan synapomorphy, it is useful to study the
264 presence of ‘preadapted’ molecular tools that were present before the emergence of animals.
265 To do so, even if the data remain scarce, comparative genomics of the closest unicellular

266 relatives of metazoans are needed: choanoflagellates as the sister group of metazoans is of
267 course of particular interest, but other members of Holozoa (such as Filasterea) and Opimoda
268 (including Opisthokonta and Amoebozoa) can be useful (Ferrer-Bonet et Ruiz-Trillo 2017;
269 Olson 2013; Richter et King 2013; Richter et al. 2018; Sebé-Pedrós, Degnan, et Ruiz-Trillo
270 2017).

271 **5.2.1. Some genes encoding for extracellular matrix proteins predate the**
272 **emergence of animal epithelia**

273 In bilaterians and cnidarians, epithelial morphogenesis has been shown to rely strongly on
274 interactions between the epithelial sheet receptors and components of the extracellular matrix
275 (ECM), including the basement membrane when it is present (Aufschnaiter et al. 2011; Dzamba
276 et DeSimone 2018; Fidler et al. 2017; Sekiguchi et Yamada 2018).

277 A few genes encoding for important extracellular matrix proteins or their cellular receptors
278 predate the emergence of Metazoa: indeed, genes encoding for integrins and integrin adhesion
279 machinery are present in unicellular relatives of metazoans (see section 2.2) (Abedin and King,
280 2010; Babonis and Martindale, 2017; Sebé-Pedrós and Ruiz-Trillo, 2010; Sebé-Pedrós et al.,
281 2010; Suga et al., 2013). In both Filasterea (*C. owczarzaki*) and Choanoflagellata (*M. brevicolis*
282 and *Salpingoeca rosetta*), several proteins including protein domains that are present in animal
283 ECM proteins (such as for example LAM G, EGF, fibronectin III) are predicted in the genomes
284 of these organisms. But these protein domains are not combined in the same way either in
285 Filasterea and Choanoflagellata, as they are in Metazoa (Fairclough et al. 2013; King et al.
286 2008; Suga et al. 2013; Williams et al. 2014). This means that most of the proteins found in
287 animal ECM and basement membrane (for instance the laminin and fibronectin key
288 components) are metazoan innovations and emerged probably in part by domain shuffling
289 (Babonis and Martindale, 2017; Fahey and Degnan, 2012; Richter et al., 2018; Suga et al., 2013;

290 Williams et al., 2014). Interestingly, most recent studies suggest that a canonical type IV
291 collagen could have been already present in the common ancestor of filastereans,
292 choanoflagellates and animals (Fidler et al. 2018; Grau-Bové et al. 2017) therefore suggesting
293 a more ancient origin of type IV collagen than previously accepted and its neo-functionalization
294 in metazoans, as it has also been suggested for integrin adhesome or cadherins (King et al.,
295 2008; Sebé-Pedrós and Ruiz-Trillo, 2010; Sebé-Pedrós et al., 2010). In other words, the
296 characterization of type IV collagen in non-metazoans challenges the previously accepted idea
297 that spongin short chain collagen (SSCC) is ancestral to type IV collagen (Aouacheria et al.
298 2006; Sally P. Leys et Riesgo 2012).

299

300 **5.2.2. Integrins: an ancient protein family**

301 As previously mentioned, (section 1.4), the key proteins shared by focal adhesions (FAs) and
302 hemidesmosomes (HDs) are integrins. Until 2010, because genes encoding for integrins were
303 not retrieved in plant, fungi and choanoflagellate genome surveys, integrins were thought to be
304 a metazoan innovation. Thanks to genomic surveys in several unicellular opisthokont species,
305 integrins were shown to be present in the last common ancestor of Filasterea and Metazoa as
306 well as actors of the integrin machinery (Focal Adhesion kinase and non-receptor protein kinase
307 Src) (Sebé-Pedrós and Ruiz-Trillo, 2010; Sebé-Pedrós et al., 2010). In addition, both integrin α
308 and β in these premetazoan taxa possess the amino acid motifs in their cytoplasmic tails
309 involved in interactions with intracellular scaffolding and signaling proteins therefore
310 suggesting that they could interact in a similar way as their metazoan homologs. Though the
311 role of the integrins in unicellular eukaryotes is currently unknown, it has been evidenced very
312 recently that the *Capsaspora* integrin are able to recruit human talin. Therefore, this result

313 suggests that the regulation of integrins via talin activity is conserved in Holozoa and predates
314 the emergence of Metazoa (Baade et al. 2019).

