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Cellulose Biogenesis in *Dictyostelium discoideum*

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1. Introduction

Organisms that synthesize cellulose can be found amongst the bacteria, protistans, fungi, and animals, but it is in plants that the importance of cellulose in function (as the major structural constituent of plant cell walls) and economic use (as wood and fiber) can be best appreciated. The structure of cellulose and its biosynthesis have been the subjects of intense investigation. One of the most important insights gained from these studies is that the synthesis of cellulose by living organisms involves much more than simply the polymerization of glucose into a (1→4)-β-linked polymer. The number of glucoses in a polymer (the degree of polymerization), the crystalline form assumed by the glucan chains when they crystallize to form a microfibril, and the dimensions and orientation of the microfibrils are all subject to cellular control (Delmer and Amor, 1995; Haigler, 1991). Instead of cellulose biosynthesis, a more appropriate term might be cellulose *biogenesis*, to emphasize the involvement of cellular structures and mechanisms in controlling polymerization and directing crystallization and deposition (Haigler, 1991; Haigler and Benziman, 1982). *Dictyostelium discoideum* is uniquely suitable for the study of cellulose biogenesis because of its amenability to experimental study and manipulation and the extent of our knowledge of its basic cellular mechanisms (as will be evident from the rest of this volume). In this chapter, I will summarize what is known about cellulose biogenesis in *D. discoideum*, emphasizing its potential to illuminate our understanding both of *D. discoideum* development and plant cellulose biogenesis.

2. Cellulose in *Dictyostelium discoideum*

The synthesis of cellulose by cellular slime molds has been known since their initial discovery (Brefeld, 1869). Cellular slime mold vegetative amoebae do not make cellulose. In some species, the amoebae can form microcysts, the walls of which contain cellulose (Raper, 1984). In species that do not form microcysts, cellulose synthesis is normally an activity of multicellular development. In *D. discoideum*, cellulose is first detected cytochemically during late aggregation (Harrington and Raper, 1968). It remains at low levels until culmination, when the amount of cellulose increases dramatically. Cellulose accounts for approximately 3-4% of the dry weight of mature culminants (Loomis, 1975; Sussman and Sussman, 1969).

Cellulose is found in the surface sheath that surrounds the multicellular structures (FIG. 1A) (Freeze and Loomis, 1977a,b; Harrington and Raper, 1968), in the slime sheath

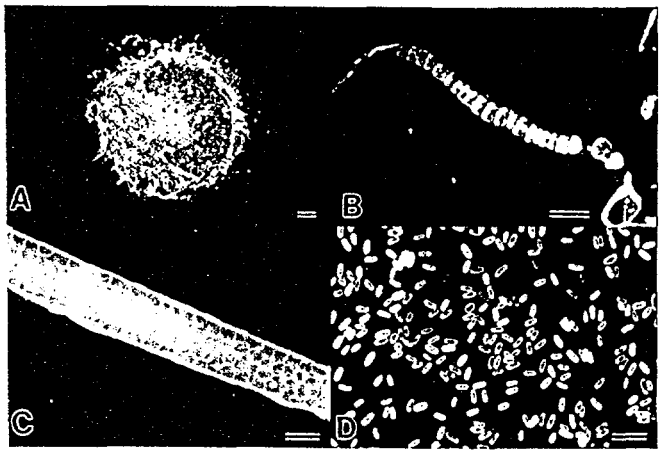

FIG. 1. *Dictyostelium discoideum* extracellular matrices that contain cellulose. A. Surface sheath forming on the surface of a consolidating aggregate. Bar = 100 μm. B. Slime trail left behind by a migrating slug. The direction of migration was from the upper left to the lower right of the micrograph. The bright hemispherical regions contain cell impressions that are regions of cellulose deposition. Bar = 100 μm. C. Stalk, consisting of a cellulosic stalk tube surrounding the stalk cells with cellullosic cell walls. Bar = 20 μm. D. Spores contain cellulose in the middle layers of their walls. Bar = 20 μm. All are epifluorescence photomicrographs of specimens treated with 0.01% (w/v) Tinopal LPW (Fluorescence Brightening Agent 28), which binds to cellulose.

