observed exact correspondence of the bands; that has been true also when we have run a single preparation in triplicate.

Waller (*2*), using ion-exchange chromatography and starch-gel electrophoresis to analyze ribosomal protein from *E. coli*, was able to detect at least 24 bands, the majority being basic proteins. Fractionation by means of polyacrylamide-gel electrophoresis has confirmed the heterogeneity of bacterial ribosomal protein (*3*). The majority of proteins—24 to 30—are basic, but several—6 to 9—are acidic (*11*). We found that protein from *E. coli* ribosomes showed, on electrophoresis at *p*H 4.5, 29 bands and a pattern different from that of mammalian ribosomal protein (Fig. 1). We do not know whether all or only some of the ribosomal proteins from *E. coli* are different from those of mammalian tissues.

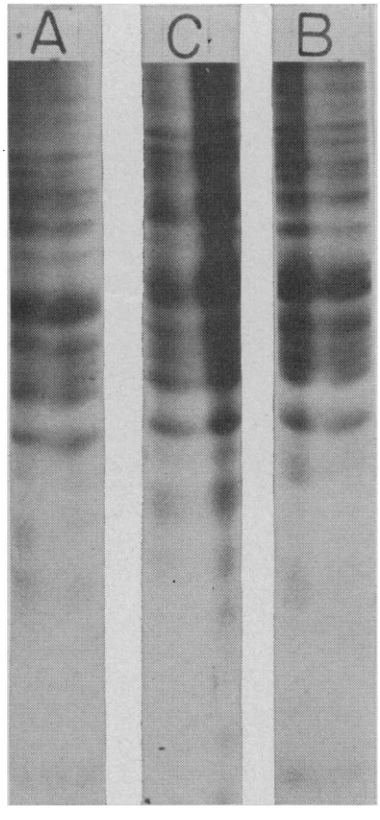

Fig. 2. Electrophoretic patterns of basic ribosomal proteins analyzed on polyacrylamide gel by the "split-gel" technique. The comparison in A is between protein from liver ribosomes (on left) and heart muscle ribosomes (on right); in B it is between protein from liver ribosomes (on left) and skeletal muscle ribosomes (on right); and in C, between protein from liver ribosomes (on left) and reticulocyte ribosomes (on right). Electrophoresis was from the top (anode) to the bottom (cathode). The concentration of separation gel was 10 percent.

No acidic protein could be consistently identified in the preparation of mammalian ribosomal protein. Occasionally one to four bands appeared when electrophoresis was carried out at *p*H 8.3, but large amounts of protein (five times the quantity ordinarily used) were required, and even then the bands were not reproducible from preparation to preparation. We cannot, therefore, be certain whether or not mammalian ribosomes contain acidic proteins. In contrast, preparations from *E. coli* always gave seven or eight acidic proteins.

There is no certainty concerning the exact number of distinct and separate proteins present in the mammalian ribosome. Possibly the preparation or the electrophoresis of the ribosomal proteins leads to breakdown of one or a few proteins to give 24 bands. The reproducibility of the electrophoretic pattern seems to indicate that this is not the case. More likely, some minor components were not resolved, and mammalian ribosomes actually contain more than 24 basic proteins. Whether electrophoresis is a valid technique for resolving heterogeneity can be confirmed only when several of the protein bands have been isolated and purified, and when their amino acid composition and sequence is determined (*3*). But whether there are few or many mammalian ribosomal proteins, they give qualitatively similar electrophoretic patterns; therefore, they must at least be derived from the same parental proteins.

There are reservations to the conclusions derived from such complicated protein patterns. At best we should only be able to detect the deletion or addition of a protein band, and not even that under all circumstances. For example, if a particular protein were present in the ribosomes of one tissue, but in an amount so small relative to the other proteins as not to be detectable, its deletion from the ribosomes of a second tissue could not be determined.

Moreover, all the minor protein components may not be resolved from the more prominent bands; the addition or deletion of such a minor band would also escape detection.

