Summary

A brief history of the literature dealing with cellulose microfibril assembly is presented, and a current summary of cellulose microfibril synthesizing complexes among eukaryotic cells is given. Terminal complexes heretofore not described include the following: linear terminal complexes (TCs) with three rows in *Eremosphaera*, *Microdictyon*, and *Chaetomorpha*; globular terminal complexes in *Ophioglossum*, *Psilotum*, *Equisetum*, and *Gingko*. Cellulose microfibril assembly in *Acetobacter xylinum* is very briefly described and compared with the process among eukaryotic cells. Particular emphasis on structures which may be involved in the spatial control of cellulose synthesis is given. Among these are cytoplasmic structures such as microtubules and microfilaments. Microfilament structures are shown to clearly surround individual microtubules which lie adjacent to the plasma membrane. Using freeze-fracture techniques, these labile associations have been shown for the first time. Microfibril orientation may be mediated through an interaction of cortical microtubules in association with microfilaments. A review of Mueller and Brown's membrane flow model for microfibril orientation is presented. Cellulose terminal complex clustering and its role in gravitropic response is covered. Definitive membrane changes with TC
clustering/disaggregation and intramembranous particle frequencies, occur within 12 min. following gravistimulation. These differences are pronounced in the cells from upper and lower hemicylinders of rapidly frozen tissue which was studied by the freeze-fracture method. A hypothesis for cellulose microfibril interaction in controlling the constraint of the growth axis is presented, and the supporting data for terminal complex clustering/disaggregation as well as fluorescent brightener inhibition of the gravitropic response support this hypothesis. The onset and regulation of cellulose microfibril assembly is presented for synchronized protoplasts generated by Boergesenia, using inhibitors of transcription and translation. These results suggest dynamic turnover of terminal complex subunits during the assembly of the cellulose microfibril. This study is concluded with a brief discussion of possible phylogenetic trends in the evolution of cellulose synthesis. A principal underlying theme is that the specific arrangement and consolidation of the terminal complex subunits determine to a large degree the size and shape of the microfibril, its crystallinity, as well as intramicrofibrillar associations. Three basic types of TCs appear among all eukaryotic cells studied so far, namely, the rosette, the globular, and the linear complex.

Introduction

Plant growth and development is ultimately a result of the expression and regulation of genes. However, our understanding of growth and development depends not only upon an unraveling of these molecular events, but also upon an interpretation of the morphology of development at the macromolecular, cellular, tissue, and organ levels.
Among these, the cell surface is one of the most important modulators of plant growth and development, for it is at the cell surface that the wall is assembled. The cell wall determines the shape of the cell, and it is involved in the control of cell elongation and expansion. Thus, an understanding of the spatial and temporal control of the structural and biochemical events leading to cell wall biosynthesis could provide insights into the overall process of plant growth and development. In this presentation, I will focus on the biosynthetic events at the plant cell surface which deal with the assembly of one of the major cell wall components, cellulose microfibrils.

The history of cellulose structure and biosynthesis has been a long one. In the field of electron microscopy, many will be surprised to learn that the first biological object examined in an electron microscope was the cellulosic wall of a cotton fiber. This was the work of Professor Ruska, who obtained electron images of cotton fibers in 1933 (Ruska 1980). The images obtained by Ruska showed for the first time a resolution which corresponded to the limit of the optical microscope, or possibly a little better. Thus, it is clearly obvious that cellulose played an important role in Ruska's efforts to continue developing and perfecting the electron microscope!

