GRAVITY EFFECTS ON CELLULOSE ASSEMBLY

R. MALCOLM BROWN, JR., 2 KRYSTYNA KUDLICKA, SUSAN K. COUSINS, AND ROBERT NAGY
Department of Botany, The University of Texas at Austin, Austin, Texas 78713-7640

The effect of microgravity on cellulose synthesis using the model system of Acetobacter xylinum was the subject of recent investigations using The National Aeronautic and Space Administration's Reduced Gravity Laboratory, a modified KC-135 aircraft designed to produce 20 sec of microgravity during the top of a parabolic dive. Approximately 40 parabolas were executed per mission, and a period of 2 × g was integral to the pullout phase of each parabola. Cellulose biosynthesis was initiated on agar surfaces, liquid growth medium, and buffered glucose during parabolic flight and terminated with 2.0% sodium azide or 50.0% ethanol. While careful ground and in-flight controls indicated normal, compact ribbons of microbial cellulose, data from five different flights consistently showed that during progression into the parabola regime, the cellulose ribbons became splayed. This observation suggests that some element of the parabola (the 20 sec microgravity phase, the 20 sec 2 × g phase, or a combination of both) was responsible for this effect. Presumably the cellulose I α crystalline polymorph normally is produced under strain, and the microgravity/hypergravity combination may relieve this stress to produce splayed ribbons. An in-flight video microscopy analysis of bacterial motions during a parabolic series demonstrated that the bacteria continue to synthesize cellulose during all phases of the parabolic series. Thus, the splaying may be a reflection of a more subtle alteration such as reduction of intermicrofibrillar hydrogen bonding. Long-term microgravity exposures during spaceflight will be necessary to fully understand the cellulose alterations from the short-term microgravity experiments.

Cellulose and lignin have played important roles in the structural support of plants as they moved from a water to a land environment throughout evolution. Moreover, the cell wall has been an important factor in the gravitropic response in plant growth patterns and habits. The β-D-glucan synthase activity has been shown to greatly increase in response to gravity stimulation in the leaf-sheath pulvinus of oat (Gibeaut et al., 1990). More specifically, significant patterns of change in cellulose assembly and in distribution of terminal cellulose synthesizing complexes have been observed in oat coleoptiles (Folsom and Brown, 1987). From these studies arises the question: how might cellulose biosynthesis be affected by gravity?

Cellulose synthesis in plants occurs in many stages over a prolonged period of time, with each stage changing the orientation of the microfibrils produced (Roelofsen and Houwink, 1953). Additionally, cellulose synthesis is closely linked to other carbohydrate production and lignification in higher plants (Eriksson, Blanchette, and Ander, 1990). Because of the complexity of diverse polymers in the cell walls of higher plants, it is difficult to analyze specifically how gravity affects cellulose biosynthesis. This study sought a simpler system, one that could be more effectively dissected with respect to changes in gravity as well as in the absence of gravity. The gram negative bacterium Acetobacter xylinum was used as a model organism for cellulose synthesis. This bacterium synthesizes pure cellulose, which can be altered during production by fluorescent dyes (Haigler, Brown, and Benzimad, 1980). In addition, the cellulose can be visualized directly by means of light microscopy (Brown, Willson, and Richardson, 1976). Furthermore, the polymerization and crystallization steps in cellulose synthesis mimic those found in other organisms, allowing comparisons to be made with cellulose synthesis in higher plants (Kuga and Brown, 1989).

Cellulose in Acetobacter xylinum is synthesized as an organized, twisting ribbon of microfibril bundles (Fig. 1). The cellulose biosynthesis process involves two successive steps: 1) the enzymatically controlled polymerization of β 1-4 linked glucan chains; and 2) the cell directed crystallization of these chains by means of intermolecular hydrogen bonding (Benzimad et al., 1980). The glucan chains form aggregates as they are extruded from terminal cellulose synthesizing complexes located in the pores in the bacterial outer membrane (Fig. 2). Outside these pores, the aggregates associate into microfibrils and ultimately into bundles that comprise the cellulose ribbon. Darkfield time-lapse video microscopy of in vivo ribbon synthesis has shown that the bacterium rotates along its long axis as it is propelled forward by ribbon elongation (Brown, Willson, and Richardson, 1976); thus, cell movement and cellulose synthesis are tightly linked.

