An epithelial tissue in Dictyostelium challenges the traditional origin of metazoan multicellularity

Daniel J. Dickinson1), W. James Nelson1)2)3) and William I. Weis1)3)4)

We hypothesize that aspects of animal multicellularity originated before the divergence of metazoa from fungi and social amoebae. Polarized epithelial tissues are a defining feature of metazoa and contribute to the diversity of animal body plans. The recent finding of a polarized epithelium in the non-metazoan social amoeba Dictyostelium discoideum demonstrates that epithelial tissue is not a unique feature of metazoa, and challenges the traditional paradigm that multicellularity evolved independently in social amoebae and metazoa. An alternative view, presented here, is that the common ancestor of social amoebae, fungi, and animals spent a portion of its life cycle in a multicellular state and possessed molecular machinery necessary for forming an epithelial tissue. Some descendants of this ancestor retained multicellularity, while others reverted to unicellularity. This hypothesis makes testable predictions regarding tissue organization in close relatives of metazoa and provides a novel conceptual framework for studies of early animal evolution.

Keywords:
- Dictyostelium; epithelium; metazoan evolution; multicellularity

Introduction

A simple epithelium consists of a two-dimensional sheet of structurally and functionally polarized cells that are often organized into a three-dimensional tube, and is a defining feature of animal body plans. An epithelium (the trophoderm) is the first differentiated tissue formed during embryogenesis, and many adult tissues are composed of epithelia [1, 2]. Epithelial cells have a polarized organization of the plasma membrane, cytoskeleton, and cytoplasmic organelles [2, 3]. The apical plasma membrane faces the lumen of an organ or the outside of the organism, while the basal (or basolateral) membrane contacts the underlying tissue. Polarized epithelial sheets regulate the directional absorption and secretion of proteins and other solutes, an essential physiological function that is often disrupted in human disease [1]. In addition, the polarized organization of cytoskeletal components in epithelial cells contributes to tissue shape changes during embryonic morphogenesis [4]. Despite the diversity of body plans and lifestyles, all metazoa share an epithelial tissue as their basic unit of organization, suggesting that the development of epithelial cell polarity was a very early event in metazoan evolution [5, 6].

Genetic, cell biological, and molecular studies in a variety of metazoa have shown that the formation and maintenance of polarized epithelial cells require cell-cell adhesion mediated by the cadherin-catenin complex [7]. Cadherins are transmembrane receptors that form homophilic and heterophilic adhesive interactions with cadherins on adjacent cells, providing a spatial cue that initiates cell polarity [8]. β-Catenin and α-catenin are cytosolic binding partners of cadherin that transduce this adhesive cue and mediate cell polarity, in part by directing the reorganization of the cytoskeleton [8, 9]. Consistent with

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its fundamental role in epithelial organization, the cadherin-catenin complex is conserved in all metazoans.

Phylogenetically speaking, metazoans belong to the unikonts, a group that also includes fungi, social amoebae, and a number of unicellular or colonial protists (see Fig. 2) [10, 11]. Historically, it was thought that multicellularity evolved independently in animals, fungi and social amoebae, and that epithelial tissue was a unique feature of animals [11–16]. However, two recent studies have established the existence of polarized epithelial tissue in the non-metazoan social amoeba *Dictyostelium discoideum* [15, 16]. This finding calls into question the notion of an independent origin of multicellularity in animals and social amoebae. Here, we propose and discuss the alternative hypothesis that all unikonts evolved from an ancestor with a simple multicellular organization.

### Identification of an epithelial tissue in *Dictyostelium*

*D. discoideum* is a representative of the social amoebae, a group of organisms that feed as single cells but undergo a transition to a multicellular form upon starvation (Fig. 1A) [17]. Starving cells secrete cyclic AMP, which acts as a chemoattractant and induces cells to aggregate into spherical mounds (see [18] for a detailed description of the aggregation process). Once aggregation is complete, the mound elongates to form a slug. After a period of migration, the slug develops into a fruiting body by a process called culmination. The developmental process can thus be divided into three phases: (1) chemotaxis and aggregation; (2) slug formation and migration; and (3) culmination. Of these three phases, chemotaxis has received the most attention, and relatively little is known about the later stages of development. However, it is during culmination that the greatest degree of complexity in tissue organization and cell type specialization is observed.