315 On the other hand, to build HD, plectin is required to form bridges between the cytoplasmic
316 keratin intermediate filament network and the integrins. To date, no plectin has been
317 characterized so far in non-metazoan lineages but other linker proteins allowing the binding
318 between integrins and the cytoskeleton are common among unikonts (Sebé-Pedrós et al., 2010).

319 Altogether, integrins predate the emergence of animal and may play an ancestral role in
320 signaling. The role in cell-ECM adhesion might have been coopted in metazoans (Sebé-Pedrós
321 and Ruiz-Trillo, 2010; Sebé-Pedrós et al., 2010).

322

323 **5.2.3. Genes encoding for Cell-cell Adhesive proteins predate the emergence of**
324 **animal epithelia**

325 In the section 1.4 we described the types of junctions found in bilaterians and their molecular
326 composition. To date, because actin- based adhesive-like junctions (Ganot et al. 2015; Magie
327 et Martindale 2008; S. L. Tamm et Tamm 1987) and adherens junctions (A. V. Ereskovsky et
328 al. 2009; Fahey et Degnan 2010; Sally P. Leys et Riesgo 2012; S. P. Leys, Nichols, et Adams
329 2009) were described in Ctenophora and Porifera respectively, it was assumed that adherens
330 junctions (AJs) constitute the ancestral type of junctions of animals. As a consequence, the
331 cadherins and catenins involved in the establishment of AJs (section 1.4) were searched for in
332 priority in unicellular relatives of animals.

333 According to the presently (scarce) available genomic data, it seems that at least three cadherin
334 families were present in the last common ancestor of Choanozoa (taxa grouping
335 Choanoflagellata and Metazoa): lefftyrins, coherins and hedglings (Nichols et al. 2012). Among
336 these families some were lost secondarily in bilaterians or earlier. To date, only one cadherin

337 gene was found in the filasterean *C. owczarzaki* (King et al. 2008; Nichols et al. 2012; Richter
338 et King 2013). It is now therefore obvious that cadherins predate metazoans. But only
339 metazoans have *bona fide* classical cadherins containing the two domains required for its
340 interaction with catenins (JMD and CBD)(Clarke et al. 2016; Miller et al. 2013; Murray et
341 Zaidel-Bar 2014).

342 As far as catenins are concerned, the Amoebozoa *Dictyostelium discoideum*, possesses catenins
343 (Aardvark and *Dda*-catenin), involved in establishing epithelial-like sheets (Dickinson, Nelson,
344 et Weis 2011, 2012). Interestingly, Aardvark-related proteins are also present in
345 choanoflagellates and filastereans (Nichols et al. 2012; Suga et al. 2013). Aardvark and *Dda*-
346 catenin are considered close relatives of beta- and alpha- catenins because of shared domain
347 features (Miller et al. 2013, 2018; Richter et al. 2018; Suga et al. 2013). As a consequence, it
348 means that the joint presence of cadherins and catenin-related proteins predate the emergence
349 of Metazoa even if they are predicted to not interact. Unfortunately, there is no experimental
350 data so far available to establish the function of these proteins in these organisms (Nichols et
351 al., 2012). But the recent acquisition of a transfection protocol in choanoflagellates is expected
352 to enable to soon fill this gap (Booth, Szmidt-Middleton, et King 2018).

353 Concerning genes encoding for proteins involved in other types of junction, none of the
354 components of gap junctions have been characterized in non-metazoans yet (Moroz et Kohn
355 2016), while, in contrast, a claudin-like gene was retrieved in *Capsaspora* and *Monosiga*
356 genomes (Ganot et al. 2015) (see figure 5).

357 **5.2.4. Do some genes encoding for members of cell polarity complexes predate the**
358 **emergence of animal epithelia?**

359 Though the typical collar cell of choanoflagellates shows an obvious basal-apical cell polarity,
360 to date the genes encoding for the three metazoan polarity complexes (CRUMBS, SCRIBBLE

361 and PAR, figure 2) remain nearly unexplored. Among the various actors involved in polarity
362 complexes (see section 1.5), only DLG, a member of the SCRIBBLE complex was shown to
363 predate the emergence of the metazoans and has been found in Choanoflagellata, Filasterea and
364 Ichthyosporea (Belahbib et al. 2018; Fahey et Degnan 2010; Ganot et al. 2015; Le Bivic 2013;
365 Murray et Zaidel-Bar 2014; Richter et al. 2018). For now, without no further contrary evidence,
366 all other components are considered metazoan innovations (figure 5.4). In addition, the key
367 pathways known to regulate these 3 epithelial polarity complexes (among which Wnt and PCP
368 pathways) are also considered as metazoan specificities (Babonis and Martindale, 2017).

