oidal nature have been shown to be secreted by the human corpus luteum.

A specific radioimmunoassay (RIA) for porcine relaxin has been developed with ^{125}I-labeled polytyrosylrelaxin (*6*). Its antiserum was found to cross-react with relaxin-like substances in serums of many species including those of humans in late pregnancy (*7*). Using this relaxin RIA (*7*), we attempted to determine the source of human relaxin in late pregnancy.

The subjects of the study were seven pregnant women at term being delivered by cesarean section; plasma samples from both ovarian veins were obtained from each of these women 20 to 30 minutes after the placenta was delivered. The right ovarian vein was always sampled first and a peripheral blood sample was simultaneously obtained. The corpus luteum was on the right side in four women and on the left side in the other three women. All ovarian vein samples were taken with syringe and needle after the utero-ovarian blood flow was excluded by digital compression to prevent cross circulation from the other ovary. The samples were assayed for relaxin. In addition, for comparison, progesterone was measured by RIA (*8*).

Plasma progesterone concentrations (Fig. 1) were higher in the ovarian vein of the corpus luteum–bearing ovary than in the peripheral plasma ($P < .025$) or the noncorpus luteum side ($P < .05$). Relaxin concentrations were likewise significantly higher in the ovarian vein of the corpus luteum–bearing ovary than in either the peripheral plasma or the plasma of the contralateral ovary ($P < .005$). When peripheral levels are subtracted from luteal side ovarian vein concentrations and relaxin was plotted again progesterone, it can be seen that increased luteal progesterone secretion was accompanied by increased relaxin secretion (Fig. 2). Roughly, an increase of relaxin (1 ng/ml) was associated with an increase of progesterone (30 to 50 ng/ml). Such concentrations of relaxin are too low to be detected by current bioassay methods.

Our studies demonstrate that a peptide is present in the serums of pregnant women at term, which competes with porcine relaxin in a specific RIA. This substance is secreted by the pregnancy corpus luteum, and its secretion correlates with luteal progesterone secretion.

GERSON WEISS
Department of Obstetrics and Gynecology, New York University School of Medicine, New York 10016
E. M. O'BYRNE, B. G. STEINETZ
Research Department, Pharmaceutical Division, Ciba-Geigy Corporation, Ardsley, New York 10502

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Cellulosic Microfibrils: Nascent Stages of Synthesis in a Higher Plant Cell

Abstract. *Freeze-fracturing of untreated plasma membrane and inner wall surfaces of stelar tissue in corn roots demonstrated the association of globular complexes with the ends of nascent microfibrils. It is proposed that the granule complexes associated with the outer leaflet of the plasma membrane coordinate the assembly of the cellulosic microfibrils.*

Elucidation of the mechanisms of cellulosic microfibril formation is important in understanding the dynamic aspects of plant cell wall functions. Cellulosic microfibrils constitute the structural framework of the cell wall. The integrity and form of the cell are determined, in part, by the site and pattern of microfibrillar deposition. Likewise, microfibrillar deposition and orientation must be understood in terms of the biosynthetic pathway, the role of membranes in the assembly process, and the nature of the synthetic complex itself (*1*).

In higher plants, it has been hypothesized that microfibril synthesis and assembly occur at the cell surface (*1*), but definitive proof for this is lacking. In certain algae, however, formation of microfibrils has been found in association with the plasma membrane (*2, 3*) as well as membranes of the Golgi apparatus (*4*).

The advent of the freeze-etching technique has enabled large areas of internal and external surfaces of membranes to be examined. Several studies of plant cell membranes by this technique have indicated the involvement of the plasma membrane in cellulosic microfibril synthesis (*5, 6*). Recent investigations have demonstrated that fixatives and cryoprotectants destroy plasma membrane–wall interfaces thereby not preserving the labile structures associated with cellulose synthesis. Willison (*7*) demonstrated the deleterious effects of fixatives and cryoprotectants on cell wall–plasma membrane interfaces in *Phaseolus* root tips. Recently, by means of electron microscopy, Brown and Montezinos (*3*) demonstrated linear complexes associated with growing cellulosic microfibrils in the unicellular green alga, *Oocystis*. These complexes were preserved only in cells which had been directly frozen in Freon. Because of the advantages recognized in the direct freezing of *Oocystis* cells, it became apparent that a similar approach with a higher plant cell might yield useful data on cellulose formation.

