semblages of organically preserved microfossils (Fig. 1): the biota preserved in chert lenses in algal laminated carbonates of the Galeros Formation (Carbon Canyon Member) is comprised of cyanophytic filaments and colonial and solitary algal unicells, 2 to 56 μm in diameter (*4, 10*); that of the cherty pisoliths of the Walcott Member of the Kwagunt Formation includes blue-green algal filaments, rare chitinozoans, and spheroidal unicells 10 to about 60 μm in diameter (*8, 10*); and, as mentioned above, the shale biota of the Walcott Member appears to be comprised solely of acritarchs and chitinozoans, the latter ranging to more than 140 μm in maximum dimension (Fig. 3). In addition, shales of the Tanner, Awatubi, and Walcott members of the sequence (Fig. 1) contain the planktonic alga *Chuaria* (*6*), a megasphaeromorphid acritarch that ranges from about 500 to more than 3000 μm in diameter (*18*). Inasmuch as all of these assemblages are of approximately the same geologic age, it seems evident that their compositional differences must largely reflect differences of facies: relatively large planktonic forms, the chitinozoans and *Chuaria*, are predominant in the biotas preserved in the shaley facies whereas the microbiotas of the cherty carbonates (sediments apparently evidencing relatively nearshore, more shallow conditions) are composed chiefly of benthonic algal filaments and small unicells. Although facies-dependent relationships of this type have heretofore been largely overlooked in the Precambrian, their recognition is important; of the various overviews of early evolution set forth in recent years (*2, 4, 19*), virtually all have been based only on those fossils detected in the relatively restricted, shallow-water, cherty carbonate facies. Thus, in light of results summarized here together with important discoveries recently reported from other Precambrian shales (*20*), it seems reasonable to conclude that shaley facies represent a promising, but as yet largely untapped, source of new evidence on the diversity, evolution, and biostratigraphic usefulness of the Precambrian biota.

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9. Locality 1: cherty pisolite bed, northeastern rim of Nankoweap Butte [at "*Chuaria*" indicated by brackets in (*8*), figure 1]; locality 2: shale underlying locality 1 by about 3 m; locality 3: shale 3 to 5 m beneath cherty pisolite bed on the northern rim of Nankoweap Butte, about 0.5 km west of locality 1 [near arrow indicating "fossiliferous cherty pisolite" in (*8*), figure 1]; locality 4: shale 3 to 5 m beneath cherty pisolite bed in slump block on the northwestern flank of Nankoweap Butte, about 1 km north-northwest of locality 1.
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Cellulases Can Enhance β-Glucan Synthesis

Abstract. *β-Glucan synthesis from uridine diphosphoglucose by pea epicotyl tissue slices is increased two- to threefold by preliminary, short-term treatment with cellulases purified from auxin-treated peas. We suggest that cellulases introduce chain ends in accessible regions of cellulose microfibrils which then act as primers for chain elongation.*

Auxin treatment of apical tissue of pea seedlings (*Pisum sativum*, L.) evokes a number of growth reactions (*1*), as well as the simultaneous deposition of large amounts of cellulose (*2*), increased glucan synthetase activity (*3*), and the development of very high levels of two β-1,4-glucanendohydrolases (*4*). The cellulases have been purified to homogeneity and measurements of their physical characteristics (*4*), kinetic properties, and substrate specificities (*5*) indicate that they are distinct proteins which merit designation as true β-1,4-glucan-4-glucanohydrolases (E.C. 3.2.1.4). They hydrolyze linkages in β-1,4-linked glucans, with preference for longer chains and with no detectable activity toward cellobiose, β-glucosides, β-1,3-glucans, or glucan containing alternating β-1,3 and β-1,4 linkages. Transglucosylase activity could not be detected (*5*). Antibodies against the purified enzymes have been used to study their subcellular location and to assay for the appearance and distribution of cellulase messenger RNA (mRNA) (*6*). Pea cellulases appear to be induced by the auxin type of growth regulator, and to be generated and transported in rough endoplasmic reticulum vesicles to the inner surface of the growing primary wall. They may become associated with β-glucan synthetase during this process (*2*). The problem is to assign a function to cellulases in cell walls when rapid net cellulose deposition and extension of the microfibrillar framework are occurring.