369 If metazoans evolved these specific means to control their epithelial cell polarity, it is obvious
370 that cell polarity is more generally necessary for proper functioning of numerous other cell
371 types such as, for example, neurons in animals, pollen tubes in plants and hyphae in Fungi. The
372 achievement of cell polarity is intimately linked to cytokinesis, orientation of mitotic spindles
373 and asymmetric separation. Some molecular elements controlling cell polarity are known to be
374 highly conserved across eukaryotes including animals, in epithelial and non-epithelial cell
375 types, such as MOB proteins and Rho GTPase (Bornens 2018; Hoff 2014; Lefèbvre et al. 2012;
376 Slabodnick et al. 2014).

377

378 **5.2.5. Main conclusion: cell adhesion and polarity may have emerged several times**
379 **during the evolution of Holozoa**

380 According to the previously cited results in Choanoflagellata and Filasterea, the proteins
381 involved in cell-cell adhesion and cell-matrix adhesion to achieve aggregative/transient
382 multicellular stages in these organisms are different from what is found in Metazoans.
383 Nevertheless, key protein domains involved in major cell-cell or cell-matrix protein interactions
384 are highly conserved in holozoans. It means that, during the evolution of Holozoans
385 aggregative/multicellular stages probably emerged thanks to intense domain shuffling of shared

386 building blocks, as Lego® bricks, and duplication-divergence events in already present gene
387 families (e.g. cadherins and catenines). Thanks to these ancestral domains and families, the
388 metazoan lineage evolved specific tools, representing metazoan synapomorphies (such as
389 polarity complexes, classical E-cadherin and signaling pathways), to achieve permanent
390 multicellular level of organization with epithelial features.

391

392 **5.3. The probably ancestral features of the metazoan epithelium**

393 **5.3.1. The composition of the ancestral basement membrane**

394 To date, comprehensive biochemical experiments to determine the protein composition of the
395 basement membrane of animals have only been carried out in cnidarians and bilaterians (Fidler
396 et al. 2014, 2018; Halfter et al. 2015). As a consequence, only partial biochemical information
397 is available for the other animal lineages, and in particular for sponges and ctenophores which
398 are considered the most basal taxa in the metazoan tree. Most of our present knowledge for
399 these two phyla therefore relies on indirect hypotheses based of transcriptomic and genomic
400 surveys.

401 A few studies have compared the extracellular matrix (ECM) genes found in the genomes of
402 early-diverging animals such as sponges and cnidarians with that conserved across bilaterians.
403 These comparisons enabled to identify an ancestral core set of ECM components, receptors and
404 degrading proteases (J. C. Adams 2013, 2018; Özbek et al. 2010; Tucker et Adams 2014;
405 Williams et al. 2014). For what is known from bilaterians and cnidarians, the extracellular
406 matrix is mainly composed of type I collagen, elastin and fibronectins but there is also a great
407 variety of glycoproteins (as laminin), proteoglycans and different types of collagens (except

408 type IV). Its density, fluidity and composition is variable between taxa and between different
409 tissues of the same taxa (Fidler et al. 2018; Hynes 2012; Kular, Basu, et Sharma 2014).

410 In contrast, the composition of the basement membrane seems less variable between taxa (even
411 though slight variation can occur between tissues). Indeed in all cases the main components are
412 laminins and type IV collagen, interconnected with mainly nidogen and perlecan and,
413 sometimes, with additional molecules such as agrin (Fidler et al. 2017, 2018; Medwig et Matus
414 2017; Pozzi, Yurchenco, et Iozzo 2017; Sekiguchi et Yamada 2018). According to genomic
415 surveys, only genes encoding for type IV collagen and laminin are present in ctenophores and
416 homoscleromorph sponges. Surprisingly, despite of the absence of the connecting proteins
417 nidogen and perlecan, they are able to build a basement membrane (at least for *Beroe ovata* and
418 *Pleurobrachia pileus*) (Fidler et al. 2017, 2018). This finding suggests that these two proteins
419 (type IV collagen and laminin) are essential and shared components of BM in animals and
420 therefore constitute the core toolkit already present in the last common ancestor of all extant
421 metazoans. This also means that the other BM components (perlecan and nidogen) emerged
422 more recently during metazoan evolution. Future experiments are needed to understand the
423 differences in the organization and mechanical properties of the BM between
424 cnidarians/bilaterians and ctenophores/homoscleromorph sponges.