left behind by migrating slugs (FIG. 1B) (Freeze and Loomis, 1977a,b; Hohl and Jehli, 1973; Wilkins and Williams, 1995; Zhou *et al.*, 1995), in the stalk tube that surrounds the stalk cell population (FIG. 1C) (Freeze and Loomis, 1978; Harrington and Raper, 1968; Raper and Fennell, 1952), in the stalk cell walls (FIG. 1C) (Blanton, 1993; Freeze and Loomis, 1978; Harrington and Raper, 1968; Raper and Fennell, 1952), and in the spore walls (FIG. 1D) (Berg *et al.*, 1988; Erdos and West, 1989; Harrington and Raper, 1968; Hemmes *et al.*, 1972; West and Erdos, 1990). Cellulose is also found in the wall layers of the macrocysts (Harrington and Raper, 1968).

It is not known which cells contribute cellulose to the surface sheath or the slime trail. Cytochemical (Bonner *et al.*, 1955; Harrington and Raper, 1968), ultrastructural (George *et al.*, 1972), and autoradiographic (George, 1969; George, 1989) evidence suggests that at least a sub-population of the prestalk cells is responsible for stalk tube cellulose synthesis. Stalk cell wall and spore wall cellulose are synthesized by the individual prestalk and prespore cells. Therefore, all cells will have contributed cellulose to at least one structure by the conclusion of development.

It is of great interest to know if there are differences in the biophysical characteristics of the cellulose found in each of the structures. If there are, then *D. discoideum* would provide a particularly useful model for exploring how cells control and change the biophysical characteristics of cellulose. Unfortunately, the published studies of *D. discoideum* cellulose structure used techniques that perhaps altered the cellulose being studied, lacked a structural

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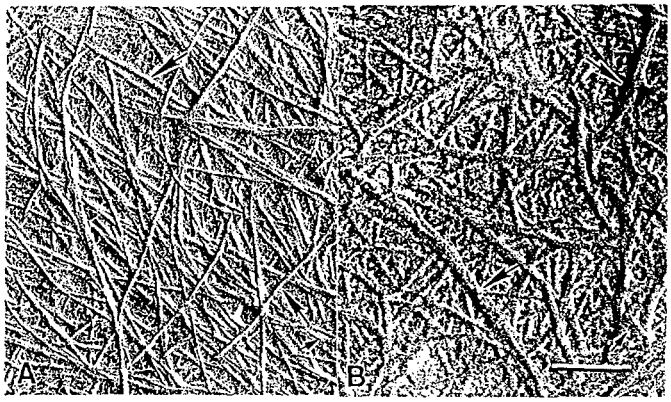

FIG. 2. Carbon/platinum replicas of cellulose microfibrils of the stalk tube and stalk cell wall. Stalk tube cellulose microfibrils (**A**, the arrows point to individual microfibrils) are smaller and more uniform in width (averaging 5.6±0.4 nM) than those of the stalk cell walls (**B**, the arrows points to single large microfibrils), which averaged 10.8±4 nM in width. The small arrow in the bottom right of each micrograph represents the direction of shadowing. Bar = 100 nM. From (Grimson *et al.*, 1997).

context, and/or did not have the resolution necessary to detect fine structural or dimensional differences in microfibrils (George and Hohl, 1969; Gezelius and Ranby, 1957; Hemmes *et al.*, 1972; Hohl and Jehli, 1973; Mühlethaler, 1956; Raper and Fennell, 1952). For instance, the X-ray diffraction studies of *D. discoideum* cellulose used bulk samples of stalks, which will have cellulose from the sheath, stalk tube, and stalk cell wall. The earliest of these studies reported the cellulose to be in the crystalline form now known as cellulose II (Gezelius and Ranby, 1957; Mühlethaler, 1956; Raper and Fennell, 1952). Most naturally occurring cellulose is cellulose I, which can be easily and unintentionally mercerized during isolation and purification procedures to form cellulose II. A later study using electron diffraction suggested that the cellulose is indeed cellulose I (Roberts *et al.*, 1989). Additional high resolution diffraction studies are required of cellulose from each structure in isolation to determine the presence of differences in the crystalline characteristics.

Microfibril dimensions can be determined by electron microscopy of negatively stained and shadow-casted specimens. One early study reported that the microfibrils of the stalk tube and stalk cell wall cellulose were the same width (Mühlethaler, 1956). However, high resolution studies of microfibrils from stalk tube and stalk cell walls revealed significant differences in their dimensions (FIG. 2A, B) (Grimson *et al.*, 1997). Similar studies have not yet been performed with cellulose from surface sheath or spore walls.

