A Polarized Epithelium Organized by β- and α-Catenin Predates Cadherin and Metazoan Origins

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exception of Rh3, other opsins could replace Rh1 (Fig. 4C). However, the transgenic flies showed significant differences from wild type when given a choice between 18° and 20° to 22°C (Fig. 4D). Another GPCR coupled to G_q [5-hydroxytryptamine ($5\text{-}HT_2$)] did not function in place of Rh1 (Fig. 1C).

The mammalian opsin that is most similar to *Drosophila* Rh1 is melanopsin (OPN4) (*21*). Expression of *Opn4* under control of the *ninaE* promoter did not reverse the phototransduction defect in adult $ninaE^{I17}$ (fig. S8). However, *Opn4* enabled the $ninaE^{I17}$ larvae to distinguish between 18°C and 24°C (Fig. 4C).

The observations that Rh1 is required for thermosensory discrimination and that OPN4 could substitute for Rh1 suggest that Rh1 and related opsins might be intrinsic thermosensors. However, the intrinsic rate of thermal activation, which is ~1/min in fly photoreceptor cells (*22*), is far too low to account for the requirement for Rh1 for thermosensation. We suggest that an accessory factor might interact with Rh1 and accelerates its intrinsic thermal activity. Finally, because rhodopsin has dual roles, it is interesting to consider the question as to whether the archetypal role for rhodopsin was in light sensation or in thermosensation.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6022/1333/DC1
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A Polarized Epithelium Organized by β- and α-Catenin Predates Cadherin and Metazoan Origins

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A fundamental characteristic of metazoans is the formation of a simple, polarized epithelium. In higher animals, the structural integrity and functional polarization of simple epithelia require a cell-cell adhesion complex that contains a classical cadherin, the Wnt-signaling protein β-catenin and the actin-binding protein α-catenin. We show that the non-metazoan *Dictyostelium discoideum* forms a polarized epithelium that is essential for multicellular development. Although *D. discoideum* lacks a cadherin homolog, we identify an α-catenin ortholog that binds a β-catenin–related protein. Both proteins are essential for formation of the epithelium, polarized protein secretion, and proper multicellular morphogenesis. Thus, the organizational principles of metazoan multicellularity may be more ancient than previously recognized, and the role of the catenins in cell polarity predates the evolution of Wnt signaling and classical cadherins.

A simple epithelium is the most basic tissue type in metazoans (multicellular animals). It is the first overt sign of cellular differentiation during embryogenesis and is important for the morphogenesis of many tissues and homeostasis in the adult (*1*). A simple epithelium comprises a cell monolayer surrounding a luminal space. The cells have a polarized organization of plasma membrane proteins, organelles, and cytoskeletal networks that together regulate the directional absorption and secretion of proteins and other solutes (*1*).

The structural integrity and functional polarity of epithelial tissues in higher animals require cell-cell adhesion mediated by classical cadherins (*2*). Adhesion provides a spatial cue that initiates cell polarization via recruitment of cadherin-associated cytosolic proteins (*3*), including the Wnt-signaling protein β-catenin (*4*) and the actin-binding protein α-catenin (*5*). Classical cadherins, which have extracellular cadherin repeats (*6*) and a conserved cytoplasmic domain that can bind β-catenin (*7*), are found in all multicellular animals, including sponges, but not in choanoflagellates (*8*–*10*), which suggests that classical cadherins are restricted to metazoans. However, the evolutionary history of the catenins is unknown, and thus how the cadherin-catenin complex evolved to mediate epithelial polarity in metazoans is unclear.

The non-metazoan social amoeba *Dictyostelium discoideum* undergoes multicellular morphogenesis in response to starvation: Single cells aggregate and undergo culmination to form a fruiting body, which comprises a rigid stalk that supports a collection of spores (Fig. 1A) (*11*). The mechanical rigidity of the stalk is due to the stalk tube, which contains cellulose and the extracellular matrix proteins EcmA/B (Fig. 1B) (*12*, *13*). Harwood and colleagues described a ring of cells surrounding the stalk tube at the tip of the culminant and speculated that these cells might contribute to stalk formation during culmination (*14*, *15*). However, the subcellular organization and function of tip cells have not been characterized.