The fruiting body consists of a rigid stalk supporting a collection of spore cells at the top (Fig. 1B). The defining event of culmination is the production of the stalk, which elevates the spores and eventually facilitates their dispersal. The stalk consists of stalk cells encased in a rigid tube made of cellulose and extracellular matrix (ECM) proteins [19, 20]. During culmination, the stalk cells increase in volume, but because they are encased in a rigid tube, their expansion is directed in the vertical direction and contributes to the lifting force that raises the spore head [21]. Stalk formation is orchestrated by the...
tip, which is a specialized structure located at the apex of the culminant [15, 16, 21–24]. Though the tip was previously thought to play an important role in culmination, its subcellular organization and function had not been studied in detail.

Confocal microscopy showed that the tip contains a highly organized cell monolayer that forms a tube surrounding the apex of the stalk (Fig. 1B, C) [15], consistent with earlier electron microscopy data [25, 26]. These cells, which we refer to as the tip epithelium, exhibit many of the hallmarks of epithelial cell polarity, including: a polarized organization of the actomyosin and microtubule cytoskeletons; a polarized distribution of cytoplasmic organelles; and division of the plasma membrane into apical and basolateral domains with distinct protein compositions (Fig. 2) [15, 16]. Most importantly, polarity of the tip epithelial cells in Dictyostelium requires homologs of β- and α-catenin, which are necessary for epithelial organization and polarity in metazoans.

Two distinct developmental functions have been identified for the tip epithelium. First, the epithelial cells secrete cellulose and ECM proteins directionally to form the rigid stalk tube on the exterior of the stalk [15]. Second, the epithelium is an actomyosin-based contractile structure that applies a

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Figure 2. Conservation of epithelial characters in unikonts. The table shows the presence (colored square) or absence (white square) of the indicated characters in the indicated species. A question mark indicates that no information is available for this particular character and species. Character descriptions and notes: (1) No molecular information about these species is available because their genomes have not yet been sequenced. (2) Solid squares indicate constitutive multicellularity; cross-hatched squares indicate facultative multicellularity. (3) Indicates a columnar monolayer of cells that morphologically resemble a simple epithelium. (4) Indicates a polarized organization of the plasma membrane, cytoskeleton, and cytoplasmic organelles. (5) Indicates direction secretional of proteins and other solutes. (6) Indicates an apical enrichment of myosin motor proteins, resulting in apical contractility. (7) Indicates the presence of cell-cell junctions linked to the actin cytoskeleton. These are represented by adherens junctions in metazoans, and by junctions of unknown molecular composition in D. discoideum. See the text for details. (8) Solid squares indicate classical cadherins that contain a cytoplasmic domain that can bind β-catenin; cross-hatched squares indicate proteins with extracellular cadherin repeats but no classical cadherin cytoplasmic domains. (9) β-catenin orthologs are very difficult to identify using sequence-based methods such as BLAST, due to the presence of armadillo repeats that are also found in many other proteins. Therefore, we conservatively use solid squares to represent proteins that are known to be functionally similar to β-catenin and cross-hatched squares to represent armadillo repeat proteins with sequence similarity to β-catenin but unknown function. (10) The α-catenin ortholog from D. discoideum has been shown to be functionally similar to α-catenin but not the related protein vinculin [15]. Members of this protein family from choanozoa and chytrid fungi are therefore inferred to be α-catenin-like. (11) Solid squares represent an essentially complete set of polarity proteins (see text). A few polarity proteins, including Lgl, Par-1, Par-4, and Par-5 are members of protein families that also include non-metazoan members.
squeezing force to the stalk, thereby limiting its diameter [16]. Although these two functions result from distinct cellular activities, they both contribute to patterning the stalk: the squeezing force applied by the epithelium causes the stalk to be tall and thin, while the secreted cellulose and ECM rigidify the stalk and ensure that its diameter remains constant even as stalk cells expand. These recent descriptions of the subcellular organization and function of the tip epithelium support the historical view of the tip as an organizing center for fruiting body morphogenesis [21–24]. In addition, it is striking that the tip epithelium is capable of polarized secretion and apical enrichment of myosin during morphogenesis, both of which are important functions of metazoan epithelia.