There is additional evidence that the biophysical nature of cellulose changes from one stage to the next. Stalk tubes, stalk cell walls, and slime sheaths dissolved at different rates in Schweitzer's reagent, which is a cellulose solvent (Raper and Fennell, 1952). Cellulose crystallinity (measured by rate of glucose release during methanolysis) was higher in the slug slime sheath than in the sheath surrounding aggregates (Freeze and Loomis, 1977a).

3. Biochemical Regulation of Cellulose Synthesis in *Dictyostelium*

In spite of the importance of cellulose in plants and intense efforts directed at the study of plant cellulose synthesis, little is known about the biochemistry of plant cellulose biosynthesis. None of the relevant proteins has been identified, much less purified. Only recently have candidates for a plant cellulose synthase gene (the first from any eukaryote) been isolated (Pear *et al.*, 1996). The reasons for the slow progress in understanding eukaryotic cellulose synthesis have been extensively discussed (Delmer, 1987; Delmer and Amor, 1995; Haigler and Blanton, 1996) and are beyond the scope of this review, other than to note that the achievement of high rates of cellulose synthesis in membrane preparations from any cellulose-synthesizing organism eluded biochemists until relatively recently. Historically, most attempts resulted either in low or undetectable activity or in synthesis of a polymer other than cellulose [for reviews, see (Blanton and Haigler, 1996; Delmer, 1987; Delmer and Amor, 1995; Haigler and Blanton, 1996)]. Claims of *in vitro* synthesis of cellulose must be bolstered by extensive and technically difficult analyses to confirm the nature of the product (Blanton and Haigler, 1996; Delmer, 1987). To date, the best-characterized *in vitro* system is that from the Gram-negative bacterium *Acetobacter xylinum* (Ross *et al.*, 1991). There have been suggestions of recent progress with *in vitro* synthesis in higher plants (Brown *et al.*, 1996). Most intriguing has been the suggestion that the substrate for the cellulose synthase, UDP-glucose, may be channeled directly to the cellulose synthase by a closely coupled sucrose synthase (Amor *et al.*, 1995).

Attempts to obtain *in vitro* cellulose synthesis in cellular slime molds include those of Ward and Wright (1965) and Loomis and Thomas (1976) with *D. discoideum* and Philippi and Parish (1981) with *Polysphondylium pallidum*. More recently, Blanton and Northcote (1990) reported a high-rate *in vitro* system in crude membranes from *D. discoideum*. Unfortunately, efforts to solubilize this membrane-bounded enzyme activity failed, precluding any efforts to purify the enzyme. However, several interesting observations emerged from continued study of the enzyme: (1) the product formed in the *in vitro* reaction was extensively characterized (Blanton and Northcote, 1990); (2) the enzyme preparation was highly active, with *in vitro* rates approaching the calculated *in vivo* rates (Blanton and Northcote, 1990); (3) the crude membrane preparations generated product in quantities readily detected by the unaided eye (FIG. 3A); (4) the *in vitro* product was in the form of rodlets, as revealed by negative staining electron microscopy (FIG. 3B). It is not unusual for *in vitro* preparations to yield rodlets rather than microfibrils (Lin *et al.*, 1985), perhaps because the membrane isolation procedure disrupts cellular organization necessary for cellulose I microfibril formation (Saxena *et al.*, 1994); (5) the *D. discoideum* activity was not activated by the novel activator of bacterial cellulose synthesis [bis-(3',5')-cyclic diguanylic acid (c-di-GMP, (Ross *et al.*, 1987)], nor could the activity be depleted by washing the membranes with chelators (conditions that deplete the *Acetobacter* membrane-associated activity; (Ross *et al.*, 1987) (Blanton, unpublished observations); (6) the enzyme activity was developmentally regulated (FIG. 4A) (Blanton and Northcote, 1990) and (7) in stalk cell monolayer cultures of the DIF-deficient strain HM44, the induction of the cellulose synthase activity depended upon the addition of DIF-1 (FIG. 4B) (Blanton, 1993).

