

## Complex Carbohydrates in the Cyst Wall of *Histiculus similis*

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A cytochemical and structural study of the cyst wall of *Histiculus similis* has been carried out. Application of cytochemical methods for complex carbohydrates indicated that the mesocyst is rich in sulphated glycosaminoglycans. The endocyst and granular layer contained neutral and sulphated glycoproteins, respectively.

### INTRODUCTION

The ultrastructure of the cyst wall has been studied in several hypotrichous ciliates. In *Oxytrichafallax* (Grimes, 1973), *Pleurotricha* sp. (Matsusaka, 1976), *Stylonychia mytilus* (Walker *et al.*, 1975), *Gastrostyla steinii* (Walker *et al.*, 1980) and *Laurentiella acuminata* (Gutierrez *et al.*, 1980), the cyst wall is composed of four layers called, from outside to inside: the ectocyst, mesocyst, endocyst and granular layers. In these ciliates a resorption of cilia and kinetosomes occurs during encystment, and the mature cyst is termed kinetosome resorbing. *Diophrys scutum* (Walker & Maugel, 1980) has a three layered cyst wall and the mature cyst is non kinetosome resorbing.

In the present work a structural and cytochemical study of the cyst wall in *Histiculus similis* was carried out by means of light microscopy and transmission electron microscopy.

### METHODS

*Organism and culture conditions.* *Histiculus similis* (a hypotrichous ciliate) was cultured at  $20 \pm 1^\circ\text{C}$  in Pringsheim's solution and fed on *ChlorogoniIm* sp. (Ammermann, 1974). The encystment was induced by placing the cell in Pringsheim's solution deprived of nutrients.

*Electron microscopy.* The cysts were fixed in 2% (v/v) glutaraldehyde in 0.1 M-phosphate buffer pH 7.0 and then postfixed in 1% (w/v) osmium tetroxide in the same buffer and embedded in Epon. Thin sections were contrast stained with uranyl acetate and lead citrate. Thick sections were stained with toluidine blue (Trump *et al.*, 1961).

*Light microscopic cytochemistry.* Thick sections of cysts fixed in 2% glutaraldehyde in 0.1 M-phosphate buffer pH 7.0 and embedded in Epon were subjected to the following treatments: the periodic acid-Schiff test for neutral mucosubstances (McManus, 1946); alcian blue 8GX pH 2.5 (Mowry, 1956); and 0.05% (w/v) alcian blue pH 5.8 in the presence of graded concentrations of  $\text{Mg}^{2+}$  for the identification and differentiation of the three negatively charged acidic groups of mucins ( $-\text{COOH}$ ,  $-\text{PO}_3\text{H}$  and  $-\text{SO}_3\text{H}$ ) (Scott & Dorling, 1965).

*Ultrastructural cytochemistry.* Thin sections of cysts fixed in buffered 2% glutaraldehyde and embedded in Epon were mounted on gold grids and stained by the periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-SP) method of Thiery (1967). Oxidized control sections were treated either with silver proteinate without previous thiocarbohydrazide treatment or with thiocarbohydrazide alone. Other sections were floated on 1% (w/v) phosphotungstic acid in 1 M-HCl for 40 to 60 min according to Fléchon (1970) and washed with 1.25 M-HCl.

Alternatively, cysts fixed in buffered 2% glutaraldehyde were stained with dialyzed iron (DI; Wetzel *et al.*, 1966) or high iron diamide (HID; Spicer *et al.*, 1978), dehydrated and embedded in Epon.

### RESULTS

A fine structural analysis of the cyst wall of *H. similis* revealed striking similarities to other kinetosome resorbing cysts. The cyst wall consisted of four layers. The outermost layer (the ecto-

*Abbreviations:* DI, dialysed iron; HID, high iron diamide; PA-TCH-SP method, periodic acid-thiocarbohydrazide-silver proteinate method.