Table 1. Preliminary treatment of tissue slices with pea cellulases significantly enhances synthesis of alkali-insoluble glucan. Slices were incubated for 20 minutes, with or without 150 units of pure BS or BI cellulase (*4*) in 0.1 ml of buffer and washed in buffer; glucan synthetase was assayed as described in Fig. 1. Reactions were performed in quintuplicate and incorporation of ^{14}C was calculated.

Preliminary incubation	Glucan synthetase activity*	
	Alkali-soluble	Alkali-insoluble
None (zero time)	9.81 ± 0.89	7.26 ± 0.56
BS (20 minutes)	11.65 ± 1.07	20.56 ± 1.27
BI (20 minutes)	8.80 ± 0.89	13.60 ± 0.84
Buffer (20 minutes)	9.26 ± 1.67	7.59 ± 0.29

*Nanomoles glucose incorporated per segment from uridine diphosphoglucose per 10 minutes ± the standard error.

There is an accumulating body of evidence to indicate that cellulose synthesis in bacteria and higher plants normally occurs at many loci at the wall-protoplast interface, and involves glucosyl transfer from sugar nucleotide to acceptor molecules which may be cellodextrin-lipid or cellodextrin-protein (*7*), or cellodextrins (*8*) or cellulose itself (or both) (*9*). Whether or not glycolipids and glycoproteins act as intermediates, the availability of β-1,4-glucan chain ends (primers) where elongation can take place is a potential limiting factor for the whole process. There is evidence (*2*) that crude fungal cellulase preparations can, paradoxically, increase the net incorporation of radioactive glucose into cellulose by pea segments, provided that the enzyme is applied at a very low concentration. The possibility is raised, therefore, that endogenous plant cellulases act constructively as an integral part of cell-surface cellulose-synthesizing complexes.

We now report that purified pea cellulases added to thin slices of pea epicotyl can enhance subsequent β-glucan synthesis from uridine diphosphoglucose (UDPG). Pea slices were first incubated with the cellulases at concentrations per unit volume of medium comparable to the concentrations reached per unit of fresh weight of auxin-treated intact tissue (*1*, *4*). After various intervals, the slices were washed in buffer to remove unadsorbed cellulase and any soluble cellodextrins which may have been generated. They were then incubated with [glucose-^{14}C]UDPG, and measurements were made of incorporation of ^{14}C into products soluble in alkali and insoluble in alkali. A preliminary incubation period of 15 to 30 minutes in either buffer-soluble (BS) or buffer-insoluble (BI) cellulase enhances rates of synthesis of alkali-insoluble glucan from UDPG (Fig. 1). Yields of alkali-soluble glucan are relatively unaffected. Preliminary incubation and washing of slices in the absence of cellulase has no effect on glucan synthesis.

After establishing this time scale (Fig. 1), the experiment was repeated in quintuplicate using a 20-minute preliminary incubation with cellulase followed by washing and a 10-minute synthetase assay. The results (Table 1) confirm that both enzymes evoke a statistically significant increase (two- to three-fold) in the capacity of slices to generate alkali-insoluble glucan from UDPG. Further tests showed that there was no synergistic interaction between the two cellulases when they were supplied together to pea slices.

We interpret these data as indicating that cellulose biosynthesis in growing pea cells is limited by the availability of primer molecules, and that partial endohydrolysis of preexisting wall components by pea cellulases can help to overcome this limitation. Analogous proposals have been made for a constructive function for low concentrations of amylases in starch- and glycogen-synthesizing systems (*10*). In present and earlier (*2*) tests, prolonged incubation in cellulase reduced cellulose deposition, suggesting that continued hydrolysis at accessible wall regions eventually results in reduced primer availability. Buffer-soluble cellulase is more effective than buffer-insoluble cellulase, perhaps because a smaller molecular size (molecular weight, 20,000 and 70,000, respectively) facilitates penetration of BS to the site of cellulose synthesis. The absence of any significant effect of cellulase treatment on the incorporation of label into alkali-soluble glucan is undoubtedly due to the fact that this component of pea walls is predominantly β-1,3-glucan.