Although the recent description and genetic analysis of the tip epithelium were restricted to the model organism *D. discoideum*, available evidence suggests that epithelia may be widespread in social amoebae. Cell monolayers with a similar appearance to the tip epithelium have been described in five other dictyostelid species, representing three of the four major phylogenetic groups of social amoebae [27–30]. In every case these tissues were located adjacent to the stalk at the tip of the culminant, suggesting a developmental function similar to that of the *D. discoideum* tip epithelium. Moreover, homologs of β- and α-catenin are found in every social amoeba whose genome has been sequenced ([15] and DJD, unpublished results). Some of these species have fruiting body morphologies that are more elaborate than *D. discoideum*, and it will be interesting to determine whether more complex morphogenetic movements of epithelia contribute to the formation of these structures.

**Dictyostelium has a minimal molecular machinery for epithelial cell polarity**

Despite the morphological, molecular and functional similarities between *Dictyostelium* and animal epithelia, there are also differences, as might be expected given the evolutionary distance involved (Fig. 2). Perhaps most importantly, *Dictyostelium* does not have homologs of classical cadherins, which are essential for recruitment of β- and α-catenin to the plasma membrane in metazoans. Nevertheless, the catenins localize to the plasma membrane in the tip epithelium [15]. Presumably an as yet unknown membrane-associated protein interacts with the catenins to recruit them to the plasma membrane, and identifying this molecule is an important goal for future studies. In addition, *Dictyostelium* lacks “polarity proteins” including the PAR proteins, Crumbs, and Scribble, which are important regulators of epithelial polarity in higher animals and may be necessary for the more complex morphogenetic movements that metazoan epithelia must execute during morphogenesis [4, 31]. Finally, metazoan β-catenin also plays an important role as a transcriptional co-factor in the Wnt signaling pathway [32], but despite the presence of a β-catenin homolog, other key Wnt pathway components are absent in *Dictyostelium*, indicating that the role of β-catenin in epithelial polarity predates its function as a signaling protein.

A more complex issue is the role of cell-cell junctions in the tip epithelium. Adherens junctions containing classical cadherins and their cytosolic binding partners, including the catenins, mediate cell-cell adhesion in animals [9]. The adherens junction associates with actin and serves to mechanically couple the cytoskeletons of adjacent cells, allowing force transmission across a tissue [33, 34]. In many (though not all) metazoan epithelial cells, the adherens junction localizes to the boundary between apical and lateral plasma membrane domains. Interestingly, transmission electron microscopy revealed actin-associated junctional structures in *Dictyostelium* tip epithelial cells that localize to the apical-lateral boundary and appear to connect contractile actomyosin bundles across several cells [15, 16, 25]. Despite this apparent similarity at the ultrastructural level, these *Dictyostelium* cell-cell junctions do not require either β- or α-catenin [15], or cadherins (which are absent in *Dictyostelium*) and, therefore, are most likely not homologous to the metazoan adherens junction. One possibility is that the presence of similar-looking adhesion structures in *Dictyostelium* and animal epithelia reflects convergent evolution. Alternatively, sequence homologies of junctional components other than the catenins might exist but be too weak to detect computationally. Determining the molecular composition of *Dictyostelium* cell-cell junctions will distinguish between these two possibilities.

Overall, it appears that the molecular machinery involved in epithelial cell polarity in *Dictyostelium* is much simpler than in higher animals (Fig. 2). Therefore, studies in *Dictyostelium* are likely to be informative for elucidating the core evolutionarily conserved machinery necessary for formation of a polarized epithelium. β-Catenin and α-catenin clearly belong to this minimal machinery, since they are required for epithelial polarity in both *Dictyostelium* and metazoans [15]. Additional molecular components have been identified in immunoprecipitates of α-catenin from fruiting bodies, including a *Dictyostelium* IQGAP homolog and its binding partner cortexillin that appear to act downstream of the catenins to regulate myosin localization and tissue morphology [16]. Available data suggest that IQGAP may also have a role in epithelial cell polarity in higher animals [35–37], although it is unknown whether the putative role of IQGAP in metazoan epithelia involves myosin regulation. Thus, experiments in *Dictyostelium* can shed light on the fundamental requirements for epithelial cell polarity and reveal new molecular mechanisms that may be relevant in other systems.