We confirmed the earlier observation (*14*) that the tip consists of an organized monolayer of cells surrounding the stalk (Fig. 1, A and B, and movie S1). Additionally, we found that these cells have a distinctive polarized organization: Centrosomes and Golgi localized to a stalk side of nuclei (Fig. 1C), and the transmembrane protein cellulose synthase [encoded by the *dcsA* gene (*12*)] localized to the plasma membrane domain adjacent to the stalk tube (Fig. 1D). Thus, *D. discoideum* tip cells have a subcellular organization that is characteristic of a simple polarized epithelium (fig. S1), and we refer to these cells as the tip epithelium.

In metazoans, β-catenin and α-catenin are essential for the formation of polarized simple epithelia (*16*, *17*). A β-catenin–related protein called Aardvark has been identified in *D. discoideum* (fig. S2) (*9*, *14*). We identified a member of the α-catenin family in this organism, which we

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named *Ddα*-catenin on the basis of structural and functional characteristics (*9*). *Ddα*-catenin is approximately 35% homologous to human α-catenins and their paralog vinculin (Fig. 2A and figs. S3 to S5). *Ddα*-catenin was expressed at low levels in single *D. discoideum* cells but was up-regulated during multicellular development (Fig. 2B). Endogenous *Ddα*-catenin localized to cell-cell contacts in the slug and fruiting body (Fig. S6 and fig. S7A) and especially in columnar cells of the tip epithelium (Fig. 2C).

We examined whether *Ddα*-catenin is similar to metazoan α-catenin or vinculin, or both (*9*). Like metazoan α-catenin, *Ddα*-catenin bound and bundled actin filaments (Fig. 2, D and E). *Ddα*-catenin bound to the *D. discoideum* β-catenin–related protein Aardvark (Fig. 2F) and mouse β-catenin (fig. S9), and its localization to cell-cell contacts in vivo was Aardvark-dependent (Fig. 2C and fig. S7). Unlike mammalian αE-catenin, but like the *C. elegans* α-catenin ortholog HMP-1 (*18*), purified *Ddα*-catenin was monomeric in solution (fig. S10), and it did not inhibit the actin-nucleating activity of the Arp2/3 complex (Fig. 2G). In contrast to its overall similarity to metazoan α-catenin, *Ddα*-catenin lacked key properties of metazoan vinculin (figs. S11 and S12) (*9*). Because *Ddα*-catenin represents the most basally branching members of the α-catenin/vinculin family (fig. S4), these data indicate that the ancestral member of this protein family was probably α-catenin-like.

To test whether *Ddα*-catenin and its binding partner Aardvark are involved in the polarized organization of the tip epithelium, we depleted *Ddα*-catenin using RNA interference (fig. S13). When *Ddα*-catenin was depleted below a level that could be detected by means of immunofluorescence, multicellular development arrested at the onset of culmination (Fig. 3A). Tip cells were disorganized, and the stalk and tip epithelium were absent (Fig. 3, A and B). Moreover, the distributions of Golgi and centrosomes were not polarized (Fig. 3C and fig. S14), and cellulose synthase was mislocalized intracellularly (Fig. 3D). Culminants with partial *Ddα*-catenin knockdown exhibited a milder phenotype: A distinct stalk and tip epithelium formed, but the epithelium appeared disorganized and was more than one cell layer thick (Fig. 3B), and organelles (Fig. 3C and fig. S14, arrowheads) and cellulose synthase (Fig. 3D) were not correctly polarized. Prestalk cell differentiation was unaffected in *Ddα*-catenin