Clearly, much remains to be done on the biochemistry of cellulose synthesis in *D. discoideum*, including more complete characterization of the *in vitro* product, renewed efforts

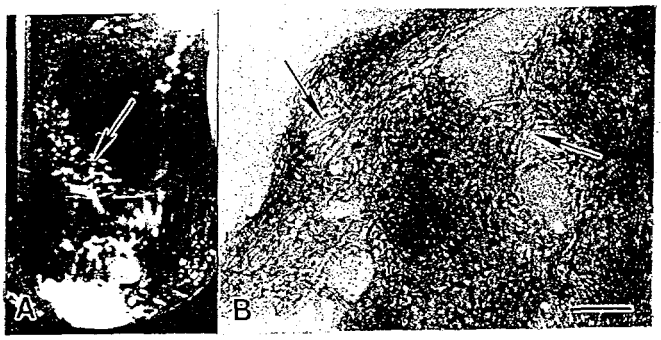

FIG. 3. *in vitro* cellulose synthesis in *Dictyostelium discoideum*. A. Microsome suspensions incubated with UDPG in standard assay conditions (Blanton and Northcote, 1990) will settle out of suspension. Removal of the microsome membranes with 1% (w/v) SDS and boiling with 1% NaOH reveals the flocculent *in vitro* material (arrow). 2.5x. B. Electron micrograph of negatively stained *in vitro* product, which appears as small rodlets (arrow). Bar = 100 nM.

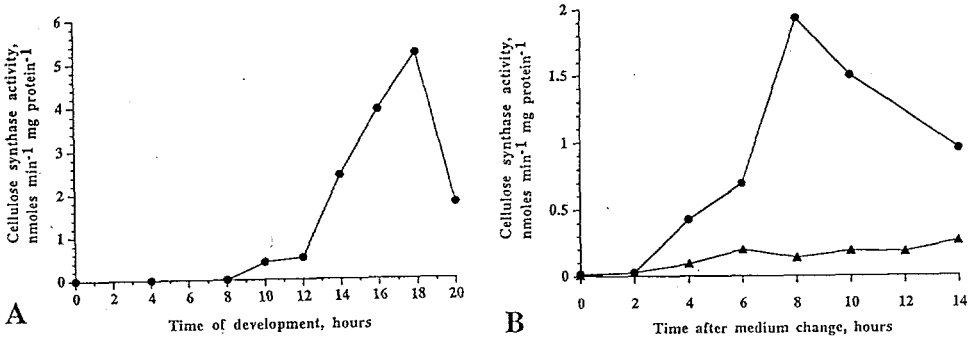

FIG. 4. Kinetics of cellulose synthase activity during development. A. Enzyme activity in crude membranes prepared from various points during synchronous development of strain NC-4 on filters. B. Enzyme activity of membranes prepared from the DIF-deficient strain HM44 in monolayers in the presence of 5 mM cAMP. At t_0 the original medium was removed, the plates washed twice with stalk salts, and new medium with 5 mM cAMP and either 0 (open squares) or 100 nM (open circles) DIF-1 added to the plates. Figure 4B from (Blanton, 1993).

to obtain a solubilized active enzyme and then to purify the enzyme, subcellular localization of the enzyme activity, and determination of the cell-type specificity of the enzyme activity (particularly in the context of a developmental time course). It would be of interest to know if there is more than one cellulose synthase and, if so, how they differ in their biochemical regulation.indexcellulose!synthesis—)

4. Roles of Cellulose in Development

By analogy with plants, we would assume that a significant role of cellulose would be to confer structural strength to sheaths, stalks, and spore walls, but it is likely that there are additional roles for cellulose. Discovering these would be helped if mutants in cellulose synthesis were available, but none have been isolated. The gene for the *D. discoideum* cellulose synthase has not yet been cloned, so gene knock-outs and/or anti-sense experiments have not been possible. None of the known inhibitors of plant cellulose synthesis appears to be effective in *D. discoideum* (Blanton, unpublished observations).

There is evidence for other roles of cellulose in development. Cellulose appears to be important in maintaining the integrity of slugs and in slug motility (although this may be simply a manifestation of its role in conferring strength). Mutants in UDPG-pyrophosphorylase were reported to be incapable of synthesizing cellulose (Dimond *et al.*, 1976; Freeze and Loomis, 1977b). The absence of this enzyme would have other effects as well, so these mutants are not proxies for cellulose synthase mutants. The mutants made slugs that were poorly motile and incapable of maintaining their integrity over time, were arrested prior to culmination, and never made spores, stalk tube, or stalk cells (Dimond *et al.*, 1976; Freeze and Loomis, 1977b).

