Development of Cellulose Synthesizing Complexes in Boergesenia and Valonia

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Received January 11, 1988
Accepted March 19, 1988

Summary
The development of linear cellulose synthesizing complexes (= TCs) of two selected siphonocladalean algae, Boergesenia forbesii and Valonia ventricosa was investigated by following the time course of the regeneration of cell walls with the freeze fracture technique after aplanospore induction. The following structural changes of TC development were examined: (1) TCs initiate de novo; (2) the first nucleation of TC subunits occurs within 2 hr in Boergesenia and 5 hr in Valonia after aplanospore induction, immediately followed by the assembly of cellulose microfibrils; (3) TCs increase their length during the assembly of randomly oriented microfibrils; and, (4) TCs stop increasing in length after the assembly of ordered microfibrils begins, with some time lag. The data demonstrate that linear TCs are not artificial products but dynamic entities which are involved in the assembly of cellulose microfibrils.

Keywords: Boergesenia forbesii; Valonia ventricosa; Freeze fracturing; Cellulose synthesizing complexes; Microfibrils.

1. Introduction
The putative cellulose synthesizing complexes are thought to consists of subunit particles which are involved in the assembly of cellulose microfibrils. Two types of terminal complexes (TCs) have been found in algae; one is a linear TC (Brown and Montezinos 1976), and the other is a rosette (Giddings et al. 1980). According to previous investigations, most linear TCs have 3 rows of subunit particles (Brown and Montezinos 1976, Itoh et al. 1984, Mizuta et al. 1987), and the TC length is different among species to species (Itoh 1987, Mizuta 1987), while the TCs in Boodlea coacta have the length different between primary wall and secondary wall synthesis (Mizuta 1985). Therefore, it is not clear whether the TC length is different among species or dependent upon the developmental stages of wall assembly. Another question relates to how the subunits of the TCs are assembled to make linear TCs. Furthermore, the question whether the TC length has some correlation with microfibril assembly is unknown. To understand the structure and function of linear TCs, we investigated the development of TCs during the various stages of cell wall regeneration in the aplanospores of both Boergesenia forbesii and Valonia ventricosa.

2. Materials and Methods
Boergesenia forbesii and Valonia ventricosa provide excellent experimental materials for the present purpose by gentle and careful handling of the cells after specified wounding. The procedure to culture and prepare the cells of Boergesenia and Valonia, and also their aplanospores are the same as those described in the previous paper (Itoh et al. 1984). The synchronized cells were grown and collected at 10–20 min intervals for Boergesenia or 1 hr intervals for Valonia during the “early stages” of cell wall regeneration from 1.5 to 3 hr after wounding (Boergesenia) or from 2 to 8 hr after wounding (Valonia). To examine further changes of TCs, another collection (Boergesenia) or the same collection (Valonia) of synchronized cells was grown and collected at 6, 10, and 20 hr after wounding (Boergesenia) or at 10, 12, 15, 20, and 25 hr after wounding (Valonia). Wall regeneration was examined with epi-fluorescence microscopy by observing the orientation of microfibrils stained with the fluorescent brightener, Tinopal LPW. For freeze fracture experiments, selected cells were pipetted onto single specimen supports and frozen by liquid propane without any cryo-protectant. Freeze fractured replicas were prepared from these materials according to the procedures described in the previous paper (Itoh and Brown 1984). The replicas were observed with a Philips 420 transmission electron microscope.

3. Results
Boergesenia and Valonia had a different time course of cell wall regeneration. It took more time for Valonia
Acknowledgements

The research of one of us (HJS) is funded by the Netherlands Organization for Scientific Research (NWO), grant nr. H. 86-88. The Trebouxia strain was kindly supplied by Dr. G. Gärtner, University of Innsbruck, Austria. We thank Dr. W. de Priester, Ms. G. E. M. Lamers, and Mr. W. Star for their assistance in the cryofixation and TEM work.