The structure of cellulose was first described by a French scientist, Anselme Payen, in the nineteenth century. Since this initial discovery, many investigators have sought the mechanism of the biosynthesis of cellulose. In 1958, Roelofsen first suggested that an enzyme, located at the tip of a microfibril, might be responsible for the polymerization of glucose, as well as its crystallization into the microfibril. Roelofsen also noted that in the crystal lattice of cellulose, the molecules cannot be organized in an anti-parallel
arrangement, lending further support to the catalytic site being at one tip of the growing microfibril. Roelofsen predicted that it might be possible with electron microscopy to visualize the cellulose synthesizing complex. In 1984, we have not only visualized the synthesizing complex for cellulose, but it has been possible to experimentally alter cellulose microfibril synthesis. Recently, the actual crystal lattice of the glucan chains within a single microfibril has been clearly visualized with ultra-high resolution microscopy (Harada, Sugiyama, Fujiyoshi, and Uyeda, 1984). At the present, we are entering into a new era in understanding cellulose microfibril assembly and orientation. The goal of this presentation is to convey the importance of the relationship of cellulose microfibril assembly and orientation to the growth and development of the plant cell. This is an area which needs much more research, and one which will eventually help to provide clues to the regulation of plant growth and development. It is ironical on one hand, that we know so much about the molecular aspects of auxin binding and hormones in plant growth, but little, if any, is known about subtle structural changes which take place during cell growth, let alone the regulation of these processes.

What do we know about the structures involved in the synthesis of cellulose microfibrils? It was Roelofsen’s (1958) initial suggestion that led to a number of important studies dealing with this question. Foremost among these, was the work of Preston (1964) who proposed the ordered granule hypothesis for cellulose microfibril assembly. This hypothesis was based largely on shadowing preparations of the innermost wall layers of the alga Chaetomorpha. Plasmolysis of Chaetomorpha produced granular aggregates which were observed lying in files, and these were in close association with the innermost microfibrils. These
ordered files led Preston to propose the following criteria for cellulose microfibril assembly: (a) the system should be able to produce microfibrils by end synthesis, as Roelofs en had earlier suggested; (b) the system of synthesis must extend over the entire cell surface; and (c) the system must be capable of producing microfibrils in all three directions, either separately or simultaneously. This latter concept envisioned a cuboidal-ordered granule with the simultaneous, multi-axial synthesis of microfibrils from a fixed point.

As will be noted, the concept of a stationary synthesizing complex was later replaced by that of a mobile synthesizing complex, especially with publication of the fluid-mosaic model for plasma membrane structure by Singer and Nicholson in 1972. Since Preston's ordered granule hypothesis was proposed, many investigators have noted "granules" associated with microfibrils (Staehelin, 1966; Robards, 1969; Frey-Wyssling, 1976). In 1972, Robinson and Preston examined cell wall biogenesis in the alga Oocystis with freeze fracture. They observed ordered granule bands and suggested that these were involved in cellulose microfibril assembly; however, their model was not based on an understanding of the fracture planes of the bimolecular leaflet of the plasma membrane.

In a theoretical paper in 1974, Heath proposed a unified hypothesis for the role of membrane bound enzyme complexes and microtubules in microfibril synthesis. Heath suggested that cellulose synthetase complexes are located in the plasma membrane and that they have an associated component which projects through the inner side of the plasma membrane which interacts with adjacent microtubules to help move and guide the complex through the membrane. Heath's model incorporated the recent Singer-Nicholson fluid mosaic model.
In 1976, Brown and Montezinos examined *Oocystis* with freeze-fracture, and they found linear terminal synthesizing complexes associated with cellulose microfibrils. These terminal complexes (TCs) were on the E-fracture face of the plasma membrane. It is now believed that the granule bands of *Oocystis*, observed earlier by Robinson and Preston, are not directly involved in the synthesis of microfibrils; however, they may influence microfibril orientation, but, if so, this appears to be a unique case among the organisms thus far studied.

Since the initial report by Brown and Montezinos in 1976, many papers have provided descriptions of terminal synthesizing complexes, and the list continues to grow year by year. Table 1 summarizes the current status of terminal synthesizing complexes.

