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Studies of the Cell Surface of *Paramecium*

CILIARY MEMBRANE PROTEINS AND IMMOBILIZATION ANTIGENS

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1. We have developed a procedure to isolate the ciliary membranes of *Paramecium* and have analysed the membrane proteins by electrophoresis on polyacrylamide gels containing either Triton X-100 or sodium dodecyl sulphate. The electrophoretic pattern on gels containing sodium dodecyl sulphate showed 12–15 minor bands of mol.wt. 25000–150000 and one major band of mol.wt. 200000–300000 that contained approximately three-quarters of the total membrane protein. 2. We present evidence that the major membrane protein is related to, but not identical with, the immobilization antigen (i-antigen), which is a large (250000mol.wt.), soluble, surface protein of *Paramecium*. The similarity of the i-antigen and the major membrane protein was shown by immunodiffusion and by the electrophoretic mobilities in sodium dodecyl sulphate of these two proteins from *Paramecium* of serotypes A and B. The non-identity of these two proteins was shown by their different electrophoretic mobilities on Triton X-100-containing gels and their different solubilities. 3. We propose that the major membrane protein and the i-antigen have a precursor–product relationship.

Many biological membranes have been analysed for their protein compositions. In a few membranes, such as those of erythrocytes, many of the proteins have been purified and functionally identified (Guidotti, 1972; Steck, 1974).

Few electrically excitable membranes have been studied, however, because of the difficulty of obtaining large amounts of a single type of membrane uncontaminated by myelin and other membranes. The electric tissues of *Electrophorus* (Klett *et al.*, 1973) and *Torpedo* (Raftery, 1973; Eldefrawi & Eldefrawi, 1972) have proved to be valuable sources of the acetylcholine receptor protein, and the garfish olfactory membrane has been studied (Grefrath & Reynolds, 1973) and used as a source of tetrodotoxin-binding protein (Benzer & Raftery, 1972).

A novel source of excitable membrane is the ciliated protozoan, *Paramecium aurelia*. The surface membrane of *Paramecium* is capable of generating receptor potential and action potentials, which govern responses to various stimuli (Eckert, 1972). Since a Ca^{2+} action potential is generated, it should be possible to isolate a voltage-dependent Ca^{2+}-gate protein from *Paramecium*.

The electrophysiology of the excitable membrane of *Paramecium* has been extensively studied, and the relationship between the behaviour of *Paramecium* and its membrane electrophysiology is well understood (Eckert, 1972). In addition, many behavioural mutants have been isolated in *Paramecium aurelia*. These behavioural mutants show abnormal electrophysiological patterns, indicating defects in their surface membranes (Kung *et al.*, 1975; Kung, 1971; Satow & Kung, 1974). Therefore a detailed study of the surface membrane of *Paramecium* should contribute to the understanding of the molecular mechanism of behaviour and membrane excitability.

No biochemical studies have been done on the surface membrane of *Paramecium*. In this paper, we present a method for the isolation of surface membranes from *Paramecium* and an analysis of the protein composition of these membranes.

Although the surface membrane of *Paramecium* has not been studied, much work has been done on the surface coat of *Paramecium*. Most micro-organisms have surface layers external to their plasma membranes. *Escherichia coli*, for example, has two outer layers, the peptidoglycan layer and the lipopolysaccharide layer; and the surface of *Acanthamoeba* is covered by a lipophosphoglycan (Korn *et al.*, 1974). The surface of *Paramecium* is covered with a layer of large soluble glycoproteins of mol.wt. 250000–300000 (Reisner *et al.*, 1969*a*; Steers, 1962). These surface proteins of *Paramecium* are called 'immobilization antigens' (i-antigens), because paramecia are immobilized when incubated with antiserum against them (Preer, 1969). The composition of the i-antigens is unusual, since they contain

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10% cysteine, with no free thiol groups, and 25% serine+threonine (Reisner *et al.*, 1969*b*; Steers, 1962; Jones, 1965).

Although the function of the i-antigens is not yet clear, the control of their synthesis is of great interest to cell biologists and geneticists. A paramecium contains genes for over 12 different i-antigens, but only one i-antigen is present on the cell surface at a time. Changes in environmental conditions and various other stimuli will cause a cell to synthesize a different i-antigen (Preer, 1969). Thus the i-antigens are useful model systems for studying gene regulation and differentiation.

We have found that, in addition to the i-antigen, there is a related protein in the surface membrane of *Paramecium* that is controlled co-ordinately with the i-antigen. We propose that there is a precursor–product relationship between these two proteins.

Materials and Methods

Materials

Polyacrylamide-gel reagents were Electrophoresis Purity Grade from Bio-Rad Laboratories, Richmond, Calif., U.S.A. Sodium dodecyl sulphate was either supplied by Bio-Rad Laboratories or recrystallized from 95% (v/v) ethanol (Hansma, 1974). Triton X-100 was supplied by Sigma Chemical Co., St. Louis, Mo., U.S.A. Electrophoresis was carried out in a Hoeffer electrophoresis chamber (Bio-Rad model 150). Cerophyl (dehydrated cereal grass leaves) was obtained from Cerophyl Laboratories, Kansas City, Mo., U.S.A.

