Cellulose microfibrils, cell motility, and plasma membrane protein organization change in parallel during culmination in *Dictyostelium discoideum*

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SUMMARY

Prestalk cells of *Dictyostelium discoideum* contribute cellulose to two distinct structures, the stalk tube and the stalk cell wall, during culmination. This paper demonstrates by freeze fracture electron microscopy that two distinct types of intramembrane particle aggregates, which can be characterized as cellulose microfibril terminal complexes, occur in the plasma membranes of cells synthesizing these different forms of cellulose. The same terminal complexes were observed in situ in developing culminants and in vitro in monolayer cells induced to synthe-size the two types of cellulose. We propose that cessation of cell motility is associated with a change in packing and intramembrane mobility of the particle aggregates, which causes a change in the nature of the cellulose synthesized. The terminal complexes are compared to those described in other organisms and related to the previous hypothesis of two modes of cellulose synthesis in *Dictyostelium*.

Key words: *Dictyostelium discoideum,* Cellulose synthesis, Microfibril terminal complex, Cell motility, Freeze fracture

INTRODUCTION

Cellulose synthesis in the cellular slime mold *Dictyostelium discoideum* is absent in vegetative and aggregating amoebae, but begins at late aggregation and continues through culmination. Cellulose is found in the surface slime sheath of multicellular stages, as well as in the stalk tube, stalk cell walls, and inner layer of the spore walls. Raper and Fennell (1952) suggested that two distinct modes of cellulose biogenesis occur during culmination. They proposed that, in analogy with synthesis of a cellulose pellicle by prokaryotic *Acetobacter xylinum*, the synthesis of the stalk tube (or sorophore sheath) was a communal effort of prestalk cells, whereas, in analogy with plant cell wall synthesis, the stalk cell wall was produced by individual prestalk cells. Brown et al. (1983) suggested that these different modes of cellulose synthesis might represent a transition between fixed site synthesis, found previously only in prokaryotes (e.g. *Acetobacter*), and mobile site synthesis, commonly found in algae and higher plants, in which the synthetic complex moves within the fluid plasma membrane. *Dictyostelium* might uniquely use both synthetic modes; prestalk cells with a fixed cellulose-synthesis site would generate cellulose that moved away from the cell to form the stalk tube, whereas those with a mobile site would form stalk cell walls (Brown et al., 1983). Before the results reported below, no data were available to test this hypothesis.

Membrane structure correlated with cellulose synthesis has been seen by freeze fracture electron microscopy in higher plant and algal cells, providing strong evidence that cellulose biogenesis occurs in association with aggregated groups of intramembrane particles (IMPs) in the plasma membrane. (Freeze fracture is a technique by which membranes are often fractured at the hydrophobic interface between leaflets of the phospholipid bilayer, exposing the interior of the bilayer as the protoplasmic fracture face (PF) and the exoplasmic fracture face (EF) and revealing included proteins and protein aggregates as IMPs.) Similarly, freeze fracture of *Acetobacter* revealed pores and aligned particles (probably deformed pore proteins) in the outer lipopolysaccharide membrane (Brown et al., 1976; Zaar, 1979), whereas biochemical studies demonstrated that cellulose polymerization occurs at the plasma membrane (Bureau and Brown, 1987).

The IMP aggregates correlated with cellulose synthesis are called cellulose microfibril terminal complexes (TCs) and may contain cellulose synthases and/or proteins required for the deposition and crystallization of cellulose outside the plasma membrane. The packing of TCs is sometimes distinct between organisms and/or developmental stages and helps to determine the characteristics of cellulose microfibrils, e.g. crystallinity, shape, width, etc. (Delmer, 1983; Brown, 1985; Haigler, 1985; Delmer and Amor, 1995). Three distinct types of TC packing are known: single linear rows, multiple linear rows resulting in a rectangular TC, and rosette or rosette/globule types composed of six IMPs arranged in a circle with, possibly, a central globule (Mizuta and Brown, 1992). In eukaryotes, single linear TCs are uncommon, having been described only

in the brown alga *Pelvetia fastigiata* (Peng and Jaffe, 1976). Long single linear TCs are found in *Acetobacter* (Brown et al., 1976; Zaar, 1979). Multiple linear TCs have been identified in algae with wide microfibrils (11-30 nm wide), although superficially similar TCs are found in algae synthesizing thin microfibrils (<4 nm wide; reviewed by Itoh, 1990; Tsekos and Reiss, 1992). TCs of the rosette/globule type are the most morphologically conserved between diverse species, ranging from zygnemetalean algae to higher plants, and are correlated with synthesis of smaller microfibrils (<6 nm wide; Hotchkiss, 1989).

Since *Dictyostelium* may use two modes of cellulose synthesis, we examined the plasma membranes of prestalk cells synthesizing stalk tube or stalk cell wall to determine if TCs were present and if their organization changed during development. Such a study would be difficult if it relied solely on *Dictyostelium* culminants because stalk-tube-synthesizing cells include only those appressed to the tube apex (George et al., 1972). Therefore, in addition to developing culminants, we used a mutant strain (HM44; Kopachik et al., 1983) that is deficient in synthesis of the stalk cell differentiation-inducing-factor, DIF-1 ({1-[(3,5-dichloro-2,6-dihydroxy-4-methoxy)phenyl]hexan-1-one}; Kay, 1987). HM44 amoebae in monolayer cultures can be induced to form stalk cells by DIF addition (Kopachik et al., 1983), and this system allowed collection of cells actively synthesizing intercellular cellulose, which may represent a stalk tube-like mode of cellulose synthesis (Blanton, 1993), or stalk cell walls.

