of rigidly fixed, regularly spaced obstacles. Polyacrylamide gels are actually elastic media. We have assumed that the network forces the DNA chain to bend as it migrates through the gel; however, if the network forces the chain to bend, then the chain must also exert a force that can deform the network. The actual local elastic free-energy barriers encountered by a DNA chain in tight gels are probably much smaller than predicted from the mean spacing of rigid fibers, a; hence the low value found for B_{eff}.

Although apparently good agreement is obtained with the mobilities of DNA fragments containing intrinsic bends near the center of the chain, the agreement is significantly worse with molecules bent near the ends. At least some of this disagreement may be due to the assumption of a rigid gel. An elastic gel is expected to accommodate larger deformations for DNA molecules bent near one end than for molecules bent near the center, thus lowering the elastic free-energy barriers for motion of chains bent near one end.

In addition, there are limitations due to modeling intrinsic curvature with a large bend located at a single position. More realistically, the natural curvature at a bending locus, such as that in kinetoplast DNA, would occur over a number of tube segments. We found, however, that distributing an effective 66° intrinsic bend without torsional constraints over two or more segments about the middle of a chain had little effect on the computed mobility. This was determined from simulations on a chain with a series of two or three torsionally unconstrained bends with equal values of θ^0 chosen to give the same free-chain $<h^2>$ values as that of the once-bent chain. Modeling the chain more realistically with several adjacent smaller bends including azimuthal phasing and torsional elasticity (as an additional parameter) encounters a number of difficulties and seems difficult to justify in view of the other approximations in this calculation, such as omission of an explicit treatment of gel elasticity.

Good agreement is also obtained with the predictions of the Lumpkin-Zimm formula (*11*) (Eq. 1) at the lowest gel concentration (Fig. 3). The data shown are values of the ratio of $<h^2>$ for bent chains to that for an unbent chain. Because this ratio pertains to the free chain and not the tube, the model cannot explain the decreasing mobility of intrinsically bent chains with increasing gel concentration.

The picture of the electrophoresis of DNA in concentrated gels presented here suggests that the anomalous mobility of intrinsically bent DNA molecules in concentrated gels such as polyacrylamide can be accounted for by inclusion of an elastic free-energy contribution to the dynamics of the chain. The numerical values of the elastic force constant that successfully fit the data are much smaller than values appropriate for DNA alone; we attribute this to elastic compliance of the gel matrix. An objective of this work is to achieve a rigorous understanding of the gel electrophoresis of bent DNA fragments so that this simple physical technique may be used to accurately characterize DNA bends in systems such as some protein-DNA complexes, which are often not amenable to analysis by electro-optical, or other, methods.

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Lignin-Like Compounds and Sporopollenin in *Coleochaete*, an Algal Model for Land Plant Ancestry

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Unusual cell wall structure and resistance to microbial degradation led to an investigation of resistant biopolymers in *Coleochaete* (Chlorophyta, class Charophyceae), a green alga on the evolutionary lineage that led to land plants. In *Coleochaete* that are undergoing sexual reproduction, vegetative cell walls contain material similar to lignin, a substance generally thought absent from green algae, and the zygote wall includes sporopollenin. Knowledge of chemically resistant compounds in *Coleochaete* may facilitate interpretation of the fossil record. Placental transfer cells in *Coleochaete orbicularis* and in the hornwort *Anthoceros* survive acetolysis and contain lignin-like compounds, implying a close relation between these taxa.

ON THE BASIS OF CYTOLOGICAL and chemical similarities, embryophytes (land plants) are thought to have evolved from charophycean green algae (*1*), and the charophycean genus *Coleochaete* is the extant alga with greatest similarity to embryophytes (*2*). *Coleochaete* (Fig. 1) is a tiny (1 mm in diameter) alga occurring in shallow, quiet freshwater habitats, which may be similar to those occupied by the algal ancestors of land plants (*3*). Transition to a land flora probably occurred during the late Ordovician or early Silurian, but interpretation of fossils of the earliest ancestors of plants is difficult because they were incompletely adapted to the terrestrial environment and thus lacked systems characteristic

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of land plants (*4, 5*). Study of the extant algal relatives of embryophytes provides information that helps understand this transition. We have found that the presence of a material qualitatively similar to lignin allows fertile *Coleochaete* thalli to endure strongly reducing treatments with much of their structure intact. There is also a thin layer of the highly resistant material, sporopollenin, in the zygote wall. Similarity between *Coleochaete* and the Silurian-Devonian fossil *Parka* has been noted by Niklas, and *Parka* has been shown to have sporopollenin localized in reproductive regions (*6*).