425 These recent findings in ctenophores and sponges also mean that the absences of BM in some
426 ctenophores, such as *Mmemiopsis leidy*, and in the placozoan *Trichoplax adhaerans* or of genes
427 encoding for laminin and type IV collagen (as in demosponges for instance) are secondary
428 losses. Interestingly, in contrast, the calcareous sponge *Sycon coactum* possesses genes
429 encoding for several BM proteins whereas a BM structure has never been observed in this
430 sponge class (Sally P. Leys et Riesgo 2012; Riesgo et al. 2014). More in-depth domain analyzes
431 and functional experiments have to be performed to fully understand this apparent discrepancy
432 between gene content and tissue structure (discussed in Renard et al, 2018).

433 **5.3.2. The ancestral toolkit to achieve cell polarity depends on non-bilaterian**
 434 **relationships**

435 Cell polarity is an ancestral feature of animal epithelia (Figure 5.3). It now remains to establish
 436 whether or not the molecular toolkit involved in the establishment of coordinated cell polarity
 437 is the same in non-bilaterian animals as in bilaterians. According to recent gene surveys
 438 (Belahbib et al. 2018; Riesgo et al. 2014), all the genes encoding for the nine proteins involved
 439 in PAR, CRUMBS and SCRIBBLE polarity complexes are present in Cnidaria, Placozoa and
 440 Porifera. Whereas the whole set of genes needed to build a CRUMBS complex is absent in
 441 Ctenophora, as well as the *Scribble* gene needed to complete the SCRIBBLE complex (table
 442 5.2, figure 5.4).

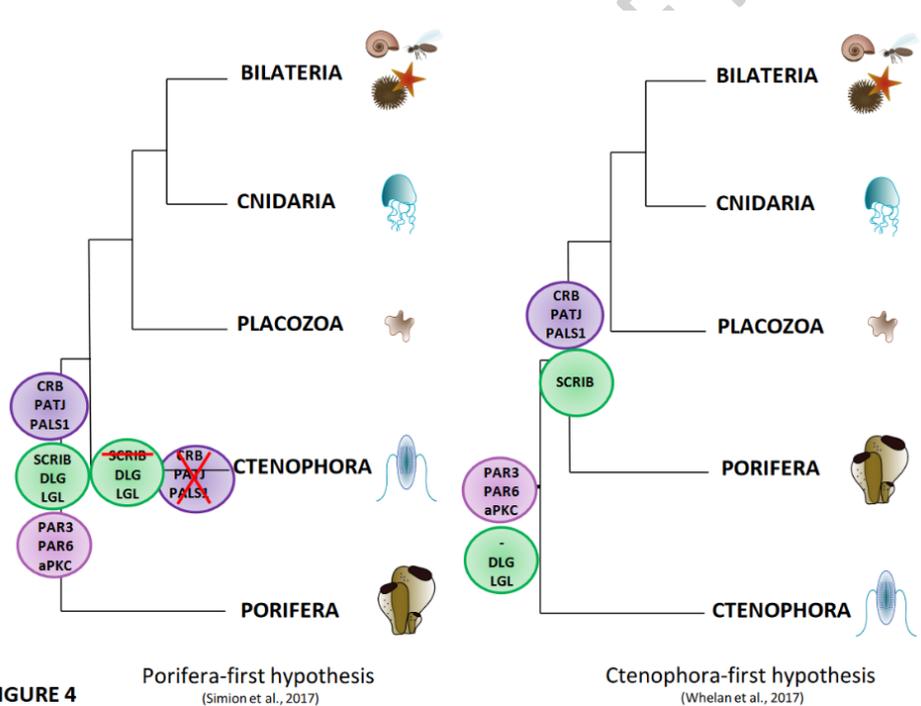
443 **Table 5.2** *Présence (x)/absence(0) in the four non-bilaterian phyla (Porifera, Ctenophora, Placozoa and*
 444 *Cnidaria) of genes encoding for proteins involved in the three cell polarity complexes, namely the CRUMBS*
 445 *complex, the SCRIBBLE complex and the PAR complex (figure 5.2), according to transcriptomic and genomic*
 446 *surveys (Belahbib et al. 2018; Le Bivic 2013; Riesgo et al. 2014)*