This paper addresses four questions regarding cellulose synthesis in prestalk cell differentiation: (1) is cellulose synthesis in the HM44 culture system analogous to that in developing culminants? (2) are different TCs associated with the plasma membranes of prestalk cells synthesizing stalk tube (or intercellular) cellulose and cell wall cellulose? (3) are different sizes or shapes of cellulose microfibrils synthesized at the two stages and do these correlate with TC organization? and (4) if different TCs exist at the two stages, what is their relationship to each other, are their shapes consistent with the fixed vs mobile site hypothesis, and how is the transition from one TC type to another controlled?

MATERIALS AND METHODS

Development
Culminants
Amoebae of *Dictyostelium discoideum* Raper strain V12M2 were grown on *Klebsiella aerogenes*, collected, washed, streaked on 2% water agar, and incubated for 2 days at 22°C with unidirectional light to initiate slug formation and migration. After migration began, the anterior 20% of the slug was removed to isolate the prestalk from the prespore population (Raper, 1940). The anterior prestalk regions were re-plated on KK_2 agar (20 mM K phosphate, pH 6.2, 2 mM $MgSO_4$, 1.5% agar) to force culmination devoid of spores (Newell et al., 1969).

HM44 monolayer cultures
HM44 monolayer cultures were prepared and induced using the two-medium-shift method (Blanton, 1993). New medium (with 5 mM cAMP and without DIF) was added at the second medium shift after which cells were collected for freeze fracture at 2-4 hours (primarily during intercellular cellulose synthesis) and after 4 hours (during stalk cell wall synthesis).

Specimen preparation for microscopy
Shadowing and negative staining
Cellulose was partially purified by boiling cells in 1 N NaOH with 1% sodium borohydride and washing in water. Some samples were further purified to leave only crystalline cellulose (Updegraff, 1969) by extraction in 1:1 (v/v) chloroform:methanol (1 minute with sonication; sonicator TSD-375, Tekmar Co., Cincinnati, OH; top setting for a microtip), washing with methanol then water, boiling (30 minutes) in 8:2:1 (v/v/v) acetic acid:H_2O:nitric acid (acetic-nitric reagent; Updegraff, 1969), and washing with water. The cellulose was attached to a Formvar/carbon coated grid and negatively stained (1% (w/v) uranyl acetate/0.2% (w/v) Bacitracin). Samples were air-dried directly onto coverslips before shadowing (details below). To observe the intercellular cellulose of the monolayer cells in position relative to the cell, the cells were picked up directly from the surface of the culture medium onto Formvar/carbon coated grids, dried, treated with 1 mg/ml proteinase K (1 hour, 37°C), washed, and shadowed. Stalk cell surfaces within developing culminants were exposed by crushing the cleaned stalk tube under a coverslip prior to shadowing.

Freeze fracture and direct shadowing
Mid-culminants were placed in yeast paste on gold specimen carriers (BAL-TEC; Middlebury, CT) and plunged into liquid propane cooled with liquid nitrogen using a plunger constructed from a design by Dr Hilton Mollenhauer (personal communication). HM44 monolayer cells were gently pelleted (28 **g**). For single replicas, they were frozen in propane as above. For double replicas, they were frozen between copper planchettes in a propane jet freezer (MF2700; RMC, Inc., Tucson, AZ).

Shadowing and freeze fracture were done in a Cressington CFE50 freeze fracture machine (Mars, PA) at −155°C under a vacuum of 3-5×10⁻⁶ torr. Both direct shadow and freeze fracture specimens were unidirectionally shadowed with 1.5 nm of platinum/carbon at 45° under control of a quartz-crystal monitor then stabilized with 3-4 nm of carbon deposited at 85°. Replicas were released in water, cleaned overnight in chromic/sulfuric acid, rinsed in distilled water, and picked up on Formvar coated grids. Samples were examined in an Hitachi HU-11E transmission electron microscope at 75 kV.

Epifluorescence microscopy
Monolayer cultures were fixed for 60 minutes in culture medium containing 0.5% (v/v) glutaraldehyde, washed, and stained with 0.1% (w/v) Tinopal LPW (a gift from Ciba Geigy, Greensboro, NC). Dishes were examined with an Olympus IMT-2 inverted microscope using the violet filter cube.

Microfibril measurements
TEM magnification was calibrated both externally and internally using ferritin and/or catalase crystals. Microfibrils measured were selected at random on photographic prints.