**An epithelium in *Dictyostelium* may indicate an ancient origin for multicellular organization**

*Dictyostelium* and metazoan epithelia are structurally and functionally similar tissues, and importantly, they share a degree of homology at the molecular level, since the catenins are required for the epithelial organization and polarity in both systems [15, 16]. What does this result mean for our understanding of animal origins? As mentioned above, the traditional view has...
been that social amoebae, animals, and fungi each evolved multicellularity independently [12, 13]. However, the molecular and functional similarities between Dictyostelium and animal epithelia may force us to re-examine this conclusion.

Is Dictyostelium an appropriate model system to study the origins of multicellularity in animals? Social amoebae diverged from the metazoan lineage earlier than other metazoan relatives (most notably choanoflagellates and sponges) [10], but molecular data indicate that they may have evolved relatively little since their divergence. Ribosomal RNA sequences from different social amoebae are approximately as diverse as those of metazoa, even though all social amoebae have a similar lifestyle and morphology [30, 38]. Moreover, the cellular slime mold Fonticula alba has a life cycle very similar to that of social amoebae, even though it is phylogenetically more closely related to fungi [39]. Together, these observations suggest that the analysis of living social amoebae can provide considerable insights into the nature of their ancient ancestors, which might have had much in common with the metazoan ancestors that lived around the same time. Of course, a caveat is that one cannot rely too heavily on data from any single species when drawing conclusions about the nature of metazoan ancestors. Observations from social amoebae, choanoflagellates, as well as intermediate-branching species such as ichthyosporeans and chytrid fungi must ultimately be integrated to yield a coherent understanding of metazoan origins.

Surprisingly, analysis of the genomes of choanoflagellates, social amoebae, and other close relatives of metazoans revealed that many of the molecules historically thought to be restricted to metazoans are found in these early-branching lineages as well (Fig. 2) [40–43]. Although in most cases, the cellular functions of these proteins are unknown, the similar functions of the Dictyostelium and metazoan catenins provides one illuminating example [15, 16]. Strikingly, Dictyostelium α-catenin can bind directly to mouse β-catenin, indicating that this protein-protein interaction may have been conserved since the common ancestor of social amoebae and animals [15]. Thus, in at least one case, proteins that are conserved between metazoans and non-metazoans also have conserved functions. We expect that when more cases are studied in detail, additional examples of functional similarity between metazoan and non-metazoan proteins will be found.

It is conceivable that the similar functions of the catenins in Dictyostelium and animals are a product of convergent evolution. However, we think that the degree of molecular, morphological, and functional similarity between epithelial tissues in Dictyostelium and animals is very unlikely to be explained by convergence. In light of the observations discussed above, we propose that at least some of the organizational principles underlying animal multicellularity predate the divergence of social amoebae from metazoans. But how are we to reconcile this rather provocative conclusion with our knowledge of other close metazoan relatives and with the view of social amoebae, fungi, and animals as independent “inventors” of multicellularity [11–13]? We suggest that an answer to this apparent contradiction can be found by re-thinking the nature of the transition to multicellularity in metazoans.

Evidence for independent origins of multicellularity is not conclusive

The idea that multicellularity originated independently in social amoebae, fungi, and animals has been supported by three main lines of evidence. First, these groups diverged approximately 600 million years before the appearance of the earliest putative metazoans in the fossil record [44, 45]. However, the absence of putative metazoans in the fossil record prior to the Ediacaran period is not definitive, as it is possible that multicellular unikonts prior to this time were either too small or too fragile to fossilize, or too morphologically different from extant animals to be recognizable [46].