In 1976, Mueller, Brown and Scott first observed terminal synthesizing complexes in a higher plant cell. More details of the structure of the higher plant terminal synthesizing complex was presented in 1980 (Mueller and Brown). A globular component is bound to the terminus of a microfibril. This globular component is located primarily on the surface of the outer leaflet of the plasma membrane, and it makes an impression contiguous with an opposing "rosette" of six particle subunits on the P-fracture or innermost leaflet of the plasma membrane. Together, the rosette and globular complex comprise what is believed to be the functional terminal complex in higher plant cells. This has been confirmed among representatives of gymnosperms and angiosperms, and now appears to be a common structure among other land plants such as *Equisetum*, Fig. 1 (Emons, 1984, in press); *Psilotum*, Fig. 2; *Ginkgo*, Fig. 3; and *Ophioglossum*, Fig. 4 (Itoh and Brown, personal communication).
In 1980, Giddings, Brower, and Staehelin also described "rosettes" associated with the termini of microfibrils in the alga, *Micrasterias*. These structures, however, while given the same name, are quite different from those described by Mueller and Brown for higher plants. The rosettes in *Micrasterias* have been described as transmembrane components, and the rosettes can be arranged in rows or hexagonal arrays, the latter producing bands of parallel microfibrils. Recently, *Spirogyra*, Fig. 5, was shown to have a hexagonal array of rosettes (Herth, 1983; Hotchkiss, Roberts, Itoh, and Brown, 1983). The hexagonal array pattern of rosettes appears to be confined to members of the Zygnematales; however, it does not appear to be present in all members of the Zygnematales. Hotchkiss and Brown (personal communication) have preliminary evidence suggesting that in the alga *Mougeotia*, the microfibrils are synthesized by a globular-rosette complex, similar to that found in higher plants.

In 1984, Itoh and Brown described a new type of linear terminal complex associated with the ends of microfibrils of the giant alga cell *Valonia macrophysa*, Figs. 6 and 7. Unlike the linear terminal complexes first observed in *Oocystis*, the subunits of the *Valonia* TCs traverse the plasma membrane. Granule bands are absent in *Valonia*. Since this recent discovery, linear terminal complexes, similar to *Valonia macrophysa*, have been found in a closely related genus, *Boergesenia forbesii*, Fig. 8, (Itoh, O’Neil and Brown, 1984, in press), as well as *Microdictyon*, Fig. 9, (Itoh and Brown, personal communication), and *Chaetomorpha*, Fig. 10, (Itoh and Brown, personal communication).

As a background toward describing a new model for the role of cellulose microfibril assembly in the differential growth mediated by the gravitropic response, I would like to recall the work of Brown,
Willison, and Richardson, 1976, in which the sites of cellulose synthesis of the gram-negative bacterium, Acetobacter xylinum, were first described. In this organism, cellulose microfibrils are synthesized from one or more rows of terminal synthesizing complexes arranged longitudinally on the cell. As the microfibrils are generated, they coalesce laterally through intermicrofibrillar hydrogen bonding to form bundles. The bundles associate with neighboring bundles to produce a composite ribbon of cellulose microfibrils. It is the ribbon which has been observed directly, using dark-field microscopy (Brown, et al., 1976). Since the number of microfibrils per ribbon is known, and the number of glucan chains per microfibril can be determined, it has been possible, using time-lapse cinematography and video microscopy, to make calculations of the rate of glucose incorporation into cellulose on a per cell basis (Brown, 1981). Furthermore, Acetobacter has proven to be a very useful organism in understanding how cellulose microfibrils can be altered by external agents which interfere with crystallization (Haigler, Brown, and Benziman, 1980; Brown, Haigler, and Cooper, 1982). In addition, it should be noted that the synthesizing complexes on the surface of Acetobacter are fixed relative to the cell surface. That is, they do not move over the cell surface as the microfibrils are generated. The consequence of these fixed sites is that the microfibrils are synthesized into the extracellular medium. A cellulosic cell wall is not formed. Instead, a distinct pellicle or membrane is generated through the combined synthesis of all of the cells at the gas-liquid interface of the Acetobacter culture.
THE SPATIAL CONTROL OF CELLULOSE SYNTHESIS

It is clear that among a diversity of plant types, only a limited number of specialized cellulose synthesizing complexes exist. These can be divided into two basic groups: (a) complexes of subunits with a rosette configuration, or (b) complexes of subunits which form a linear pattern (see Table 1). While these terminal complexes have yet to be isolated and confirmed unequivocally to be the actual cellulose synthetases themselves, the circumstantial evidence for the role of these structures as cellulose synthetases is compelling indeed. How have some of the structural studies conveyed a better understanding of the role of cellulose synthetases in plant cell growth and development?