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Table 1. *Time course of TC length during the regeneration of cell wall in the aplanospores of Boergesenia forbesii*

<table>
<thead>
<tr>
<th>Hr after wounding</th>
<th>TC number measured</th>
<th>Mean TC length (nm)</th>
<th>Max TC length (nm)</th>
<th>Min TC length (nm)</th>
<th>TC density (μm²)</th>
<th>MF imprints on the cell wall</th>
<th>MF imprints on the plasma membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 H &amp; 30 Min</td>
<td>No TCs occur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 H &amp; 50 Min</td>
<td>No TCs occur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 H</td>
<td>87</td>
<td>114 ± 31.0</td>
<td>194</td>
<td>65</td>
<td>0.6</td>
<td>ND*</td>
<td></td>
</tr>
<tr>
<td>2 H &amp; 10 Min</td>
<td>42</td>
<td>151 ± 44.3</td>
<td>295</td>
<td>73</td>
<td>1.3</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2 H &amp; 22 Min</td>
<td>87</td>
<td>149 ± 35.6</td>
<td>249</td>
<td>73</td>
<td>1.3</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2 H &amp; 40 Min</td>
<td>62</td>
<td>171 ± 43.2</td>
<td>310</td>
<td>78</td>
<td>1.7</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3 H</td>
<td>53</td>
<td>256 ± 64.1</td>
<td>400</td>
<td>128</td>
<td>2.2</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4 H &amp; 30 Min</td>
<td>118</td>
<td>510 ± 109.1</td>
<td>818</td>
<td>242</td>
<td>1.7</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6 H</td>
<td>80</td>
<td>608 ± 141.7</td>
<td>867</td>
<td>233</td>
<td>2.4</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10 H</td>
<td>28</td>
<td>656 ± 144.5</td>
<td>900</td>
<td>360</td>
<td>ND*</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>20 H</td>
<td>33</td>
<td>665 ± 147.8</td>
<td>918</td>
<td>209</td>
<td>2.0</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* ND = no data.

to regenerate the cell wall. Thus, the development of TCs is described separately. It has been demonstrated that both species have linear TCs on both E- and P-fracture faces of the plasma membrane (Itoh et al. 1984, Itoh and Brown 1986). With the double replica technique, the subunit morphology as well as the length of a Boergesenia TC on the P-fracture face is identical to that of the complementary E-fracture face (Mizuta 1985). Therefore, the present investigation on the development of TCs has focused on a comparison of P-fracture faces of the TC at various stages of development of the young aplanospore.

3.1. Development of TCs in Boergesenia

In Boergesenia, fragments of the protoplasm became spherical and generate aplanospores within 1 to 1.5 hr after wounding. These cells began to assemble random microfibrils within 2 hr after wounding, continuously synthesizing random microfibrils between 2 to 4 hr after wounding. The cells began to assemble ordered microfibrils within 5 hr after wounding.

The development of TCs in the “early stage” of cell wall regeneration from 1.5 to 20 hr after wounding is shown in Table 1. After 1.5 hr post-wounding, the cells showed neither TCs nor specific arrangements of particles on the plasma membrane as shown in Fig. 1. Randomly arranged membrane particles were the only structural components which could be observed on both E- and P-fracture faces of the plasma membrane. We have also observed the plasma membrane in cells obtained between 1 and 1.5 hr after wounding. During this stage, aplanospores were not yet established. Neither TCs nor any ordered arrangement of membrane particles were observed at this stage.

Several different structural entities were observed in the cells between 1.5 and 2 hr after wounding. One was a circular structure which includes many particles, suggesting the recent fusion of a vesicle membrane with the plasma membrane (Fig. 2). Another was a large cluster of particles as shown in Fig. 3. By 2 hr post-wounding, small elongate groups of particles appeared (Fig. 4). The smallest cluster was composed of only 10 particles. These elongate particle clusters represent the earliest recognizable morphology attributable to a developing TC. These structures had a mean length of 114 ± 31.0 nm (87 measurements). At this stage, some of the clusters had a random arrangement of particle subunits, while others had structure with greater linear order. The shortest nascent TC was only 65 nm in length, while the longest one was 194 nm in length. These nascent TCs were distributed randomly on the plasma membrane at an average density of 1.0 TC/μm² (= 189 TCs measured over 183.6 μm²). No imprints of microfibrils were observed on the plasma membrane at this stage.