RESULTS

Comparisons between prestalk cells synthesizing two types of cellulose in situ and in vitro
Prestalk cells as listed below were compared in developing culminants (in situ) and in monolayer cultures (in vitro): (1) in situ cells synthesizing stalk tube; (2) in situ cells synthesizing stalk cell wall; (3) in vitro cells synthesizing intercellular cellulose (proposed to be analogous to stalk tube; Blanton, 1993); and (4) in vitro cells synthesizing stalk cell wall. It was possible to establish unequivocally the spatial and developmental context of the in situ prestalk cells because the integrity of the culminant was routinely retained in the replicas (Fig. 1) by improved
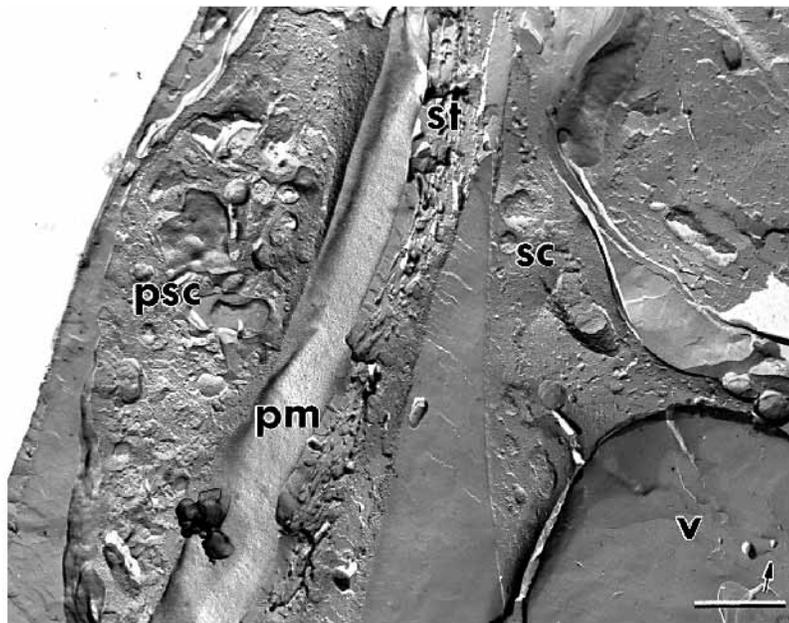
Fig. 1. Freeze fracture replica of a rapidly frozen intact mid-culminant of *Dictyostelium* showing preservation of spatial integrity. For this and all subsequent freeze fracture or shadowed micrographs, the arrow in the lower right-hand corner indicates the direction of shadow. psc, prestalk cell; sc, stalk cell; st, stalk tube; pm, plasma membrane; v, vacuole. Bar, 1 μm.

methods (M. J. Grimson and C. H. Haigler, in preparation). The in situ prestalk cells first synthesize extracellular stalk tube cellulose while moving up the exterior of the stalk tube in the developing culminant by pseudopodial motion. Then they synthesize individual cell walls once they turn into the stalk tube and become stationary. They also increase in size inside the stalk tube due to enlargement of a newly-formed central vacuole (Raper and Fennell, 1952; George et al., 1972; see Fig. 1). The in vitro cells were analyzed during two modes of cellulose synthesis that occur in temporal sequence as seen by fluorescence microscopy after staining cellulose with Tinopal LPW (Fig. 2). First a trail of intercellular cellulose was synthesized by each cell while it was moving by pseudopodial motion (Fig. 2, arrow). Then, the monolayer cell stopped moving, and an individual cell wall containing cellulose was laid down (Fig. 2, arrowhead). Parallel cultures were always observed by fluorescence prior to rapid freezing to ensure high enrichment in the expected modes of cellulose synthesis. All the results described below for cellulose microfibril size and freeze fracture were identical between in situ cells synthesizing stalk tube and in vitro cells synthesizing intercellular cellulose. Similarly, all results were identical between in situ and in vitro cells synthesizing cell walls. Therefore, for brevity results are shown below only for in vitro cells.

Comparison of cellulose microfibrils synthesized at two stages of prestalk cell development

Shadowing confirmed that intercellular cellulose microfibrils extended away from the cell group (Fig. 3A), whereas cell wall cellulose surrounded the cell (Fig. 3B). Intercellular and cell wall cellulose microfibrils appear different at higher magnification (Fig. 3C,D). Twists in both types of cellulose microfibrils were rare (Fig. 3C,D), indicating that twisting was probably not part of the biogenetic mechanism. Differences in sizes of cellulose microfibrils synthesized at the two stages were confirmed (Fig. 4) by examining them after negative staining (not shown). (The same measurements were obtained from shadowed samples after subtracting the 1.5 nm shadow

thickness, except that the smallest microfibrils were obscured by the shadow.)

The intercellular cellulose contained abundant wide microfibrils (averaging 5.6±0.4 nm) with a narrow dimension of 2.3-2.8 nm (as revealed by twists that probably resulted from specimen preparation). It also contained numerous microfibrils only 1.6±0.3 nm wide (Fig. 4), and some microfibrils were splayed at the ends to reveal subfibrils that were 1.8±0.2 nm wide (data not shown). The wider microfibrils had an overall rod-like shape (Fig. 3C). Microfibrils aggregated to form larger bundles up to 13±4 nm wide, although 5.6-5.8 nm fibrils could be clearly distinguished within them (Fig. 3C; arrows).

The cell wall cellulose contained only wide microfibrils (averaging 10.8±4 nm) and exhibited more variable widths than the intercellular cellulose (Fig. 4). The narrow axis was 2.5-2.8 nm as measured at rare twists. Bundles existed with average widths up to 29±4.3 nm (Fig. 3D, arrows). The cell wall microfibrils had a more ribbon-like appearance (Fig. 3D) than the intercellular microfibrils (Fig. 3C). Microfibrils with splayed ends revealed subfibrils with a width of 1.5-1.9 nm (data not shown).