Since the cell wall is composed of a fibrillar, and a non-fibrillar phase, the expansibility of the wall and its general structural features are controlled to a large degree by the positioning and interaction of the various polymer species which occupy the cell wall. As a background to the spatial control of cellulose assembly, there are several a priori assumptions to be made:

A. Cellulose microfibrils are the reinforcing and constraining structures of the plant cell wall;

B. The specific orientation of cellulose microfibrils can determine the directionality of differential constraint and, therefore, cell expansion; and

C. The driving force for cell expansion and cell growth is turgor.

These assumptions postulate that cellulose microfibril biosynthesis and orientation is an essential component for the multitude of events involved in the spatial control of directed plant cell growth. In eukaryotic cells, the synthesizing complexes are free
to move in the plane of the fluid membrane. The advantages of a mobile site are that the complexes can move over the surface of the cell as the microfibrils are being generated, thus encasing the protoplast within a cell wall network of microfibrils of great length. This model differs from Preston's ordered granule hypothesis, in which he proposed that many complexes spread over the cell surface are fixed. The mobile site model introduces an additional complication, namely, the orientation of microfibrils. How can mobile sites of cellulose synthesis be oriented? This is a question which has not been satisfactorily answered, but microtubules and actin-like microfilaments, in close association with the membrane must surely be involved (Quader 1984). Freeze-fracture data, Fig. 11, have indicated that microtubules are either parallel to the most recently synthesized layer of microfibrils (Mueller and Brown, 1982), or perpendicular to the most recent layer of microfibrils synthesized (Itoh and Brown, 1984). Thus, the question of the role of microtubules in the orientation of cellulose microfibrils is a complicated one and needs further research (Emons, 1984, in press; Lloyd and Barlow, 1982). It is not possible to describe precisely how cellulose microfibrils are oriented, but it is interesting to note that in 1982, Mueller and Brown first suggested that a directed membrane flow might be involved. Their evidence was indirect and based on the positioning of microfibrils around stationary pit fields. In this model, the microfibril, being anchored into the wall on one end, but having a flexible synthesizing terminus embedded in the plasma membrane on the other end, is free to move in the membrane in the direction of synthesis; however, the membrane flowing at a rate faster than that of synthesis would influence the orientation of microfibrils parallel to the axis of flow,
much like parallel orientations of logs in a rapidly moving stream. The requirements for such a membrane-mediated flow would include unidirectional synthesis, and this was indeed the case for longitudinal walls. The presence of microtubules and actin-like molecules at the cell surface in association with the plasma membrane, as well as microtubules, lends support to the model that the driving force for unidirectional membrane flow could be mediated by a membrane-associated actin-myosin system. Even if such a system is discovered, this would not answer the question of what specifically may regulate the spatial control of cellulose synthesis; nevertheless, it would provide an interesting new conceptual framework for analysis of dynamic events which occur within a short time span. Recent studies by Mizuta (personal communication) have shown unidirectional accumulations of intramembranous particles on one side of TCs, suggesting the influence of membrane flow as a "steering current" for the orientation of microfibrils. These studies, to my knowledge, provide the first evidence in support that directed membrane flow orients cellulose microfibrils.

THE GRAVITROPIC RESPONSE

With the background of information about the relationship of the terminal synthesizing complexes in *Acetobacter* to the hierarchical aggregation of microfibrils into bundles and the ribbon, it became interesting to see if similar aggregation patterns might exist in a "dynamic" or regulatory sense in eukaryotic cells; thus, we have investigated the role of cellulose synthesis in the gravitropic response. The work to be described has been done in collaboration with
my graduate student, Debra Brown. We have been investigating the
gravitropic response of the *Avena* coleoptile (Brown and Brown, 1983;
Brown and Brown, 1984). The gravitropic response in this tissue is well
known and has been examined in great detail. Using the freeze-fracture
technique, we have analyzed the patterns of cellulose microfibril
assembly during gravistimulation, and we have compared these patterns
with the upright grown control plant. The success of our experimental
protocol has been due to the rapid freezing of living tissue in the
absence of cryoprotectants. It has been possible to split the
coleoptiles longitudinally immediately prior to freezing, both before
and after gravistimulation. Thus, cells from both hemicylinders have
been examined for structural differences which might relate to the
differential growth observed during the gravitropic response.

