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Diverse evolutionary paths to cell adhesion

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Abstract

The morphological and physiological diversity of animals, fungi, plants, and other multicellular organisms stems from the fact that each lineage acquired multicellularity independently during the course of evolution. A prerequisite for the origin of multicellularity in each lineage was the evolution of mechanisms for stable cell-cell adhesion or attachment. Recent advances in genomics and phylogenetics allow comparative studies that provide critical insights into the evolutionary foundations of cell adhesion. Reconstructing the evolution of cell junction proteins in animals and their unicellular relatives exemplifies the roles of co-option and innovation during evolutionary transitions to multicellularity. Comparative studies of volvocine algae reveal specific molecular changes that accompanied the evolution of multicellularity in *Volvox*. Finally, comparisons between animals and *Dictyostelium* indicate the extent to which commonalities and differences in the biology of their unicellular ancestors influenced the evolution of adhesive mechanisms in each lineage. Understanding the unicellular ancestry of cell adhesion helps illuminate the basic cell biology of multicellular development in modern organisms.

Separated at Birth: Multicellular Lineages and Their Unicellular Relatives

The structural integrity of multicellular organisms depends upon the establishment and maintenance of stable cellular connections. In fact, multicellular life would not have been possible without the *de novo* evolution of mechanisms for attaching ancestrally solitary cells together. Detailed studies of intercellular interactions in plants and animals have revealed dramatic differences, both in the types of molecules used to mediate cell adhesion and in the ways those molecules interact with neighboring cells and the extracellular environment [1,2].

The disparity in cell biology between different multicellular lineages provided some of the first evidence that they each evolved independently from distinct unicellular ancestors. The convergence of comparative cell biology, genomics, and phylogenetics now demonstrates that there have been multiple independent origins of multicellularity [3,4]. The many transitions can thus be viewed as repeated experiments that illuminate the role of historical contingency in the evolution of new morphologies [5]. In addition, recent comparisons among diverse unicellular and multicellular organisms reveal the genome content and cell biology of long-extinct unicellular ancestors and may help elucidate the cellular, molecular, and evolutionary foundations of modern multicellularity and development [6–8].

If the now extinct unicellular progenitors of multicellular organisms lived today, their study might reveal the molecular changes that accompanied the evolution of cell adhesion. Instead, one must look to extant unicellular organisms to identify genes that were potentially important for the evolution of their multicellular sisters. To this end, a clear understanding

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of the phylogenetic relationships among diverse unicellular and multicellular eukaryotes is critical.

The phylogenetic distribution of multicellular lineages shows that most have close unicellular relatives with similar cell biology (Figure 1). This has two important implications. The first is that different multicellular lineages evolved stable cell adhesion independently. The second is that the presence of extant unicellular relatives for many multicellular lineages holds the promise of revealing the nature of their unicellular progenitors.

Comparisons among three sets of lineages within this phylogenetic framework have shed light on the diverse evolutionary history of cell adhesion mechanisms (Figure 1). In the first, insights into the genetic and cell biological bases of animal origins have emerged through the analysis of diverse animals and their closest living relatives, the choanoflagellates (Figure 2) [6,9]. Second, comparisons made between two unrelated multicellular lineages, animals and dictyostelids (slime molds) (Figure 1), reveal common themes in cell adhesion mechanisms that evolved independently. Last, multicellular transitions in a class of green algae, the Volvocaceans, are illuminated by comparisons among unicellular *Chlamydomonas* (Figure 1) and other taxa within the Volvocaceans that may represent transitional forms in multicellular evolution (Figure 4) [10]. These data provide a window into the evolution and diversification of multicellular forms.

Co-option and Innovation During Animal Cell Junction Evolution

One feature that distinguishes animals from all other multicellular lineages is the epithelium, a single layer of tightly packed, polarized cells held together by a distinctive set of stable cell junctions [11]. Each junction serves a different function within the epithelium and contains a unique collection of proteins. Together, these proteins provide adhesive and structural support to protect against mechanical stress, barrier function to block particles from crossing the epithelial layer, and communicating function allowing small molecules to pass directly between neighboring epithelial cells. Variations in the diversity and organization of junctions, as well as differences in junctional protein composition, contribute to the development and differentiation of discrete tissue types and likely played a role in the evolution of novel animal forms. By studying the phylogenetic distribution of junctions and their associated proteins, we can reconstruct the evolutionary history of cell junctions and illuminate the connection between epithelia and body plan evolution in animals.