Two Acetobacter xylinum strains were used in this study; strain NQ 5 (ATCC 53582) and strain AY 201 (ATCC 23769). Both strains were studied on agar surfaces as well as in liquid culture. Strain NQ 5 is noted for production of compact cellulose and is the one more commonly used for cellulose synthesis studies (Lin and Brown, 1989). Agar colonies of this strain are unique in that they contain cellulose synthesized in tunnels (Thompson et al., 1988: Fig. 3). Strain NQ 5 also is noted for periodic reversals in the direction of cellulose synthesis (Lin and Brown, in press). A. xylinum strain AY 201 is noted for its ability to form a variety of different colony morphologies (Roberts, Saxena, and Brown, 1989). This unique ability is believed to be a result of differences in the number of

1 Received for publication 29 February 1992; revision accepted 23 July 1992.

The authors thank the personnel at NASA for assistance in the planning of this study, especially Dr. Glenn Spaulding, Linda Billica, Dr. David Wolf, Lynette Bryan, Ray Schwartz, Bob Williams, Jesse Deming, David Tsao, and Tinh Trinh; and Richard Santos of the University of Texas for technical assistance. Research was supported by NASA grant NAG 9-397 to RMB.

2 Author for correspondence.
glucan chains synthesized per terminal complex, resulting in less crystalline cellulose (Kudlicka and Brown, 1991). The variety of strain AY 201 known to produce rough colonies on agar produces noncrystalline cellulose of tubular morphology in high concentrations of Tinopal LPW® (Haigler and Chanzy, 1988). Furthermore, this noncrystalline cellulose can be induced to crystallize by dye removal by photoisomerization by the addition of acid (Cousins and Brown, 1991) or with acidic washes (Haigler and Chanzy, 1988).

A recent set of experiments was designed to analyze whether the effect on crystallization due to microgravity would manifest itself in another form by preventing the crystallization step or by inducing the crystallization step abiotically in the microgravity environment. To explore the effect of gravity changes on cellulose assembly, bacterial cellulose synthesis was studied under microgravity conditions using The National Aeronautics and Space Administration's (NASA's) Reduced Gravity Laboratory, a modified KC-135 aircraft designed to produce 20 sec of microgravity during the top of a parabolic dive. Twenty sec of $2 \times g$ is integral to the pullout phase from each dive. Although abiotic protein crystallization experiments performed under microgravity conditions are now well defined (DeLucas et al., 1989), our experiments represent the first examination of a biologically controlled crystallization of a polymer.

MATERIALS AND METHODS

Experimental design—Two types of experiments were performed using NASA's modified KC-135 aircraft, each requiring different design specifications. The first type of experiment consisted of a group of studies aimed at analyzing the ultrastructure of the cellulose ribbons synthesized under microgravity conditions (Table 1). An aluminum pallet with recesses for 17 petri dishes was designed and custom built (Fig. 4). The basic syringe assembly for initiation and termination of cellulose synthesis (Fig. 5) consisted of a sterilizable dual nipple petri dish secured to the pallet with clamps. One-ml Tuberculin syringes were mounted on blocks and held in place with additional metal clamps, and pinch valves were used for positive