At least 24 distinct basic proteins can be resolved from mammalian ribosomes by electrophoresis on polyacrylamide gels. Since the patterns of the proteins are similar, it seems reasonable to conclude (subject, of course, to the reservations already discussed) that the same cistron or cistrons direct the synthesis of ribosomal protein in the several mammalian tissues and that one or more different cistrons direct the synthesis of bacterial ribosomal protein.

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Glucans of Oomycete Cell Walls

Abstract. *The cell walls of selected oomycetous fungi are composed primarily of glucans, and cellulose constitutes a relatively small proportion of the total glucan. The noncellulosic constituents consist of acid-soluble glucan or glucans and insoluble glucan or glucans. These noncellulosic glucan fractions contain β-(1→3) glucosidic linkages and apparently β-(1→6) linkages also.*

Cell walls of fungi are known for their chitinous nature which distinguishes them from the cellulosic walls of green plants (*1*). The Oomycetes (*2*), however, may be readily distinguished from the majority of fungi since their walls do contain cellulose, and appar-

ently chitin is lacking (*3–5*). In previous studies (*3, 4*) of cell walls of the Oomycetes, demonstration of cellulose was difficult, and Parker and his coworkers (*4*) claimed that, in four genera of the order Saprolegniales, noncellulosic polysaccharides were present in greater quantities than cellulose was. These workers reported that the monosaccharide constituents of the noncellulosic polysaccharides were primarily glucose and mannose, but did not describe other features. Bartnicki-Garcia's recent investigations (*5*) showed that cellulose was indeed a minor constituent in *Phytophthora cinnamomi* and *P. parasitica*. He found that the noncellulosic polysaccharides were mainly glucans; mannose occurred in trace amounts only. Initially, chromatography of acid hydrolyzates of *Phytophthora* walls yielded small amounts of laminaribiose and gentiobiose in addition to glucose and cellobiose; this was confirmed later by using appropriate chemical criteria. These results seemed to indicate the presence, in addition to cellulose, of a glucan or glucans composed of β-(1→3) and β-(1→6) linkages, which was supported by the isolation of acid-soluble glucan that had a negative optical rotation.

Our recent investigations of *Atkinsiella* (*6*), a marine fungus, and of *Pythium* (*7*) revealed notable similarities between the structure of the walls of these two genera and of *Phytophthora*. Less extensive investigations of *Achlya* and a species of *Phytophthora*, not examined by Bartnicki-Garcia, gave no evidence of any basic differences, in the structure of the wall, between them and the species examined in greater detail. Results of our work suggest that β-(1→3) and β-(1→6) glucans are the predominant polysaccharides in cell walls of *Atkinsiella dubia* and *Achlya ambisexualis* in the order Saprolegniales, and of *Phythium debaryanum* and *Phytophthora cactorum* in the Peronosporales. Since these orders include the most familiar and widely occurring Oomycetes, it is conceivable that the structures of cell walls reported here may be found in most fungi in this assemblage.