Mutants in β-*N*-acetylhexosaminidase were shown to synthesize cellulose of a lower crystallinity than wild-type and produced slugs that were poorly motile and fragmented into smaller slugs (Freeze and Loomis, 1977b). Secretion of β-*N*-acetylhexosaminidase into the sheath could play a role in the cellular alteration of cellulose crystallinity (Freeze and Loomis, 1977b; Freeze, 1992).

A mutant of *Dictyostelium mucoroides* has been isolated in which the slug slime sheath, stalk tube, and primary macrocyst wall cannot form (Larson *et al.*, 1994). The mutant still makes cellulose, but the slugs do not migrate and the stalks (consisting of stalk cells only) are curled and weak (Larson *et al.*, 1994).

Cellulose-containing structures have been determined to play key roles in developmental events. For instance, the dimensions of the stalk tube determine the final form of the sorocap (Raper and Fennell, 1952). The formation of the surface sheath on tight aggregates determines the size of the mound (Loomis, 1972). The surface sheath and slug slime sheath are important in slug motility (Wilkins and Williams, 1995), determination of slug polarity (Garrod, 1969; Loomis, 1972), response to external stimuli (Loomis, 1972), and cell differentiation (Farnsworth and Loomis, 1975; Loomis, 1972). The integrity of the surface sheath may protect the multicellular structures from predators (Kessin *et al.*, 1996). However, in each of these instances there is no evidence of cellulose doing anything other than providing strength.

In an intriguing series of experiments, Farnsworth (1974) inserted small tubes of cellulose nitrate into slugs and found that the cells within the tubes differentiated to form stalk cells. He suggested that the role of cellulose was to create a diffusion barrier, resulting in

the increased local concentration of compounds responsible for directing cell differentiation (Farnsworth, 1974). He ruled out a direct effect of cellulose itself, demonstrating that stalk and sheath fragments and capillaries lined with cellulose did not cause similar effects (Farnsworth, 1974). It is possible, however, that the presence of cellulose stimulates cells to secrete either a soluble signal or a protein that becomes immobilized on the cellulose network and serves a signaling function.

There is good evidence that cellulose provides a framework for the assembly of extracellular matrix proteins. A number of proteins are released from the slime sheath by cellulase treatment, suggesting a close association with cellulose (Grant and Williams, 1983). Among these are a family of oligomeric proteins called the sheathins, which co-localize with cellulose in the cell print zones in the slug sheath (Zhou *et al.*, 1995). The sheathins are glycoproteins whose *O*-linked glycosylation is dependent upon the *modB* locus (Wilkins and Williams, 1995). In *modB* mutants, the sheathins and cellulose are deposited normally, but the slugs are altered in their morphology and migratory behavior (Wilkins and Williams, 1995).

Other glycoproteins depend upon the *modB* locus for their glycosylation, including those in the PsB multiprotein complex, which is found in the prespore vesicles. Upon secretion of the prespore vesicles, the PsB complex contributes to the formation of the spore coat and the spore matrix (Watson *et al.*, 1994). McGuire and Alexander (1996) determined the order of assembly of the proteins in the complex and demonstrated that the complex had a specific cellulose binding activity. These results suggested a role for cellulose in the localization of the spore coat proteins (McGuire and Alexander, 1996). Cellulose-binding capacity of sheathins was also demonstrated, suggesting a possible role for the *modB*-dependent glycosylation for interaction with cellulose (McGuire and Alexander, 1996; Wilkins and Williams, 1995).

Cellulose-glycoprotein interactions are, therefore, important in the structuring of the spore coat and the slug slime sheath. It is likely that similar interactions are involved in structuring the surface sheath, the stalk tube, and the stalk cell walls. The differences in the glycoprotein populations of the various extracellular matrices formed by *D. discoideum* (West and Erdos, 1988) and differences in cellulose structure and organization may result in matrices of different function.

What is learned from exploring the assembly of the various *D. discoideum* extracellular matrices may provide insights into the assembly of plant cell walls, about which very little is known (Varner and Lin, 1989). Plant cell wall biologists do not yet enjoy the range of gene-knockout mutants, well-characterized monoclonal antibodies, and sensitive assays employed by McGuire and Alexander (1996).