Fig. 2. A single monolayer cell with trailing intercellular cellulose (arrow) synthesized while the cell was motile, and a cell wall (arrowhead) produced after cessation of cell movement. Note that the intercellular cellulose is polarized to one end of the cell as a result of cell movement. Bar, 20 μm.
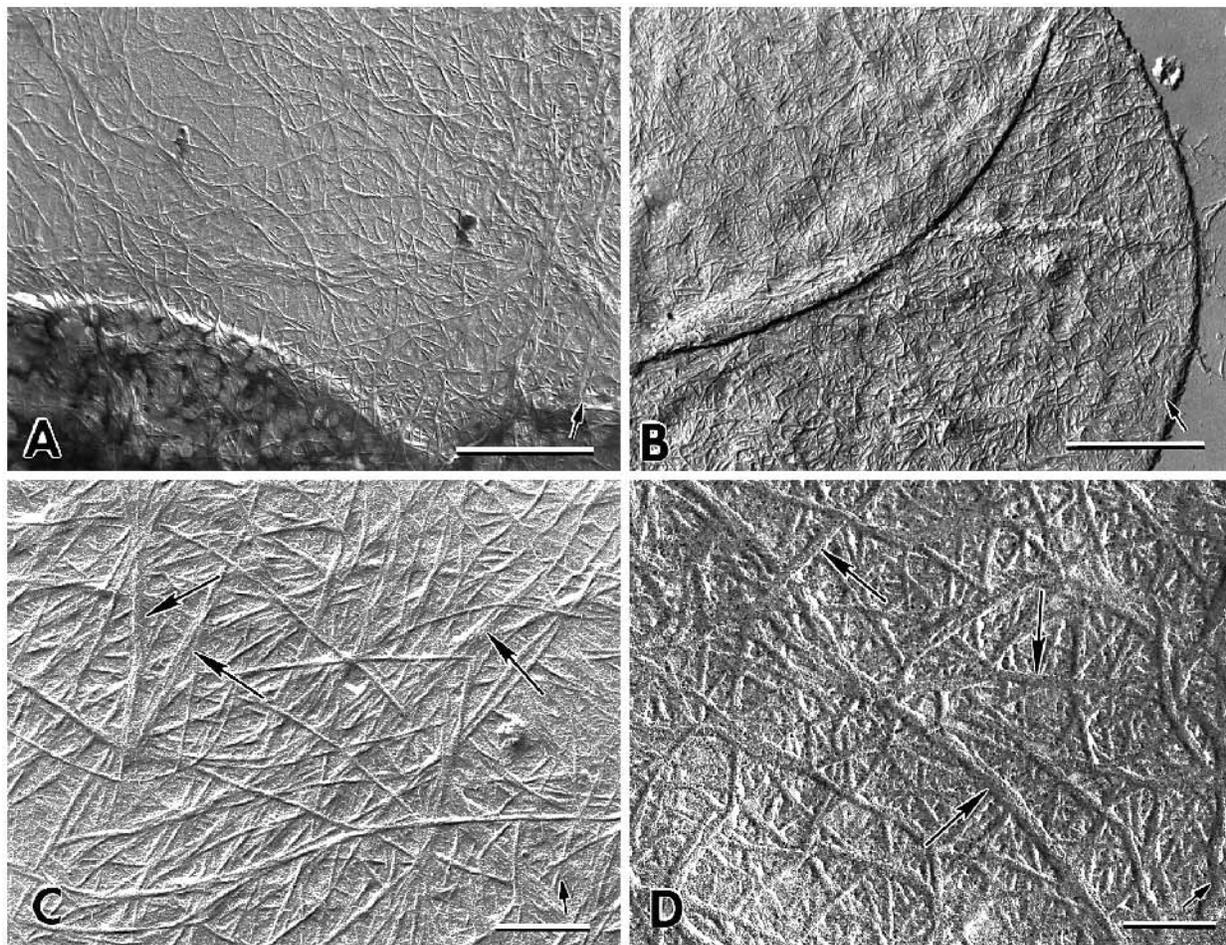
Fig. 3. Shadowed intercellular (A,C) and cell wall (B,D) cellulose microfibrils of in vitro prestalk cells. Arrows indicate bundles of microfibrils. For comparison of the two types of cellulose, the magnifications are the same for A,B (bar, 1 µm) and for C,D (bar, 100 nm).

Comparison of plasma membrane protein organization at two stages of prestalk cell development

The plasma membranes of all prestalk cells had IMP aggregates similar to those in other cellulose-synthesizing organisms (see below) and with arrangements that correlated with measured cellulose microfibril sizes. Therefore, we will refer to these aggregates hereafter as TCs.

The membranes of prestalk cells synthesizing intercellular cellulose showed no organized structure on the PF (Fig. 5A), whereas single linear TCs (20-95 nm long) of 3-12 IMPs (7.0±0.3 nm diameter) were observed on the EF (Fig. 5B) in complementary replicas. (The fracture face was identified in whole cell images observed at lower magnification.) Microfibril impressions on the EF were not commonly observed, which is consistent with fibrils moving away from the cell.

The membranes of prestalk cells synthesizing cell walls had clusters of poorly ordered IMPs (7.1±0.5 nm diameter; up to 48 in a group) on the PF (Fig. 5C) and complementary ordered arrays of pits (4 nm diameter; 12-35 in a group) on the EF (Fig. 5D). The EF was also characterized by microfibril impressions (visible mainly in lower magnification micrographs), which were consistent with the expanding vacuole appressing the plasma membrane against the cell wall. Pits occurred in straight

rows of 2-6 running diagonally across the array width. If a long axis of the pit array was evident, the maximum array width was 35-40 nm (until 'super clusters' formed, see below). Some pit arrays had scattered 7 nm IMPs superimposed on them.
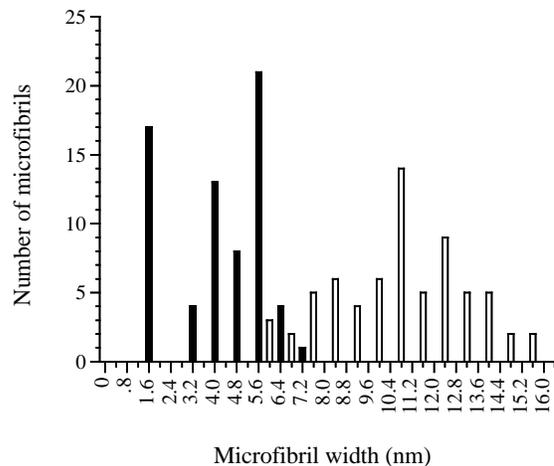