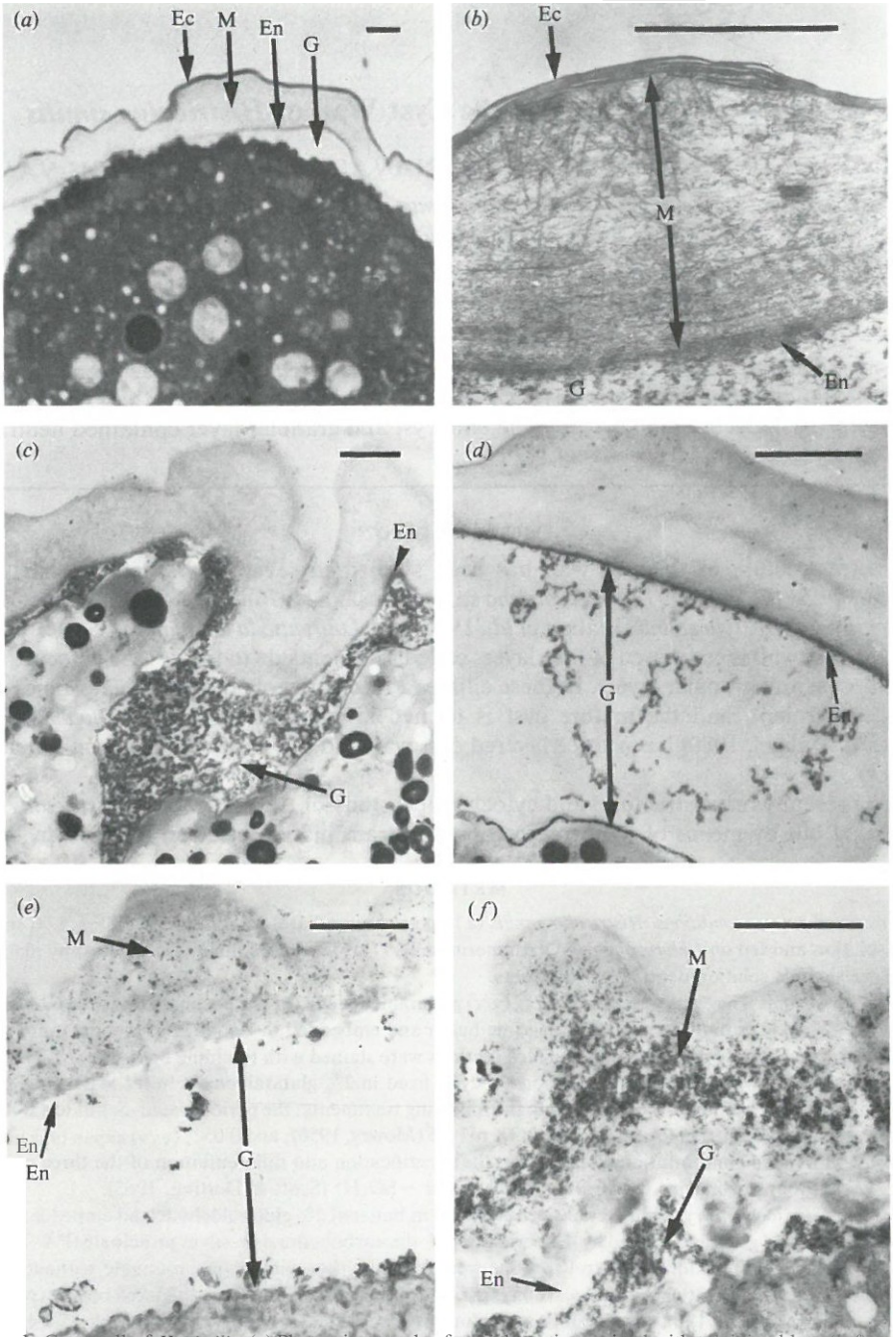


Fig. 1. Cyst wall of *H. similis*. (a) Photomicrograph of a thick section stained with toluidine blue. (b-f) Electron micrographs of ultrathin sections: (b) resting cyst wall; (c) cyst treated by the PA-TCH-SP method; (d) cyst wall stained with phosphotungstic acid at low pH; (e) DI staining; (f) HID staining. Ec, ectocyst; M, mesocyst; En, endocyst; G, granular layer. The bar markers represent 1 μm.

cyst) presented an electron dense lamellar structure forming concentric plates, while the second layer (the mesocyst) was formed by a stratum of interwoven fibres. The endocyst was an electron dense fibrillar thin layer and the innermost layer (granular layer) had an extremely variable thickness and appeared to be composed of a granulated and electron dense material. (Fig. 1a,b).

Both the endocyst and granular layers had an intense periodic acid-Schiff reaction which did not disappear after digestion with  $\alpha$ -amylase. The mesocyst exhibited alcianophilia at pH 2.5 that was completely abolished after methylation at 60 °C; this alcianophilia was reversed completely after a methylation-saponification-alcian blue (pH 2.5) sequence of treatment. The staining with alcian blue at pH 5.8 in the presence of 0.1 M-MgCl<sub>2</sub> gave intense staining of the mesocyst; this intense reaction also occurred in 0.5 M-MgCl<sub>2</sub> but decreased in 0.8 M- and 1 M-MgCl<sub>2</sub>. The intense alcianophilia of the mesocyst could mask any reaction of the endocyst with alcian blue; therefore, the reactivity of the endocyst towards cationic reagents was not determined by light microscopy. The ectocyst appeared unreactive in techniques used for characterizing glycoconjugates.

In agreement with the above results, the PA-TCH-SP technique revealed the existence of glycoconjugates in both endocyst and granular layers (Fig. 1e). Treatment with phosphotungstic acid at low pH produced similar results: precipitates of phosphotungstic acid appeared in the endocyst and granular layer (Fig. 1d). Finally, acidic mucosubstances were tested by electron microscopy using the DI and HID procedures. Both the mesocyst and granular layers showed DI and HID affinity (Fig. 1e, J).

#### DISCUSSION

The characteristics of complex carbohydrates determined at the electron microscopic level agreed with those found by light microscopic cytochemistry. According to Spicer *et al.* (1979), mucosubstances which possess PA-TCH-SP reactivity but which lack DI and HID affinity are neutral glycoproteins, while mucosubstances which are DI or HID positive but which lack PA-TCH-SP reactivity can be regarded as glycosaminoglycans. Substances which possess PA-TCH-SP reactivity and also DI and/or HID affinity are acidic glycoproteins. These can be separated further into sulphated glycoconjugates which have affinity for both DI and HID, and carboxylated glycoproteins which have affinity for DI but not for HID.

Cytochemical results obtained from the cyst wall of *H. similis* indicate that the mesocyst is rich in sulphated glycosaminoglycans, the endocyst contains neutral glycoproteins and the granular layer contains sulphated glycoproteins. The ectocyst was unreactive in the techniques used for characterizing glycoconjugates. However, the ectocyst precursors are PA-TCH-SP positive in the hypotrichous ciliate *Laurentiella acuminata* (J. C. Gutierrez & A. Torres, unpublished data). The lack of PA-TCH-SP staining in the ectocyst might be due to a blockage of vicinyl-glycol groups occurring during the encystment.

Sulphated glycosaminoglycans in the mesocyst form a hydrophilic region that would play an important role in the transport of solutes and in the retention of water. Acidic glycoproteins of the granular layer, in contact with the cytoplasm of the cyst, are good candidates for mediating cyst-environment interactions.

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