To test the resistance of *Coleochaete* thalli to chemical degradation, fertile and vegetative thalli of *C. orbicularis* Pringsheim and fertile thalli of *C. pulvinata* Braun were subjected to acetolysis (*7, 8*). Vegetative thalli were destroyed. Fertile thalli of both *C. orbicularis* and *C. pulvinata* survived acetolysis, implying general occurrence of resistant material within the genus. *Coleochaete orbicularis* thalli were virtually intact; cells were completely cleared of their contents, but preservation of cell walls allowed many structural features to be identified (Fig. 2a). The pattern of deposition of acetolysis-resistant material in *Coleochaete* and destruction of vegetative thalli suggest that the substance is not produced constitutively, but rather is induced by the formation of a zygote, probably mediated by a diffusible substance. Only cells near zygotes survived acetolysis; in *C. orbicularis* the central portion of the thallus, composed of older cells, and recently formed marginal cells, were destroyed. In *C. pulvinata*, gametophytic cell walls nearest the zygote had a thicker deposition of acetolysis resistant material than did more distal cell walls (Fig. 2b).

To explore the possibility that acetolysis-resistant material in *Coleochaete* was sporopollenin, Fourier transform infrared (FTIR) spectra were obtained from acetolysed *C. orbicularis* and compared to a known source of sporopollenin (Fig. 3, a and b). Sporopollenin does not form the bulk of acetolysis-resistant material in *Coleochaete* cell walls, but is present surrounding the zygote. In electron micrographs a thin, electron-dense layer is visible in the inner wall of mature zygotes (Fig. 2, c and d). This layer was isolated by moderate base treatment which destroyed the dominant acetolysis-resistant material (*9*). Electron microscopic characteristics, resistance to both acetolysis and base treatment, and FTIR spectra indicate that this layer is sporopollenin (Fig. 3c). Sporopollenin in zygote walls is of interest in the study of the origin of embryophyte spores; the layer may be homologous to a "membrane" around spore tetrads in late Ordovician and early Silurian sediments (*10*).

Standard qualitative tests indicate the primary acetolysis-resistant material present in the thalli of fertile *Coleochaete* is a phenolic biopolymer (a lignin-like substance). Acetolysed *Coleochaete* (not base-treated) autofluoresced under ultraviolet light (*11*), suggesting a phenolic compound. The Mäule test, a qualitative test for lignins containing syringyl groups, produced a positive reaction, staining acetolysed *C. orbicularis* a deep brown and quenching autofluorescence. Phloroglucinol did not stain acetolysed thalli. If present in angiosperm tissue, these characteristics would be considered diagnostic of a lignin or lignin-like compound (*12*). Suberin is a persistent material, found in embryophytes, and thought to be a combination of lignin-like polymers and lipids, but qualitative tests for lipids associated with suberin (*12*) were negative in unacetolysed thalli of *Coleochaete*.

Lignin and lignin-like compounds are generally considered absent from the algae (*14, 15*), but such a material has been reported in the charophycean green alga *Staurastrum*, where it confers resistance to microbial attack (*16*). A lignin-like material probably serves an antimicrobial function in *Coleochaete* as well. *Coleochaete* retains zygotes on parental thalli (even if the parental thalli are dead) until the subsequent growing season. Lignin-like biopolymers in fertile thalli