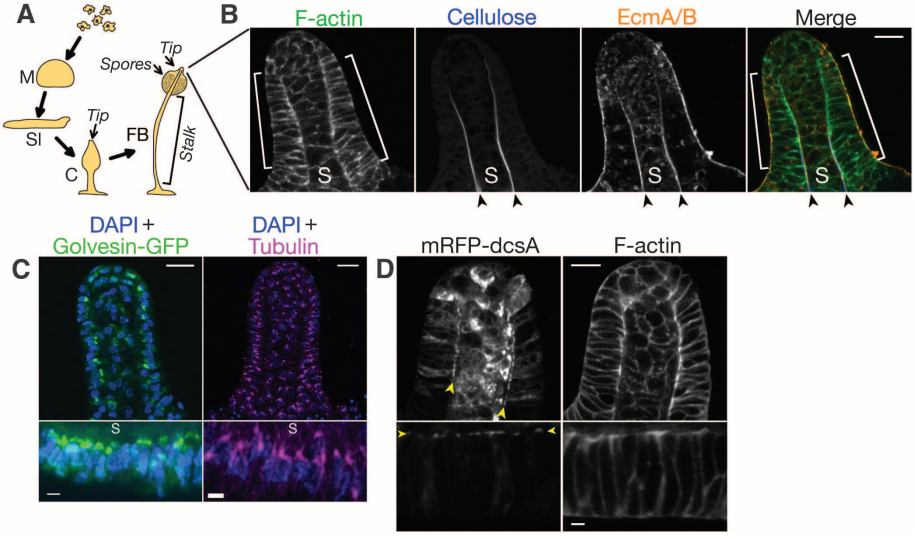

Fig. 1. (**A**) *D. discoideum* developmental process. M, mound; Sl, slug; C, culminant; FB, fruiting body. (**B**) Confocal section of the tip of a wild-type culminant. Brackets indicate the tip epithelium; arrowheads indicate the stalk tube; S indicates the stalk. (**C**) Maximum-intensity projections showing Golgi (left), centrosomes (right), and nuclei (4′,6′-diamidino-2-phenylindole stain) in the entire tip (top) or tip epithelium (bottom). (**D**) Confocal section of the tip (top) and tip epithelium (bottom) in a wild-type culminant expressing cellulose synthase (mRFP-dcsA). In tip epithelial cells, mRFP-dcsA localizes to the tip epithelial cell membrane adjacent to the stalk (arrowheads). mRFP-dcsA is also expressed in the stalk cells. Scale bars, [(B) to (D)] 10 μm in lower-magnification views and [(C) and (D)] 2 μm in higher-magnification views. In views of the tip epithelium, the top of the images faces the stalk.