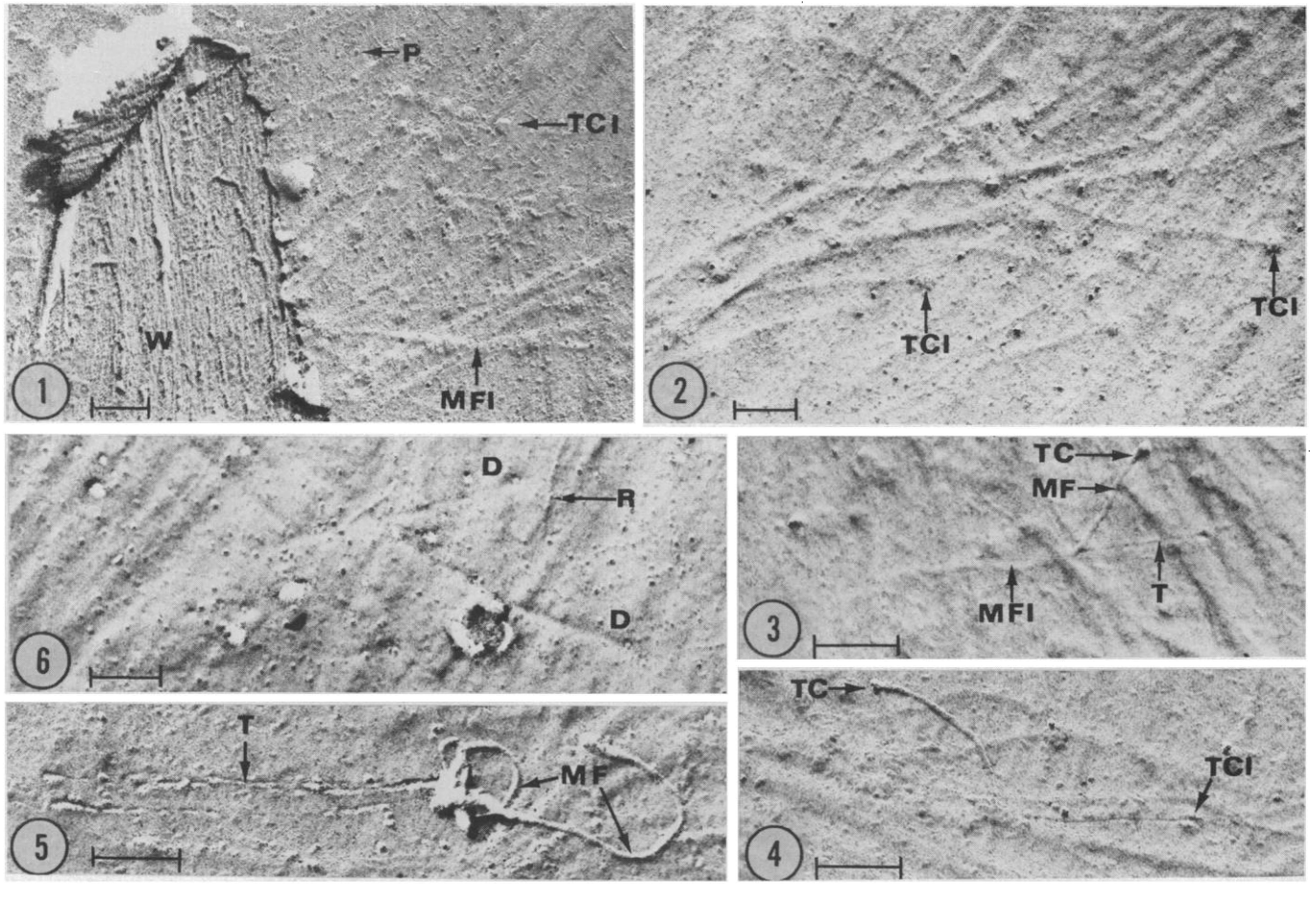
The freeze-etch study described here was conducted on stelar tissue of 3-day-old *Zea mays* cv. Burpee snowcross roots. Corn seeds were surface sterilized in 2 percent Clorox, soaked overnight, and then germinated in the dark at 26°C ± 1°C on moistened filter paper in petri plates. Just prior to use, 1.0-mm portions of the stele were removed 12 mm ± 1 mm back from the tip, placed immediately on gold specimen holders and, without any prior treatment with fixative or cryoprotectant, quickly frozen in Freon 22, maintained at liquid nitrogen temperature, then transferred to liquid nitrogen for storage. A Balzers BA 360M freeze-etch apparatus was used, and specimens were etched at −106°C for 2 minutes prior to shadowing with platinum-carbon and coating with carbon. The replicas were cleaned in Clorox and then in 75 percent H_2SO_4. They were examined with a Hitachi HU 11E-1 electron microscope.