5. Developmental Regulation of Cellulose Synthesis during Stalk Formation

The stalk consists of two distinct structures: the stalk tube, which is synthesized by the prestalk cell population, and the stalk cell walls, which are synthesized by the individual prestalk cells once they have entered the stalk tube (FIG. 1C). Raper and Fennell (1952) first made the observation that stalk tube cellulose synthesis was analogous to cellulose synthesis by the Gram-negative bacterium *Acetobacter xylinum*, where the bacterial cells contribute cellulose to a communal pellicle. They further noted that stalk cell wall cellulose synthesis was similar to that of plant cells, where cellulose is synthesized around a particular cell.

In most organisms, cellulose synthesis is associated with the plasma membrane and distinct intramembrane particle arrays can be seen by freeze fracture electron microscopy to be associated with cellulose synthesis [reviewed in (Delmer and Amor, 1995; Haigler, 1985)]. The cellulose synthesizing sites were grouped into two categories, fixed and mobile, based upon their hypothesized mobility in the plane of the plasma membrane (Brown *et al.*, 1983). A fixed complex would tend to synthesize cellulose that extended away from a cell (as in *Acetobacter*, where the crystallization of cellulose actually drives the cell forward in the medium; Brown *et al.*, 1976). A mobile complex would tend to synthesize cellulose that formed a cell wall (Brown *et al.*, 1983). Stalk cellulose synthesis in *D. discoideum* seemed to present the unique situation of an organism using both modes of cellulose synthesis, fixed for the stalk tube and mobile for the stalk cell walls (Brown *et al.*, 1983). How the transition occurs from one mode to the other is of great importance not only to the developmental biology of *D. discoideum*, but also to an understanding of how cells in general may control cellulose biogenesis.

Cellulose microfibrils are added to the outside of the stalk tube by prestalk cells as they migrate upwards (George *et al.*, 1972; Mühlethaler, 1956; Raper and Fennell, 1952). The microfibrils are oriented parallel to the long axis of the stalk, probably resulting from the upward migration of the cells along the tube (George *et al.*, 1972). Once the prestalk cells turn into the open apex of the stalk tube, their migration is arrested. At this point, they continue to contribute cellulose to the inside of the stalk tube, but this is now randomly ordered since the cells are not moving (George *et al.*, 1972; Mühlethaler, 1956). The cells begin to vacuolate and enlarge and synthesize walls of randomly ordered cellulose microfibrils (George *et al.*, 1972; Mühlethaler, 1956). As noted previously, the microfibril dimensions of the stalk tube and stalk cell walls differ (FIG. 2A, B) (Grimson *et al.*, 1997).

Stalk cell monolayer cultures displayed the same transition seen in intact culminants. Amoebae were plated in monolayers in tissue culture dishes with stalk cell induction medium (Kay, 1987). Cells committed to the stalk cell pathway migrated in the dishes, depositing behind them cellulose microfibrils with dimensions identical to those in the stalk tube (FIG. 2A) (Grimson *et al.*, 1997). The cells stopped migrating and formed a stalk cell wall composed of cellulose microfibrils with dimensions identical to those in intact stalk tube (FIG. 2B) (Grimson *et al.*, 1997). Monolayer cultures of the DIF-deficient strain HM-44 were induced to form stalk cells by addition of synthetic DIF-1. If cAMP were present during late stalk cell differentiation, stalk cell wall formation was delayed and there was an increased synthesis of the 'stalk-tube" cellulose (FIG. 5A); in the absence of cAMP stalk cell walls were formed much more quickly with little synthesis of the 'stalk tube cellulose" (FIG. 5B) (Blanton, 1993).

The shift in modes of cellulose synthesis parallels the expression patterns of prestalk cell specific genes. The interior of the stalk tube is marked by the expression of the *ecmB* gene (Jermyn and Williams, 1991); its expression is enhanced by DIF-1 and repressed by cAMP (Berks and Kay, 1988, 1990). Similarly, stalk cell wall cellulose synthesis in monolayer cultures was delayed by cAMP and enhanced by DIF-1 (Blanton, 1993). The tip of the developing culminant outside the stalk tube is characterized by the expression of the *ecmA* gene (Jermyn and Williams, 1991); its expression is enhanced by cAMP (Berks and Kay, 1988, 1990). Similarly, stalk tube cellulose synthesis in monolayer cultures was enhanced by cAMP (Blanton, 1993).