A second argument for independent origins of multicellularity in social amoebae, fungi and animals has been the very different lifestyles of organisms in these taxa. These groups were thought to have little in common at the level of tissue organization, which is one reason that the finding of epithelial tissue in Dictyostelium was so surprising. It is important, however, to avoid confusing the structure of a tissue with its physiological purpose. At the organismal level, epithelial tissue has a different purpose in Dictyostelium than in animals. Animal epithelia have functions that include forming a protective barrier, directional absorption and secretion, and execution of morphogenetic movements during development [1, 2, 4]. The function of the Dictyostelium tip epithelium is to pattern the stalk, a role not obviously related to that of any metazoan epithelial tissue. Nevertheless, at the cellular level, Dictyostelium and animal epithelia are very similar: they both have a monolayer organization, secrete proteins and other solutes directionally, exhibit apical contractility, and require the catenins for their organization and polarity (Fig. 2). Thus, organismal lifestyle may not be a good predictor of tissue structure and function, and similarly organized tissues can have quite different roles in different organisms. The unikont lineages that branch between social amoebae and animals have unique lifestyles and ecological niches, but tissue organization in these lineages has generally not been well studied. Closer examination of these species may well reveal unexpected similarities to social amoebae or animals.

Third, the phylogenetic clade that includes social amoebae, fungi, and animals also contains some unicellular organisms [10, 11], and it was considered more parsimonious to postulate independent origins of multicellularity than a loss of multicellular organization in multiple unicellular lineages. We now propose an alternative model that can account for both the putative homology of epithelial tissues in metazoans and social amoebae, as well as the apparent loss of multicellularity in many unikonts.

Multicellularity as a plastic trait in metazoan ancestors

A polarized epithelium is the first tissue formed during metazoan development,
Hypotheses

amoebae are becoming multicellular. Hence, social amoebae can replicate indefinitely as single cells, and in nature they may go many generations without becoming multicellular. Hence, social amoebae are facultative multicellular organisms that adopt multicellularity only for a specific purpose. This mode of multicellularity is different from the constitutive multicellularity that defines metazoans.

Multicellularity in metazoans can be considered a fixed state, since all metazoans are multicellular throughout their lives (with the exception of gametes). Apparently, adaptations associated with constitutive multicellularity have deprived animal cells of the potential to survive and reproduce on their own, or at least to effectively compete with other unicellular organisms. In contrast, facultative multicellularity in social amoebae can be viewed as a continuum: some species, including Dictyostelium, readily enter the multicellular state when subjected to starvation, while other species preferentially respond to starvation by forming unicellular cysts and are only rarely multicellular [38, 46]. The amount of time spent in the multicellular state is thus governed both by environmental cues and by the organism’s response to those cues. Given this scenario, it is easy to imagine how a facultative multicellular organism could revert to a unicellular state, simply by adapting to a novel environment that favored single cells. The variable preference for aggregation versus encystation in different species of social amoebae [38, 46] provides evidence that evolution can indeed modulate the extent of facultative multicellularity, and solitary amoebae such as Acanthamoeba could be examples of species that have abandoned multicellularity entirely.

Other unikonts also exhibit facultative multicellularity, and the amount of time spent as a multicellular organism similarly varies within each group. For example, the choanoflagellate Salpingoeca rosetta can exist either as a single cell or as small colonies [47]. The physiological significance of colony formation is unknown, but it has been suggested that unicellular and colonial states are each advantageous under different environmental conditions. Consistent with this idea, colony formation and morphology are influenced by presence of a specific bacterial prey species [47]. Another group of metazoan relatives, the ichthyosporeans, also transitions between a unicellular amoeboid state and simple spherical colonies [48, 49]. The cellular slime mold Fonticula alba exhibits facultative multicellularity very similar to Dictyostelium, despite being more closely related to fungi than to social amoebae [39]. Finally, many species of fungi exhibit facultative multicellularity, switching from unicellular (yeast-like) to multicellular (pseudohyphal or filamentous) growth in response to specific environmental conditions [50]. Multicellularity in fungi is particularly plastic, having been lost completely and then re-invented on several occasions [13]. Taken together, these observations indicate that facultative multicellularity is widespread in unikonts, whereas constitutive multicellularity is characteristic of metazoans.

We propose that the common ancestor of all unikonts was a facultative multicellular organism in which some aspects of cell or tissue polarity were controlled by the conserved catenin complex. Because of the inherently plastic nature of facultative multicellularity, some of the lineages descended from this ancestral unikont reverted to the unicellular state, while others retained a facultative multicellular lifestyle. In one lineage (metazoans), multicellularity became constitutive. This hypothesis accounts for the presence of an epithelial tissue in Dictyostelium, is consistent with our knowledge of unikont natural history and phylogeny, and is sensible given the widespread distribution of facultative multicellularity among unikonts. The idea that multicellularity existed prior to metazoan origins may also help to explain the finding that components of key metazoan signaling pathways can be found in other unikonts.