Fig. 4. Bar graph of the frequency of microfibril widths in intercellular (solid bars) and cell wall (white bars) cellulose.
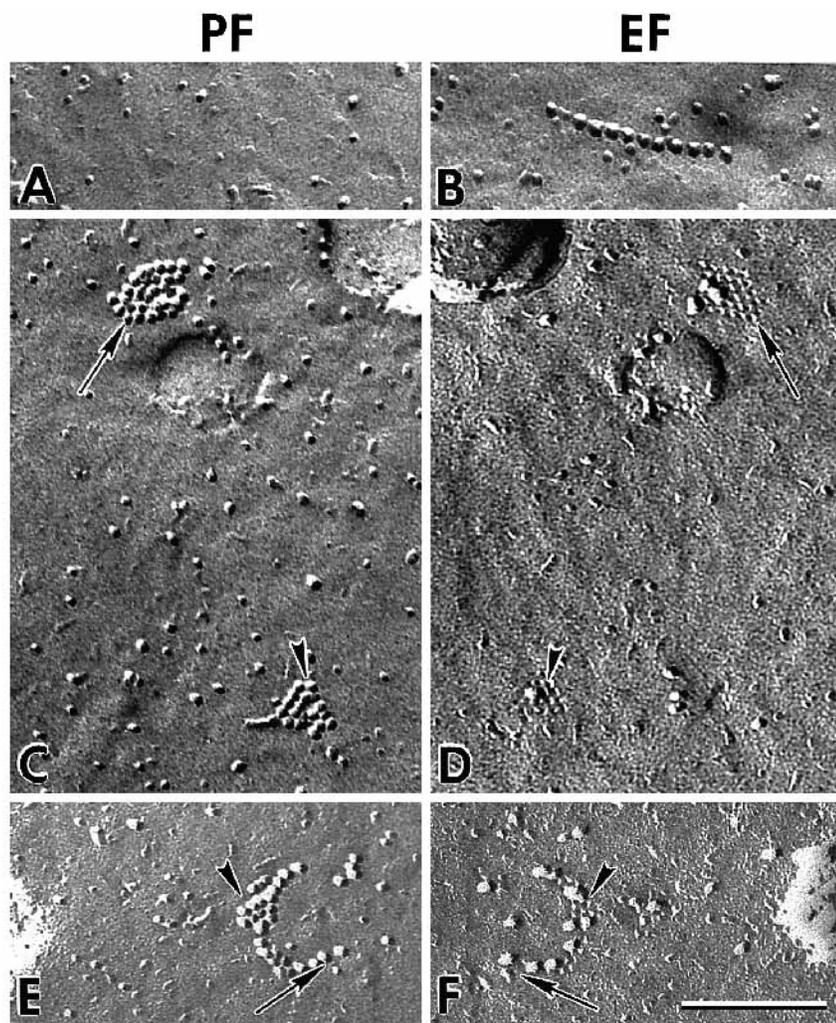
Fig. 5. Freeze fractured plasma membranes of prestalk cells with PF and EF faces in the left and right columns, respectively. During intercellular cellulose synthesis, the PF (A) shows no structure complementary to single linear TCs on the EF (B). During cell wall cellulose synthesis, clustered IMPs on the PF (C) are complemented by ordered pit arrays on the EF (D). Two complementary areas are shown by paired arrows and arrowheads in C and D. During the transition from intercellular to cell wall cellulose synthesis, single linear TCs collapse. Only clustered PF IMPs (E, arrowhead) are complemented by pits (F, arrowhead), whereas adjacent regions of linear IMPs show no complementary pits (E,F, arrows). Bar, 100 nm.

Some membranes examined during the temporal transition between intercellular and cell wall cellulose synthesis showed single linear TCs on the PF that appeared partially collapsed (Fig. 5E, arrowhead). The regions of clustered IMPs showed complementary pits on the EF (Fig. 5F, arrowhead) even though no detectable pits complemented adjacent single linear rows of IMPs (compare arrowed region in Fig. 5E,F). Note that upon apparent partial collapse, the single linear TC remained with the PF rather than the EF upon fracture (compare Fig. 5A).

In cell wall-synthesizing cells, pit arrays were about 25% smaller than their corresponding IMP clusters, and there were more PF IMPs than EF pits. Although high angle of shadow and deformation of the IMPs could have hindered accurate counting in some cases, these phenomena could also be due to filling in of pits due to lipid heating during the fracturing and/or shadowing processes (Sleytr and Robards, 1977). Probably the peripheral layer of pits in an array was preferentially obscured, which would also explain why single linear TCs do not have complementary pits (Fig. 5A) even when they are adjacent to clustered IMPs that are complemented by pits (Fig. 5E,F). Similarly, pit diameter (4 nm) was smaller than IMP diameter (7 nm), which is another indication of lipid changes during sample processing.

Evidence for formation of multiple linear TCs from single linear TCs

Cells collected 2-4 hours post-induction of HM44 cultures showed a variety of aggregated IMPs including single linear TCs (Fig. 6A), apparently collapsing single linear TCs (Fig. 6B), and clustered IMPs (Fig. 6C,D). The variation was due to the semi-synchronous transition in vitro from intercellular cellulose to stalk cell wall synthesis (Blanton, 1993). However, TCs on any particular membrane were all the same type. In contrast, cells collected after 4 hours (synthesizing stalk cell walls) showed only clustered IMPs and pit arrays, which are indicative of multiple linear TCs (see discussion). After the multiple linear TCs formed, they appeared to collide at random, forming TC super clusters shown by PF IMP groups (data not shown) and EF pit arrays (Fig. 6E,F). Note that central depressions were sometimes visible on EF IMPs (Fig. 6B, arrows), but never on PF IMPs (Fig. 6C,D).