Cell cultures

Paramecium aurelia, syngen 4, of the following strains were used: wild type, 51s; and the 'pawn' mutants, d4-94 and d4-133. The pawns are behavioural mutants isolated from stock 51s (Kung *et al.*, 1975). The pawn mutants were used because populations of these paramecia often contained both serotype A cells and serotype B cells, although this does not appear to be a direct effect of the pawn mutation.

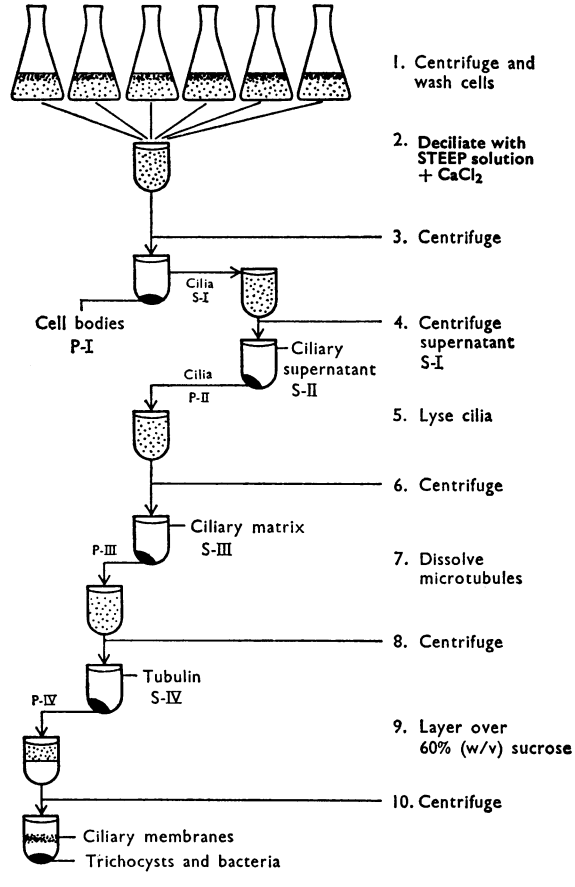
Cells were cultured in autoclaved Cerophyl medium inoculated with *Enterobacter aerogenes* by the procedure of Sonneborn (1950). The cells were harvested and concentrated by centrifugation for 2min at 350*g* in an oil-testing centrifuge (IEC model HN-S).

Isolation of ciliary membranes (see Scheme 1)

The concentrated cells were washed once by centrifugation in Dryl's (1959) salt solution. Cilia were detached and separated from the cell bodies by the method developed by Gibbons (1965) for *Tetrahymena* (Hansma, 1974). To detach the cilia, cells in Dryl's (1959) solution were mixed with 4vol. of STEEP solution [150mM-sucrose, 15mM-EDTA, 11% (v/v) ethanol, 30mM-KCl, adjusted to pH8.3 with HCl], and $CaCl_2$ was added to a final concentration of 10mM (Gibbons, 1965) (Scheme 1, step 2). The suspension was centrifuged (5min at 1500*g*, 4°C) to sediment the cell bodies (P-I, Scheme 1).

The cilia were then sedimented by centrifugation (15min at 12000*g*, 4°C), and the pellet (P-II) was lysed by shaking in a solution of 1mM-Tris–HCl–0.1mM-EDTA, pH8.3. The lysed cilia were centrifuged as described above, and the pellet (P-III) was suspended in 0.6M-KI to dissolve the microtubules (Gibbons, 1965). This suspension was re-centrifuged as described above. The resulting pellet (P-IV) contained ciliary membranes, trichocysts and bacteria, as determined by phase-contrast microscopy.

Pellet P-IV was suspended in 2ml of Tris buffer (10mM-Tris–HCl, pH7.8) (Witman *et al.*, 1972),



Scheme 1. *Procedure for the isolation of ciliary membranes from Paramecium*

For details of fractions see the text.

layered over 3ml of 60% (w/v) sucrose in Tris buffer and centrifuged in a preparative ultracentrifuge (Beckman L2-65B with SW-65 rotor; 1h, 65000*g*, 4°C). Ciliary membranes banded at the sucrose–buffer interface and were collected with a syringe.

Isolation of immobilization antigens

Immobilization (i) antigens were isolated by the method of Preer (1959). Cells of known serotype (see below) were extracted with a salt–ethanol solution (Preer, 1959). This extract was fractionated by acid precipitation at pH2. The supernatant was adjusted to pH7 and further fractionated by $(NH_4)_2SO_4$ precipitation. The i-antigens precipitated in the $(NH_4)_2SO_4$ interval of 35–61% saturation (23°C). The precipitate was redissolved and dialysed against a solution of 1mM-Tris–HCl–0.1mM-EDTA, pH8.3. The dialysed i-antigens were stored frozen.