The cells after 2 hr and 10 min post-wounding had a mean TC length of 151 ± 44.3 nm (42 measurements). The shortest TC was 73 nm, while the longest one was 295 nm. A number of random microfibril imprints on both the outer surface of the cell wall and the plasma membrane were detected in a limited number of cells, indicating that microfibrils had been synthesized at this stage and were already being incorporated into the growing thin cell wall. The cells at 2 hr and 22 min post-
Fig. 9. Many tetrads (circled) and one TC (arrow) are shown on a P-fracture face of the plasma membrane in a 2 hr cell of *Boergesenia forbesii*.

Fig. 10. Both microfibrils and TCs (arrowheads) are shown adjacent to one another on the P-fracture face of the plasma membrane. Note that a microfibril ends at the ES face. CW cell wall; PF P-fracture face of the plasma membrane; ES Exoplasmic surface of the plasma membrane; MF microfibril
Table 2. *Time course of TC length during the regeneration of cell wall in the aplanosores of Valonia ventricosa*

<table>
<thead>
<tr>
<th>Hr after wounding</th>
<th>TC number</th>
<th>Mean TC length (nm)</th>
<th>Max TC length (nm)</th>
<th>Min TC length (nm)</th>
<th>TC density per μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td>No TCs occur</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>No TCs occur</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>No TCs occur</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>96</td>
<td>100 ± 23.1</td>
<td>153</td>
<td>47</td>
<td>2.1</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>170 ± 61.7</td>
<td>367</td>
<td>107</td>
<td>3.3</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>212 ± 64.2</td>
<td>320</td>
<td>84</td>
<td>ND*</td>
</tr>
<tr>
<td>8</td>
<td>27</td>
<td>251 ± 79.7</td>
<td>417</td>
<td>100</td>
<td>2.1</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>344 ± 82.8</td>
<td>483</td>
<td>233</td>
<td>4.2</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>419 ± 91.0</td>
<td>483</td>
<td>250</td>
<td>3.3</td>
</tr>
<tr>
<td>15</td>
<td>80</td>
<td>474 ± 141.5</td>
<td>850</td>
<td>167</td>
<td>3.7</td>
</tr>
<tr>
<td>20</td>
<td>12</td>
<td>500 ± 111.2</td>
<td>683</td>
<td>367</td>
<td>3.0</td>
</tr>
<tr>
<td>25</td>
<td>23</td>
<td>471 ± 142.2</td>
<td>700</td>
<td>200</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* ND = no data.

wounding had a mean TC length of 149 ± 35.6 nm (87 measurements). The shortest TC was 73 nm, while the longest one was 249 nm. As demonstrated earlier, randomly oriented imprints of microfibrils were observed on the outer surface of the cell walls as well as the plasma membrane. Some of the TCs at this stage showed a dispersed structure, having 2 to 4 distinctive rows, with some intervals between each row. The cells at 2 hr and 40 min post-wounding had a mean TC length of 171 ± 43.2 nm (62 measurements). The shortest TC was 78 nm, while the longest one was 310 nm. Randomly oriented imprints of microfibrils were observed on the plasma membrane as well as on the outer surface of the cell wall. The mean TC length of 256 ± 64.1 nm (53 measurements) was measured in the cells at 3 hr post-wounding (Fig. 5), ranging from 128 to 400 nm. At this stage, the TCs had a well ordered arrangement of subunits. Many imprints of microfibrils with random arrangement were observed on the outer surface of the cell wall as well as on the plasma membrane. The random imprints of microfibrils represent a stage of active synthesis of the primary wall. The cells at 4.5 hr post-wounding had a mean TC length of 510 ± 109.1 nm, ranging from 242 to 818 nm (Fig. 6). Most of the TCs were arranged in parallel, suggesting that the cells were starting to assemble ordered microfibrils at this stage. Note that the density of TCs had increased to 1.7 TCs/μm² (= 211 TCs in 122.8 μm²). The cells at 6 hr post-wounding had a mean TC length of 608 ± 141.7 nm (80 measurements), ranging from 233 to 867 nm (Fig. 7). The cells at 10 hr post-wounding had a mean TC length of 656 ± 144.5 nm (28 measurements), ranging from 360 to 900 nm. The cells at 20 hr post-wounding had a mean TC length of