During the freeze-etch process, biological membranes fracture in the plane of the median hydrophobic interface (*8*). At the fractured surfaces of thin, elongate cells within excised corn steles, microfibril impressions bearing pronounced terminal globules were frequently observed within discrete regions. When the inner leaflet of the plasma membrane of this tissue is torn away, the fractured face of the outer leaflet is revealed (Fig. 1). This is termed the EF face (*9*). Typical randomly scattered membrane particles are present. Microfibrillar impressions are visible as well as associated granules measuring about 160 to 200 Å.

In some cases during the fracturing process, the plasma membrane and adjacent microfibrils are displaced to reveal part of an inner wall layer (Fig. 1). The wall microfibrils measure about 80 Å in width. There are differences in orientation between the earlier-formed microfibrils and the innermost microfibrillar impressions visible through the outer leaflet of the plasma membrane. Microfibrillar impressions with their associated terminal globular complexes are illustrated at higher magnification in Fig. 2, a view of the EF fracture face. The terminal complex appears roughly spherical and seems to be composed of subunits. Clusters of microfibrils with terminal complexes show a predominant unidirectional synthesis within a given bundle. Individual microfibrils appear to associate rapidly to form a bundle soon after synthesis. The marked relief of the microfibrillar impressions and their associated terminal complexes clearly differentiates the layer of active synthesis from previously deposited layers of microfibrils.

Figure 3 demonstrates that the wall microfibrils are responsible for the impressions on the EF fracture face since the tear mark through the outer plasma membrane leaflet is continuous with the impression. In Figs. 3 to 5, the complex remains attached to the end of the microfibril as it is torn through the membrane. The terminal complex is better resolved in the torn microfibril and appears to have a globular terminus associated with a thicker, subtending cylinder. The complex might be envisioned as a tapering club. In Fig. 6, a PF fracture face, the complementary depressions of the microfibril and its terminal complex are revealed. Several rows of particles are visible, similar to those previously reported and briefly described in corn (*10*) and in other higher plants (*6*, *7*). Since these particles frequently are arranged in rows parallel to the microfibril orientation it is possible that they are involved with the orientation of microfibrils (*7*).

Our evidence further supports the model of cellulose synthesis by which a granular enzyme complex adds glucose units to a developing microfibril end, originally hypothesized by Roelofsen (*11*) and later by Preston (*12*), for which clear structural evidence has been presented by Brown and Montezinos (*3*). In corn roots the globular complexes appear to be intimately associated with the outer leaflet of the plasma membrane. Furthermore, it seems likely that the terminal globular complex moves during microfibril formation [see (*13*)]. The polymerization of glucose residues and the forces of crystallization (*3*) at the microfibrillar terminus could result in the lateral movement of the synthesizing complex if the recently made portion of the microfibril remained embedded in the wall.



Figs. 1 to 6. The direction of shadow is from the bottom of the page to the top (scale bars, 0.1 μm). Fig. 1. An EF fracture face with microfibrillar (*MFI*) and terminal complex impressions (*TCI*). Typical randomly scattered particles are also present (*P*). Note the "window" of adjacent wall microfibrils (*W*). Fig. 2. An EF fracture face with impressions of a cluster of microfibrils. Each microfibril bears a terminal complex (*TCI*). Fig. 3. An EF fracture face of a microfibrillar tear through the outer leaflet of the plasma membrane demonstrating continuity of the tear (*T*), the microfibril (*MF*) and its terminal complex (*TC*), and the microfibrillar impression (*MFI*). Fig. 4. An EF fracture face with terminal complex impression remaining in association with the tear. Note the clublike morphology of the complex. Fig. 5. An EF fracture face with two microfibrillar tears. Note the prominent clublike terminal complex with globular terminus. Fig. 6. A PF fracture face showing complementary depressions (*D*) of microfibril and associated complex. Note the rows of particles (*R*) commonly associated with this fracture face.

The mechanism of microfibrillar orientation is not completely understood. Microtubules have been proposed as an orienting force for microfibrils (*14*), but in this study, microtubules have not been observed. Perhaps the partial orientation of microfibrils could occur as a result of intermittent binding between the surfaces of microfibrils. This is consistent with the evidence presented in Fig. 2.

Although alkali insoluble β-1,4 glucans can be synthesized in vitro, the apparent necessity for the presence of the intact plasma membrane has made it thus far impossible to demonstrate in vitro synthesis of microfibrillar cellulose (*15*). It is interesting that a chitin-synthesizing system has been shown to make microfibrils in vitro (*16*). A globular enzyme complex is associated with the tip of a growing chitin microfibril, similar to what has been observed in this study.