Counts of IMPs in both TC types were compared from a random collection of membranes. Groups of 3-4 or 12 IMPs predominated in the EF single linear TCs (Fig. 7A). The longer TCs were most commonly observed in small patches on cells adjacent to the stalk tube, whereas the shorter TCs were common in cells remote from it. Groups of 7-12, 19-24, or 31-
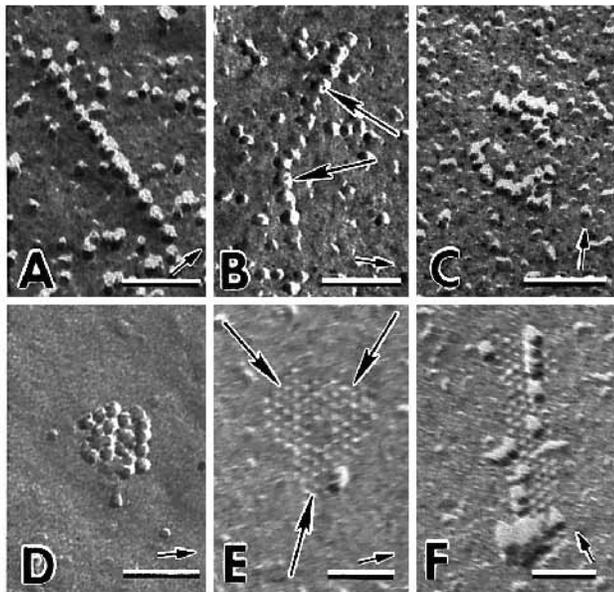
Fig. 6. Proposed transition from single linear TCs to multiple linear TCs during prestalk cell differentiation. Single linear TCs (A) on the EF of motile cells synthesizing intercellular cellulose begin to collapse (B) at 3-4 particle intervals immediately after cessation of forward cell movement (arrows indicate central depressions on IMPs). After the linear aspect is lost, the IMPs fracture to the PF and continue to aggregate (C,D). Some IMP 'super clusters' eventually form (not shown). These are large multiple linear TCs as indicated by geometrical packing, including diagonal pit rows, of complementary large EF pit arrays (E,F). Formerly individual arrays are indicated by arrows in E. Bars, 50 nm.

Fig. 7. (A) Bar graph showing the frequency of single linear TCs with certain numbers of IMPs. (B) Bar graph showing the frequency of multiple linear TCs with certain ranges of IMP number.

36 IMPs predominated in the multiple linear TCs (Fig. 7B). (Ranges of IMP number help compensate for counting inaccuracies that may have resulted from plastic deformation, varied angles of shadowing, or pulling of IMPs to the EF.)

DISCUSSION

These results demonstrate that prestalk cells of *Dictyostelium discoideum* have two TC types at different developmental stages, corresponding to synthesis of two distinct sizes of crystalline cellulose microfibrils. Induced monolayer cultures and developing culminants are identical in terms of the two modes of cellulose synthesis, supporting the contention that intercellular cellulose in monolayer cultures is analogous to stalk tube cellulose and that monolayer cultures are valid models with which to study cellulose synthesis (Blanton, 1993). These results are consistent with fixed and mobile sites of cellulose synthesis in *Dictyostelium*, as discussed below.

Single linear TCs, their microfibril product, and comparison to the fixed *Acetobacter* TC

Motile prestalk cells have single linear rows of about 12 IMPs associated with synthesis of 5.6×2.5 nm microfibrils, whereas rows of 3-4 IMPs correspond to 1.5-1.8 nm wide subfibrils. Since the minimum crystallite size of true cellulose I is 2.8×2.8 nm contained within an approximately 3.5 nm microfibril (Chanzy et al., 1978; Haigler, 1985), these thin microfibrils
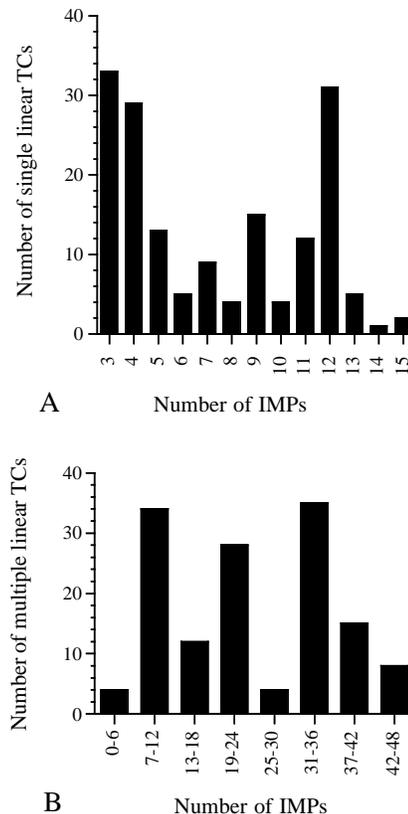
suggest a low crystalline order. This possibility is consistent with X-ray diffraction (Gezelius and Rånby, 1957), although the microfibrils resist digestion in acetic/nitric reagent, which reputedly dissolves all but crystalline cellulose (Updegraff, 1969).