The clear requirement for cell adhesion in multicellular organisms suggests that the metazoan ancestor must have possessed, at a minimum, basic cellular connections. If so, what form did those connections take? Evidence from the most basal extant animals has shed light on this question. Adherens junctions, which physically tether adjacent cells to one another [12], are found in rudimentary form in sponge epithelia (Box 1) [13]. From studies in later-branching lineages, we know that the core molecules providing adherens junctions with their adhesive function are the classical cadherins. The presence in sponges of a cadherin with diagnostic features of classical cadherins [14,15] suggests that the molecular foundations for adherens junctions were in place in the last common ancestor of all animals (Figure 2). Experiments to test whether cadherins localize to sponge junctional structures and mediate adhesion would provide important insights into the earliest stages of adherens junction evolution.

In addition to adhesion, epithelial tissues exhibit barrier properties that prevent passage of particles such as ions and other solutes across the epithelial cell layer. In invertebrates and some mammals, septate junctions provide occluding function and evidence suggests that these junctions may extend back to sponges [16–21], raising the possibility that the barrier

capacity of epithelia also evolved early in animal history. Addressing this prospect will require further characterization of putative septate junctions in sponges. More generally, sponges offer the opportunity to study the most basic functions of epithelia, those that were likely in place in the last common ancestor of animals.

The arrival of new, more complex animal morphologies was coincident with the appearance of pannexin-based gap junctions (characterized by the presence of pannexin proteins) that afford epithelial cells the ability to communicate through the passage of small molecules between cells [22]. The earliest branching organisms with pannexin-based gap junctions are cnidarians such as sea anemones and *Hydra*, which also have clearly identifiable adherens and septate junctions [23–25]. The emergence of gap junctions in organisms with tissue-level organization suggests that the evolution of intercellular communication in epithelia may have contributed to the elaboration of eumetazoan body plans during the Cambrian radiation.

The phylogenetic distribution of adherens, septate and pannexin-based gap junctions reveals that core functions of epithelia were in place before the evolution of Bilateria. Subsequently, new types of cell junctions evolved in different lineages. Chordates evolved junctions that have surprisingly similar functions to those that already existed. Septate junctions appear to have been replaced in most chordates by tight junctions [26–28] that, until recently, were thought to have independent evolutionary origins. Although most of the molecules that comprise septate junctions are not shared with tight junctions, recent studies indicate that one primary component of tight junctions – claudins – serve barrier functions in septate junctions of *Drosophila* (fruit fly) epithelia [29–31]. This suggests that tight and septate junctions may share a common ancestry or, alternatively, that the use of claudin-family members in *Drosophila* septate junctions and the tight junctions of chordate lineages is convergent.

Chordates also evolved a second class of gap junction containing connexins rather than pannexins. Interestingly, unlike tight and septate junctions, connexin and pannexin-based gap junctions may work alongside each other, as they are often expressed in the same tissues [25]. Despite functional similarities, pannexin and connexin protein families have no recognizable sequence similarity, indicating either that they are not evolutionarily related or that they have diverged beyond recognition.

The final major elaboration of epithelial junction diversity came in a new form of adhesive junction called the desmosome. Desmosomes, which are restricted to vertebrates, support epithelia against mechanical stress and contain two specific types of cadherins; desmocollins and desmogleins [12]. The absence of desmosomes in other animals implies that they might serve a function specific to vertebrate biology or simply that their emergence was coincident with the evolution of vertebrates. Indeed, the evolution and diversification of junctional proteins in later branching lineages may have contributed to the evolution of highly specialized tissues. For example, the vertebrate-specific clustered protocadherins are expressed in complex patterns during brain development and may be involved in the differentiation of specific neuronal cell populations [32,33].