Atkinsiella was grown in liquid culture medium by methods described elsewhere (*6*). The other three species were grown in Emerson's GY5 medium (*8*) supplemented with 1.25 g of peptone (Difco) per liter. The fungi were harvested and washed free of culture medium, and excess moisture was removed by gentle suction on a Büchner funnel. Cell walls were isolated chemically (*9*) by treating mycelia in hot methanolic-KOH (5 g of KOH per 100 ml of 80 percent methanol); five extractions of 15 to 20 minutes duration were used. The walls were then washed with 80 percent methanol until they were free of alkali, and the resultant residue from each of the four genera was subjected to a series of chemical treatments, which yielded, in each case, three polysaccharide fractions. Fraction I was extracted from chemically isolated walls by five treatments in 0.1*N* HCl at 70°C; the extracts were collected by filtration through a fritted-glass funnel. The combined extracts were added to ethanol to give a final concentration of 85 percent ethanol. Precipitated polysaccharide was collected by centrifugation, washed with ethanol, slurried in H_2O, and freeze-dried. Fraction I polysaccharide that was isolated from all four fungi formed opalescent solutions in H_2O; reactions with I_2KI were negative. Reprecipitation with ethanol did not occur readily, but small amounts of NaCl induced flocculation. The preparation of partial hydrolyzates was carried out in sealed vials containing 2 mg of polysaccharide and 0.5 ml of concentrated HCl (37 to 38 percent). After hydrolysis for 1 hour at 22°C, the hydrolyzates were chilled and diluted with 5 ml of H_2O. Insoluble material was removed by centrifugation and the supernatant fluids were dried in a stream of N_2 at 40°C; acid removal was accomplished by four to five dryings under N_2. Mono- and disaccharide components of the partial hydrolyzates were identified by thin-layer chromatography on 0.25-mm cellulose layers. Chromatograms were irrigated with a mixture of *n*-propanol, ethyl acetate, and H_2O (7 : 1 : 2), and spots were detected with alkaline $AgNO_3$. Spots corresponding to glucose, laminaribiose (*10*), and gentiobiose were detected in all fraction I partial hydrolyzates. Chromatographic results obtained with *Achyla* are shown in Fig. 1 and are representative of those obtained with the other genera. Composition of the partial hydrolyzates indicated that the parent polysaccharides were glucans containing β-(1→6) and β-(1→3) linkages. The β-(1→6) linkages, estimated from intensity of spots on the chromatograms, may have been present in greater proportions, but small quantities of gentiobiose probably were formed through acid reversion (*11*).

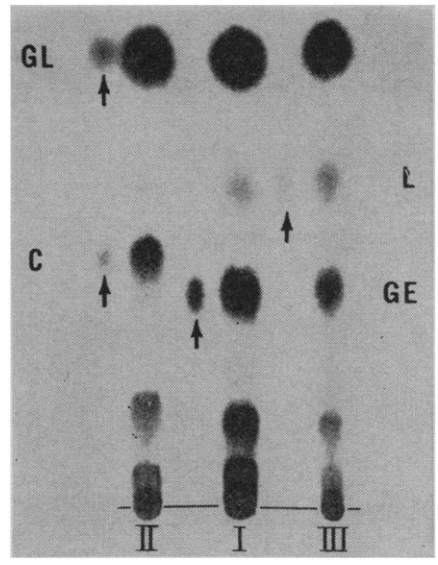

Fig. 1. Thin-layer chromatogram comparing partial hydrolyzates of fractions of cell walls from *Achlya* with authentic mono- and disaccharides. Lettered symbols (left and right margins) identify the sugars in the corresponding horizontal rows. *GL*, glucose; *C*, cellobiose; *L*, laminaribiose; and *GE*, gentiobiose. Arrows indicate the authentic compounds. Roman numerals below the starting line designate the partial hydrolyzates of wall fractions that have corresponding numbers in the text.

After extraction of fraction I, cell wall residues were given five 30-minute treatments in cuprammonium hydroxide (Schweitzer's reagent) to dissolve cellulose. The pooled extracts were slightly acidified by addition of acetic acid, and the precipitate of regenerated cellulose was collected by centrifugation, washed with 1*N* acetic acid, washed with H_2O until free of acid, and freeze-dried, giving fraction II. After the cellulose extraction, the residues were washed three times with 1*N* acetic acid, washed with H_2O, and finally freeze-dried, yielding fraction III. The wall fractions that we investigated, therefore, can be identified on the basis of their solubilities as: I, soluble in hot 0.1*N* HCl (H_2O soluble after isolation); II, soluble in Schweitzer's reagent (cellulose); and III, insoluble residue.

Partial hydrolyzates of fractions II and III were prepared by extending the duration of hydrolysis to 1.5 hours. The hydrolyzates were chromatographed, and the mono- and disaccharides were identified. As anticipated, fraction II gave spots corresponding to glucose and cellobiose upon partial hydrolysis (Fig. 1). At times, traces of

laminaribiose and gentiobiose appeared, indicating that the cellulose was slightly contaminated with β-(1→3) and β-(1→6) glucan. This occurred because these substances are slightly soluble in alkali (*4, 6, 7*), and it demonstrates the lack of specificity of Schweitzer's reagent if it is not used in conjunction with other methods for detection of cellulose. The partial hydrolyzates of fraction III (Fig. 1) were almost identical to those of fraction I, except for an apparent decrease in the proportions of gentiobiose (β-1,6-linkage).

