Nascent Stage of Cellulose Biosynthesis

Abstract. *Freeze-etching of never-dried pellicles or of incubated suspensions of both* Acetobacter xylinum *and* Acetobacter acetigenum *show a nascent form of the cellulose microfibril which has a core surrounded by an amorphous sheath. Drying of the pellicle or suspension reduces the diameter of the sheath and changes the form of the microfibril to the one usually seen. This nascent form of the cellulose microfibril is consistent with previous postulations of an intermediate polymer or polymers in the biosynthesis of cellulose.*

Cellulose is the most abundant biological polymer (*1*) but its molecular structure has only been deduced recently (*2*), the chemical pathway of biosynthesis is just beginning to be understood (*3*), and almost nothing is known about the physics of formation of the basic morphological unit, the microfibril (*4*). Apropos of the last problem, earlier workers proposed the formation of an intermediate polymer or polymers, followed by association and crystallization of these chains to form the microfibril (*5*). There were serious conceptual and observational objections to this scheme (*4*) and not enough early experimental evidence to support it conclusively. However, additional evidence for transient, intermediate polymers has come recently from density-gradient centrifugation of cell-free particulate-enzyme preparations which synthesize cellulose (*6*). We report here new evidence for a hitherto unrecognized stage in cellulose biosynthesis, which is consistent with intermediate polymers, from freeze-etch electron microscopy of never-dried cell suspensions and pellicles.

Cellulose-free cell suspensions of *Acetobacter xylinum* and *Acetobacter acetigenum* were prepared as described by Hestrin and Schramm (*7*) to yield a final concentration of about 5×10^9 viable cells per milliliter. One volume of suspension was added to an equal volume of 0.01 percent glucose in 0.01*M* phosphate, 0.003*M* citrate buffer, *p*H 6, and the mixture was incubated at 35°C for 0, 10, or 20 minutes. This incubation produced cellulose microfibrils rapidly (*7*). At the appropriate time, droplets of the mixture were frozen by plunging them into liquid Freon 12 cooled by liquid nitrogen. For examination of never-dried pellicles from both species, a bacterial culture was incubated at 28°C for 48 hours, the filmy pellicle cut with scissors into approximately 1-mm cubes, and the cubes frozen immediately as above. For dried pellicles, the film was washed free of medium with distilled water, stretched gently over the concave side of a watch glass, and dried overnight above anhydrous $CaCl_2$ under vacuum. After drying, the film was rewetted, cut into 1-mm squares, and frozen as above. All freeze-etching for both films and cell sus-

