challenges of defining the roles of various proteins in mechanical force transduction, apical constriction, and apical-basal polarity, *D. discoideum* has many of the core properties of a multicellular tissue, but with a highly reduced number of components. This, in combination with the ability to genetically manipulate the organism and its 24-h developmental cycle, makes it ideal for such investigations.

**D. discoideum Contains a True Epithelial Tissue**

*D. discoideum* is a single-celled amoeba that feeds on bacteria. When food runs out, ~100,000 cells aggregate and begin a process that culminates in a fruiting body composed of a stalk bearing a spore mass (Fig. 3). At the tip of the developing fruiting body, a single layer of cells surrounds the stalk (10, 18). These cells secrete components of the stalk tube, and cells within the stalk tube are compressed as the fruiting body grows; it is thought that compression of the stalk cells by the rigid tube results in anisotropic vertical expansion, which lifts the spore head off the ground (6, 7, 34). The entire process, starting from starvation to formation of a mature fruiting body, takes ~24 h in the laboratory.

Analysis of tip cells reveals that they are wedge-shaped, with the smaller edge bordering the stalk tube (10). The cells have a polarized organization, with centrosomes and the Golgi apparatus on the stalk side of the nucleus and the transmembrane protein cellulose synthase, which is responsible for secreting cellulose of the stalk tube, on the membrane adjacent to the stalk tube (10). Thus these cells have all the hallmarks of a polarized metazoan epithelium, with an apically constricted side bordering a tube; we call this tissue the tip epithelium (Fig. 3).

Transmission electron microscopy of the tip revealed electron-dense structures between cells that morphologically resemble metazoan AJs, and it was reported that knockout of the β-catenin-like protein Aardvark produced morphological defects in the fruiting body and a loss of these AJ-like structures (18). The primary structure of Aardvark has features of mammalian β-catenin essential for cell-cell adhesion, including a positively charged groove characteristic of the β-catenin binding site for classical cadherins and a sequence just NH2-terminal to the armadillo domain that is homologous to the α-catenin binding site of mammalian β-catenins (1, 10, 19, 33). In contrast, Aardvark lacks the NH2- and COOH-terminal “tails” that mediate interactions of metazoan β-catenins with Wnt signaling pathway components, which is consistent with the absence of almost all the other key Wnt pathway components in *D. discoideum* (10).

The *D. discoideum* genome also encodes an α-catenin/vinculin homolog that biochemically resembles metazoan α-catenin: it binds Aardvark and can bind murine β-catenin, and it binds and bundles F-actin (10); we call this protein Ddα-catenin. Similar to the Aardvark knockout, small interfering RNA-mediated knockdown of Ddα-catenin produces defects in the fruiting body. Loss of either protein produces a disorganized, multilayered tissue at the tip of the stalk, rather than the regular monolayer present in the wild-type fruiting body (10). Importantly, and in contrast to earlier reports (18), we found AJ-like structures connecting the epithelial cells in strains deficient in Aardvark/β-catenin or Ddα-catenin (10). Thus it appears that, at a molecular level, these structures are not equivalent to metazoan AJs. This would also be consistent with the observation that *D. discoideum* lacks detectable homologs of classical cadherins. Whether this indicates convergent evolution to a structure responsible for connecting the cytoskeletons of adjacent cells requires further investigation. Nonetheless, given that the catenins affect morphogenesis and organization of the tip epithelium, we examined their roles more closely.

**D. discoideum Catenins Control Cell Polarity**

Aardvark/β-catenin and Ddα-catenin localize to the lateral membrane of the tip epithelial cells. Epistasis experiments using the Aardvark/β-catenin knockout strain or small interfering RNA-mediated knockdown of Ddα-catenin showed that Aardvark/β-catenin is required for the membrane localization of Ddα-catenin (10, 11). This is similar to the requirement of...
β-catenin to localize α-catenin to the plasma membrane in higher Metazoa. Remarkably, loss of Aardvark/β-catenin or Dda-α-catenin disrupts the polarity of tip epithelium cells: centrosomes and Golgi are now randomly distributed, and cellu-lose synapse appears to be mistargeted and mostly intracellular. Moreover, Sec15, a component of the exocyst complex required for polarized membrane trafficking and secretion, is also mislocalized. Thus the catenins have a critical role in polarized cell organization, membrane trafficking, and localized secretion (10).

How do the catenins contribute to the formation of a polarized epithelial monolayer? Visualization of myosin revealed that it is enriched at the apical side of the epithelial cells, and a cross section through the tip revealed an actin-rich myosin ring surrounding the stalk, the appearance of which is reminiscent of the contractile actomyosin ring in some metazoan tubular epithelia (11). In Aardvark-knockout and Dda-α-catenin-depleted cells, the stalk tube is significantly wider, and myosin is no longer enriched at the apex. These observations indicate that the catenins contribute to apical myosin localization and, consequently, we speculate, formation of a contractile ring needed to produce normal stalk tube morphology. However, it has not been established whether this ring corresponds to the fibrous intercellular junctional structures observed via electron microscopy (10, 18).

To understand how the catenins regulate apical myosin localization, we took a proteomics approach to find interacting partners of Dda-α-catenin (11). Analysis of Dda-α-catenin immunoprecipitates revealed the presence of IQGAP1 and cortexillins I and II (11). IQGAPs are known to bind to cortexillins, and these complexes are involved in regulating myosin distribution in dividing single cells (2, 14, 15, 21, 25, 26, 35, 42). Dd IQGAPs have a RasGAP homology region also found in yeast and metazoan IQGAPs but do not have the NH2-terminal actin-binding and IQ repeats in other IQGAPs. Ddcortexillins contain actin-binding and coiled-coil domains (but no IQ repeats), suggesting that the fungal and metazoan IQGAPs represent fused versions of the DdIQGAP-cortexillin complex (10). Epistatic analysis of DdIQGAP1 and Ddcortexillin knockout strains revealed that these proteins 1) are localized to the lateral membrane by the catenins and 2) antagonize myosin localization to the lateral membrane (11) (Fig. 4). Interestingly, knockout of DdIQGAP1 or Ddcortexillin results in mislocalization of myosin but does not affect other aspects of cell polarity, indicating that the influence of the catenins on polarity is likely mediated by other factors.