Our hypothesis has significant implications for views on the origins of modern metazoans. If it is correct, we must regard the evolutionary event that gave rise to metazoans not as a transition to multicellularity per se, but as a transition from facultative to constitutive multicellularity. This event could be viewed in one sense as an abandonment of the unicellular stage that characterizes the life cycles of facultative multicellular organisms (although animals still have unicellular gametes that may serve as a buffer against “cheater” mutations [51]). By definition, facultative multicellular unikonts rely on the unicellular stage for at least one essential function; e.g., in social amoebae, both feeding and cell proliferation are confined to the unicellular phase of the life cycle. Presumably, then, the transition to constitutive multicellularity was triggered by the appearance of new signaling pathways that allowed a greater degree of coordinated cellular movements and differentiation, thus permitting different essential functions (e.g. feeding and reproduction) to occur simultaneously in a single multicellular individual. This view of the transition to constitutive multicellularity is in fact not very different from other proposals for the transition to multicellularity in animals (e.g. [5, 12, 52]); the novelty of our hypothesis concerns the nature of the ancestral organism in which this transition occurred.

Testable predictions of the “facultative multicellularity” hypothesis

Our hypothesis predicts that if tissue polarity mediated by the catenin complex predates the divergence of Dictyostelium from metazoans, then we should expect to find polarized cells that require the catenin complex in some of the organisms that diverged from the metazoan lineage after social amoebae. Given our current knowledge of unikont phylogeny and the availability of sequenced genomes, the most informative lineages to examine would be choanoflagellates, ichthyosporeans, and chytrid fungi (see Fig. 2). Almost nothing is known about multicellular tissue organization in these organisms,
all of which have been studied much less than Dictyostelium. However, the fact that all of these organisms have homologs of α-catenin [15] is encouraging, and we would predict that studying the function of α-catenin in these species would be likely to yield considerable insight into metazoan evolution. Exciting progress has been made toward establishing choanoflagellates and ichthyosporeans as genetically manipulable experimental systems ([53]; I. Ruiz-Trillo, personal communication), and we are hopeful that future studies will address the nature and genetic requirements of multicellular organization in these organisms.

A second important prediction of our hypothesis is that the catenins should influence epithelial structure and polarity in Dictyostelium and metazoans by similar mechanisms. The alternative convergent evolution hypothesis makes the opposite prediction, that the mechanisms of epithelial organization by the catenins should be very different between metazoans and social amoebae. One similarity between Dictyostelium and metazoans is that β- and α-catenin interact physically and genetically and are required for epithelial polarity in both systems. However, beyond these facts, we do not yet have a sufficiently comprehensive understanding of how the catenin complex contributes to epithelial organization in either Dictyostelium or metazoans to be able to say whether its mechanism of action is similar or dissimilar overall. The finding that IQGAP homologs that act downstream of the catenins to regulate myosin in the Dictyostelium tip epithelium [16] is a start, and an important next step is to determine whether this mechanism is also relevant in metazoan epithelia. Additional cell biological studies of epithelial polarity in both Dictyostelium and animal model systems are likely to shed more light on this issue.

Conclusions

We have proposed a novel intellectual framework for the study of early metazoan evolution, based on the surprising finding of polarized epithelial tissue in a non-metazoan social amoeba. Similar to metazoan simple epithelia, the Dictyostelium tip epithelium consists of a monolayer of cells with a distinct apical-basal polarity that is dependent on homologs of β- and α-catenin. This tissue contributes to multicellular morphogenesis through both directional secretion of proteins [15] and apical localization of contractile actomyosin structures [16]. To explain these results, we hypothesized that metazoans originated from an ancestor that was facultatively multicellular, rather than from a unicellular ancestor as was previously proposed. The key predictions of this hypothesis are that cell polarity mediated by the conserved catenin complex contributes to epithelial organization in these organisms.

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