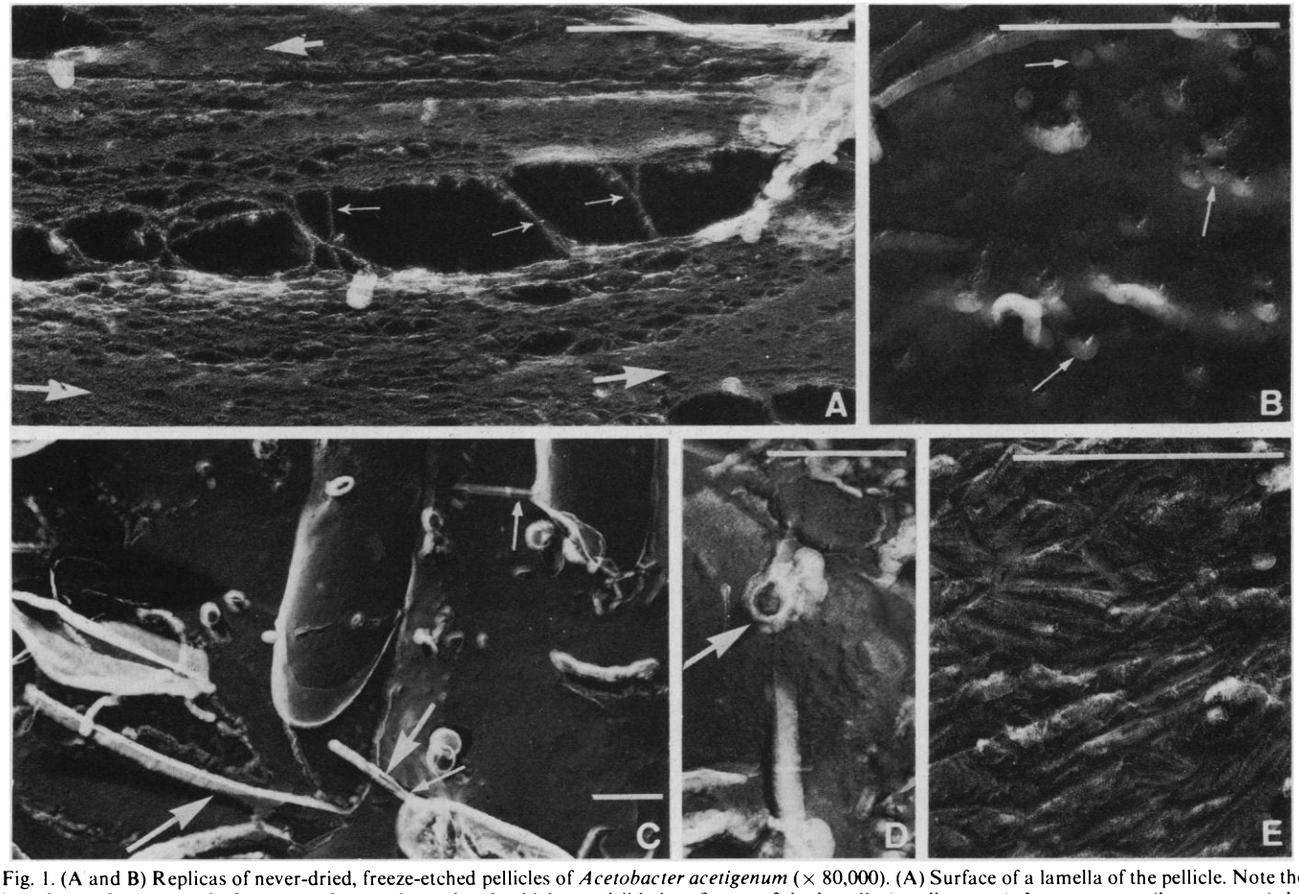

Fig. 1. (A and B) Replicas of never-dried, freeze-etched pellicles of *Acetobacter acetigenum* (× 80,000). (A) Surface of a lamella of the pellicle. Note the broad strands composed of a core and amorphous sheath which are visible in a fissure of the lamella (small arrows). In some areas (large arrows) the strands fuse to form a structureless matrix. In other areas, the cores form a reticulum or may extend many hundreds of nanometers. (B) Portion of the surface of a lamella where the strands have been broken by fracture. The ends of the strands rise above the surrounding ice surface, and the ends of the cores project from the ends of the strands. Note the line of demarcation of the material of the sheath from the surrounding ice surface (arrows). (C and D) Replicas of freshly incubated cells of *Acetobacter xylinum*. (C) Strands of the core and sheath that extend from and appear to be attached to the bacterial cell wall (small arrows). Note the strands that are arched over and free from the surrounding surface but still show the core and amorphous sheath (large arrows) (× 20,000). (D) A strand that lies on the surface of the cell wall and appears to extend from a protrusion (arrow) (× 40,000). (E) Replica of a dried and rewetted pellicle of *Acetobacter acetigenum*. Note the presence of segments of typical cellulose microfibrils (× 80,000). Scale bars = 0.5 μm.

pensions was done in a Balzers BA 360 M apparatus, as described by Moor (*8*). Examination of the carbon replicas of the surfaces was by an AEI-6B and a Siemens 101 electron microscope.

Replicas of surfaces of lamellae of never-dried, freeze-fractured pellicles from both species of bacteria which produce cellulose show little evidence of the characteristic microfibrillar morphology (Fig. 1A). Instead, a lamella is composed of broad strands (frequently 30 to 40 nm in diameter, as opposed to the 10- to 12-nm microfibrils revealed by other techniques) of an amorphous material within which there is sometimes a core. In some parts of the pellicle there is no perceptible evidence for cores, only a mass of strands, but both core and sheath are readily perceived when the strands are pulled apart (Fig. 1A). In other areas, the cores may extend hundreds of nanometers. When strands are broken by fracturing, the replicas show that the ends of the cores often extend above the sheath and that there is a visible separation between the material of the sheath and the surrounding ice (Fig. 1B). A sheath and its core may be seen attached to or apparently extending from a bacterial cell incubated for 10 or 20 minutes (Fig. 1C). Sometimes the proximal tip can be seen within a fracture plane of the bacterial cell envelope, and sometimes this tip extends into the external milieu from a protrusion on the surface of the cell wall (Fig. 1D). Some strands intimately associated with freshly incubated bacterial cells have a diameter of almost 100 nm. The discreteness of the sheath (and its core) is made particularly visible when extended etching of the fracture surface leaves the sheath partially elevated above the surface of its ice matrix (Fig. 1C).