During our investigations a number of chromatograms were run with several authentic sugars in addition to those found in hydrolyzates of cell-wall fractions. Consequently, we were able to eliminate galactose and mannose, among the hexoses, as constituents of the polysaccharides. We eliminated α-linked di- and oligosaccharides by infrared analyses (discussed below), which gave a clear indication of β-linkages but not of α-linkages.

In view of previous studies (*4, 5*) that showed a preponderance of noncellulosic polysaccharides in oomycete walls, we noted the relative proportions of fractions I, II, and III. The percentages (of total recovered polysaccharide) of the wall fractions are given in Table 1. However, our data do not represent a quantitative analysis of wall components, since we determined only the recovered amounts of each fraction and did not concern ourselves with the effects of alkaline degradation (in Schweitzer's reagent) of the glucans, which most certainly occurred to some extent (*12, 13*). Nevertheless, the tabulated values demonstrate that the walls are composed primarily of noncellulosic glucans.

Table 1. Percentages of three polysaccharide fractions recovered from the walls of oomycetous fungi.

Fungus	Fractions (% recovered)		
	I	II	III
Atkinsiella	7	31	62
Achlya	22	11	67
Pythium	13	12	75
Phytophthora	2	7	91

To substantiate our initial conclusions concerning the structures of the noncellulosic glucans, we tested the sensitivity of fractions I and III to β-(1→3)-glucan glycanohydrolase (*10, 14*). The enzyme employed was an exoglucanase, since it catalyzed the release of glucose units from the ends of polysaccharide chains (*15*); it was used at a concentration of 0.5 mg/ml in 0.01*N* ammonium acetate buffer, *p*H 5. One milliliter of enzyme solution was mixed with 2 mg of polysaccharide in each case, and the mixtures were incubated at 35°C for 48 hours. The reaction mixtures were then centrifuged, and the supernatant fluids were concentrated. Monosaccharides in the supernatants were detected by thin-layer chromatography as described above; enzyme activity on the polysaccharide fractions was observed in all instances. Glucose was the major product, but there were traces of unidentified oligosaccharides. Enzyme activity on cellulose was not detected. The enzymolysis of the fractions, therefore, confirmed the presence of β-(1→3) linkages. Since similar confirmations for the presence of β-(1→6) linkages are lacking at the present time, we regard our identification as tentative. However, the recent work of Wang and Bartnicki-Garcia (*5*) demonstrated synthesis of β-(1→6) linkages in the walls of *Phytophthora cinnamomi*, which lends considerable support to our tentative identification.

We also studied the infrared absorption of the various glucans. Spectra were recorded on a Perkin-Elmer Infracord spectrophotometer (*10*) with the use of the pressed-KBr disc technique (*16*); representative spectra are shown in Fig. 2. Our initial purpose was to confirm the presence of β-linkages in the noncellulosic glucans. This was accomplished since all spectra showed an absorption peak at approximately 890 cm^{-1} (*16*). Absence of an absorption peak at 844 cm^{-1} indicated the absence of α-linked glucans (*16*). In addition, in some instances the region of the spectra between 950 and 1150 cm^{-1} was indicative of the general nature of the polysaccharide. In this region, spectra of the celluloses that we studied showed three relatively strong absorptions (two of these appear as shoulders on the illustrated *Pythium* spectrum, rather than as easily identified peaks). In cotton cellulose these peaks occurred at 1023, 1047, and 1106 cm^{-1}; in *Pythium* cellulose they appeared at 1023, 1056, and 1109 cm^{-1} (Fig. 2). In the same spectral region, noncellulosic polysaccharides showed two principal absorptions. We employed laminarin (*10*) as a basis for comparison, since, in this polysaccharide, the β-(1→3) and β-(1→6) linkages are well established (*17*). Spectra obtained

