Cell wall architecture of the fission yeast *Schizosaccharomyces pombe*[1]

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Summary. β-Glucan and α-galactomannan were detected by immunocytochemical techniques in *S. pombe*. Except in the cell plate, both polysaccharides were found to be interwoven in the cell wall. In the cell plate, α-galactomannan was associated with the secondary septa, β-glucan with the primary septum. On the cell surface, in exact opposition to α-galactomannan, β-glucan was detected only on walls generated by fission but not on those growing by extension. These results indicate that β-glucan is directly implicated in the fission process.

Key words. α-Galactomannan; β-glucan; immunoelectron microscopy; *Ricinus communis* lectin; *Schizosaccharomyces pombe*, yeast cell wall.

The microarchitecture of yeast cell walls is largely hypothetical[2] because a) the bulk of the components appear to be structurally amorphous, b) the walls have a low electron density, c) the polymers presumably interweave to a considerable degree and d) specific immunocytochemical methods are lacking for detecting β-glucan, the main cell wall constituent and the most important structural polysaccharide since it confers rigidity, tensile strength and shape to the cell. Indeed β-glucan is not antigenic and no lectin is known to react exclusively with it.

This fission yeast *Schizosaccharomyces pombe* has been the subject of extensive research efforts concerning its cell cycle[3,4], morphogenesis[5] and genetics[6]. More recently, advances in molecular biology have led to the cloning and sequencing of *S. pombe* genes[7,8] as well as the establishment of a *S. pombe* transformation system[9]. The fact that *S. pombe* can serve as a host organism for the cloning and expression of foreign genes[10] has significantly augmented the interest and potential usefulness of this microorganism. The further development of *S. pombe* as a tool in molecular biology will depend on a detailed understanding of the processes governing the cell division cycle and the structure of the cell wall as a function of the cell cycle. *S. pombe* cell walls contain branched (1→3)-β-glucan (46–54%), linear (1→3)-α-glucan (18–28%) and α-galactomannan (9–14%). The galactomannan consists of a (1→6)-linked backbone with (1→2)-linked mannose side chains having a terminal galactose residue[11,12]. Unlike the budding yeast *Saccharomyces cerevisiae*, *S. pombe* does not contain chitin[11].

The purpose of this report is to describe the cell surface and also the subcellular localization of β-glucan of *S. pombe* by an immunocytochemical technique using antibodies raised against laminaribiose (3-O-β-D-glucopyranosyl-D-glucose) conjugated to edestin. We have also extended previous observations[13] in the detection of cell surface and intracellular α-galactomannan and related its localization and distribution to that of β-glucan, the major cell wall component.

Material and methods. Laminaribiose, derivatized with β-(p-aminophenyl) ethylamine was coupled to edestin via its diazonium salt[14]. Derivatized edestin, containing 29 moles of hapten per mole of protein, was used to immunize rabbits. Derivatized edestin (0.4 mg) was dissolved in 0.5 ml saline, pH 10 (NaOH) and injected with 0.5 ml complete Freund's adjuvant in a rabbit, intracutaneously at two sites. After 2 weeks, the animal received at two sites a s.c. injection. The intracutaneous injection was repeated after two further weeks. 2 months after the beginning of the immunization schedule, a blood sample was taken for the radioimmunoassay. The antiserum (25 µl) diluted 1:20 in 0.15 M NaCl – 0.02 M Tris, pH 7.4 (TBS) containing 1% bovine serum albumin was added to serial dilutions of inhibitor (25 µl) in the buffer. After 1 h *Saccharomyces rouxii* NRRL Y-2547 cells (25 µl, $A_{650nm} = 12$) were added; the yeast was grown in liquid medium containing yeast extract (3%), peptone (0.5%), malt (0.3%) and glucose (3%), thoroughly washed with saline and fixed for 15 h at room temperature in saline containing 4% glutaraldehyde. After 3 h incubation, the yeast cells were washed three times with the buffer and incubated with ^{125}I-protein A (25 µl, 2.3×10^4 cpm). The cells were washed three times with the buffer and the radioactivity counted. The values were corrected for blank experiments using the preimmune serum. The experiment was made in triplicate and the quantity of sugars to achieve 50% inhibition was determined from the inhibition curves.