The role of the DdIQGAP1-cortexillin complex as a downstream effector of Dda-α-catenin in myosin localization is interesting in light of what is known about IQGAPs in other organisms. In fission yeast, IQGAP organizes actin filaments in the contractile ring during cytokinesis (40) and works with other proteins, including myosin, to correctly position the cytokinetic ring (24, 30). The function of IQGAP in mammals is poorly understood, but it is known to promote cell migration, tumor invasion, and disruption of cell-cell adhesion, likely by stabilizing the active forms of the small GTPases Rac1 and Cdc42, which regulate the actin cytoskeleton (16, 22, 23, 43). Importantly, mammalian IQGAP1 and αE-catenin bind competitively to β-catenin (23), which would seem to be different from the colocalization of these proteins in D. discoideum, and further work is needed to understand how these proteins interact and regulate distributions. The interaction of mammalian IQGAP1 with β-catenin is inhibited by the active, GTP-bound forms of Rac1 and Cdc42 (16).

A model for myosin localization that emerges from these studies (Fig. 4) is that Aardvark/β-catenin and Dda-α-catenin localized to the lateral membrane recruit the DdIQGAP1-
cortexillin complex, which prevents myosin from binding at those sites, resulting in selective localization of myosin to the apical cortex. Importantly, knockdown of Ddx-catenin leads to uniform localization of the DdIQGAP-cortexillin complex around the cell cortex, indicating that the interaction with Ddx-catenin is not required for its recruitment to the cortex per se. Rather, it indicates that the ability of the complex to interact with cortical actin is somehow enhanced by the Aardvark/β-catenin-Ddx-catenin complex, thereby producing the lateral localization. This might be due to a Ddx-catenin-mediated change in local F-actin conformation in a manner that enhances cortexillin affinity or recruitment of another factor that can affect IQGAP1/cortexillin localization. Candidates for the latter include Rac family small GTPases, which are known to associate with IQGAP (13). Again, further work is needed to identify how these proteins interact and regulate their distributions and the localization of myosin.

Conclusions

Apical actomyosin activity mediates apical constriction and, therefore, morphogenesis of epithelial tubes in Metazoa, but the molecular mechanisms that underlie myosin accumulation at the apical membrane are poorly understood (36). Our study of the *D. discoideum* fruiting body has provided unique insights into this problem, in particular, by defining a molecular pathway that connects Aardvark/β-catenin and Ddx-catenin localized to the lateral membrane to selective localization of myosin at the apical cortex.

The metazoan AJ lies at the boundary of the apical and lateral membranes, defining the apical-lateral membrane boundary in epithelia, and, therefore, it is intimately tied to the functional polarity of the tissue. In mammals, dysregulation of cell-cell contacts and loss of apical-basal polarity are defining events in cancer cell metastasis, including loss of contact inhibition, epithelial-to-mesenchymal transition, and cellular invasiveness (5, 27). Despite their importance in development and disease, little is known at the molecular level about how cell-cell contacts bear mechanical forces and transduce them into biochemical signals or how these structures determine epithelial polarity. Our findings that Aardvark/β-catenin and Ddx-catenin also control the polarized organization of the tip epithelium indicate that this organism will therefore be extremely valuable in understanding the interplay of cell-cell junction formation and apical-basal polarity. As noted above, however, the apparent junctional structures that connect epithelial cells in *D. discoideum* and Metazoa are not molecularly equivalent. Key unresolved questions include 1) how Aardvark/β-catenin and Ddx-catenin are targeted to the lateral membrane of the tip epithelium, as classical cadherins are absent in this organism, and 2) which molecules constitute the cell-cell junctions observed by electron microscopy.

Study of *D. discoideum* fruiting body morphogenesis has provided novel insights into the evolution of multicellularity. 1) The fact that this organism has a polarized simple epithelium was a surprise, as epithelial tissue had previously been thought to be characteristic of only Metazoa. 2) The β-catenin-α-catenin complex appears to represent a basal machinery for organizing epithelial cells for apical-basal polarity, both for polarized membrane trafficking and apical myosin localization for generation of a contractile ring, in the absence of cadherins, Wnt signaling, and most of the polarity proteins thought to be essential for apical-basal polarity in Metazoa. Further studies are needed to examine how these basal functions became associated with cadherin-based adhesion and Wnt signaling upon the emergence of Metazoa, or if these properties represent convergent solutions to the problem of epithelial morphogenesis. We have argued previously (12) that, to understand this problem, we must go beyond genome-wide surveys for the presence or absence of homologous proteins that might be involved in particular physiological roles. Detailed biochemical analysis will be required, as significant variations in protein-protein interactions and allosteric regulation have been found between even very closely related proteins. These differences can provide great insight into how proteins and their interactions change during evolution to meet the particular demands of the tissue and its environment.

GRANTS

This work was supported by a National Science Foundation Graduate Research Fellowship (D. J. Dickinson) and National Institute of General Medical Sciences Grants GM-035527 (W. J. Nelson) and GM-56169 (W. I. Weis).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

W.I.W. and D.J.D. prepared the figures; W.I.W. drafted the manuscript; W.I.W. approved the final version of the manuscript; W.J.N. and D.J.D. edited and revised the manuscript.

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