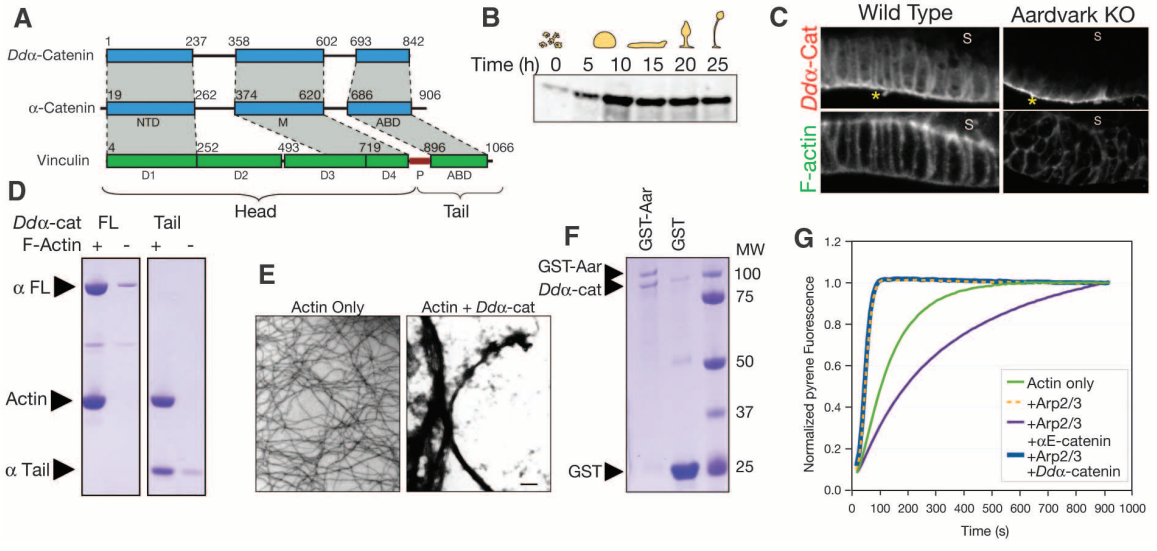

Fig. 2. (**A**) Primary structures of *Ddα*-catenin and human α-catenin and vinculin. Regions of homology are shaded gray. NTD, N-terminal domain; M, M-domain; ABD, actin-binding domain; P, proline-rich region. (**B**) Western blot for *Ddα*-catenin at the indicated developmental time points. (**C**) Confocal sections of the tip epithelium in a wild-type culminant and an Aardvark knockout (*14*). Asterisks indicate nonspecific signal on the exterior of the culminant (fig. S8). (**D**) High-speed pelleting assay demonstrating binding of 5 μM full-length (FL) or the isolated tail domain of *Ddα*-catenin to 5 μM F-actin. (**E**) Negative-stain electron micrographs of actin filaments in the absence or presence of 5 μM *Ddα*-catenin. Scale bar, 500 nm. (**F**) Bead-bound fractions from a glutathione *S*-transferase (GST) pull-down assay demonstrating binding of *Ddα*-catenin (10 μM) to GST-Aardvark (~0.3 μM). 5 μM GST is a negative control. (**G**) Pyrene actin polymerization assays were performed in the presence of N-WASp VCA domain and the indicated additional proteins. αE-catenin or *Ddα*-catenin concentrations were 5 μM.

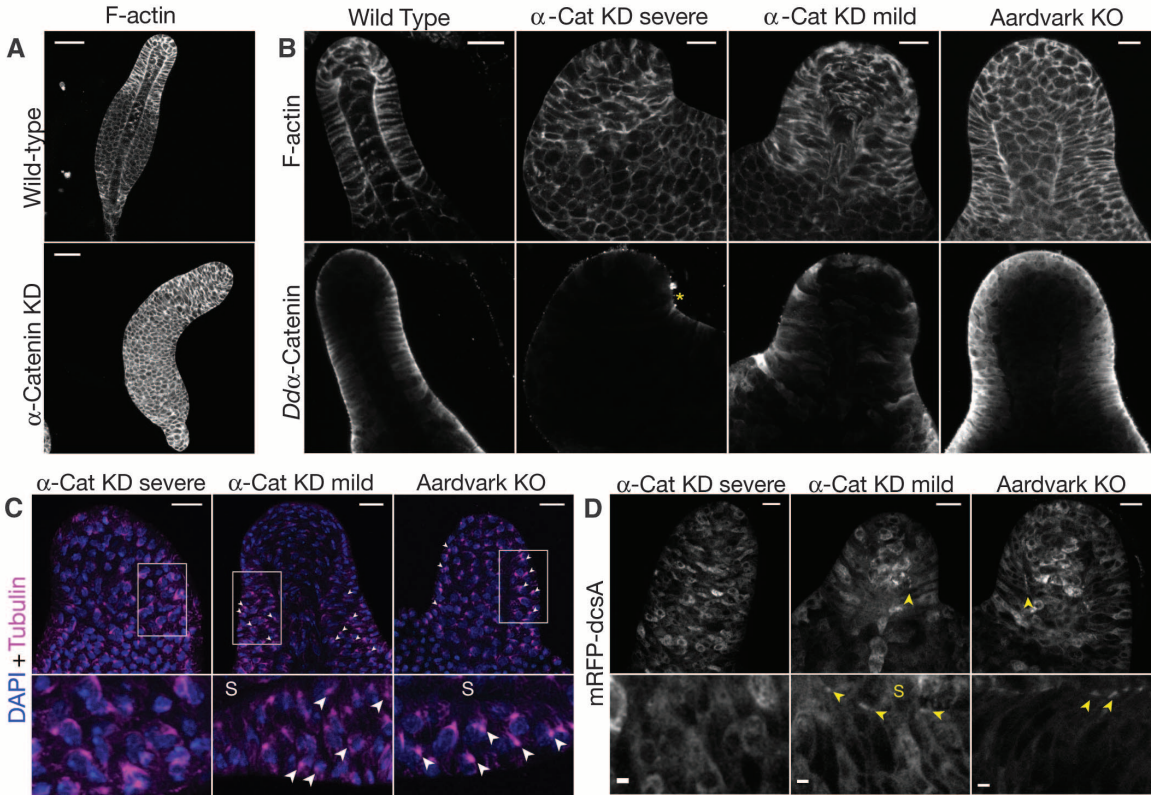

Fig. 3. (**A**) Early culminants formed by wild-type and *Dd*α-catenin knockdown cells. (**B**) Confocal sections of the tip in culminants of the indicated cells. Severe and mild *Dd*α-catenin knockdown phenotypes are distinguished by the absence or presence, respectively, of a nascent stalk. Asterisk indicates nonspecific signal on the exterior of the culminant (fig. S8). (**C**) Maximum intensity projections showing centrosomes and nuclei. Arrowheads indicate centrosomes that are mislocalized relative to wild type (Fig. 1C). (Bottom) Higher-magnification views of the boxed regions. (**D**) Confocal sections of the (top) tip and (bottom) tip epithelium in culminants of the indicated cells expressing mRFP-dcsA (cellulose synthase). Arrowheads indicate residual localization of mRFP-dcsA in mild *Dd*α-catenin knockdowns and Aardvark knockouts. Scale bars, (A) 25 μm, [(B) to (D)] 10 μm in lower-magnification views, or [(C) and (D)] 2 μm in higher-magnification views.