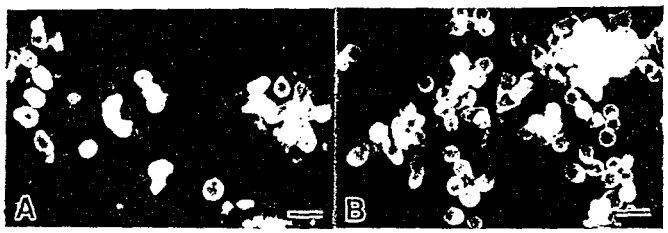

FIG. 5. Cellulose synthesis in induced monolayer cultures of HM44. Cells committed to the stalk cell pathway in monolayer cultures were exposed to 5 mM cAMP alone **A** or 100 nM DIF alone **B**. In **A**, a few stalk cell walls formed, but there was also abundant material between the cells that has been shown to be the monolayer equivalent of stalk tube cellulose (Grimson *et al.*, 1997). In **B**, stalk cell formation progressed much further and little stalk tube cellulose was made. Bar = 10 μm. The photomicrographs were obtained as described (Blanton, 1993).

Freeze-fracture electron microscopic studies of plasma membranes of organisms that synthesize cellulose revealed the presence of characteristic complexes of intramembrane proteins (IMPs) that appear to be associated with cellulose synthesis. These complexes (called terminal complexes, or TCs) range from single linear rows of particles in *Acetobacter*, to multiple linear rows in some algae, to rosettes of six particles in higher plants and some green algae (Mizuta and Brown, 1992). Terminal complex morphology is distinctive between organisms, developmental stage (in some cases), and is somehow involved in determining the characteristics of the cellulose microfibrils (Brown, 1985; Delmer, 1983; Delmer and Amor, 1995; Haigler, 1985). Therefore, it was of interest to determine the type of TC present during stalk tube and stalk cell wall formation. A long single linear TC was observed in *D. discoideum* membranes, but no developmental context was provided for the single published micrograph (Brown, 1990).

Modern methods of cryopreservation and newly developed methods to preserve the integrity of the freeze fracture replicas (thereby permitting the critical determination of the spatial localization of a particular membrane in a culminant) were employed to examine membranes of intact culminants and cells in monolayer cultures (Grimson *et al.*, 1997). Characteristic single linear rows of particles were seen in cells synthesizing stalk tube cellulose (FIG. 6A). Cells synthesizing stalk cell walls had TCs consisting of multiple linear rows (FIG. 6D-F). Some membranes could be observed to have intermediate forms, where the single rows appeared to be collapsing upon themselves to form the multiple linear rows (FIG. 6B, C). The distinct differences in microfibril dimensions observed (FIG. 2A, B) could be correlated with the respective TC types and dimensions (Grimson *et al.*, 1997). The spatial location of the different TCs is illustrated diagramatically in FIG. 7.

To summarize, the stalk tube mode of cellulose synthesis involves prestalk cells that are moving, longitudinal orientation of microfibrils of a characteristic size, and single linear TCs. The stalk cell wall mode of cellulose synthesis involves non-motile cells, random orientation of microfibrils of a dimension different from that of the stalk tube, and multiple linear TCs. How does the cell control the change in packing of the TCs? One intriguing possibility is that the motile state of the cell determines the TC packing and hence the microfibril size and mode of cellulose synthesis (Grimson *et al.*, 1997). The combination of forward cell movement and