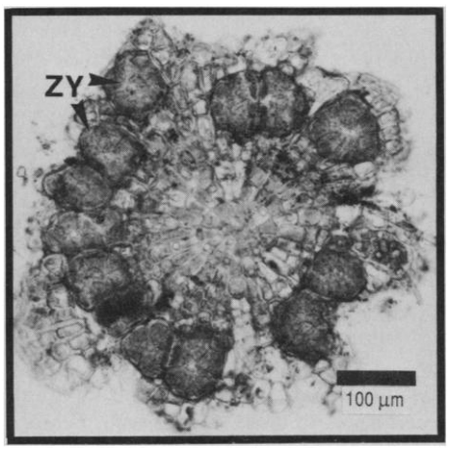

Fig. 1. *Coleochaete orbicularis* with mature zygotes (ZY). In this species the thallus is a monostromatic disk of parenchyma that grows radially. Central cells are oldest; vegetative growth occurs in marginal cells. Internal cells may differentiate into sperm, zoospores, or hair cells. Oogonia (egg cells) develop from marginal cells and are surpassed by growth of adjoining tissue, some cells of which later generate a cortical layer over the zygote. Some species of *Coleochaete* (for example, *C. pulvinata*) are cushions of branched filaments. *Coleochaete*, like other charophycean algae, differs from embryophytes in life history. *Coleochaete* exhibits zygotic meiosis; vegetative thalli are gametophytic (1*N*). The zygote is the only diploid stage in the life history, and serves as an overwintering structure.

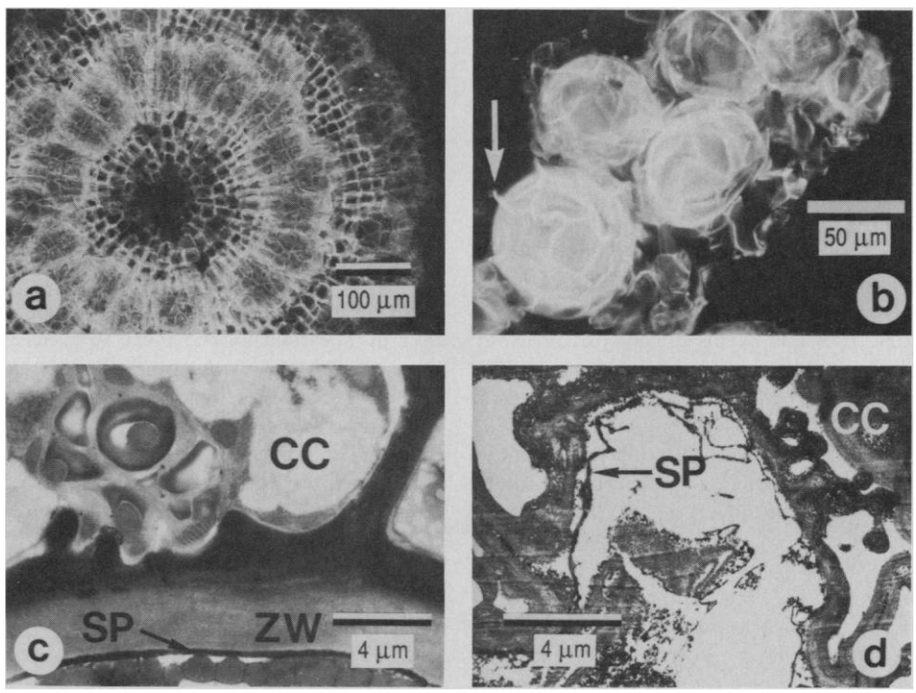

Fig. 2. (**a**) Acetolysed *C. orbicularis* autofluorescing. Visible are gametophytic cells and zygotes. In this thallus there were two periods of initiation of oogonia, resulting in two concentric rings of zygotes. Zygotes near the center are older and fully developed. (**b**) Acetolysed *C. pulvinata* autofluorescing. Arrow points to resistant cortical cell wall adjacent to zygote. (**c**) High-voltage electron micrograph of unacetolysed *C. orbicularis* zygote and adjacent cortical cells (*20*). (**d**) Electron micrograph of acetolysed *C. orbicularis* zygote showing sporopollenin layer after acetolysis. Note that the central region of the zygote wall has been destroyed (SP, sporopollenin; ZW, zygote wall; CC, cortical cell with wall ingrowths).