Fig. 11. A P-fracture face of the plasma membrane in a 4 hr cell of Valonia. Neither TCs nor any particular arrangement of particles are seen

Fig. 12. Circular arrangement of particles from a 5 hr aplanosore of Valonia

Fig. 13. A nascent TC (indicated by arrowhead) is shown on a P-fracture face of the plasma membrane of a 5 hr Valonia aplanosore

Fig. 14. Three TCs (indicated by arrowheads) are shown at the end of microfibril imprints on a P-fracture face of an 8 hr Valonia aplanosore. Note the random orientation of those TCs

Fig. 15. Several TCs (indicated by arrowheads) are shown at the end of microfibril imprints on a P-fracture face of the plasma membrane in a 10 hr Valonia aplanosore. Note the random orientation of those TCs

Fig. 16. Several TCs (indicated by arrowheads) with a parallel orientation are shown on a P-fracture face of a 12 hr Valonia aplanosore

Fig. 17. Several TCs (indicated by arrowheads) with a parallel orientation are shown on a P-fracture face of 15 hr Valonia aplanosore. Note the two TCs indicated by double arrowheads are closely lined up, suggesting the merger one another

Fig. 18. Several TCs (indicated by arrowheads) with a parallel orientation are shown on a P-fracture face of the plasma membrane in 25 hr Valonia aplanosore
665 ± 147.8 nm (33 measurements), ranging from 209 to 918 nm (Fig. 8). The TCs showed a fairly ordered arrangement in the cells from 6 to 20 hr after wounding. Consequently, the nascent TCs occurred in the cells at 2 hr post-wounding. These TCs increased in length from 2 to 6 hr post-wounding mainly throughout primary wall synthesis. TCs ceased to increase their length by 6 hr post-wounding. Any further significant increase in TC length was not observed from 6 hr to 20 hr post-wounding.

During these structural changes, especially at the onset of TC assembly, we often observed the tetrads which are composed of four particle subunits (Fig. 9). In some cases, the plasma membrane was covered with many tetrads. We observed both tetrads and TCs on the same fractured plane of plasma membrane (i.e., P-fracture face). Another important observation was that TCs were seen adjacent to the ends of microfibrils with both in the same orientation (Fig. 10). It is worthwhile to note that the uppermost microfibril ended on the outer leaflet of plasma membrane which happened to be left after freeze fracturing. Therefore, we are able to see four different structures, namely, TCs on the P-fracture face, the outer leaflet of plasma membrane, microfibrils and the outermost surface of the cell wall in a limited area.

3.2. Development of TCs in Valonia

In Valonia, spherical aplanospores were produced within 1.5 hr after wounding. These cells began to assemble microfibrils within 5 hr after woundings with continuous synthesis of random microfibrils during primary wall synthesis between 5 to 10 hr after wounding. The cells began to assemble ordered microfibrils at 10 to 12 hr after wounding. The development of TCs during the complete stage of cell wall regeneration is shown in Table 2.