Cells of *Schizosaccharomyces pombe* Lindner CBS 251, grown in 1% yeast extract, 6% glucose, were fixed overnight at room temperature in saline containing 2% glutaraldehyde and embedded in Lowicryl K4M, a highly hydrophilic cross-linked acrylate-based resin[15]. Gold colloids (Au_{12} to Au_{56}) were prepared and labeled with protein A according to standard procedures[16,17]. The subscript refers to the average diameter of the particle in nm when measured from electron micrographs. For localizing β-glucan by transmission electron microscopy (TEM), thin sections were a) floated on TBS containing 1% bovine serum albumin (2 h); b) on the anti-laminaribiose antiserum diluted 1:20 in the same buffer (1 h); c) rinsed three times on the buffer; d) floated on protein A-Au_{13} diluted in TBS containing 0.5 mg/ml Carbowax 20-M (Union Carbide) ($A_{520nm} = 5$); e) rinsed three times on the same buffer and three times on water. The thin sections were examined with a Philips EM 300. For SEM, the glutaraldehyde-fixed yeast cells (100 µl, $A_{650nm} = 6$) were a) incubated in the anti-laminaribiose antiserum diluted 1:10 in TBS containing 1% bovine serum albumin; b) washed in TBS containing 0.5 mg/ml Carbowax-20-M; c) incubated overnight in 150 µl of the same buffer containing 25 µl protein A-Au_{47} ($A_{540nm} = 43$). The cells were examined after critical point drying in a Philips SEM 505 without metal coating. Controls for specificity included a) the replacement of the diluted antiserum by normal rabbit serum; b) incubation with the diluted antiserum containing 3 mg/ml laminarin and c) incubation of the sections of the cells with gold-labeled protein A alone. For localizing α-galactomannan by SEM, *Ricinus communis* lectin 120 (RCA_I) was adsorbed onto Au_{56} particles as described previously[16]. A cell suspension ($A_{650nm} = 5$, 100 µl) was incubated for 4 h with 0.5 ml RCA_I-Au_{56} in TBS containing 0.5% Carbowax 20-M. The cells were then dehydrated and examined as described elsewhere[16]. For TEM, thin sections were floated on RCA_I-Au_{12} ($A_{520nm} = 4$) for 2 h. For control experiments, the markers contained 0.2 M lactose.

Inhibition of anti-laminaribiose antibody binding to *Saccharomyces rouxii* cells by sugars