Underlying the epithelium in tissue-grade animals is the basal lamina, a dense layer of collagen-rich extracellular matrix (ECM) that acts as an attachment substrate to anchor epithelial cells and helps maintain cell polarity. As is the case for adherens junctions, the molecules required for cell-substrate attachment emerged early in animal evolution (Figure 2). Integrins, the receptors that link cells to the ECM to form hemidesmosomes and focal adhesions, and the proteins that comprise the basal lamina are found in all major animal phyla, including some sponges, despite the fact that most sponges lack a true basal lamina

[19,34–38]. Remarkably, integrin subunits and their predicted intracellular binding partners are also found in the genome of a unicellular relative of animals, *Capsaspora owczarzaki* [39]. The early emergence of integrins and ECM components suggests that the association of cells with a common substrate such as the basal lamina may have contributed to the transition to animal multicellularity.

The characterization of junctions in animals provides important insights into animal epithelial evolution, but how can we understand the deepest roots of epithelia? An instructive example can be found in the study of cadherins in the closest known relatives of animals, the choanoflagellates [6,40]. Cadherins were long thought to be an animal innovation not found in other eukaryotes. However, genomic analysis of the choanoflagellate *Monosiga brevicollis* has uncovered an unexpected diversity of cadherins, on a par with some animals, despite its apparently simple, single-celled morphology [15]. The presence of cadherins in a unicellular species of choanoflagellate, as well as the localization of two *M. brevicollis* cadherins to the choanoflagellate feeding structure, suggests that the ancestral functions of cadherins may have been for sensing and responding to extracellular cues. Furthermore, comparisons of cadherin protein domain composition in *M. brevicollis* and diverse animal cadherins suggest a link between ancestral cadherins and intracellular signal transduction, such as tyrosine kinase and hedgehog signaling. Only after the divergence of the choanoflagellate and animal lineages were cadherins co-opted in animals to form stable junctions between epithelial cells.

The phylogenetic distribution of cell junctions across animals suggests that many of the major epithelial junctions evolved early in animal evolution. The epithelial cells of the metazoan ancestor likely possessed adhesive function, substrate attachment capabilities and possibly barrier properties, with junction-mediated communication emerging later. As animals evolved, so too did their epithelia, acquiring new proteins and eventually new junctional types that allowed the development of more specialized tissues. In some cases, the evolutionary path of junctional proteins appears to be one of convergence as for the pannexins and connexins that compose gap junctions and, possibly, septate and tight junctions. In other cases, such as that of the cadherins, the molecules related to junctional proteins evolved before the junction itself, suggesting that co-option to new functions may have been a driving force for animal evolution. Sponges, apparently lacking some of the well-defined junctions present in later-branching lineages may preserve aspects of the ancestral state of epithelia and further study of sponge epithelia will yield valuable insights into basic epithelial biology. It is also possible that heretofore undiscovered cell junction types may exist in the less well-studied early-branching lineages. Continued research into the molecular foundations of cell biology in choanoflagellates will bring to light critical steps in animal evolution and a more complete picture of the origin and evolution of animal cell junctions will emerge.

Parallels Between *Dictyostelium* and Animal Adhesion

Multicellular organisms typically develop in one of two ways, either through division without cell separation or through cell aggregation [41]. The first mode of multicellular development is exemplified by organisms like plants, animals and fungi while the second mode, a less common strategy among eukaryotes, is nicely illustrated by the dictyostelid slime molds. Dictyostelids exist as unicellular amoeboid cells that, when deprived of nutrients, initiate a morphogenetic program ultimately leading to the formation of a multicellular fruiting body that facilitates spore dispersal. Like animal cells, amoeboid cells from dictyostelids lack a cell wall, allowing their cells to change position within the multicellular organism. The absence of a cell wall also allows them to make dynamic changes in their adhesive properties during development through the regulated expression of

diverse adhesion molecules. The parallels between animal and dictyostelid cell biology as well as the development of dictyostelid fruiting bodies through cell aggregation predict that dictyostelids can yield unique insights into the evolution of cell adhesion mechanisms.