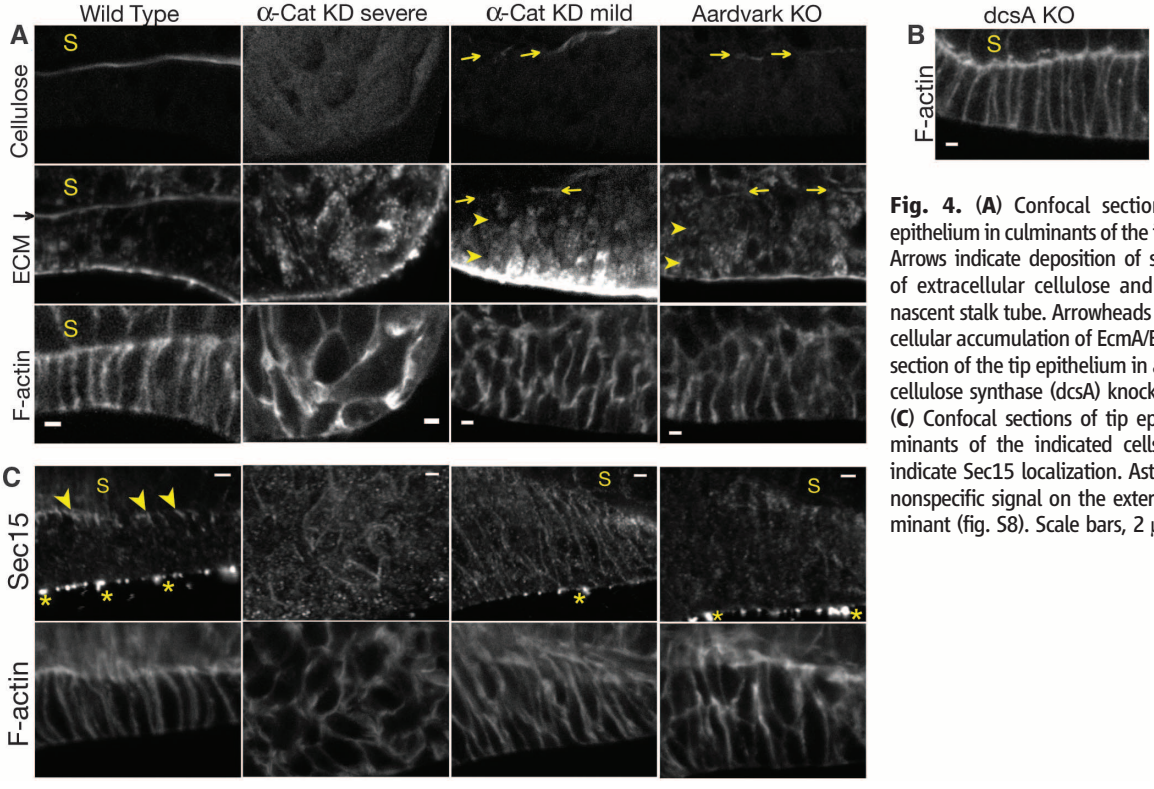

Fig. 4. (**A**) Confocal sections of the tip epithelium in culminants of the indicated cells. Arrows indicate deposition of small amounts of extracellular cellulose and EcmA/B in a nascent stalk tube. Arrowheads indicate intracellular accumulation of EcmA/B. (**B**) Confocal section of the tip epithelium in a culminant of cellulose synthase (dcsA) knockout cells (*12*). (**C**) Confocal sections of tip epithelia in culminants of the indicated cells. Arrowheads indicate Sec15 localization. Asterisks indicate nonspecific signal on the exterior of the culminant (fig. S8). Scale bars, 2 μm.

knockdowns, indicating that the lack of a stalk was not due to a failure of the developmental program to correctly specify cell types (fig. S15).