The presence of globular terminal complexes in oat coleoptiles has
been confirmed, and the structure of these complexes appears to be
similar to those observed by Mueller, *et al.* (1976). The innermost
layer of cellulose microfibril deposition is transverse to the
longitudinal axis of the coleoptile. In the control plants, which were
grown upright, the transverse microfibrils are highly ordered and exist
in bundles or aggregates of twisted cables, Fig. 12. An examination of
the E and P-fracture face of the plasma membrane from such cells
reveals highly clustered, globular complexes and rosettes, Figs. 13 and
14. These complexes are found primarily in the growing tip (0.5 to 2 cm
from the apex). In the regions beyond the elongation zone, no globular
complexes are found. Thus the presence of complexes correlates well
with regions where there is expected cellulose synthesizing activity.

In gravistimulated coleoptiles, the cells in the upper hemi-
cylinder show bundles of twisted cables of microfibrils similar to
those found in cells from both hemicylinders of the control upright plant, Fig. 15. Furthermore, the globular complexes in cells from the upper hemicylinder are clustered.

The situation is quite different in cells from the lower hemicylinder following gravistimulation. Remarkable changes appear within twelve minutes following gravistimulation. The microfibrils, instead of being parallel to one another and oriented transverse to the longitudinal axis of the coleoptile, are more randomly dispersed, Fig. 16. In addition, the terminal synthesizing complexes are not tightly clustered.

The distribution of intramembranous particles (IMPs) from the E-fracture face of the plasma membrane shows dramatic changes in cells following gravistimulation for only 15 minutes. The average distribution of IMPs of the E-fracture face in the control plant is 1,466 ± 55 IMP's/μ². The IMP distribution on the PF-face of the control plants is 2,434 ± 111 IMPs/μ². Thus, there is a significant partitioning of IMPs in the plasma membrane. The E-fracture faces of the plasma membrane from cells within the lower hemicylinder, after fifteen minutes of gravistimulation, show a great reduction in the number of IMPs. Here, only 777 ± 29 IMPs/μ² are present. Cells from the upper hemicylinder of the same plant show an average distribution of 1,461 ± 78 IMPs/μ², and this correlates favorably with the IMP counts from the control non-gravistimulated plants.

The freeze-fracture studies of the Avena coleoptiles show definitive changes occurring within minutes of gravistimulation. How can these changes be interpreted? We have proposed (Brown and Brown, 1984) that the clustering of cellulose synthesizing complexes, in regulating the proximity of cellulose microfibril synthesis, as well as
intermicrofibrillar hydrogen bonding, could lead to the establishment of a constraining axis. When TC clustering is disrupted, a change in the constraining axis might lead to a change in the growth rate. At the tissue level, differential growth could result from the specific events associated with the cellulose microfibril assembly. What data tend to support this hypothesis?

First, the time course of events is rapid enough to fall within range of the observed response; namely, terminal complex changes occur within 10 - 15 minutes following gravistimulation, and TC disaggregation occurs only on the cells from the lower hemicylinder. It should be emphasized that this event precedes the visualization of the early bending response. Thus, the presence of a constraining axis could lead to reinforcement, and thus control the rate of cell wall expansion. If the kinetic data from Acetobacter are used as a basis for the rate of microfibril elongation in Avena, it would take approximately 15 min for terminal synthesizing complexes to synthesize one completed loop on the transverse axis of the cell. This time course supports the hypothesis of Richmond (1984) that the innermost layer of microfibrils controls the elongation of the internodal cell of Nitella. Are there other data which support the involvement of the innermost layer of microfibrils in the differential growth response? The optical brighteners Calcofluor and Tinopal have been very useful probes to study microfibril assembly (Haigler and Benziman, 1982). Tinopal is known to prevent crystallization of glucan chains into microfibrils, but not to impede the polymerization process (Haigler, et al., 1980). These agents have no effect on microfibril structure when applied after biosynthesis and crystallization are completed. To test the effects of brighteners on the gravitropic response, Avena coleoptiles were
subjected to treatment in 0.01% Tinopal for 20-25 minutes. Under these
conditions, the gravistimulated coleoptiles continued to elongate at
0.8 mm/hr. for up to 24 hours; however, no differential bending
occurred when the coleoptiles were in contact with the fluorescent
brightening agent. When Tinopal was removed, the gravitropic response
resumed within 20 min.