To draw comparisons between animals and dictyostelids, an understanding of basic dictyostelid development is necessary. The best-studied dictyostelid, *Dictyostelium discoideum*, has a defined lifecycle consisting of both unicellular and multicellular states (Figure 3). In the vegetative state, *D. discoideum* remains unicellular; if nutrients become low and starvation ensues, the amoeboid unicells aggregate to form a motile multicellular slug. Once a nutrient-rich environment is found, the slug will develop into a fruiting body that consists of a long stalk bearing a spore-filled sac at its tip. Spatial and temporal regulation of diverse adhesion molecules occurs throughout development and correlates with dramatic morphological changes (Figure 3).

Cell adhesion mechanisms employed during *D. discoideum* morphogenesis have striking similarities with animal cell adhesion. Many cell-cell and cell-matrix adhesion genes that have been characterized in *D. discoideum* appear to be tightly regulated, becoming activated at specific stages of development (Figure 3). The calcium-dependent adhesion molecule DdCAD-1 provides one example that highlights the similarities between animal and *D. discoideum* adhesion. Homophilic adhesion among membrane-bound DdCAD-1 proteins early in *D. discoideum* development helps to initiate cell contacts and has been proposed to play a role similar to that of animal cadherins, an unrelated class of calcium-dependent proteins that also helps establish stable cell junctions through homophilic interactions [42–44]. The DdCAD-1 protein consists of two β-sandwich domains; the N-terminal domain, which has a βγ-crystallin fold, mediates homophilic adhesion, and the C-terminal immunoglobulin-like domain contributes both to homophilic binding function and tethering of the protein to the cell membrane through interactions with cell surface-associated proteins [45]. In both animals and *D. discoideum*, expression of specific adhesion genes is frequently limited to a subset of cells within the multicellular organism and this spatial regulation is critical for proper development [2,33,46]. During the slug stage, after cell contacts have been established, DdCAD-1 localizes to the periphery of the slug while other adhesion molecules are expressed in the interior [47]. Furthermore, deletion of the DdCAD-1gene leads to defects in morphogenesis, delayed development and aberrant cell sorting indicating that, like many animal adhesion molecules, DdCAD-1 has important developmental functions [33,48–50]. In animals, the phosphorylation state of an adhesion protein often has dramatic affects on its adhesive properties [51]. Similarly, ras-dependent dephosphorylation of DdCAD-1 increases DdCAD-1-mediated adhesion of cells [52].

Other animal-like features of *D. discoideum* cell adhesion not to be overlooked are structures resembling adherens junctions in the fruiting body [53]. Not only do these structures have the appearance of adherens junctions in electron micrographs, but, like animal adherens junctions, they are actin-rich. Furthermore, a protein called aardvark localizes to the junctions and has been implicated in cell signaling. Aardvark belongs to the protein superfamily that also contains the animal protein β-catenin, a cytoplasmic component of animal adherens junctions that organizes the junction-associated actin cytoskeleton and has additional important roles in cell signaling [54].

Orchestration of *D. discoideum* development requires regulated cell adhesion and the mature organism possesses stable cell junctions associated with the actin cytoskeleten. The molecular basis for this adhesion comes from a diverse set of proteins expressed throughout morphogenesis, each with a specific function within the organisms. The adhesive properties of *D. discoideum* are remarkably similar to those of animals yet most *D. discoideum* adhesion molecules have little sequence similarity to animal proteins. This suggests that

their cell adhesion molecules have distinct evolutionary origins due to the unique unicellular ancestors from which they evolved (Figure 1). Although the genome composition of dictyostelids is different from that of animals, the cell biological characteristics that they share, such as motility and the absence of a cell wall, may have lead to the convergence of adhesive mechanisms. An alternative explanation is that the cell adhesion proteins in animals and dictyostelids are homologous but have diverged beyond detection at the molecular level and have been lost in all of the unicellular and multicellular organisms that branch between these two lineages. Continued investigation into the molecular underpinnings of *D. discoideum* adhesion will expand our understanding of dictyostelid multicellularity and its relationship to animal biology.

The Volvocine Algae: From Cell Walls to Extracellular Matrix

Many of the multicellular lineages present on earth today evolved from unicellular ancestors with rigid cell walls. Indeed, the cells of land plants, fungi, and red and brown algae are all bound by a cell wall. For these cells, adhesion is a passive process very different from the intimate cellular associations found in animals and Dictyostelids; physical connections are established as new cells form and the resulting attachments between cells are stabilized and maintained throughout life. This type of multicellular development, in which cells divide and remain linked by their shared cell wall, has important implications for the developing organism as the cells cannot reposition themselves after cytokinesis.