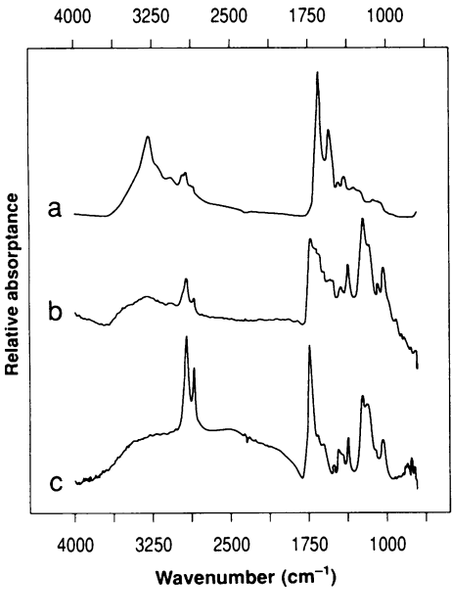

Fig. 3. Infrared spectra of (a) a thallus of acetolysed *C. orbicularis*, and (b) sporopollenin (*25*). Differences between the spectra include absorption at 1727 cm^{-1} and peak shifts in the 1000 to 1400 cm^{-1} region. (c) A thallus of *C. orbicularis* that has been both acetolysed and treated with base. Peak locations and intensities correlate with acetolysed pollen (*26*).

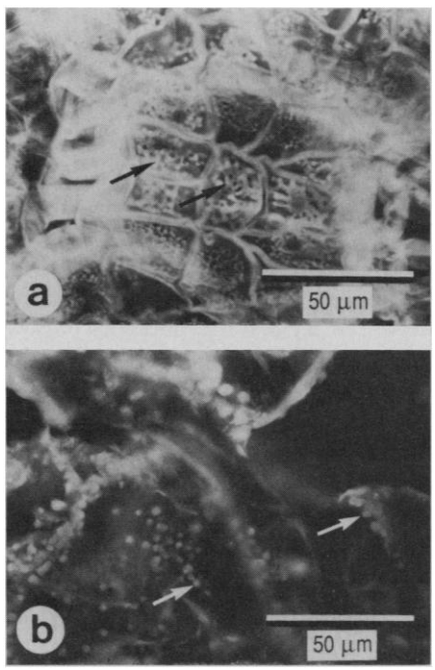

Fig. 4. (**a**) Putative placental transfer cell wall ingrowths in cortical cells covering a zygote of *C. orbicularis* (acetolysed). (**b**) Acetolysed *Anthoceros* transition zone showing placental transfer cell wall ingrowths. As in *Coleochaete*, some vegetative cells lacking wall ingrowths also survived acetolysis.

of *Coleochaete* would retard microbial degradation of the parental thallus (*16, 17*), thus promoting retention of zygotes, which therefore germinate in a favorable environment for attachment and growth of resulting meiospores. In contrast, the vegetative thallus of the extant charophycean alga *Chara* (*18*), which does not retain its zygotes until they germinate, is not impregnated with lignin-like material and was completely degraded by acetolysis. Zygotes of *Chara*, which are known to be surrounded by sporopollenin (*19*), survived acetolysis. Presence of lignin-like compounds in both *Coleochaete* and *Staurastrum* implies that they may occur in other charophycean algae, and in turn, indicates that lignin's role as an antimicrobial agent predated its role as a structural cell wall component.

Among phylogenetically significant structures that survive acetolysis in *Coleochaete orbicularis* are wall ingrowths in cortical cells surrounding zygotes (Fig. 4a). These are similar to wall labyrinths in placental transfer cells of bryophytes and other embryophytes and are known only from this species among the algae (*3, 20*). Placental transfer cells function in the transfer of photosynthate and nutrients to the zygote. Since hornworts are an embryophyte group considered to be phylogenetically close to charophytes (*2, 21*), the placental region of *Anthoceros* (*22*) was isolated by dissection, subjected to acetolysis (*7*), and examined by fluorescence microscopy. Placental transfer cell walls of *Anthoceros* survived acetolysis (Fig. 4b) and were qualitatively similar to acetolysed *Coleochaete* (*23*). Presence of lignin-like compounds in placentae of *Anthoceros* may reflect descent from a *Coleochaete*-like ancestor. In view of the phylogenetic importance of lignin (*24*), placental regions of other embryophytes need to be examined for lignin-like compounds.

Presence of lignin-like material in the placenta and sporopollenin in zygotes of *Coleochaete* support the cladistic placement of *Coleochaete* as a sister taxon to embryophytes. Many characters used to determine phylogeny in extant organisms cannot be studied in the fossil record (*4*), and study of resistant structures in *Coleochaete* may simplify comparisons between extant and fossil organisms. A useful marker identifying early embryophytes in the fossil record may be placental transfer cells, which survive acetolysis and are easily identified by light microscopy. *Coleochaete* is also a potential model system for research into the biochemical evolution of phenolic polymers and developmental regulation of their deposition in cell walls.

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