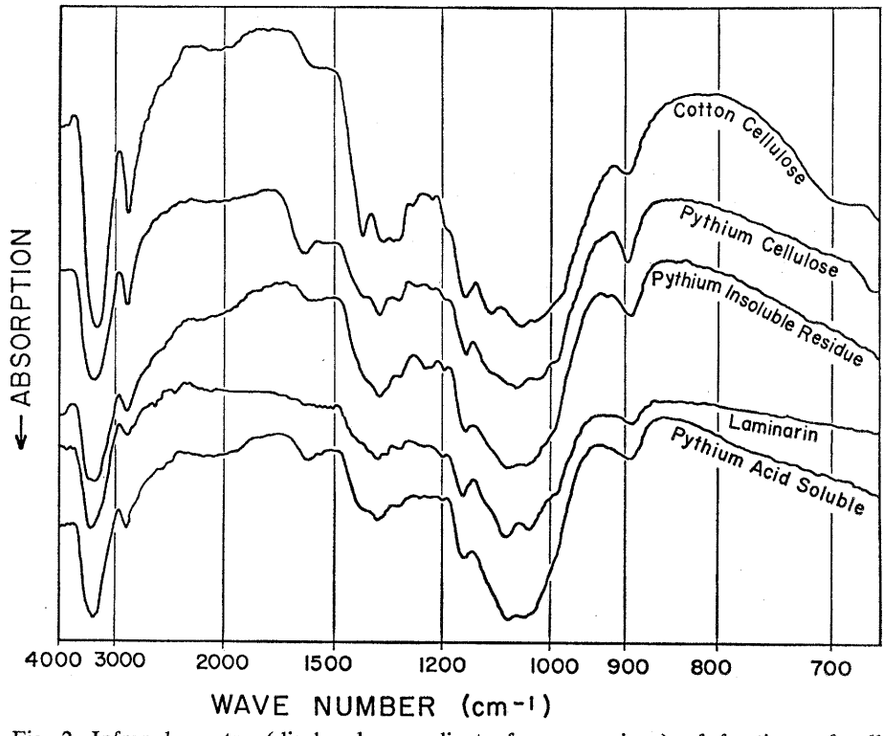

Fig. 2. Infrared spectra (displaced on ordinate for comparison) of fractions of cell walls from *Pythium* and of reference polysaccharides. The spectra labeled "*Pythium* acid soluble," "*Pythium* insoluble residue," and "*Pythium* cellulose," correspond, respectively, to fractions I, III, and II in the text.

from fungal noncellulosic glucans were similar to the spectrum of laminarin; an absorption at 1072 cm^{-1} occurs in laminarin and both fungal glucans (Fig. 2). The second principal absorption appears at 1042 cm^{-1} in the *Pythium* spectra, and the corresponding laminarin absorption occurs at 1040 cm^{-1}. We do not suggest that these observations alone provide a basis for distinguishing between different types of cell-wall polysaccharides, but they do constitute corroborative evidence for glucan heterogeneity in oomycete walls. Moreover, it should not be inferred that there is unqualified similarity in chemical constitution between laminarin and the fungal glucans, since mannitol is a known constituent of the former (*17*) and apparently is absent in the latter (*6*).

Our investigation, along with that of others (*4, 5*), therefore, reveals a significant coherence among selected Oomycetes with respect to the structure of their cell walls. The walls of other Oomycetes in the orders Leptomitales and Lagenidiales have not been investigated in detail in any instance. Since criteria based on morphology have led to the belief that members of these little-investigated taxa are closely allied with the fungi that we and others have examined, one could predict features of the composition of cell walls in these other groups. On the other hand, more complete quantitative investigations (*5–7*), employing mechanically isolated walls, show that studies such as ours do not demonstrate all the salient features of cell-wall structure. Therefore, investigations of the structure of cell walls in the Leptomitales and Lagenidiales are clearly warranted.