The intermediate forms that reveal important steps in most multicellular transitions have been wiped away by evolution and extinction. An exception to this is found in the volvocine algae, with diverse species reflecting everything from unicellular to colonial to multicellular morphology. The graded complexity within the volvocine algae provides a superb opportunity to explore the evolution of cell adhesion in organisms with cell walls (Figure 4).

The phylogenetic relationships among the volvocine algae provide a framework for examining the genomic and cell biological changes that occurred during their evolution (Figure 4). The group contains organisms ranging from the unicellular flagellate *Chlamydomonas reinhardtii* to the fully differentiated multicellular *Volvox carteri* (*Volvox*), a spherical organism containing somatic cells and reproductive cells. Molecular phylogenetic studies indicate that *Volvox* and its close relatives (family Volvocaceae) form a monophyletic group that shares a recent common ancestor with *Chlamydomonas* [55]. Branching between *Volvox* and *Chlamydomonas* are *Gonium pectorale* (*Gonium*) and *Pandorina morum* (*Pandorina*), whose morphologies appear to represent intermediate forms, suggesting that multicellular *Volvox* may have evolved though progressive increases in size and complexity (Figure 4).

Unlike animals, which adhere through membrane-bound proteins and typically use ECM as an attachment substrate, volvocine algae achieve multicellularity by embedding their cells in a common ECM (Figure 4). Indeed, characterization of cell wall and ECM components in the volvocine algae indicates that the conversion of cell wall structures to ECM and the subsequent expansion of this ECM was critical during the transition to multicellularity [56]. The cell wall of unicellular *Chlamydomonas* can be broken into two main parts: the highly organized outer cell wall or `tripartite layer', named after its striped appearance in electron micrographs, and the less structured inner cell wall (Figure 4) [57]. In colonial *Gonium* the cell wall resembles that of *Chlamydomonas*, with each cell surrounded by tripartite layer. *Gonium* cells in colonies are connected by specialized cell wall structures, potentially preserving an early stage in the evolution of the cell wall into ECM [56]. Unlike in *Gonium*, the tripartite layer in *Pandorina* and *Volvox* encases the whole multicellular organism and is

important for holding the cells together once cytoplasmic connections have broken down [58].

The inner cell wall has also changed throughout volvocine evolution, appearing as an amorphous region below the outer cell wall of *Chlamydomonas* and growing into a voluminous ECM that provides a structural network in which cells are embedded in *Pandorina* and *Volvox*. The dramatic change in inner cell wall volume has been so great that each *Volvox* cell has approximately 10,000 times as much ECM as a *Chlamydomonas* cell does [56]. The morphological differences in cell wall organization among the volvocine algae may reflect its evolution from a simple protective layer into a scaffold that helps organize, support, and protect cells within colonial and multicellular contexts.

Molecular studies corroborate morphological evidence that the cell wall was transformed into ECM during volvocine evolution, revealing that co-option and diversification of cell wall protein families to ECM may have played an important part in the transition to multicellularity. Both the inner and outer cell wall of *Chlamydonomas* are comprised primarily of hydroxyproline-rich glycoproteins (HRGPs). The functional equivalency of the *Chlamydomonas* outer cell wall and the tripartite layer of colonial and multicellular volvocine species has been demonstrated experimentally [59] and the homology of cell wall proteins has been asserted due to high sequence similarity. For instance, homologs of the *Chlamydomonas* outer cell wall protein GP2, a HRGP, have been identified in *Volvox* and *Pandorina* and shown to localize to the tripartite layer [60]. Additionally, the protein ISG (inversion specific glycoprotein), which is critical for ECM assembly during *Volvox* development [61], has a close relative in *Chlamydomonas* called VSP-3 that is found in the outer cell wall [62].