		Porifera	Ctenophora	Placozoa	Cnidaria
PAR	PAR 3	x	x	x	x
	PAR 6	x	x	x	x
	aPKC	x	x	x	x
CRUMBS	CRB	x	0	x	x
	PAT J	x	0	x	x
	PALS1	x	0	x	x
SCRIBBLE	SCRIB	x	0	x	x
	LGL	x	x	x	x
	DLG	x	x	x	x

447
 448 Because the relative phylogenetic position of Ctenophora and Porifera is still uncertain (Simion
 449 et al., 2017; Whelan et al., 2017; reviewed in King and Rokas, 2017) two scenarios are possible:
 450 either the nine polarity genes (DLG predates the emergence of Metazoa) were present in
 451 common metazoan ancestor then some were lost secondarily in ctenophores, or only 5 of these

452 genes were ancestral and the other 4 genes would represent innovations acquired later during
 453 animal evolution (Figures 5.4 and 5.5). Moreover, whatever the scenario considered there is
 454 no experimental evidence so far that neither these proteins are able to form complexes *in vivo*
 455 in Placozoa and Porifera (even if residue and domain analyzes tend to support this hypothesis,
 456 see Belahbib et al., 2018) nor on their function in these phyla. Interestingly, a very recent
 457 immunolocalization of the PAR proteins in the ctenophore *Mnemiopsis leydii* strongly
 458 questions the functional conservation of this protein outside the cnidarian-bilaterian lineage
 459 (Salinas-Saavedra et Martindale 2019).

460



461 **FIGURE 4**

Porifera-first hypothesis
(Simion et al., 2017)

Ctenophora-first hypothesis
(Whelan et al., 2017)

462 **Figure 5.4: Two scenarios for evolution of the three polarity complexes (CRUMBS, PAR and SCRIBBLE complexes)**
 463 **taking into account the uncertainty of phylogenetic relationships at the base of the animal tree (Porifera or**
 464 **Ctenophora as sister group of all other metazoans): either the 3 polarity complexes were already present in the**
 465 **last common ancestor of all extant animals or partially two of them were present.**

466

467

468 **5.3.3. Were adhesive junctions present ancestrally?**

469 According to the analyses of the different types of junctions present in bilaterians and
470 cnidarians, it is clear that their common ancestor must have had AJs, gap junctions and SJs, but
471 the origin of these major innovations remains unknown:

472 *5.3.3.1. Communicating junctions*

473 In bilaterians, gap junctions are either composed of innexin (invertebrates) or connexin
474 (vertebrates). Whatever the evolutionary origin of these two proteins (homology vs.
475 convergence), the gap junction they compose show amazing structural and functional
476 similarities (Skerrett et Williams 2017). In cnidarians, innexin also composes gap junctions
477 (Alexopoulos et al. 2004; Takaku et al. 2014), it therefore means that innexin-gap-junctions
478 were already present in the last ancestor of bilaterians and cnidarians. In ctenophores, gap
479 junctions have been reported (Satterlie et Case 1978) and, interestingly, a gene encoding for
480 innexin has been characterized in *Pleurobrachia* (Moroz et Kohn 2016). Nevertheless, given
481 that innexin can participate to cell-cell communication via the establishment of either gap
482 junctions or hemichannels (Güiza et al. 2018), the localization of the innexin protein at the level
483 of gap junction is needed to establish the possible function conservation of innexin in
484 ctenophores. In contrast, until now, neither the structure nor the innexin genes were reported
485 yet in sponges and in placozoans (Moroz et Kohn 2016; Smith et Reese 2016). In sponges, a
486 very different type of communicating junction was described in glass sponges (Hexactinellida),
487 named plug junctions, clearly unrelated to gap junctions found in other animals (S. P. Leys,
488 Cheung, et Boury-Esnault 2006; S. P. Leys, Mackie, et Reiswig 2007). Therefore, if we assume
489 1) that the innexin protein is involved in the formation of gap junctions in ctenophores, and 2)
490 that the Ctenophora-first hypothesis is the right one, then, gap junctions were present ancestrally

491 in animals and lost secondarily in sponges and placozoans. Because none of these hypotheses
492 have so far been validated, it seems premature to draw such a conclusion.