The significance of this report is that for the first time in higher plants, the cellulosic microfibril and its presumptive synthesizing complex have been demonstrated at the ultrastructural level. Furthermore, we believe that the morphological evidence represents a true picture of the assembly process free from artifactual distortion.

SUSETTE C. MUELLER
R. MALCOLM BROWN, JR.
TOM K. SCOTT

Department of Botany,
University of North Carolina,
Chapel Hill 27514

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Revertants of Human Cells Transformed by Murine Sarcoma Virus

Abstract. *Revertants of nonproducer human osteosarcoma (NP/KHOS) cells induced by Kirsten murine sarcoma virus were isolated after incubating at high temperature (40.5°C) overnight and subcloning at 36°C. The morphologic variants, from which murine sarcoma virus could no longer be rescued, had growth properties similar to those of the nontransformed, parent human osteosarcoma cells and did not release RNA-dependent DNA polymerase activity. These revertants were nontumorigenic in nude mice. The revertants supported leukemia virus growth and showed an enhanced sensitivity to murine sarcoma virus superinfection. Thus, the revertants were from human cells transformed by an oncogenic RNA virus.*

Cells transformed by murine sarcoma virus (MSV) can revert to variant forms in which their morphology and function resemble that of normal cells (*1, 2*). It has been reported that MSV transformed mouse cells termed S^+L^- (sarcoma-positive, leukemia-negative) (*3*) spontaneously gave rise to morphologic variants from which MSV could no longer be rescued (*1*). However, no such spontaneous reversion was found in human S^+L^- cells (*4*). Another type of morphological revertant (*2*), which contained rescuable MSV genome, has been observed at a low frequency from the MSV-induced rat nonproducer (NP) cells by single cloning procedures. Frequency of reversion has sometimes been increased by first treating the cells with sublethal doses of halogenated pyrimidines (*5*) or by adapting them to high temperature (*6*). Nonproducer, human osteosarcoma (KHOS) cells isolated from transformed foci induced by Kirsten murine sarcoma virus (Ki-MSV) (*7*) produce neither infectious virus nor murine leukemia virus (MuLV) antigens, but they contain the MSV genome which can be rescued by superinfection with MuLV. The NP cells produced tumors when transplanted subcutaneously into athymic (nude) mice (*8*). We now report the isolation and characterization of revertants from Ki-MSV transformed human NP cells. The revertants were isolated after incubation at high temperature (40.5°C) overnight and subcloning at 36°C. The morphologic variants, from which MSV could no longer be rescued, had growth properties similar to those of the nontransformed, parent human osteosarcoma (HOS) cells and did not release RNA-dependent DNA polymerase activity. These revertants were nontumorigenic in nude mice. To our knowledge, this is the first observation of revertants from human cells transformed by an oncogenic RNA virus.

The human NP (KHOS) cells cloned from a transformed focus (*7*) were maintained under liquid medium (*8*) for 30 passages to "fix" the premanently transformed state before use in our study. One-day-old cultures of KHOS were incubated overnight at 40.5°C and maintained at 36°C for two passages. During this period, transformed cells were detached and decanted from the cultures.

Table 1. Properties of revertants from human nonproducer cells (KHOS).

Properties	HOS cells	KHOS cells	Revertants from KHOS	
			240S	312H
Morphology	Flat	Transformed	Flat	Flat
Saturation density* ($\times 10^5$/cm^2)	1.7	4.8	0.85	0.75
Cell aggregates†				
Size	Small	Large	Small	Small
Viability of cells ($\times 10^5$)	1.3	6.2	0.5	0.4
Plating efficiency (%) in soft agar	1.5	9.4	1.1	1.1
Type C virus particles	Negative	Negative	Negative	Negative
CF titers of MuLV gs antigen	< 2	< 2	< 2	< 2
Reverse transcriptase‡	Negative	Negative	Negative	Negative
Tumorigenicity in nude mice§	Negative	Positive	Negative	Negative
MSV rescued by type C viruses‖	None	Present	None	None

*Maximum number of cells obtained after initial planting with 5×10^3 cell/cm^2 and then incubating at 36°C under conditions where growth medium was changed every 3 days. †Cell aggregates formed after 3 days with an agar static system. Viability of cell aggregates determined by 4 days after planting 2×10^5 cells per plate initially. ‡RNA dependent DNA polymerase activity was measured by incorporation of [^{3}H]thymidine triphosphate into acid-precipitable materials in 100× concentrated supernatant of cultures (*8*). §Five million cells inoculated into each nude mouse. ‖Supernatants from type C virus infected cultures were taken at 14 days, were passed through an 0.4-μm HA filter, inoculated into horse skin cells and human embryonic skin and muscle cells and examined for foci.