Similar results were obtained with an Aardvark knockout strain (*14*) (Fig. 3, B to D, and fig. S14), indicating that both *Dd*α-catenin and Aardvark are required to organize and polarize the tip epithelium during culmination. Harwood and colleagues reported that Aardvark was necessary for formation of actin-associated cell-cell junctions in tip cells that appeared similar to adherens junctions at the ultrastructural level (*14*, *15*, *19*). However, we found that Aardvark knockouts formed junctions similar to wild-type, as did *Dd*α-catenin knockdowns (fig. S16). Because these junctions do not require *Dd*α-catenin or Aardvark, and *D. discoideum* does not have classical cadherins, we conclude that these junctions are unlikely to be molecularly equivalent to metazoan adherens junctions (*9*) and are not involved in the developmental phenotypes described above.

To better understand the developmental mechanism underlying impaired stalk formation in *Dd*α-catenin knockdowns and Aardvark knockouts, we examined whether the stalk tube components cellulose and EcmA/B were correctly distributed. Accumulation of cellulose and EcmA/B in the stalk tube was absent in severe *Dd*α-catenin knockdowns and was strongly reduced in mild *Dd*α-catenin knockdowns and Aardvark knockouts (Fig. 4A and fig. S17, A and B) (*19*). Cellulose synthase (compare Figs. 3D and 1D) and EcmA/B (Fig. 4A and figs. S17, A and B, arrowheads) were mislocalized intracellularly in tip epithelial cells but were unchanged in stalk cells, indicating that tip epithelial cells are the primary source of secreted cellulose and EcmA/B in the stalk tube. Confirming this interpretation, we observed rare cases in which half of the tip epithelium was better organized than the other half, and in those culminants cellulose and EcmA/B accumulated in the stalk tube adjacent to the better-organized tip epithelial cells (fig. S18). In cellulose synthase knockouts, which do not form a stalk tube (*12*), the tip epithelium was morphologically normal, and EcmA/B were secreted (Fig. 4B and fig. S17C), demonstrating that tip epithelial polarity is genetically upstream of stalk tube formation.

Because tip epithelial cells appear to secrete cellulose and EcmA/B directionally to form an organized stalk tube, we tested whether the secretory pathway was polarized in wild-type and mutant strains. Sec15, a component of the Exocyst complex involved in polarized exocytosis in diverse systems (*20*), localized adjacent to the stalk tube (Fig. 4C)—reminiscent of Exocyst localization in polarized mammalian epithelial cells (*21*)—and this distribution was strongly disrupted in *Dd*α-catenin knockdowns and Aardvark knockouts (Fig. 4C). The molecular mechanisms underlying the polarized organization of the Exocyst in *D. discoideum* are unknown, but the catenins have been reported to associate in a complex with Exocyst components in mammalian cells (*22*).

Taken together with earlier results (*14*), our work shows that the non-metazoan *D. discoideum* has a bona fide polarized epithelium consisting of a single layer of structurally and functionally polarized cells that secrete proteins into a luminal space (fig. S1). Epithelial polarity in both metazoans and *D. discoideum* requires homologs of α-catenin and β-catenin, indicating a close evolutionary relationship between *D. discoideum* and metazoan epithelia. Because *D. discoideum* lacks cadherins, Wnt-signaling components, and polarity proteins of the PAR, Crumbs, and Scribble complexes (*9*), the conserved catenin complex appears to be an ancient functional module that mediates epithelial polarity in the absence of the more complicated machinery found in metazoans (*1*).

The fact that the catenin complex is essential for epithelial polarity in both *D. discoideum* and metazoans indicates that this complex probably functioned in cell polarity before the divergence of social amoebae and metazoans. It is possible that the catenins evolved initially to mediate cell polarity in a unicellular organism and then were used to organize cell polarity in a multicellular context in both social amoebae and metazoans. Alternatively, the last common ancestor of social amoebae and metazoans may have formed a polarized epithelial tissue organized by the catenin complex, but epithelial polarity was lost in some intervening lineages (*9*). In either case, our results identify unexpected similarities in tissue organization between two groups of distantly related organisms that were thought to have independently evolved multicellularity (*23*), and thus reveal molecular factors and organizational principles that may have contributed to the early evolution and diversification of animals.

References and Notes

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Supporting Online Material

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