What do these data mean in relation to a control of the
differential growth response? Clearly, gravity is the stimulus, and the
differential expansion of the cell walls in the two hemicyinders of
Avena coleoptile is the response.

The results with Tinopal suggest the involvement of only a very
small portion of the total wall in the gravitropic response. This is a
compelling argument to support the necessity for crystalline cellulose
microfibrils and their capability for intermicrofibrillar hydrogen
bonding as crucial to the establishment of the differential growth
response. What precisely mediates the spatial control of cellulose
microfibril assembly is a matter of conjecture at present; however, it
would be interesting now to consider events which might regulate the
clustering of terminal complexes. Such events could include proton
efflux, auxin redistribution, or calcium-ion redistribution.

Auxin has frequently been postulated to be involved in the
gravitropic response (Wilkins, 1979). Most published accounts of the
time-course of direct auxin effects are in the range of 30 min. - 1 hr.
We have observed microfibril reorientation within 10 - 15 min. after
gravistimulation. Preliminary experiments in our laboratory with
exogenous auxin applications show no effects on terminal complex
clustering or microfibril reorientation within 15 min. Thus, auxin may
not be directly responsible for the gravitropic response, but it may
mediate the activities of other components such as proton efflux or calcium redistribution.

THE ONSET AND REGULATION OF CELLULOSE MICROFIBRIL ASSEMBLY

Another interesting problem which has only recently been considered is that of the dynamics of the cellulose synthesizing machinery. The work to be discussed below has been done in collaboration with Dr. Takao Itoh of the Wood Research Institute, Kyoto, Japan, Mr. Rory O'Neil of our Department, and Dr. Raymond Legge. The experimental organism is *Boergesenia forbesii*, a close relative of the Siphonocladalean alga, *Valonia*. Wounding of these large cells induces the formation of a multitude of protoplasts which synthesize new walls in near synchrony (Itoh and Brown, 1984). The cell wall of *Boergesenia* contains numerous highly crystalline microfibrils 300 nm in diameter. Linear terminal complexes synthesize these microfibrils (Itoh, O'Neil, and Brown, 1984).

In order to consider the question of turnover and the regulation of proteins during the production of protoplasts, we followed the developmental sequence of TC formation leading to cell wall regeneration. We also studied the effects of cycloheximide and actinomycin D. The window for effective inhibition by actinomycin D is restricted to the first hour following wounding. Thus, it seems likely that many of the genes must be activated as part of the repair mechanism following wounding, especially those coding for terminal complexes. Results from actinomycin D treatment suggests that the first hour following wounding is apparently critical in terms of DNA transcription, and if the appropriate repair mechanisms are not
activated within this period, the wounding proves fatal. In contrast, protoplasts are capable of recovering from cycloheximide treatment. Protoplasts incubated for one hour after wounding, and followed by a four hour cycloheximide treatment, were devoid of a cell wall. Freeze-fracture of the plasma membrane indicated a corresponding absence of terminal complexes. If these cells were incubated in a normal medium for one hour after the four hour cycloheximide treatment, neither cell walls nor terminal complexes were observed; however, a very thin cell wall was observed after 1.5 - 2 hours of recovery, and a 9 hr. recovery period provided sufficient time for the establishment of a typical secondary wall. Whenever wall microfibrils were observed by freeze fracture, terminal complexes were observed associated with microfibrils. The tight correlation between the appearance of the terminal complex and the appearance of cell wall microfibrils is important to dispel any doubt that the terminal complexes of the linear form in Boergesenia are responsible for microfibril synthesis and deposition.