Neither TCs nor imprints of microfibrils were observed on both E- and P-fracture faces of the plasma membrane of the cells at 2, 3, and 4 hr after wounding (Fig. 11). Similar to Boergesenia but at a later time after wounding, circular clusters of particles were observed (Fig. 12). A significant change in the structure on the plasma membrane occurred in cells at 5 hr post-wounding. Here, many short linear clusters of membrane particle subunits were observed on the plasma membrane. Only a single cluster of subunits is shown in Fig. 13. As with Boergesenia, these clusters are believed to represent nascent TCs. Some of the clusters showed a random arrangement of subunits within a cluster, leading to a width with more than 4 subunits. Other TCs showed a fairly ordered array of 3 rows of subunits. The mean length of these nascent TCs was 100 ± 23.1 nm (96 measurements), ranging from 47 to 153 nm as shown in Table 2. The nascent TCs were distributed evenly on the plasma membrane, averaging 2.1/μm². The orientation of the TCs was random, and no imprints of microfibrils were observed on the plasma membrane. The TC length increased in cells from 6 to 10 hr after wounding (Figs. 14 and 15). Throughout these stages, TCs were oriented randomly, indicating that the cells in these stages are involved in active synthesis of the primary wall, although parallel patterns of TCs were observed in some of the cells at 10 hr post-wounding. The orientation of TCs was largely parallel in the cells at 12 hr post-wounding (Fig. 16). The cells at 15 hr post-wounding had fairly elongated TCs (Fig. 17). The mean length of those TCs was 474 ± 141.5 nm (80 measurements), ranging from 167 to 850 nm. By 12 hrs post-wounding, the orientation of TCs was parallel in all cells examined. Two TCs indicated by double arrow-heads in Fig. 17 appeared lined up, suggesting the possibility of a merger of two smaller TCs into a large one. The cells at 20 and 25 hr post-wounding did not show any significant increase in TC length (Fig. 18; Table 2). Consequently, the nascent TCs increased in length in the cells during primary wall synthesis from 5 to 15 hr after wounding. TCs had no further increase in length after 15 hr post-wounding.

4. Discussion

The present investigation demonstrates that the TCs in both Boergesenia and Valonia initiate de novo, and increase their length during the synthesis of the primary wall, becoming more than 5 to 6 times longer than the nascent TCs. The evidence that the TCs increase in length indicates that TCs are not artificial products, but dynamic entities which are involved in the assembly of cellulose microfibrils.

In Boergesenia, the nascent TCs from 2 hr and 10 min post-wounding are fairly short, with a mean TC length of 151 nm, although the imprint of microfibrils was observed on the plasma membrane. This evidence demonstrates that even such short, nascent TCs are capable of synthesizing cellulose microfibrils. The mean TC length increases gradually, accompanied by an increase in length of both shortest and longest TCs up to 3 hr post-wounding. The imprints of microfibrils are observed in all stages onward from 2 hr and 10 min post-wounding.
It is worthwhile to estimate the TC number per unit area to get an accurate value of how soon the surface of the plasma membrane becomes covered by the new synthesis of microfibrils during the developmental changes of cell wall regeneration. The present results in _Boergesenia_ showed a TC density of 1.0 TC/μm² at 2 hr post-wounding during which nascent TCs were being activated. On the other hand, the density of TCs increases to 1.7 TC's/μm² at 2 hr and 40 min after wounding. Thereafter, no further increase of TC density was recognized, although the pattern of microfibril orientation begins to change from random to parallel within 4 to 5 hr after wounding. This correlation between the TC density increase may be responsible for the increase in microfibril density within a limited period right after the initiation of TCs. That is, more active TCs are available per unit area to synthesize a greater number of microfibrils.

The developmental processes of the TCs of _Valonia_ during the "early stage" of cell wall regeneration are more or less similar to those of _Boergesenia_. The circular arrangement of subunits into clusters is observed on the plasma membrane in both _Boergesenia_ and _Valonia_. The evidence suggests that TC subunits may be conveyed by cytoplasmic vesicles from the cell interior to the plasma membrane. Once the particle subunits of the vesicles are discharged into the plasma membrane as a large cluster, it forms into small clusters by making a crosslinkage of particle subunits, eventually giving rise to a nascent TC. Recent investigation for the transport of rosettes particles from the Golgi-derived vesicles to the plasma membrane in isolated mesophyll cells of _Zinnia elegans_ (HAGLER and BROWN 1987) may support the present result.

The developmental changes of TC structure common to both _Boergesenia_ and _Valonia_ during the "early stage" of wall regeneration are summarized as follows: (1) the pre-established TCs disappear from the plasma membrane of the mother cell immediately after wounding, thus any structural entities other than the structural membrane proteins are not observed (ITOH and BROWN 1985); (2) the nucleation of membrane particles into linear complexes occurs, giving rise to nascent TCs consisting of approximately 10 subunits; the nucleation of TCs in _Boergesenia_ and _Valonia_ occurs within 2 hr and 5 hr after wounding, respectively; (3) the nascent TCs increase their length throughout the synthesis of the primary walls in both algae. These evidences will give us further informations how the linear TCs are initiated and assembled to develop into more active form to synthesize cellulose microfibrils, although the difference of TC length between primary and secondary wall synthesis has been reported briefly by MIZUTA (1985).