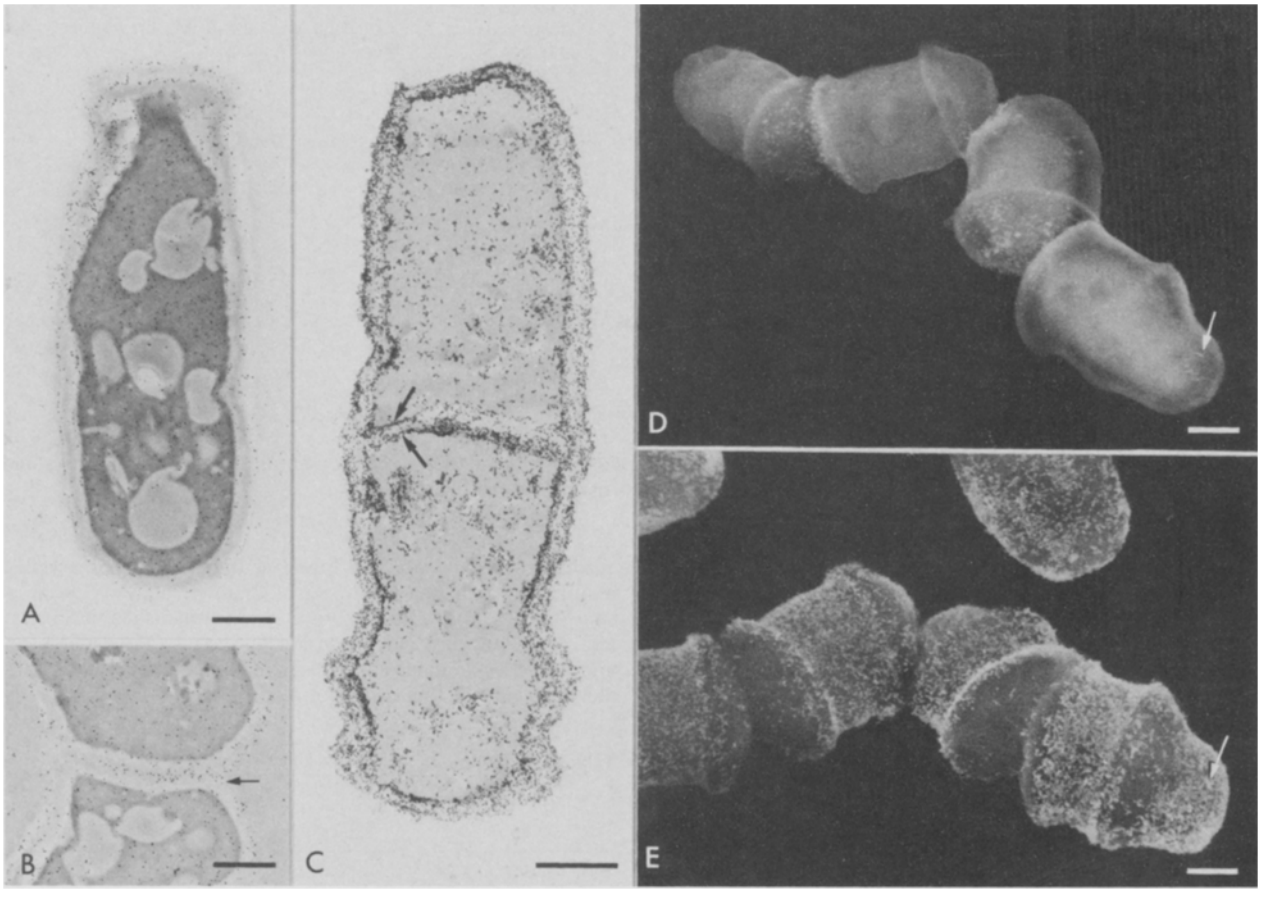
Inhibitor	Quantity required for 50% inhibition (nmoles)	Inhibition with 2.5 mM (%)
Laminarin	0.75*	100
p-Nitrophenyl-β-D-glucopyranoside	400	80
p-Nitrophenyl-α-D-glucopyranoside	< 2500	25
Methyl-β-D-glucopyranoside	< 2500	36
Methyl-α-D-glucopyranoside	< 2500	25

*100% inhibition was observed in the presence of 6 nmoles laminarin (as glucose).

Results and discussion. As judged from the table, the anti-laminaribiose antiserum possessed a high degree of specificity directed against the terminal nonreducing β-laminaribiosyl end group as found with a laminaribiose-azo bovine serum albumin conjugate obtained by Allen et al.[18]. Although the ultrastructure of the yeast was not very well preserved, due to a limited penetration of the resin, specific staining of β-glucan was obtained on thin sections throughout the cell wall and in the newly-forming cell plate. No staining was observed when the cells were embedded in the Spurr medium[13]. In the cell wall (fig. A), marking was denser in the more electron-lucent area (near the periphery of the cell and close to the plasmalemma). In the cell plate (fig. B), marking was denser in the area of the primary septum. Specific immunoreactive sites were also localized in the cytoplasm (fig. A). In the controls, nonspecific staining was practically absent. The presence of β-glucan was observed by SEM only on walls newly established by fission (fig. D). When stereopairs were examined most of the marking appeared on a network of filaments. Marking was almost absent in the growing new end of the yeast and totally absent in the remaining cell walls growing by extension. In control experiments, no marking was observed.

α-Galactomannan was detected on thin sections by a direct method using gold particles labeled with *Ricinus communis* 120 lectin which is specific for terminal galactose residues (fig. C). The marking density observed was much higher than that reported previously by an indirect method[13]. α-Galactomannan was present throughout the cell wall with a higher density at the periphery and near the plasmalemma. In the cell plate, α-galactomannan appeared to be localized mostly in the area corresponding to the secondary septa. Contrary to earlier observation where a different embedding resin was used[13], marking was also observed in the cytoplasm (fig. C). In exact opposition to β-glucan (fig. D), α-galactomannan was found by SEM on all cell walls growing by extension (fig. E). Marking extended some distance from the wall in a hair-like fashion thus supporting the suggestion that the hairs observed by others[19] contain α-galactomannan. Similar observations were made both by TEM and SEM using concanavalin A, a lectin binding to α-galactomannan but not to β-glucan (figures not shown).