---

Figs. 1–3. 1. An electron micrograph of a negatively stained *Acetobacter xylinum* cell (strain AY 201) with a ribbon of cellulose parallel to its longitudinal axis. This ribbon twists with an average periodicity of 0.72 μm, × 8,250. Bar = 1 μm. 2. A schematic diagram of cellulose synthesis in *Acetobacter xylinum*. Glucan chains are polymerized at terminal cellulose synthesizing complexes located in the cytoplasmic membrane. These chains are extruded through pores in the bacterial envelope, each pore producing a subfibril. Approximately three subfibrils comprise a microfibril by hydrogen bonding. The microfibrils ultimately form the cellulose ribbon. Encircled is a diagram of a proposed cellulose synthase complex with the subcomponents as follows: A. UDP-glucose binding subunit. B. Cyclic di-GMP binding subunit. C. Glucan chains. D. Product holding subunit, possibly involved in the crystallization step. E. Cellulose subfibril. 3. An agar colony of *Acetobacter xylinum* strain NQ 5 viewed using Nomarski optics. The entire colony was transferred to the EM grid for the electron microscopy studies, but only the outer edge of the colony was viewed because it was less compact than the center of the colony. Note the extensive circular "tunnels" of cellulose characteristic of this strain (Thompson et al., 1988), × 500. Bar = 1 μm.
control of injectable liquids. In operation, the top of the petri dish was secured to the bottom with parafilm. An electronic control package consisting of temperature regulation, gravity level monitors, and parabolic counting circuitry was also installed.

The second type of experiment performed using the modified KC-135 aircraft was the direct microscopic visualization of cellulose synthesis during parabolic flight. A second pallet (Fig. 6) was developed to sample real-time cell movement as a result of cellulose ribbon synthesis with the hypothesis that a change in the cellulose assembly process in parabolic flight may be accompanied by a change in cellular movement. Video images were obtained using a Philips 540 × 750 element CCD chip camera coupled to a Zeiss Universal Light Microscope. A 32× Neofluor objective lens, a 1.6× optivar lens, and a 10× ocular lens were used to magnify the brightfield image of the cells as it entered the camera. The video image was enhanced with a Tandy Pro-camp Unit and then recorded onto HI-8 videotape using a Canon A-1 HI-8 Camcorder. The videosignal was split from the image enhancer to a local 7 inch monitor for focusing. A Peltier solid stage device that was “temperature sinked” to the main pallet using 1/8 inch copper tubing and silicone conductive gel was used to maintain the petri dish stage temperature at 30 C. Water was pumped through the system with a small peristaltic pump.

The KC-135—The first pallet was used on five different missions aboard the KC-135 aircraft; the second pallet was used only once. In both cases, the KC-135 performed 40-50 parabolic dives, with every tenth parabola interrupted by 5 min of level flight as a break period for the crew.

Typically, the modified KC-135 aircraft began parabolic flight (Fig. 7) after 15-20 min of level flight to the designated parabolic flight region. At that point, the plane was at 26,000 feet, 525 knots, and thrust was applied to attain a 45° pullup. Thrust was then reduced until it equaled drag, and a 0 x g pushover was performed at 325 knots. This maneuver put the plane into a 45° 0 x g dive for approximately 24-30 sec. Then a 2 x g pullout phase was performed, placing the plane back at 26,000 feet and 525 knots, ready for the next parabola. At the end of the parabolic flight regime, the plane flew level for another 15-20 min before landing.