The HRGP constituents of the inner wall have been modified, diversified and specialized over time to serve as ECM in the later branching algae. Pherophorins are a class of HRGP found throughout the ECM of *Pandorina* and *Volvox* [63]; pherophorin homologs have also been identified in *Gonium* and *Chlamydomonas* [64]. The expression of some pherophorins changes in response to wounding and is also modulated by pheromones that trigger sexual development in *Volvox*, indicating that pherophorins are a developmentally regulated class of proteins. Phylogenetic analyses of pherophorins suggest that the unicellular volvocine ancestor possessed a relatively diverse array of pherphorin proteins and that the protein family has further diversified in *Volvox*, potentially adopting new developmental functions [7]. Together, these data indicate that the early cell-wall-associated HRGPs of the basal volvocine algae evolved into a class of proteins with ECM function in colonial and multicellular lineages. Furthermore, comparison of HRGPs from diverse species suggests that gene duplication, divergence and domain shuffling played an important role in volvocine evolution [56,62,64].

The volvocine algae represent a well-characterized example of how a unicellular organism can evolve mechanisms of cellular attachment through the co-option of cell wall proteins to ECM. The expansion and diversification of the HRGPs correlates with the appearance of ECM and homologs of *Chlamydomonas* cell wall-associated HRGPs localize to ECM-rich structures in colonial and multicellular species, suggesting that this protein family played an important role in the evolution of multicellularity. The transformation of cell wall to ECM was a critical step in the evolution of multicellularity in Volvocaceae and represents one of many ways that cells can evolve stable attachments. Investigation of cell adhesion machinery in other multicellular cell wall-bound organisms indicates that despite similarities in cell biology, adhesion mechanisms can differ greatly from one lineage to the next. For example, cells of land plants, which share a closer ancestry with the volvocine algae than most other multicellular lineages (Figure 1), adhere using pectin polysaccharides found

throughout the cell wall and enriched in the middle lamellae and tricellular junctions [1,65]. Cellulose, hemicellulose and lignin are also important structural elements found in the cell walls of many plant lineages that are absent in the cell wall volvocine algae [66]. Fungi, whose cell wall composition differs greatly from plants, use entirely different molecules to maintain intercellular connections [67]. These differences in cell adhesion reflect the unique evolutionary history of each multicellular lineage and suggest how the biology and genomic content of the unicellular ancestor exert strong influences on the evolution of multicellular forms.

Contingency and Chance in the Evolution of Cell Adhesion

What explains the existence of dramatically different modes of cell adhesion in each of the different multicellular lineages? Part of the answer derives from the disparate cell biology and genome composition of the different unicellular progenitors of each lineage. For example, we can infer that the unicellular progenitor of animals was a heterotrophic flagellate that lacked a cell wall. Likewise, animal cells lack cell walls, permitting them to adhere dynamically and reorganize into complex tissues and organs during development. In contrast, the last common ancestor of *Chlamydomonas* and *Volvox* was encased in a cell wall, a feature that provides structural integrity but prohibits cell rearrangement. The genome contents of the distinct progenitors of animals and *Volvox* may have also predisposed the two lineages to certain forms of multicellularity. Through comparative genomics and cell biology one can identify those genes that represented preadaptations for cell adhesion in each lineage [68]. HRGP cell wall components in the unicellular progenitor were co-opted for use in the ECM of *Volvox*, just as cadherins in the unicellular progenitor of animals were co-opted to function as adhesion receptors in epithelia. An outstanding question is whether these molecules and those used in other lineages (e.g. *Dictyostelium*) were especially suited for mediating cell-cell interactions, or whether their recruitment to function in cell adhesion was primarily through chance [5,69].

Understanding the unicellular ancestry of cell adhesion mechanisms will reveal fundamental aspects of cell biology in multicellular organisms and provide insights into the development and evolution of morphologically complex eukaryotes. Progress on this front will require expanded comparisons of genomes from diverse multicellular organisms paired with their closest single-celled relatives. With advances in genome sequencing technology, coupled with improvements in genetic manipulation in non-model organisms, we can elucidate critical genomic and functional changes that launched eukaryotic transitions to multicellularity. The approach proposed, while most directly relevant to the origin of cell adhesion, holds the promise of further illuminating the roles of historical contingency, biological constraint, and chance during evolution.