493 5.3.3.2. *Sealing/Occluding junctions*

494 As far as sealing junctions are concerned, septate junctions are present in Cnidaria. And despite
495 ultrastructural variations observed by electron microscopy and incomplete information about
496 their molecular composition, bilaterian and cnidarian SJs are currently considered to be
497 evolutionary related (Ganot et al. 2015). Again, as for the other structures examined in this
498 chapter, our knowledge from the three other non-bilaterian phyla is too scarce and therefore
499 prevents us from elaborating a clear evolutionary scenario for the origin of SJs. In Placozoa,
500 ladder-like structures reminiscent of the SJs were described (Ruthmann, Behrendt, et Wahl
501 1986b) but more precise observations are needed to conclude whether or not these structures
502 are *bona fide* SJs (Ganot et al. 2015; Smith et Reese 2016). In ctenophores, no SJs have been
503 reported so far, although “atypical” junctional structures (without septa) have been
504 characterized (Hernandez-Nicaise, Nicaise, et Reese 1989; Magie et Martindale 2008). Albeit
505 sponge epithelia clearly have sealing properties (E. D. M. Adams, Goss, et Leys 2010a), SJs
506 have been reported by only one study in the calcisponge *Sycon ciliatum* and later studies failed
507 to confirm this feature (Eerkes-Medrano et Leys 2006; Ledger 1975; S. P. Leys, Nichols, et
508 Adams 2009). Interestingly, despite the absence of unquestionable SJ outside the Cnidaria-
509 Bilateria lineage, genes encoding for core components of SJs are present in placozoans and
510 some of them in sponges and in ctenophores (Ganot et al. 2015; Riesgo et al. 2014): four
511 proteins (Claudin-like, neuroglian, contactin, neurexin IV) considered as major structural
512 components of SJs would have predated the emergence of the Bilateria-Cnidaria lineage (figure
513 5.5). Considering the incongruences between the different analyzes performed so far in
514 ctenophores and sponges, it is currently impossible to decipher whether these genes were

515 present ancestrally or not (Chapman et al. 2010; Fahey et Degnan 2010; Ganot et al. 2015; Sally
516 P. Leys et Riesgo 2012; Riesgo et al. 2014; Suga et al. 2013).

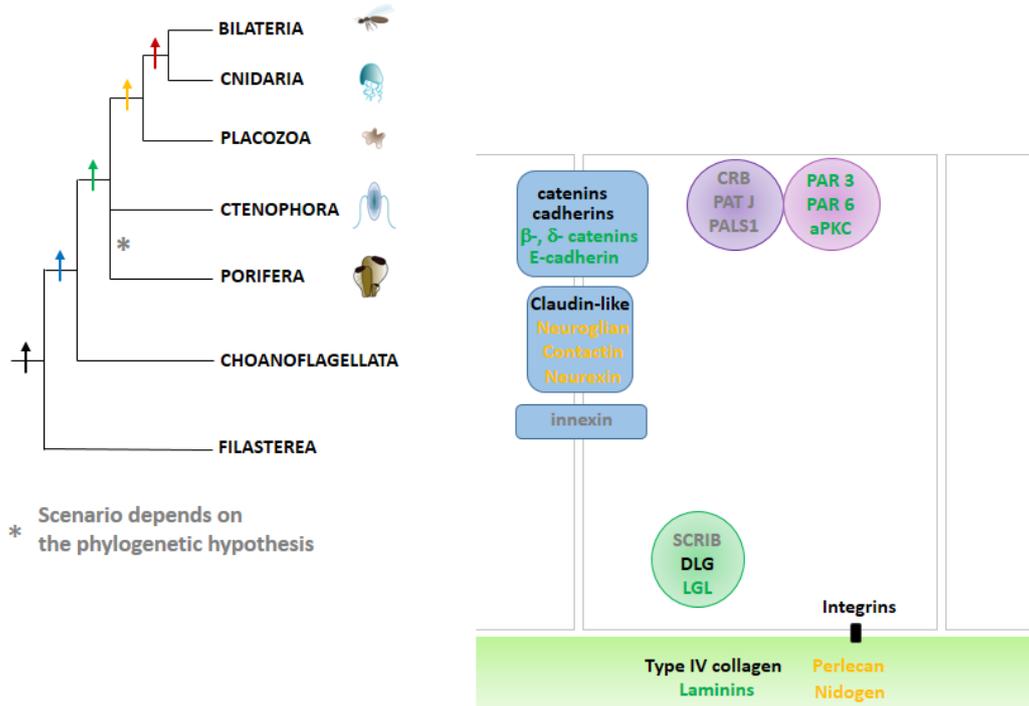
517 Thus, much remains to be explored in order to 1) understand how these three non-bilaterian
518 phyla, namely Placozoa, Ctenophora and Porifera, achieve the sealing of their epithelium, 2)
519 establish the roles of retrieved genes, and 3) be able to trace back the origin of bilaterian-
520 cnidarian septate junctions.