The *Dictyostelium* single linear TC is similar to the longer, fixed TC in *Acetobacter*. The force of microfibril crystallization causes the bacterium to rotate and move through its medium, resulting in a twisting extracellular cellulose ribbon (Brown et al., 1976). It is interesting that: (a) the average 5.6 nm stalk tube microfibrils are close to the minimum width of *Acetobacter* ribbon subfibrils (6 nm); (b) the length of the long single linear TC (95 nm) is close to the length of subsets of the *Acetobacter* TC (120 nm, with more space between IMPs); and (c) the number of IMPs in the long single linear TC (typically 12) is within the range for subsets of the *Acetobacter* TC (8-16 IMPs) (Haigler, 1985). Therefore, *Dictyostelium* may use a fixed TC similar to one subset of the *Acetobacter* TC to make stalk tube cellulose, perhaps because biophysical constraints favor this arrangement to synthesize cellulose that moves away from the cell. The stalk-tube-synthesizing cell is assumed to move via a pseudopodium, and in vitro cells also move during intercellular cellulose synthesis. Therefore, cell movement is correlated with this single linear TC as it is in *Acetobacter*. However, the crucial difference between the two organisms is that *Dictyostelium* amoeboid movement may cause synthesis of non-wall cellulose (see below), whereas *Acetobacter* cell motility is the result of cellulose biogenesis. However, single linear TCs

are not obligatorily fixed sites generating non-wall cellulose because similar TCs probably synthesize cell wall in *Pelvetia* embryos (Peng and Jaffe, 1976). The relationship, if any, of the single linear TCs shown here to much longer rows of IMPs in *Dictyostelium* cells (Brown, 1990) would have to be established by observing the longer arrays in their developmental context.

In *Dictyostelium* stalk tube cellulose synthesis, assuming all TC IMPs are active, each IMP is associated with synthesis of 3-4 glucan chains as shown by correlating IMP number, microfibril size, and space occupied by each glucan chain (Sugiyama, 1985). The glucan chain aggregate from 3 IMPs would then contain 9-12 chains forming a 1.8-2.4 nm wide subfibril, and small fibrils were observed in this study. In the cellulose I crystal, 9 chains is the smallest number that allows one fully-surrounded central chain (Sugiyama, 1985), although such small microfibrils form cellulose IV with poor lateral but good longitudinal order (Chanzy et al., 1979). A biophysical preference for forming a glucan chain aggregate of at least this size from closely spaced synthetic sites may explain why single linear TCs with 3-4 IMPs are often observed and why longer single linear TCs tend to collapse with this periodicity. A single linear TC with 12 IMPs would be associated with synthesis of 36-48 glucan chains, which would coalesce and crystallize to form the 5.6×2.3-2.8 nm microfibrils (theoretically containing 36-50 chains; Sugiyama et al., 1994) observed in the in vitro intercellular and in situ stalk tube cellulose.

Long single linear TCs are scarce in situ and in vitro, probably because they are localized in situ to a small region of the prestalk cell membrane that is appressed to the cylindrical stalk tube. Their scarcity in in vitro cells would suggest that localization to a small region is a cell-autonomous event not requiring a multi-cellular context (see below). However, these long TCs occur in large groups when found, suggesting a membrane domain specialized for cellulose synthesis. Consistent with this hypothesis, the number of 5.6 nm intercellular microfibrils is greater than the number of 1.6 nm microfibrils even though the number of 12 IMP single linear TCs is only about one half of the number of 3-4 IMP single linear TCs.

Multiple linear TCs, their microfibril product, and comparison to mobile algal TCs

Non-motile prestalk cells have clusters of up to 48 IMPs associated with the synthesis of cell wall microfibrils with heterogeneous widths (6-16 nm) and more uniform thickness (2.5-2.8 nm). The PF IMPs, which must have become disordered upon fracture, are complemented by highly ordered EF pit arrays, which probably reflect in vivo TC organization. Similarly to some algal TCs (Itoh, 1990), the width of a typical TC is 35-40 nm. Therefore, although typically shorter than those in algae, these TCs are of the mobile, multiple linear type (Itoh, 1990).

Occasionally, scattered IMPs remains superimposed on the EF pit array, indicating that the IMPs contain transmembrane proteins in situ, which is consistent with using cytoplasmic UDP-glucose to generate insoluble microfibrils outside the plasma membrane (Delmer, 1987). The central depressions observed in EF IMPs (the cytoplasmic side of the protein complex is exposed) but not in PF IMPs (the extracytoplasmic side of the protein complex is exposed) suggest either the existence of a central pore only on the cytoplasmic side or obscuring of the pore on the extracytoplasmic side by a residual product.

The cell wall microfibrils are similar to the thin microfibrils described in *Erythrocladia* (10-30×1.8 nm) (Tsekos and Reiss, 1992; Okuda et al., 1994) and *Vaucheria* (10-26×1.5-3.0 nm) (Mizuta et al., 1989). However, unlike these algal microfibrils (Mizuta et al., 1989), no striations were observed in individual, untreated, unbroken *Dictyostelium* microfibrils by negative staining or freeze fracture. Therefore, the multiple linear TC of *Dictyostelium* must synthesize a unified microfibril, whereas diagonal rows of IMPs are thought to synthesize distinct subfibrils in *Erythrocladia* (Mizuta et al., 1989).

The more heterogeneous width of cell wall microfibrils compared to stalk tube microfibrils correlates with greater variation in IMP number within the multiple linear TCs than in the single linear TCs. Similarly, for *Vaucheria*, variable numbers of packed diagonal rows of IMPs correlate with heterogeneous microfibril width (Mizuta et al., 1989; Okuda et al., 1994). Two abundant cell wall microfibril widths are 10.8 and 12.4 nm, containing about 90 and 105 glucan chains, respectively, since they are 2.5-2.8 nm thick (Sugiyama et al., 1994). If a single IMP were associated with synthesis of 3 or 4 chains, these microfibrils would correspond to TCs with 22-35 IMPs, and multiple linear TCs with 19-24 and 31-36 IMPs were abundant. Perhaps no peak is observed in microfibril width corresponding to the 7-12 IMP multiple linear TC because these clusters are transient after collapse of the single linear TCs and before fusion with others (see below).