**Progressive parabola study on agar with strain NQ 5**—This study compared cellulose synthesis using dense cell suspensions incubated either in liquid SH medium or in 40 mm glucose buffered with 0.25 mm sodium phosphate, pH 7.0. Centrifuge controls were repeated using 1:1 dilutions of cell suspension to SH medium by placing parafilm on top of the Eppendorf tube’s agar, laying a formvar coated grid on top of the parafilm, and allowing the cells to collect on the grid by centrifugal force. Cellulose synthesis controls were also repeated during the plane’s level flight before and after the parabolic regime. A final control of cellulose synthesis initiated and terminated in the lab and then flown on the plane through each phase of the flight (level flight before parabolas, during the parabolic regime, and level flight after parabolas) was also performed. We refer to this control as the pre-
The progressive parabola and altered crystallization studies used this particular pallet design, seen here being loaded onto the KC-135 aircraft for parabolic flight. Note the video camera, G-meter box (right arrowhead), and petri dishes with wrapped syringes (left arrowhead). A single 5.5-cm petri dish syringe assembly, illustrated in detail, was used for controlling initiation and termination of cellulose synthesis during flight for ultrastructural analysis. Typically, growth medium in the left syringe would flood the surface of agar colonies to initiate cellulose synthesis, or alternatively, a dense cell suspension could be used to flood liquid growth medium. The experiment was terminated by injecting the fixative (EtOH or sodium azide) from the right syringe. The video microscopy pallet for direct analysis of cellular movement viewed here has just passed the final inspection and is ready for parabolic flight. Note the agar petri dish (lower arrowhead) on the temperature-controlled stage that was used to support the cells on agar visible on the video monitor (right arrowhead). Note also the small CCD camera (upper arrowhead) used to acquire those images. This diagram of the KC-135 parabolic cycle was photographed off the side of the actual KC-135 aircraft used in this experiment. During each cycle the plane undergoes 20 sec of $0 \times g$ and 20 sec of $2 \times g$ (for details of the parabolic configuration, see Materials and Methods).

synthesized cellulose control because it was used to indicate whether the observed effects were due to the process of cellulose synthesis during parabolic flight, or to a physical phenomenon affecting the cellulose structure itself.

Experiments included cellulose synthesis initiated at the beginning of the $2 \times g$ phase of the parabola and allowed to continue for 1, 4, 20, and 40 parabolas before termination by the addition of 2.0% sodium azide. All controls and experimental cells were negatively stained and observed with TEM as stated previously.

**Progressive parabola study with strain AY 201**—This study was performed using 2-d-old agar "rough" colonies of strain AY 201 and repeated using AY 201 dense cell suspension of the same substrain inoculated into 40 mm glucose buffered with 0.25 mM sodium phosphate. Level flight controls before and after parabolic flight were performed as well as experiments that underwent cellulose synthesis for 10 and for 40 parabolas.

**Crystallization studies with strain AY 201**—Strain AY 201 variant "rough colony" cell suspension was incubated in 125 μM Tinosol LPW® for 10 and for 20 parabolas and compared with controls of tubular cellulose synthesis.
during level flight before and after the parabolic regime. Tinopal-altered cellulose synthesized in the lab was also induced to crystallize during parabolic flight over a period of 10 and 40 parabolas. A final control of tubular cellulose synthesized on the ground and terminated before being flown along with the other experiments was also performed.

**Direct visualization of cellulose synthesis** — This study, which used the second pallet, was to observe cellular movement during parabolic flight. The edge of a 3-d-old NQ 5 agar colony was continually monitored by bright-field light microscopy and recorded on high resolution videotape. This study was compared with previous ground studies on cellulose biosynthesis on agar surfaces using this strain of *A. xylinum* (Eisner and Brown, personal communication). Other controls included three petri dishes of NQ 5 agar colonies initiated for cellulose synthesis during flight. One of the plates was initiated during level flight and terminated before the parabolic regime, another was initiated during parabolic flight to verify that splayed microfibrils were observed, and the final plate was flown throughout the flight without the addition of SH medium to verify that splaying occurs during parabolic flight even on an unflooded agar surface, like the plate observed under the microscope. Another presynthesized cellulose control was also performed in buffered glucose. After the flight, the colonies of the three control plates, the colonies observed in the microscope, and the cellulose in the vial were negatively stained and observed in the electron microscope, as noted previously. Postflight processing also included time compression and single frame analysis of the in-flight recorded video.