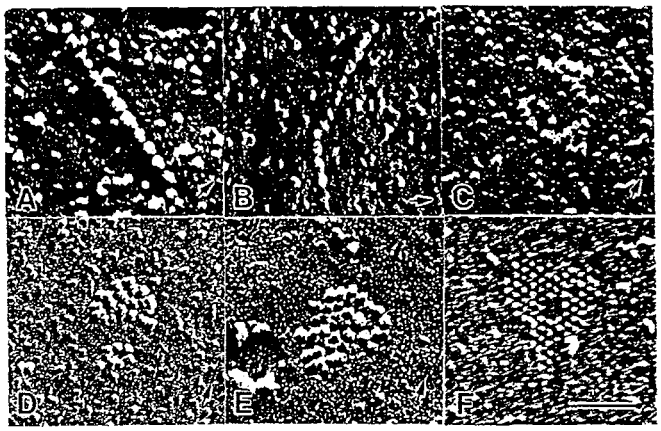

FIG. 6. Freeze fracture electron micrographs showing the TCs of prestalk cells engaged in cellulose synthesis. Cells migrating up the exterior of the stalk tube and contributing cellulose to it have single linear TCs **A**. As these cells move over into the open top of the stalk tube, they cease migration and the complexes begin to collapse upon themselves (**B**,**C**) until they form multiple linear arrays of particles (**D**,**E**). The packing of the multiple linear arrays can be best seen on the opposite fracture face (**F**). FIGS. **A**,**B**, **F** show the EF face (the inside of the outer leaflet of the plasma membrane). FIGS. **C**, **D**, and **E** show the PF face (the inside of the inner leaflet of the plasma membrane). The small arrows in the bottom right corner of each micrograph represent the direction of shadowing. Bar = 50 nM. From (Grimson *et al.*, 1997).

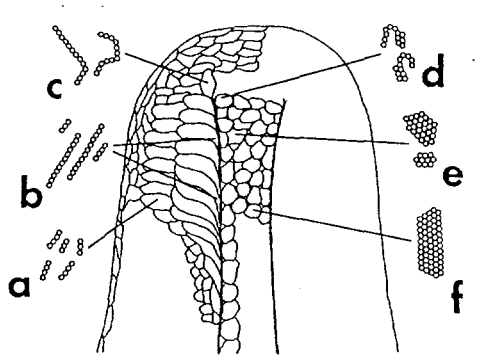

FIG. 7. Diagram illustrating the changes in TCs in the spatial context of a developing culminant tip. Prestalk cells not appressed to the stalk tube have single linear arrays of 3-4 IMPs (**a**). Those appressed to the stalk tube and moving up it have single linear TCs of 3-4 or 12 IMPs, which are associated with stalk tube cellulose synthesis (**b**). As the cells move into the stalk tube and become non-motile, the single linear TCs begin to collapse in units of 3-4 (**c**, **d**) to form multiple linear arrays (**e**) that are associated with stalk cell wall cellulose synthesis. Very large multiple linear arrays can form in later stages (**f**). From (Grimson *et al.*, 1997).

a product immobilized in the stalk tube (or stuck to the tissue culture dish) can result in a linear arrangement of IMPs in the fluid plasma membrane. Upon cessation of cell movement, the crystallization of the product would force the complex forward in the membrane, resulting in it collapsing upon itself to form the multiple linear complexes (see (Grimson *et al.*, 1997) for further discussion of this hypothesis).

Interestingly, the movement of a cell, associated with stalk tube cellulose synthesis, and its arrest, associated with stalk cell wall cellulose synthesis, occurred in the monolayer cultures in the absence of multicellular development. This suggests that the stopping of the cells involves something more than simply the physical block of the packed cells inside the stalk tube.

6. Future Directions

The most serious deficiency in the study of cellulose biogenesis in *D. discoideum* is the lack of a cloned cellulose synthase gene. This has not been for lack of trying and there are reasons why it has been a particularly difficult problem (see (Haigler and Blanton, 1996). Still, the gene must be cloned if the potential of *D. discoideum* as a model for cellulose biogenesis studies is to be fulfilled.

The hypothesis that cell motility state controls the mode of cellulose synthesis is fascinating and is testable, given the wide range of cell motility mutants available. It will be interesting to determine what role, if any, cell motility plays in cellulose deposition in the surface and slug sheath. The HM44 monolayer culture system is the only truly inducible cellulose-synthesizing system available from any organism and can be further exploited to unravel the developmental regulation of cellulose synthesis. The overall place of cellulose synthesis in *D. discoideum* development can be explored by examining cellulose synthesis patterns in various cell signaling mutants and by determining which cells are responsible for contributing cellulose to various structures.

Dictyostelium discoideum is just beginning to contribute fundamental insights into the cellular control of cellulose biogenesis, the role of cellulose in development, and the role of cellulose in extracellular matrices. The specific mechanisms may or may not be applicable to cellulose synthesis in plants, but certainly the ideas and approaches developed with *D. discoideum* will suggest new approaches or provide basic insight into the mechanisms of plant cellulose biogenesis.

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