The molecular aspects of cell wall synthesis and cell wall growth which are intimately related to the cell division cycle are relatively well known in yeast[2]. A model for extensive growth of *S. pombe* walls and for cell division has been proposed[5] based on an integration of enzymatic and autoradiographic studies, morphometric analyses and electron microscopic observations. Briefly, the extension process seems to involve endoglucanase activity very closely coordinated with β-glucan synthetase activity. Cell division consists of the formation of a primary septum and of secondary septa both sides of the primary septum growing centripetally. After completion of the cell plate, fission occurs by the progressive dissolution of the primary septal template presumably through the action of β-glucanases. Our own observations fit well into this scheme: α-galactomannan is exposed at the external surface of the wall growing by extension, and overlays β-glucan. Both polysaccharides are present throughout the wall and are interwoven to a considerable extent



Immunogold marking of β-glucan on *S. pombe* thin section showing positive reaction in the cell wall, the cytoplasm *(A)* and the primary septum of the cell plate (*B*, arrow). *D* Surface marking of β-glucan appearing only on wall newly generated by fission. Marking of the growing end is very weak (arrow). *C* Marking of α-galactomannan on thin section showing positive reaction in the cell wall, the secondary septa of the cell plate (arrows) and the cytoplasm. *E* Surface marking of α-galactomannan. Marking is in exact opposition to that found for β-glucan being present only on old and new (arrow) walls growing or having grown by extension. The bar represents 1 µm.

except in the cell plate. The primary septum contains β-glucan but little or no α-galactomannan. This feature appears to be directly implicated in the subsequent fission process as suggested above. The small amount of cell surface β-glucan that is still detected could be the remains of incompletely digested primary septa (fig. D).

In the budding yeast *S. cerevisiae*, it has been hypothesized that α-mannan is elaborated intracellularly and β-glucan only on the plasmalemma[2]. Our evidence indicates that in *S. pombe* both α-galactomannan and β-glucan or immunoreactive precursors are synthesized intracellularly.

Using a similar experimental approach with the budding yeasts *S. cerevisiae* and *Saccharomyces rouxii*, α-mannan and β-glucan were found to intersperse to a considerable degree in the cell wall, the septum and the bud scar. In analogy with *S. pombe*, β-glucan was overlaid by α-mannan over the whole cell surface including the bud scars where most of the chitin was present (unpublished observations).

By specifically locating β-glucan and other polysaccharides[20,21], our experiments provide a basis for a better understanding of the architecture of yeast cell walls. Although different layers have been observed in yeast cell walls using fixatives and nonspecific reagents[22], our results rather indicate areas enriched with different polysaccharides leading to a highly cohesive structure for the whole depth of the wall as suggested by Cabib and Roberts[2].

Finally, this is another example of the multiple use of the protein A-gold technique which allows the intracellular[23] as well as the cell surface localization[24] of antigens by TEM and SEM, respectively.