Replicas of the fracture surfaces of pellicles dried before freezing show microfibrils one-fourth to one-third as wide as the strands in the never-dried pellicles (Fig. 1E). There is little evidence for the strands found in never-dried pellicles. Instead, these replicas suggest an interlacing, felt-like structure of discrete threads similar to those recognized previously (*4*). In brief, the process of drying (which was applied to all previously examined specimens) converted the strands from a broader, unconsolidated form to a smaller, more condensed thread.

Because there is no evidence of extracellular, fibrillar material other than cellulose in cultures of either bacterium, it is reasonable to assume that the observed strands in the lamellae of the pellicles or in the suspensions of cells are a form of nascent cellulose microfibril, hitherto unrecognized. With this assumption, a strand consisting of a sheath of amorphous gel about a central core is an initial stage in the formation of the microfibril: this stage is physically altered by drying or chemical digestion of the film. Details of the process are unknown but it is plausible to suppose that water molecules are progressively removed from between the polyglucan chains, allowing the chains to associate irreversibly and to form the entity that has been recognized as the cellulose microfibril for at least 25 years (*4*). Even when drying or digestion does not occur, this association may take place slowly, leading to formation of a consolidated fibril which becomes the standard microfibril. The presence of such an early, unconsolidated sheath is consistent with the notion of intermediate, transient polymers in the biosynthesis of cellulose (*5, 6*), with the suggestion of multiple, glucan-synthetase activities (*9*), and with earlier, indirect inferences about hydration of cellulose (*4*, p. 61).

The presence of the sheath does pose additional problems of microfibril genesis because if the sheath were simply a cellulose-water gel, past experience suggests that the dried material would adopt the cellulose II lattice. If small cellulose I aggregates were already present these might assemble to give a cellulose I microfibril. However, there is as yet no evidence in the core or sheath for the crystalline, elementary fibrils which have been postulated from x-ray diffraction or electron microscope investigations (*10*).

Nonetheless, irrespective of molecular interpretations in detail, the presence of a transient gel sheath about a core represents a new, additional stage in the physical formation of the cellulose microfibril.

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Incisor Size and Diet in Anthropoids with Special Reference to Cercopithecidae

Abstract. *In 57 species of anthropoids relative size of incisors is highly correlated with diet. Anthropoids that feed primarily on large food objects (large fruits) have larger incisors than those that feed on smaller food objects (berries or leaves). This difference reflects a need for more extensive incisal preparation of larger food objects before mastication. Extensive incisal preparation causes increased tooth wear; therefore, enlarged incisors are probably an adaptive response to increase their wear potential.*

The relationship between incisor morphology and diet has been investigated in both African apes and baboons (*1*). Within these two groups of primates, the more frugivorous forms (*Pan* and *Papio*) tend to have larger incisors than the more folivorous or graminivorous forms (*Gorilla* and *Theropithecus*). The adaptive significance of this finding is thought to be related to differential tooth use. Extensive incisal preparation prior to mastication (cutting, tearing, or pulping of food objects before chewing them with the postcanine teeth) ordinarily is not necessary for leaves, stems, berries, grasses, seeds, buds, or flowers; however, it is necessary when ingesting large, tough-skinned fruits. The increased frequency and duration of incisal preparation in both *Pan* and *Papio* causes increased amounts of attrition and abrasion of the anterior dentition, and therefore their enlarged incisors represent an adaptive response to delay dental obsolescence under these conditions. Accordingly, anthropoids whose diets consist primarily of large, tough fruits should have larger incisors than anthropoids which eat leaves, grass, or berries. The purpose of this study