521 5.3.3.3. *Adhesive junctions*

522 In contrast to the two previous types of junctions, because of the clear description of adherens
523 junctions or adhesive belts in some sponges (Homoscleromorpha and Calcarea), placozoans
524 and ctenophores (A. V. Ereskovsky et al. 2009; Fahey et Degnan 2010; Ganot et al. 2015; Sally
525 P. Leys et Riesgo 2012; S. P. Leys, Nichols, et Adams 2009; Magie et Martindale 2008;
526 Ruthmann, Behrendt, et Wahl 1986b; Smith et Reese 2016; Smith et al. 2014; S. L. Tamm et
527 Tamm 1987), until recently it was taken for granted that adhesive junctions represented the
528 most ancestral type of junctions. Nevertheless recent transcriptomic and genomic surveys
529 (Belahbib et al. 2018; Riesgo et al. 2014) and deep analyses of key functional domain and
530 residues (Belahbib et al. 2018) started casting doubt on this common view. Indeed, whereas
531 only two classes of sponges harbor adherens junctions (Homoscleromorpha and Calcarea), all
532 four sponge classes possess the genes encoding for the four proteins involved in adherens
533 junctions namely E-cadherin, alpha, beta-, and delta-catenins (CCC complex, figure 1).
534 Moreover, the key domains of the E-cadherin needed to establish the CCC complex (GBM and
535 JMD) are well conserved in three of these classes (while divergent in glass sponges) (Belahbib
536 et al. 2018); and the first recent biochemical experiments conducted in a demosponge (devoid
537 of adherens junctions) suggest that this canonical complex is formed *in vitro* (Schippers,
538 Nichols, et Wittkopp 2018). Despite the fact that these new data are very exciting and represent

539 an undeniable step forward for sponge biology, they unfortunately fail to explain the functional
540 role of the CCC complex in sponge classes that are devoid of adherens junction-like structures.
541 It will be important to explore the homology of adherens junctions of homoscleromorph
542 sponges and of cnidarians/bilaterians. Recently, the involvement of vinculin in cell-cell and
543 cell-ECM contacts in *Oscarella pearsei* has been proposed following its tissue localization by
544 immunolocalization (Miller et al. 2018). This is highly reminiscent of what is known in other
545 animals (Carisey et Ballestrem 2011; Carisey et al. 2013; Miller et al. 2018), but until E-
546 cadherin is not localized in this class of sponge we will not be able to make the link between
547 the structure (AJ) and its protein composition.

548 The most surprising result of the analyses conducted by Belahbib et al (2018) was the high
549 divergence of the key cytoplasmic domains of the E-cadherin of Ctenophores: these divergences
550 prevent to predict a possible interaction of this protein with catenins, and casts doubt on the
551 formation of a CCC complex in this phylum. Again, as for sponges, biochemical and functional
552 experiments are needed to confirm/infirm *in vitro* and *in vivo* these *in silico* predictions. If this
553 is confirmed, and depending on the phylogenetic hypothesis considered, this may challenge the
554 idea that the last common ancestor of animals was already able to form CCC complexes
555 involved in the formation of adhesive junctions. Indeed the possibility of neofunctionalization
556 of cadherins and catenins is well illustrated by the case of the emergence of desmosomes in
557 vertebrates (for review see Green et al., 2020).



558
 559 **Figure 5.5: Diagram summarizing the emergence** (colored arrows) **of genes involved in key epithelial features**
 560 (junctions, basement membrane and polarity complexes) according to present available data. In the figure,
 561 proteins were located in order to remind their function in cnidarians and bilaterians, even though there is no
 562 evidence of the functional conservation of these proteins outside the cnidarian-bilaterian lineage. The reader
 563 should also note that as far as genes encoding for Neuroglian, Contactin and Neurexin are concerned, their
 564 presence in sponges and ctenophores is unclear because of inconsistencies between analyzes : it makes it hard to
 565 trace back their evolution, but there is no doubt about their presence in the last common ancestor of
 566 Placozoa/Cnidaria and Bilateria.