As has been described earlier (ITOH and BROWN 1986), tetrads were observed on the plasma membrane. We do not know the role of tetrads whether they are involved in the assembly of TC subunit or not; however, tetrads have been observed on the vacuolar membrane of marine red algae (TSEKOS et al. 1985). It is worthwhile to note that they suggest these tetrads may be involved in the synthesis of wall polysaccharides.

A microfibril having its end on the outer leaflet of plasma membrane as shown in Fig.10 suggests that the assembly (probably crystallization) of the microfibril occurs on the outer surface of the plasma membrane (PS face), right after the synthesis of each glucan chain. The finding is in good coincidence with our model presented earlier for the assembly of cellulose microfibril (ITOH and BROWN 1984).

Further changes of TC structure during wall regeneration show that TCs in _Boergesenia_ and _Valonia_ increase their length rapidly during first 6 hr (Boergesenia) or 15 hr (Valonia) after wounding. Thereafter, no significant increase in TC length is seen. A series of results on the development of TC's obtained from both _Boergesenia_ and _Valonia_ is schematically illustrated in Fig.19. The assembly of microfibrils from a random to an ordered pattern changes within 4 to 5 hr in _Boergesenia_ and 10 to 12 hr in _Valonia_. It is suggested that the cessation of the increase in TC length may be closely related to the alteration of microfibril arrangement from a random to a parallel orientation; however, the present data show that there is some time lag between the change of microfibrillar pattern and the cessation of the increase in TC length. It has been proposed that each subunit of the terminal complex has a capacity to produce either a single, or multiple glucan chains (BROWN and MONTEZINOS 1976). If this proposition is correct, the number of glucan chains per single microfibril which contribute to its dimension (both width and depth) should increase according to the increase in TC length. Thus, short TCs will produce smaller microfibrils, and long TCs will produce larger microfibrils. At present, it is not concluded from this study how the TC length may actually control the dimensions of microfibrils.

As described in the results section, we have observed a variety of TC lengths. Such a variation has been reported in the case of _Valonia macrophysa_ (ITOH and BROWN 1984). We have seen many small TCs much shorter than those TCs with an average length even
during the synthesis of the secondary wall. Some of the short TCs have an approximate length of 200 nm. We often observed TCs lined up in rows with some interval between each row in the very early stage of cell wall regeneration in both *Boergesenia* and *Valonia*. We suspect that the subunits of nascent TCs are assembled loosely at the very beginning of nucleation. Once the subunits are assembled together in a tight configuration, nascent TCs are capable of synthesizing cellulose microfibrils, even with a length less than 200 nm. Consequently, both short and long TCs during the deposition of the secondary wall should be capable of synthesizing microfibrils. Once the TCs are established, they will get longer by the merger of two TCs and/or by the incorporation of small number of particle subunits which may be conveyed newly by the Golgi vesicles to the plasma membrane. We have observed fairly long TCs with an approximate length of 1 μm in *Boergesenia* cells at 20 hr post-wounding. All of the subunits in the TC might not be involved in the synthesis of glucan chains. This question remains unanswered until more structural data for the microfibrils are obtained (Kuga and Brown 1987, Sugiyama and Harada 1986).

It is possible that the TC increase in length could be closely correlated with the change in microfibril orientation. If the main factor involved in the orientation of microfibrils is “membrane flow” as proposed by Mueller and Brown (1982) and Mizuta (1985), long TCs could be more effectively controlled in their direction than short TCs so that they would become oriented in parallel during the synthesis of secondary walls. TCs may have a threshold length beyond which the direction of TCs will be fixed in one way by the membrane flow. It is possible that cytoskeletal structures such as microtubules, microfilaments, MAPs, etc., may actively control microfibril orientation, but this question remains for future investigation.

**References**


T. ITOH and R. M. BROWN JR: Development of Cellulose Synthesizing Complexes in *Boergesenia* and *Valonia*