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9. We used chemically isolated walls in the present study for convenience. Mechanically isolated walls of *Atkinsiella* and *Pythium* (see *6* and *7*) yielded polysaccharide fractions with the same properties as fractions obtained from chemically isolated walls.
10. We thank W. Z. Hassid for a sample of authentic laminaribiose, H. J. Potgieter for a sample of β-(1→3)-glucan glucanohydrolase, R. B. Park for giving us access to equipment for infrared analyses, and B. J. D. Meeuse for a generous supply of laminarin.
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13. With *Pythium*, 70 percent of the initial wall sample was recovered. If some manipulative losses and the fact that degradation in Schweitzer's reagent affects both cellulose and noncellulosic glucans are taken into consideration, our conclusions are not in jeopardy.
14. Number 3.2.1.39 in *Enzyme Nomenclature, Recommendations of the International Union of Biochemistry* (Elsevier, New York, 1965); commonly this enzyme has been referred to as β-(1→3)-glucanase [for example, H. J. Potgieter and M. Alexander, *Can. J. Microbiol.* **11**, 122 (1965)] and, as indicated by Chesters and Bull (see *15*), inappropriately as laminarinase.
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Iodide Transport: Inhibition by Agents Reacting at the Membrane

Abstract. *Accumulation of iodide by thyroid tissue is inhibited by two phospholipase A-free proteins from cobra venom, filipin, crude phospholipase C, and lysolecithin. The venom proteins decrease K^+ in tissue but do not significantly affect incorporation of phosphorus-32 into phospholipid or stimulation of this process by thyrotropin. However, filipin and crude phospholipase C, like thyrotropin, do increase phospholipid formation.*

Phospholipids may play an important role in transport processes across cell membranes. In the thyroid, a possible carrier role of phospholipid (*1*) has been proposed, and sensitivity of the synthesis of phospholipid to such membrane-active reagents as digitoxin (*2*) and thyrotropin (TSH) (*3*) has been demonstrated. We therefore investigated the action of several substances, known to affect membrane phospholipids, on I^- transport into beef thyroid slices. Brief preincubation with crude venom from the cobra (*Naja naja*) as a source of phospholipase A, crude phospholipase C (from *Clostridium perfringens*), lysolecithin, and filipin—a polyene antibiotic—all blocked the subsequent accumulation of $^{131}I^-$ by thyroid slices. Column chromatography of cobra venom showed that the inhibiting activity was independent of phospholipase A but was associated with two distinct, late-eluting protein fractions. The effects of these proteins and the above agents on accumulation of I^-, incorporation of ^{32}P into lipid, and concentration of K^+ in tissue, have been compared.

Phospholipase A activity was determined by titration according to the method of Rodbell (*4*), but at a *p*H of 7.7 and a temperature of 24°C. Accumulation of iodide in beef thyroid slices was measured as previously described (*5*); formation of lipid-^{32}P and the effect of TSH (20 units/mg) were determined according to Kögl and van Deenen (*3*). Tissue cations were determined by flame photometry after heating the tissue in 1.0*M* H_2SO_4 for 15 minutes at 100°C. Fractionation of lyophilized *N. naja* venom on carboxymethyl cellulose was performed by a modification of the method of Yang *et al.* (*6*). Rechromatography of the concentrated eluates was carried out on the same column and yielded two active fractions which we labeled proteins A and B.

Slices were preincubated with the inhibitors in Krebs-Ringer phosphate buffer at 37°C for 15 to 30 minutes. In some experiments 3 percent albumin and additional Ca^{++} were present. Filipin was added in dimethyl sulfoxide. Inhibitors were removed by decanting, and the slices were rinsed twice with 5 to 10 ml of 0.154*M* NaCl and once with 5 to 10 ml of Ringer-phosphate medium; they were then incubated for 15 minutes in Ringer-phosphate medium to complete the wash. All subsequent tests were carried out on such slices.

Except for purified phospholipase A, all the compounds tested interfered with accumulation of iodide by thyroid slices (Table 1). Proteins A and B were approximately two to four times as potent as crude venom but contained < 0.1 percent of the phospholipase ac-