**RESULTS**

**Progressive parabola studies** — The ground controls showed typical cellulose ribbons composed of compact microfibril bundles (Fig. 8), as did the level flight control before parabolic flight (Fig. 9). Whether incubated on agar, in liquid SH medium, or in buffered glucose suspension, cellulose synthesis of both strains of *A. xylinum* became increasingly disorganized in all the experiments through progressive parabolic cycles. This disorganization was visualized in the form of a separation of microfibril bundles from the compact cellulose ribbons (Figs. 10–12). This splaying phenomenon increased percentage-wise the longer the exposure to parabolic flight (Fig. 16). Furthermore, level flight after parabolic flight produced different results depending on the composition of the support medium for cellulose synthesis. Splaying was observed in the cellulose synthesized in the buffered glucose suspension (Fig. 13), but normal compact microfibril bundles were observed in the cellulose synthesized in the liquid growth medium and on the agar (Fig. 14). The presynthesized cellulose control also showed typical compact microfibril bundles (Fig. 15). A summary of controls and experiments that produced splayed vs. normal cellulose ribbons is provided in Table 2.

**Crystallization studies with strain AY 201** — When incubated in Tinopal LPW®, typical tubular cellulose was synthesized (Fig. 17). The Tinopal-altered cellulose synthesized during parabolic flight had the same tubular morphology as did the ground control (Fig. 18). The helical orientation of the microfibrils, typical of ground controls (Fig. 19), were also observed in the tubular cellulose synthesized on the ground and induced to crystallize by acid washing during parabolic flight (Fig. 20).

**Direct visualization of cellular movement during parabolic flight** — The 2 × g pullout phase of the parabolic flight placed enough stress on the optical system that focusing on cells became very difficult. Level flight was less shaky, but clear images of cells were still difficult to obtain. Only during the 20 sec of the 0 × g period was a clear image of cells available. Still video frames were used to deduce and analyze cellular movements during the 0 × g phase as well as just before and immediately after the 2 × g phase. Level flight was also observed for movement and photographed using still video frames.

Unidirectional movement of *Acetobacter* cells was observed/deduced in all three stages—2 × g pullout, 0 × g, and 1 × g level flight. Cellulose seemed to be synthesized in conjunction with typical cell movement. One reversal in the direction of cellulose synthesis was recorded during the 2 × g pullout between the 0 × g phases of parabolas 29 and 30. Although the exact moment of the reversal cannot be shown due to the focusing problem at 2 × g flight, the change in directional movement may be deduced from the differences in the cell position in the 0 × g phases before and after the reversal (Figs. 21–26). The capacity of the cells to move during parabolic flight is thus verified as a qualitative observation; however, the percentage of cells moving during parabolic flight cannot

---

Figs. 8–11. A typical progressive parabola study using agar colonies of strain NQ 5 (other progressive parabola studies show the same results). Bars = 100 nm. 8. The ground control showed normal cellulose ribbons with tight associations of bundles of microfibrils, ×164,000. 9. Compact and twisting cellulose ribbons were observed in the level flight control before parabolic flight, ×98,000. 10. After exposure to two parabolas, approximately 20% of ribbons showed slight changes where single microfibril bundles were loosely separated or split from the ribbon (arrowheads), a phenomenon termed splaying, ×90,000. 11. After exposure to 20 parabolas, approximately 95% of the cells have synthesized splayed cellulose ribbons with less compact microfibril bundles, ×90,000.

Figs. 12–15. Illustrations of the progressive parabola study (continued). Bars = 100 nm. 12. After 39 parabolas, the percentage of ribbon splaying has leveled off and continues to be high, ×98,000. 13. Splaying was also observed in the sample from a level flight control after parabolic flight of cells in buffered glucose solution, thus suggesting a requirement of protein synthesis to maintain normal compact ribbon synthesis (for details, see text), ×85,750. 14. The level flight control after 40 parabolas in growth medium showed normal compact ribbons, ×63,000. This supports the argument of a protein synthesis requirement (for details, see text). 15. Bacterial cellulose synthesis was initiated and terminated on the ground. Then this cellulose was flown aboard the KC-135 during parabolic flight and was referred to as the “splay-synthesized cellulose control.” This material demonstrated no observable changes as a result of flight (for details, see text), ×183,750.
Fig. 16. This graph illustrates the relationship between the estimated percentage of splayed ribbons formed as a result of increased exposure to parabolic flight in the progressive parabola study on agar, using strain NQ 5.

be accurately assessed because such a small sample size prohibits use of statistical analysis.