Model for interconversion of TC types

The diagram in Fig. 8 places the data presented in their spatial and developmental context. Many multiple linear TCs have approximately 12, 24, or 36 IMPs, i.e. multiples of 12, suggesting a relationship to single linear TCs with 12 IMPs. There are numerous short and long IMP rows in vacuolar membranes of *Dictyostelium* prestalk cells during all stages of development (data not shown), as was also observed in *Pelvetia* (Peng and Jaffe, 1976). Therefore, single linear TCs are probably inserted into the plasma membrane during all phases of prestalk cell differentiation. Single linear TCs are maintained (by unknown mechanisms) in cells that are moving up the stalk tube,
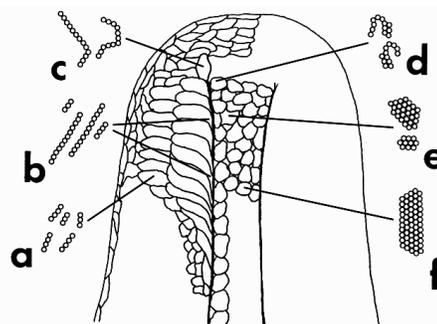

Fig. 8. Diagram of changes in TCs in the prestalk region of the developing culminant tip. Prestalk cells not appressed to the stalk tube have single linear TCs with 3-4 IMPs (a). Motile prestalk cells appressed to the stalk tube have single linear TCs with 3-4 or 12 IMPs, representing fixed sites of cellulose synthesis (b). As cells enter the stalk tube, they become non-motile and long single linear TCs collapse and aggregate to form arrays with diagonal rows of IMPs (c,d). Multiple linear TCs, representing mobile sites of cellulose synthesis, synthesize cell wall (e). A few very large multiple linear TCs form late in prestalk cell development (f).

although the movement itself combined with the relationship of the forming cellulose to the stalk tube may help to maintain this form (see Saxena and Brown, 1995). This possible explanation is supported by the predominance of 3-4 IMP TCs remote from the stalk.

When the prestalk cell turns into the stalk tube, it stops moving, and the single linear TCs collapse at 3-4 IMP intervals due to the force exerted by continued cellulose biogenesis. This would allow some tube cellulose synthesis to occur inside as suggested by George et al. (1972). Collapse of single linear TCs continues until multiple linear TCs form. Since forward movement no longer negates the force of cellulose biogenesis, the TCs begin to move through the fluid plasma membrane resulting in cell wall deposition. Small multiple linear TCs rearrange upon contact with others to form a unified TC with precise packing order; the random nature of this process correlates well with diverse sizes of multiple linear TCs and cell wall microfibrils. As the number of multiple linear TCs decreases, the chance of contact with others is reduced so that only a few 'super clusters' were observed.

We cannot explain why TC IMPs are associated with the EF (single linear TCs) then the PF (multiple linear TCs). As soon as collapse of single linear TCs begins, the IMPs remain preferentially with the PF. In algae, linear TCs have been observed on the EF, PF, or both after fracture (Itoh, 1990); these differences may have little functional consequence.

Implications of single linear and multiple linear TCs for *Dictyostelium* development

The presence or absence of cell motility may determine two modes of *Dictyostelium* cellulose synthesis, but other subcellular controls may also exist. For example, single linear TCs may be inserted in one membrane location, as proposed for rosette TCs delivered via fusing Golgi vesicles in the moss *Funaria* (Reiss et al., 1984). Then interaction of cellulose with the stalk tube might maintain single linear TCs in a membrane patch appressed to the tube. Alternatively, single linear TCs might be inserted at random, becoming localized to a patch by membrane flow and interaction of their cellulose product with the tube. There is precedent for membrane protein localization caused by cell movement (Ishihara et al., 1988), membrane flow (Bretcher, 1988), and interaction with the extracellular matrix (Gumbiner and Louvard, 1985) or solid substrate (Gingell and Owens, 1992). An underlying cytoskeletal network of actin or other proteins (Gumbiner and Louvard, 1985; Ishihara et al., 1988) might be responsible for the transient immobilization of fixed cellulose synthesis sites during synthesis of the stalk tube (see speculation of Brown et al., 1983). Microfilaments occur on the basal side of actively-motile *Dictyostelium* prestalk cells (George et al., 1972) and also in motile, cellulose-synthesizing cells of two related cellular slime molds, *Acytostelium leptosomum* (Hohl et al., 1968) and *Planoprotostelium aurantium* (Spiegel et al., 1979). However, it is not known whether such actin arrays are involved with cellulose synthesis indirectly through generation of cell motility or perhaps directly by anchoring the cellulose synthase complex.

Comparison to developmental control of cellulose synthesis in other organisms

The role of cell motility in regulating two modes of cellulose synthesis is uniquely proposed here, but changes in TC packing that correlate with different microfibrils at different developmental stages have been observed previously. For example, in *Micrasterias*, the rosette-type TCs exist singly during primary wall synthesis, but they aggregate during secondary wall synthesis into hexagonal ordered complexes that result in synthesis of bands of thicker microfibrils (Giddings et al., 1980). Similarly, the mean length of multiple linear TCs increases during secondary wall synthesis in numerous algae (Itoh, 1990). Furthermore, these data support the model of cell-directed, self-assembly of cellulose microfibrils (Haigler and Benziman, 1982) in which membrane protein organization determines microfibril size due to the gap between polymerization and crystallization.

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