Box 1. Sponge Tissues: Structure and Function

Sponges, often erroneously depicted as undifferentiated aggregates of cells, are bona fide animals. Fertilized sponge eggs develop to form larvae, consisting of polarized epithelial cells, which first swim and then settle on a solid surface before metamorphosing into a juvenile sponge. Adult sponges contain spatially-differentiated epithelia in which different regions serve discrete functions in prey capture, protection from the environment and maintenance of the structural integrity of the organism (see below). The epithelia surround a seemingly unstructured ECM-rich compartment, the mesohyl.

Larval epithelium

Sponge larvae are ellipsoidal and composed of multiple layers of cells. The outer layer, called the larval epithelium, consists of tightly packed cells that form a columnar

epithelium with the basal surface facing inward (see diagram). Each cell typically possesses a single, motile cilium at its apical end that facilitates larval dispersal.

Pinacoderm

The external surface and water channels of the sponge are comprised of pinacocytes that make up the endo- and exo-pinacoderm (inner and outer epithelium, respectively). The basic cell morphology of the pinacocytes within the endo- and exo-pinacoderm is similar in many sponges (see diagram). The pinacoderm functions as a stable sheet of cells with the pinacocytes maintaining their positions and intercellular connections over long periods of time [21]. Intercellular junctions between these cells have been visualized by electron microscopy but their molecular components and specific functions (e.g. barrier or communication) have not yet been determined [21].

Choanoderm

The choanoderm (feeding epithelium) is composed of choanocytes that display an ovoid cell body and a single flagellum surrounded by a ring of microvilli (see diagram). The choanocytes form a round chamber and beat their flagella to draw water from the incurrent canals into the chamber where bacterial food are filtered by the microvilli. The water is then expelled through the osculum.

Mesohyl

The mesohyl is composed primarily of ECM that contributes to the gross morphology of the sponge. Distributed sparsely within the dense matrix are amoeboid-like cells that secrete the ECM material and multipotent archeocytes that can differentiate into any other sponge cell-type.

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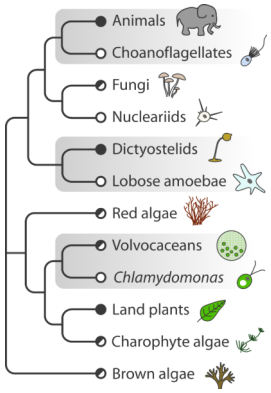


Figure 1. Diverse multicellular eukaryotes and their closest unicellular or colonial relatives

The phylogenetic relationships among select unicellular, colonial and multicellular eukaryotic lineages indicate that multicellularity evolved multiple times. Some lineages are strictly multicellular (filled circle) and some are unicellular or display simple/ undifferentiated colonies (open circle), while others have a mix of unicellular or colonial and multicellular forms (half filled circle). Comparisons among multicellular lineages and their closest unicellular relatives provide insights into the mechanisms underlying transitions to multicellularity. Lineages discussed in this review are highlighted in grey.

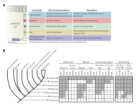


Figure 2. Phylogenetic distribution of epithelial junctions based on ultrastructural, genomic and functional data

A) Schematic representation of animal epithelial cell junctions. Each type of junction, comprised of a unique set of structural proteins, is restricted to a specific region of the epithelial cell membrane and serves a distinct function (indicated in table). (B) Evolutionary history of epithelial cell junctions. The four main functions of animal epithelia are adhesion, barrier, signaling and anchoring. Elements of adherens, septate, pannexin-based gap and anchoring junctions [hemidesmosomes (Hemides.), focal adhesion (Focal Adh.) and the basal lamina] were likely present in the last common ancestor of animals while desmosomes, tight and connexin-based gap junctions emerged later in animal evolution. For a series of ancestors representing stages in animal evolution, the presence (filled box) or absence (open box) of evidence for (1) genes diagnostic of each junction type ("Gene"), (2) junction morphology detected by electron microscopy ("Morph") and (3) experimental support (e.g. stable cell adhesion, barrier function, or protein localization; "Expt") in extant lineages is indicated [11,13–23,25,34–38,70,71].