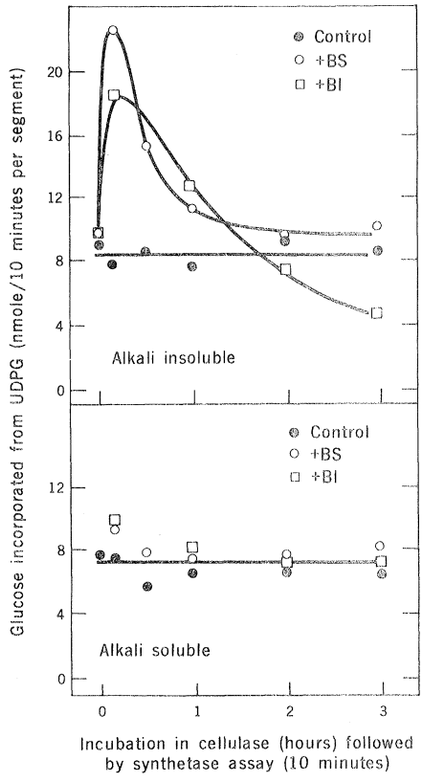

Fig. 1. Brief preliminary treatment of pea tissue slices with pure pea cellulases enhances subsequent β-glucan synthetase activity. Purified (*4*) buffer-soluble (*BS*) and buffer-insoluble (*BI*) cellulases (300 units in 0.1 ml of 0.02*M* sodium phosphate, *p*H 6.2) were shaken at 35°C with 20 transverse tissue slices cut from two 5-mm epicotyl segments (*3*). At intervals, slices were washed with 3 ml of 0.05*M* morpholinopropanesulfonic acid (MOPS), *p*H 7.25 and then supplied directly with a medium suitable for assaying surface glucan synthetase activity (*3*); that is, the total volume (250 μl) contained 5 m*M* [glucose-^{14}C]UDPG (New England Nuclear, 0.225 μc), 10 m*M* $MgCl_2$, 5 m*M* dithioerythritol in 0.05*M* MOPS, *p*H 7.25. Mixtures were shaken for 10 minutes at 35°C, during which time incorporation of ^{14}C into glucan was linear (*3*), and reactions were terminated by heating at 100°C. Whatman cellulose powder (10 mg) was added, and the mixture was washed with hot (85°C) water. Water-insoluble products were separated into alkali-soluble (extracted three times with 5 ml of 1*M* NaOH–0.1 percent $NaBH_4$ at 85°C) and residual alkali-insoluble fractions. These were neutralized, and radioactivity counted in PCS scintillation fluid (Amersham-Searle Corp., Arlington Heights, Illinois) at 90 percent counting efficiency.

Auxin-induced cellulases may play a role in the "wall-loosening" process, long thought to be an essential preliminary step in the sequence of events leading to cell wall expansion (*11*). However, during cell expansion there is a net deposition of new cellulose, and this occurs such that the microfibrillar framework of cells maintains its integrity (*2*). It is evident therefore that any hydrolysis of preexisting cellulose which takes place during growth must be followed closely or accompanied by overwhelming rates of synthesis. Thus, a concerted action of hydrolases and synthetases can be envisaged which would enable microfibrils to extend at multiple loci along their lengths. Globular complexes observed at cell surfaces (*9*), which may represent multienzyme sites for cellulose synthesis, occur as often astride microfibrils as at apparent fibril ends. One role of auxin may be to regulate effective hydrolase:synthetase ratios (*1–3*) in such complexes, thereby controlling insertion of new glucan into the wall framework.

Regardless of the variety of intermediates or primers for cellulose synthesis which may exist, our results reinforce the view that cellulose synthesis in growing plant cells occurs at or near the protoplast surface and that cellulase has the potential to enhance cellulose synthesis.

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Batesian Mimicry: Selective Advantage of Color Pattern

Abstract. *Field studies of releases and recaptures of diurnal moths painted with yellow to resemble the edible tiger swallowtail and of black moths that resemble a toxic species of swallowtail produced these results: (i) A greater proportion of the black moths were recaptured; (ii) daily trapping for a week after each release showed that the black moths survived longer than the yellow-painted moths; (iii) an analysis of wing injuries shows that most attacks can be attributed to birds and that the yellow-painted moths were attacked more often, more vigorously, or more persistently than the black moths. These results are interpreted as showing a greater predation pressure on the yellow-painted than on the black moths and, therefore, as confirming the Batesian theory of mimicry.*

The concept of Batesian mimicry has aroused interest for more than a century. Observational, theoretical, and laboratory studies with caged predators have demonstrated the apparent effectiveness of Batesian mimicry in protecting butterflies, flies, and beetles from birds, toads, and lizards (*1*). Other experiments with caged predators and artificially created models and mimics (*2*) also support the assumption that Batesian mimicry is effective in the wild. These studies constitute a strong but not conclusive case. The obvious next step is the experimental demonstration and measurement of the selective advantages of Batesian mimicry in nature, but, because of difficulties with experimental design, this has yet to be accomplished.