As for correlation with the electron microscope control studies, the in-flight control colonies and the presynthesized cellulose control showed normal cellulose production. On the other hand, the colonies initiated during parabolic flight synthesized splayed microfibril bundles within the cellulose ribbons.

**DISCUSSION**

Based on the various controls performed, both on the ground and during flight, the splaying effect appears to be a result of parabolic flight. Moreover, this effect is probably due to either the $0 \times g$ phase of the parabola alone or to a combination of the frequent changes between the $0 \times g$ phase and the $2 \times g$ phase. The splaying is not due to the $2 \times g$ phase alone or to alterations between $1 \times g > 2 \times g > 1 \times g$ because ground-based centrifuge tests at these simulated gravity forces have shown normal compact microfibril bundles within the cellulose ribbons. Only a prolonged period of microgravity will elucidate whether splaying of microfibril bundles occurs during the $0 \times g$ phase alone.

The splaying phenomenon seems to affect the cellulose biosynthesis process, rather than the structure of cellulose itself. This conclusion is evidenced by the observation that cellulose synthesized on the ground and then flown aboard the KC-135 remained unaltered by parabolic flight. Furthermore, the splaying of the microfibril bundles was due to some effect on the crystallization of the glucan chains by hydrogen bonding, not on the polymerization of the glucan chains themselves, as evidenced by the production of Tinopal-altered cellulose showing the same tubular morphology as shown by the ground controls. The tubular morphology indicates that the glucan chains were still polymerized, but the complete inhibition of the crystallization step by excessive amounts of Tinopal LPW® prevented any observable effect of parabolic flight on cellulose synthesis. Therefore, the effect of parabolic flight on crystallization must be a subtle alteration.

We hypothesize that the splaying phenomenon is a result of weakened ribbon structure, an artifact of fewer hydrogen bonds between microfibril bundles than in normal compact cellulose (Figs. 27–29). We speculate further that this decrease in hydrogen bonding is caused by damage to the cell’s crystallization machinery, part of which probably requires protein synthesis. We base these hypotheses on several lines of evidence. First, the splaying phenomenon appears to represent a dosage effect (the greater the number of parabolas, the greater the percentage of splaying) until a saturation point is reached at approximately 20 parabolas, at which point splaying occurs at a very high percentage (Fig. 16). This observation suggests that splaying is an accumulative phenomenon, which correlates well with the hypothesis that splaying is a result of either damage to or rearrangement of the cell machinery directing cellulose crystallization during parabolic flight. Furthermore, this change in the cell’s machinery seems to be reversible. After parabolic flight has ended and the plane has returned to level flight, cells initiating cellulose synthesis in SH medium resume normal crystallization of microfibril bundles, but cells in glucose buffer exhibit altered crystallization. Thus, at least some of the necessary components for repair of cellulose crystallization machinery require active and continuous protein synthesis. Precisely which proteins are altered and repaired is unknown. Finally, the change to the crystallization machinery could be manifested as decreased hydrogen bonding between the microfibril bundles. A decrease in hydrogen bonding might thus allow both sufficient minimum numbers of interbundle hydrogen bonds to maintain ribbon cohesiveness and cell movement in the wet state, but...
produce the splaying effect when the cellulose is dried onto the grid in preparation for electron microscopy. During the drying process, microfibrils might separate as a result of weakened ribbon structure. As seen in Fig. 11, the uranyl acetate is observed between microfibrils in the splayed area. Such a phenomenon is not possible if uniform hydrogen bonding occurs between the microfibrils. If cellulose assembly in *Acetobacter xylinum* is similar
in its steps to cellulose assembly in plants, how can microgravity conditions affect cellulose assembly in higher plants? The gravitropic response results in differential growth by an asymmetric change in the proximity of cellulose synthesis terminal complexes (TC’s) to one another on the upper and lower hemicylinders of a gravistimulated plant (Folsom and Brown, 1987). This change in TC orientation was theorized to affect hydrogen bonding between microfibrils and thus leads to the lower hemicylinder being less ordered and therefore less constrictive. This change in hydrogen bonding from gravistimulation in higher plants is a response that appears similar to changes in crystallinity by microgravity perturbations in Acetobacter xylinum cellulose synthesis. Furthermore, both gravistimulated plants and bacteria exposed to microgravity failed to show a response when incubated in a fluorescent dye known to interrupt hydrogen bonding at the time of cellulose synthesis. These arguments suggest that, in plants exposed to a microgravity environment, the asymmetry of TC reorientation would be lost and that both hemicylinders would undergo equal elongation due to a symmetrical decrease in ordering. In both cases, active protein synthesis is postulated to maintain the response.