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2 Cabib, E., and Roberts, R., A. Rev. Biochem. *51* (1982) 763.
3 Mitchison, J.M., Meth. Cell Physiol. *4* (1970) 131.
4 Beach, D., Durkacz, B., and Nurse, P., Nature *300* (1982) 706.
5 Johnson, B.F., Calleja, G.B., and Yoo, B.Y., in: Eucaryotic Microbes as Model Developmental Systems, p.212. Eds D.H. O'Day and P.A. Horgen. Marcel Dekker, New York 1977.
6 Egel, R., Kohli, J., Thuriaux, P., and Wolf, K., A. Dev. Genet. *14* (1980) 77.
7 Mao, J., Schmidt, O., and Söll, D., Cell *21* (1980) 509.
8 Russell, P.R., and Hall, B.D., Molec. Cell Biol. *2* (1982) 106.
9 Beach, D., and Nurse, P., Nature *290* (1981) 140.
10 Russell, P.R., and Hall, B.D., J. biol. Chem. *258* (1983) 143.
11 Bush, D.A., Horisberger, M., Horman, I., and Würsch, P., J. gen. Microbiol. *81* (1974) 199.
12 Manners, D.J., and Meyer, M.T., Carbohydr. Res. *57* (1977) 189.
13 Horisberger, M., Vonlanthen, M., and Rosset, J., Archs Microbiol. *119* (1978) 107.
14 Zopf, D.A., Tsai, C.M., and Ginsburg, V., Meth. Enzym. *50C* (1978) 163.
15 Kellenberger, E., Carlemalm, E., Villiger, W., Roth, J., and Garavito, R.M., in: Low denaturation embedding for electron microscopy of thin sections. Ed. Chemische Werke Lowi GmbH, P.O. Box, D-8264 Waldkraiburg, Federal Republic of Germany.
16 Horisberger, M., and Rosset, J., J. Histochem. Cytochem. *25* (1977) 1074.
17 Horisberger, M., and Tacchini-Vonlanthen, M., Histochemistry *77* (1983) 37.
18 Allen, P.Z., Goldstein, I.J., and Iyer, R.N., Immunochemistry *7* (1970) 567.
19 Walther, P., Müller, M., and Schweingruber, M.E., Archs Microbiol. *137* (1984) 128.
20 Horisberger, M., and Rosset, J., Experientia *32* (1976) 998.
21 Horisberger, M., and Vonlanthen, M., Archs Microbiol. *115* (1977) 1.
22 Poulain, D., Tronchin, G., Dubremetz, J.F., and Biguet, J., Ann. Microbiol. (Inst. Past.) *129A* (1978) 141.
23 Roth, J., Bendayan, M., and Orci, L., J. Histochem. Cytochem. *26* (1978) 1074.
24 Horisberger, M., and Tacchini-Vonlanthen, M., in: Lectins, vol.3, p.189. Eds T. C. Bog-Hansen and G.A. Spengler. Walter de Gruyter, Berlin 1983.

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A new chromosome race of *Sorex araneus* L. from Northern Poland

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Summary. G-band patterns were studied in the chromosomes of two Polish populations of the common shrew. Two chromosome races were established in Poland, differing by various combinations of chromosome arms.
Key words. Shrew; *Sorex araneus;* chromosome race.

The two chromosome races of *Sorex araneus* L. originally described by Meylan[2] have proved now to be separate sibling species. The Western European monomorphic race of the shrew was identified as *Sorex coronatus* Millet, 1828[3]. The whole range of *Sorex araneus* L. (Central and Eastern Europe together with the British Isles and Scandinavia, and Siberia up to the River Yenisei and Lake Baikal) is inhabited by the shrew populations exhibiting a polymorphic system of Robertsonian translocations. Six (pairs 3–8) of the nine pairs of autosomes in *S. araneus* are subject to polymorphism. In different parts of the range these pairs are composed of different arms. It has been recommended[4] that the term 'chromosome races' should be applied to shrews with varying combinations of arms in pairs 3–8. In this respect three chromosome races of *S. araneus* have been distinguished in Scandinavia[4,5], three races in England[6], two further chromosome races in Continental Europe; one in the Ulm region (Baden-Württemberg)[7] and a second in the Białowieża Primeval Forest (Northeastern Poland)[4,8]. This same chromosome race was also found in the River Biebrza valley about 150 km to the northwest of Białowieża[9]. Two chromosome races of *S. araneus*[10,11] were also distinguished in Siberia, and one of them, occurring in lowland areas (Novosibirsk region), has a combination of arms in four pairs of autosomes identical with the 'Białowieża' race [12].

Table 1. Material used for chromosome studies

Locality	2N	2Na	Sex	Designation of morphs	No. of animals studied
Wytyczno	23	20	♂♂	A_8–	2
near Włodawa	23	21	♀	A_8H_5–	1
	24	21	♂	A_8H_5–	1
Drużno Lake	27	25	♀♀	$A_8A_7A_6H_5$–	3
near Elbląg	28	25	♂♂	$A_8A_7A_6H_5$–	4
	28	26	♀	$A_8A_7A_6A_5$–	1