Because cell wall regeneration in Boergesenia protoplasts is sensitive to cycloheximide and actinomycin D, this suggests that wall regeneration is accomplished through terminal complexes synthesized de novo following wounding, rather than the alternative of cell wall regeneration through pre-existing terminal complexes. The turnover of terminal complexes needs to be considered in relation to the dynamics of cellulose microfibril assembly. A freeze-fracture electron micrograph represents only one moment of time during which the complex was active. One cannot distinguish from such evidence whether the linear complex and its subunits are all moving together as one unit as the microfibril is being generated or, possibly, whether the subunits
are being lost from the forward end of the complex and being replaced at the rear end of the complex. Such a concept of turnover would necessitate a very precise mechanism to insure continuity of glycosylation. Because the degree of polymerization (DP) of many celluloses has never been found to be greater than 20,000, either the analytical procedures for determining DP are subject to an intrinsic artifact which decreases the DP, or the lower DPs may reflect the continuous cycling of new subunits to the growing microfibril. All of this is speculative, but these preliminary experiments with cycloheximide and actinomycin D do suggest that turnover should be seriously considered.

A better understanding of cellulose microfibril assembly would be possible if the terminal complex could be effectively isolated, and the subunits primed into generating glucan chains which form microfibrils similar in structure to those present in the native state. Biochemists and others have been working for decades on this problem without apparent success. Usually, the results include evidence to suggest that, indeed, glycosidic linkages are possible under in vitro conditions, but no one to my knowledge has successfully demonstrated the in vitro synthesis of cellulose microfibrils. This is a major challenge in understanding the regulation of cellulose synthesis and the role of cellulose microfibril assembly in plant growth and development.
SOME THOUGHTS ON THE PHYOGENETIC SIGNIFICANCE OF CELLULOSE MICROFIBRIL ASSEMBLY

This presentation will conclude by a brief discussion and comparison of the various terminal complexes which have been found among eukaryotic cells to date (see Table 1). It must first be recognized that this is a preliminary analysis at best; however, it could be useful to gain an overall understanding of the phylogeny and evolution of cellulose synthesis. There are certain trends which seem to be common and which have interesting implications. A basic trend in common with all systems studied is that the basic subunit of the cellulose synthetase probably makes only a few glucan chains or perhaps even a very minute microfibril of low crystallinity, but it is the arrangement and consolidation of the subunits which determines to a large degree: (a) the size and shape of the microfibril; (b) the crystallinity of the microfibril; and (c) association of microfibrils into complex bands or aggregates. The oat coleoptile system demonstrates that perhaps a temporal association/disassociation phenomenon is a dynamic means of regulating differential growth, whereas the programmed consolidation of rosettes among certain members of Zygnematales would suggest that microfibrils and their aggregates provide a greater strength for the control of cell shape. There are several pathways of consolidation, and from this, it is apparent that the patterns are quite simple in terms of the evolution of a rosette pathway and a linear pathway. So far, no other variations on these two themes have been found. Linear aggregation of subunits, be they single subunits or rosettes themselves, leads to greater microfibril size and/or greater microfibrillar interaction. Temporal association of individual rosettes with their neighbors leads to a greater
intermicrofibrillar association, but one which is more temporal and short lasting relative to the cell cycle. Thus, this type of regulation appears to have the possibility for influencing differential cell growth.

In conclusion, one must not underestimate the importance of mechanisms for the orientation of cellulose microfibrils. It is obvious from this Conference and from numerous reports, that microtubules have an influence on orientation. It should also be evident that the control of microfibril assembly itself could have a direct influence on the direction of cellulose synthesis. Microfilaments could induce membrane flow and thus influence microfibril orientation. Thus, at present, it is not possible to make definitive statements on precisely how microtubules or microfilaments might control the directed orientation of microfibrils. This will only come when technological advances allow a more precise and definitive technique for recognizing in vivo the molecular species involved. The increasing use of monoclonal antibodies to specific molecular probes, coupled with cell-microinjection and the use of video microscopy and digital image processing to carry the limits of resolution beyond conventional microscopic approaches, makes these tools excellent candidates for continuing progress in this field. Until progress is made using more dynamic approaches, the biochemical, genetic, and structural data will continue to augment our knowledge on cellulose microfibril assembly, but we will not be in a position to thoroughly understand these integrated phenomena in a living cell. At this stage of our understanding, it is apparent that cellulose microfibril assembly and orientation is of paramount importance as a cell surface phenomenon to the regulation and control of plant cell growth and differentiation. Further studies in this field will be
important in the link for study of these fascinating problems in plant cell and molecular biology.