Another interesting point is the possible relationship of this study to the thigmotropic or “touch” genes recently discovered in Arabidopsis (Braam and Davis, 1990). These genes are induced by mechanical stimulations that lead to thigmomorphogenesis. They show a strong similarity to calmodulin genes and have been suggested to play a role in thigmomorphogenic responses by way of inducing Ca++ messengers that would act by the dissociation of microtubules for reorientation of the cytoskeleton. We suggest that thigmomorphogenesis may have a similar stimulation-response pathway to gravitropism (Table 3). Both pathways are postulated to start with the induction of gene expression leading to protein synthesis. These proteins are responsible for a change in deposition of cellulose microfibrils, either directly or indirectly, which leads to alterations in hydrogen bonding. This change in hydrogen bonding would change the rigidity of the cell wall and ultimately a change in growth. Whereas wall rigidity in the gravitropism pathway may be asymmetrically decreased via cellulose-cellulose interactions, this reduction is suggested to occur in conjunction with the older layers of the cell wall being reduced in rigidity by some other method, possibly by cellulases (Fry, 1989). This hypothesis is proposed to stimulate interest in the processes of varied plant responses and the role of cellulose biosynthesis. By no means complete, these comparisons make it clear that some parts of the pathway have many elements in common while other elements are very specific. The role of cellulose biosynthesis in differential growth and the gravitropic response awaits further investigation of enzyme purification and characterization, for it is through these techniques that we will discover the precise genetic regulatory mechanism for this process.

Table 3. A comparison of the effects on cellulose synthesis from the thigmotropic response and from the gravitropic response

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Thigmotropism</th>
<th>Gravitropism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation</td>
<td>Wind, touch, rain, etc.</td>
<td>Change in gravity</td>
</tr>
<tr>
<td>Gene expression</td>
<td>Touch genes?</td>
<td>Genes?</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>Calmodulin and related proteins</td>
<td>Cellulose synthase and related proteins?</td>
</tr>
<tr>
<td>Change in association</td>
<td>Microtubules reoriented</td>
<td>Asymmetric TC aggregation</td>
</tr>
<tr>
<td>Hydrogen bonding</td>
<td>Increased between microfibrils</td>
<td>Asymmetric change between microfibrils</td>
</tr>
<tr>
<td>Change in cell wall</td>
<td>Wall rigidity increased</td>
<td>Wall rigidity asymmetrically decreased</td>
</tr>
<tr>
<td>Change in growth</td>
<td>Reduced elongation</td>
<td>Differential elongation</td>
</tr>
<tr>
<td>Observed response</td>
<td>Stunted growth</td>
<td>Gravitropic bending</td>
</tr>
</tbody>
</table>
LITERATURE CITED