567

568 **5.3.4. Epithelial regulatory signatures**

569 Comparing similar cell types across different species by sc-RNAseq is in progress since recent
 570 years. This approach is expected to provide insight into the evolution of cellular lineages
 571 (reviewed in Marioni and Arendt, 2017). The general principle of this approach is that: 1) a cell
 572 type is defined by a unique combination of transcription factors, the terminal selectors, that
 573 form a core regulatory complex (CoRC) needed to regulate cell type-specific effector genes
 574 (Arendt et al. 2016; Hobert, Carrera, et Stefanakis 2010); 2) evolutionary relatedness between
 575 cell types should be visible through the sharing of similarities of CoRCs and effectors.

576 So far, no study has focused on the evolution of epithelial cell types. Nevertheless a few
577 transcription factors seem to be highly conserved in some epithelial cells across the animal
578 kingdom and may be expected to be part of the CoRC needed to regulate the cell fate of at least
579 some epithelial cell types. This is for example the case of epithelial specific Ets factors (ESE)
580 that regulate epithelial processes, such as epithelial proliferation and differentiation, in different
581 mammalian epithelial cell types, notably by modulating terminal differentiation pathways
582 (Feldman, Sementchenko, et Watson 2003), and that also seem to be specific markers of one of
583 the epithelial layers found in sponges (pinacocytes forming the pinacoderm layer) (Sebé-Pedrós
584 et al. 2018). In the same way, the transcription factor *Grainyhead* that may play a role in
585 regulating domain-specific effector genes for some epithelial cell types (Achim et al. 2018;
586 Boglev et al. 2011) has been found recently to be expressed specifically in some epithelial cell
587 types in the ctenophore *Mnemiopsis leydii* and the choanoderm (formed by choanocytes) of the
588 sponge *Amphimedon queenslandica* (Sebé-Pedrós et al. 2018).

589 Present data are obviously insufficient to decipher evolutionary relationships between epithelial
590 cell types between animal lineages, but ongoing projects are expected to provide clues in the
591 future years. The main difficulties to deal with at such a large evolutionary scale are: 1)
592 establishing the precise orthology and paralogy relationships of all expressed genes (Altenhoff,
593 Glover, et Dessimoz 2019), 2) acquiring high-quality reference genomes and gene annotation
594 for all invertebrate lineages which is far from being the case (Renard et al. 2018), 3) a better
595 knowledge of cell types especially in non-bilaterian phyla (Sebé-Pedrós et al. 2018) and of their
596 ability to transdifferentiate (Sogabe, Nakanishi, et Degnan 2016).

597

598

599

600 **5.4. Conclusions and future challenges**

601 It is now clear that the main features of bilaterian epithelia predate the emergence of the
602 bilaterian lineage: indeed all these features are clearly shared with cnidarians, meaning that the
603 last common ancestor of cnidarians and bilaterians already had an epithelium with cell polarity,
604 basement membrane and 3 types of junctions, and that these features are achieved with the same
605 molecular toolkit. To trace back the origin of these features it is therefore needed to pay
606 attention to the three other non-bilaterian phyla, namely sponges, ctenophores and placozoans.
607 Unfortunately, the knowledge available on these three phyla mainly relies 1) on classical
608 electron microscopy and 2) on gene surveys. These data describe major discrepancies between
609 gene content and observed histological features (reviewed in Renard et al., 2018). The other
610 great lesson learnt in the last years is that even though the basement membrane was probably
611 present ancestrally, it was lost several times during animal evolution. This finding challenges
612 the common dogma on the absolute requirement of the presence of this structure to compose
613 and pattern an epithelium. Indeed, it has been shown in demosponges that the achievement of
614 epithelial properties can be reached without the presence of a basement membrane (E. D. M.
615 Adams, Goss, et Leys 2010a). Nevertheless, the different regeneration processes found in
616 different sponge species (Borisenko et al. 2015; A. V. Ereskovsky et al. 2015) suggest that the
617 presence or absence of a basement membrane may influence the dynamics of epithelial
618 morphogenetic processes. Much remains to explore from a functional point of view to
619 understand the consequences of BM secondary losses in several species.

620 The present state of the exciting quest is that biochemical and functional experiments are needed
621 to establish the exact role of the proteins in these three non-bilaterian lineages. Recent papers
622 show that there are promising growing efforts in this direction. Only these proteomic and
623 functional data will enable us to decipher whether the basement membrane and the cell polarity

624 already present in the last common ancestor of all extant animal relied on the same molecular
625 actors, and to determine which types of junction were present ancestrally.

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