ACKNOWLEDGEMENTS

My sincere thanks go to colleagues who have been instrumental in conducting the research reported in this presentation. Especially noteworthy are Dr. Takao Itoh and Dr. Raymond Legge, for their collaboration on the studies with Boergesenia, and to Debra Brown for collaboration and studies with the gravitropic response. Also, I wish to thank Eric Roberts and Takao Itoh for providing photographs and data to be used in the table and for discussions regarding the topics. I also wish to thank Richard Santos, who printed most of the pictures, prepared the plates, carefully edited the manuscripts, and provided thoughtful comments. I thank Pamela Goff for typing and preparing the manuscript.

This research was supported in part by the National Science Foundation Grant PCM-8242213, and a research grant from Johnson & Johnson.
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Plate 1

Figure 1. E-fracture face, globular terminal complex of *Equisetum*. Scale bar, 100 NM; Magnification, 130,000x.

Figure 2. E-fracture face, with two globular terminal complexes (arrows) of *Psilotum*. Scale bar, 100 NM; Magnification, approx. 90,000x.

Figure 3. E-fracture face, globular terminal complexes of *Ginkgo* (arrows). Scale bar, 100 NM; Magnification, 83,000x.

Figure 4. E-fracture face, globular terminal complex of *Ophioglossum*. Scale bar, 100 NM; Magnification, 100,000x.

Plate 2

Figure 5. P-fracture face, array of rosettes in *Spirogyra*. Scale bar, 100 NM; Magnification, 250,000x.

Figure 6. E-fracture face, linear terminal complex of *Valonia macrophysa*. Scale bar, 100 NM; Magnification, Approx. 80,000x.

Figure 7. P-fracture face, linear terminal complex of *Valonia macrophysa*. Scale bar, 100 NM; Magnification, Approx. 95,000x.

Figure 8. P-fracture face, three linear complexes of *Boergesenia forbesii*. Scale bar, 100 NM; Magnification, 140,000x.
Plate 3

Figure 9. E-fracture face, linear terminal complex of Microdictyon. Scale bar, 100 NM; Magnification, 83,000x.

Figure 10. E-fracture face, linear terminal complex of Chaetomorpha. Scale bar, 100 NM; Magnification, 120,000x.

Figure 11. E-fracture face (right) and P-surface view (left) of Psilotum. Note microfibril impressions on the E-fracture face, and prominent microtubules on the surface of the inner leaflet of the plasma membrane. Note also that the microtubules are surrounded by parallel cables of filaments which have periodic structure similar to actin. Scale bar, 1 NM; Magnification, 10,000x.

Plate 4

Figure 12. E-fracture face from an upright-grown Avena coleoptile. Note the ordered bundles of microfibrils which lie in the transverse plane. Scale bar, 100 NM; Magnification, 50,000x.

Figure 13. Detail of an E-fracture face of an Avena coleoptile, (arrows) showing globular terminal complexes clustering. This is an upright-grown controlled plant. Scale bar, 100 NM; Magnification, 100,000x.

Figure 14. P-fracture face of upright-grown (control) Avena coleoptile, showing clustered rosettes (arrows). Scale bar, 100 NM; Magnification, 130,000x.
Plate 5

Figure 15. E-fracture face of cells from an upper hemicylinder taken from an *Avena* coleoptile which had been gravistimulated for 15 min. Note that the globular terminal complexes are clustered, as in the control upright plant.

Scale bar, 100 NM; Magnification, 170,000x.

Figure 16. E-fracture face from cells of the lower hemicylinder of an *Avena* coleoptile, 12 min. after gravistimulation.

Note the disorder of the innermost layer of microfibrils which have separated into single components. Several separate globular TCs are shown (arrows). Scale bar, 100 NM; Magnification, 83,000x.