Jones (*3*) and Kettlewell (*4*) showed that the color patterns of insects can affect the predatory responses of insectivorous birds in the field. Jones offered dead insects of various species to wild birds, finding that insects with aposematic color patterns were rejected and that such insects were often distasteful. Kettlewell's studies on industrial melanism in moths demonstrated that birds act as selective agents on insect populations with color morphs that have different adaptive values as camouflage.

Brower and his co-workers did a series of field experiments on Trinidad in order to test experimentally the Batesian mimicry theory (*5, 6*). They released black diurnal males of the edible North American saturniid moth *Callosamia promethea* which had been painted with various patterns on the upper surface of the wings. Some had been painted to resemble unpalatable butterflies found on Trinidad; others had similar quantities of black paint applied to the black areas of the wings so as not to change their appearance. They made the reasonable assumption that differential recaptures indicated differential predation. They concluded that their experiments had not demonstrated that mimics have a selective advantage in the field.

However, Waldbauer and Sternburg (*7*) contended that the experiments by the Brower group can be interpreted differently and that similar techniques could demonstrate mimetic advantage. They argued that the presumed control moths, although edible and essentially unaltered in appearance, were protected from predation by their similarity to the toxic, aristolochia-feeding *Battus* spp. of Trinidad, especially the abundant *B. polydamas*. That is, the Brower group actually compared artificial mimics of one model with mimics of another model. According to this interpretation, the Brower group's results (*5*) would be expected if promethea males are actually mimics of *Battus* spp.

Table 1. Number of moths of each painted type recaptured on each day after their release. The difference between the number of yellow- and black-painted moths recaptured is significant ($\chi^2 = 14.7$, d.f. = 6, $P < .025$).

Category	Days after release							Total
	0	1	2	3	4	5	6	
Black-painted	54	26	12	4	2	2	2	102
Yellow-painted	54	7	3	5	3	3	0	75

We now report that, with the proper controls, the release and recapture of painted promethea can demonstrate the selective advantage of one color pattern over another under natural conditions. Furthermore, because our controls were painted to resemble a palatable butterfly found in the experimental area, our results strongly support the Batesian mimicry hypothesis.

Waldbauer and Sternburg (*7*) presented evidence that male promethea belong to a well-known Batesian mimicry complex for which *Battus philenor* is the model. This complex includes as mimics all females of *Papilio troilus*, *P. polyxenes*, and *Speyeria diana*; the black females of *P. glaucus*; and both sexes of *Limenitis arthemis astyanax* (*8*). *Battus philenor* is moderately abundant in central Illinois, and all of the mimetic butterflies in the complex are common here except for the more southern *S. diana*.

We released and recaptured painted male promethea (*9*). Pupae were stored during the winter in an outdoor insectary. The moths emerged between mid-May and late August. A limited supply necessitated a design with only two groups of males. Those of one group were painted to resemble the yellow morph (a color form) of the palatable tiger swallowtail, *P. glaucus*; the others, resembling the toxic blue swallowtail, *B. philenor*, were essentially unaltered in appearance although they bore a comparable amount of black paint (Fig. 1). The flight of male promethea closely resembles that of the swallowtails, but near-perfect realism is impossible in painting the moths to resemble yellow tiger swallowtails. However, birds generalize patterns and avoid potential prey that only vaguely resemble the unpalatable model (*10*). Therefore, we applied a suggestive pattern (Fig. 1), which deceives the human eye at a distance of 5 or 6 m. The standard release point was the center of a circle (1.6 km diameter) of seven evenly spaced traps. Since native promethea are rare (*11*) in this area, there was little interference from wild, pheromone-releasing females.

On 12 days between late June and early September we released a total of 436 moths in groups of between 14 and 50 consisting of equal numbers of the two painted types. The traps were checked daily for at least 7 days after each release. From the total number recaptured (177), we calculated recapture