Numbered boxes indicate observations of note: **1**, reports of desmosomes observed in non-vertebrates by electron microscopy are controversial [11]; **2**, septate junctions have been identified in mammals but not in other chordates [16]; **3**, evidence for septate junctional proteins in sponges is based solely on BLAST analysis of EST data [19]; **4**, tight and connexin-based gap junctions are found in Urochordates but not Cephalochordates [22,26–28]; **5**, a single example of tight junctions has been reported in the spider central nervous system [72]; **6**, pannexins are present in *Hydra* but absent from the *Nematostella* genome suggesting that they are not found in all cnidarians [23,25]; **7**, while cell biological and biochemical investigations of integrin function in cnidaria is absent, preliminary research in the jellyfish *Podocoryne carnea* indicates that transcripts of integrin-α and -β subunits have been detected in the same cells as talin, a focal adhesion protein [73]; **8**, although all sponges have ECM, only some sponges display structures that resemble basal lamina and only homoscleromorph sponges have been reported to possess ECM comprised of type-IV collagen, a major component of the basal lamina [34–37]; **n/d**, not determined.

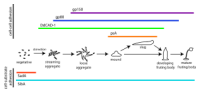


Figure 3. Cell adhesion proteins are dynamically expressed during *D. discoideum* development

Upon starvation, a complex developmental program is initiated involving temporal regulation of diverse cell-cell and cell-substrate adhesion proteins. Colored bars indicate timing of gene expression. The cell-cell adhesion protein DdCAD-1 is expressed when aggregation initiates and is followed by gp80 and then gp150. PsA expression is initiated at the mound stage and persists until fruiting body development. The cell-substrate adhesion protein SadA is active only during vegetative growth while SibA is constitutively expressed. Adapted from [46].

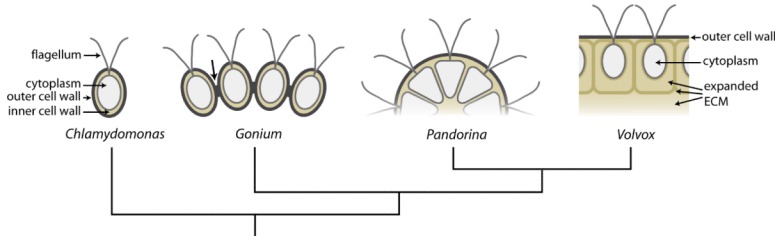
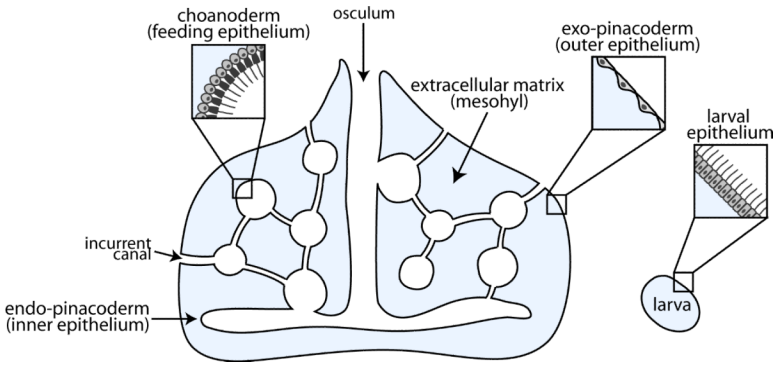


Figure 4. Evolution of cell wall into ECM in the volvocine algae

The graded complexity of volvocine algae coupled with insights into their phylogenetic relationships provide the opportunity to identify the molecular foundations of an evolutionary transition to multicellularity. *Chlamydomonas* possesses an HRGP rich cell wall consisting of an outer cell wall (dark grey) with a characteristic tripartite structure (three fibrous layers, termed the `tripartite layer') and a relatively amorphous inner cell wall (brown) surrounding the cell membrane and cytoplasm. Cells in *Gonium* colonies have similar cell walls that are attached to neighboring walls by ECM-based structures (arrow). In *Pandorina*, the outer cell wall surrounds the entire colony and the cells are embedded in a common ECM comprised of inner cell wall HRGP homologs. Multicellular *Volvox* has a voluminous ECM derived from diverse HRGP family members that have specialized functions. Adapted from [56].



box 1.