Serotype determinations

The serotype of a *Paramecium* culture refers to the type of i-antigen on the cells. The serotypes of live cells were determined from the degree of immobilization of cells incubated for 2h at 28°C with various dilutions of different antisera (Sonneborn, 1950; Macindoe & Reisner, 1967). The rabbit antisera to paramecia of serotypes A, B, C and D were kindly supplied by Professor T. M. Sonneborn, Indiana University, Bloomington, Ind., U.S.A.

Immunodiffusion

Immobilization-antigen preparations and detergent-solubilized ciliary membranes were made to react with antisera by double immunodiffusion, by using standard procedures (Clausen, 1969). Slides were stained for protein with Amido Black.

Protein determination

The protein content of membranes and i-antigens was determined by a modified Folin–phenol method (Lowry *et al.*, 1951).

Electrophoresis in sodium dodecyl sulphate–polyacrylamide gels

Membranes, i-antigens and other samples were incubated for 1–2h at room temperature (23°C) with 1% (w/v) sodium dodecyl sulphate and 1% (v/v) mercaptoethanol in Tris–acetate buffer [40mM-Tris, 20mM-sodium acetate, 2mM-EDTA, adjusted to pH7.4 with acetic acid (Fairbanks *et al.*, 1971)]. Polyacrylamide gels were prepared as described by Fairbanks *et al.* (1971) with 0.1% sodium dodecyl sulphate and 3.4 or 5.6% (w/v) acrylamide. Electrophoresis was carried out for 2–2½h at room temperature, 6mA/gel. For further details see Hansma (1974).

Gels were stained for protein with Coomassie Blue (Fairbanks *et al.*, 1971) and scanned at 550nm in a Beckman Acta III spectrophotometer. Some gels were stained for carbohydrate with periodate–Schiff stain (Fairbanks *et al.*, 1971). Peptide molecular weights were calculated from the standard curve of peptides of known molecular weight. The standard peptide mixture was RNA polymerase from *E. coli*, purified by Mr. G. Bitter by the procedure of Burgess & Travers (1971). This protein contains subunits with mol.wts. of 165000, 155000, 95000 and 39000.

Electrophoresis in Triton X-100–polyacrylamide gels

Membranes, i-antigens and other samples were incubated in 1% (w/v) Triton X-100 at 37°C for 1–2h. Polyacrylamide gels were prepared as described by Davis (1964) and Shore & Shore (1968) with 5% (w/v) acrylamide, 0.33mg of *NN'*-methylenebisacrylamide/ml and 0.5% Triton X-100. Spacer and sample gels were prepared as described by Davis (1964) with 0.5% Triton X-100. Electrophoresis was carried out in Tris (3g/l)–glycine (14.4g/l) buffer, pH8.4, for 2½–3h at 3–4mA/gel. Gels were stained for protein with Coomassie Blue (Fairbanks *et al.*, 1971). For further details see Hansma (1974).

Results

Isolation of ciliary membranes

Deciliation is a process involving membrane fusion. Thus the cell bodies remain intact even after the cilia are removed, so that there is little or no contamination of the cilia by the other membranes of *Paramecium*. Gibbons (1965) reported that the isolated cilia of *Tetrahymena* were contaminated with only about 0.1% (by number) of mitochondria. *Paramecium* cilia are also contaminated by trichocysts and bacteria (Plate 1*a*), which are removed in the final stage of membrane isolation (Scheme 1, step 10). Trichocysts are needle-shaped protein filaments that are ejected from *Paramecium* in response to a variety of stimuli, including the deciliation procedure.

Ciliary membranes isolated by the procedure of Scheme 1 appear in the phase-contrast microscope as a clean preparation of vesicles of variable size, with diameters of 0.1–1 μm (Plate 1*b*). C. Omoto & D. Nelson (personal communication) have also shown by electron microscopy that the membranes contain little or no non-membrane material.

The homogeneity of the ciliary membrane fraction was further tested by layering the crude membrane

pellet P-IV (Scheme 1) over a step gradient of 30, 40, 50 and 60% (w/v) sucrose in Tris buffer. Three membrane fractions were present: one-half of the membrane protein banded on top of the 60% sucrose layer, three-tenths banded on the 50% sucrose layer, and two-tenths banded on the 40% sucrose layer. The three membrane fractions had very similar patterns on sodium dodecyl sulphate–polyacrylamide gels (Plate 2*a*). These results suggest that the ciliary membranes isolated by the procedure of Scheme 1 are reasonably homogeneous with respect to protein composition. Since the ciliary membranes did not band at a single position on the sucrose gradient, however, they must be heterogeneous with respect to size or density.

Ciliary membrane proteins

Plate 2(*b*) shows the electrophoretic pattern in sodium dodecyl sulphate of the ciliary membrane proteins of wild-type *Paramecium*, isolated by the procedure of Scheme 1. Some 12–15 fairly reproducible bands can be identified. The most striking feature of the membrane is the very large band of high molecular weight (250000–300000), which is approximately three-quarters of the total protein, on the basis of densitometer traces of gels stained for protein with Coomassie Blue. This band also stains for carbohydrate with periodate–Schiff stain.

In addition to the major protein band, there are 10–15 minor bands of mol.wt. 25000–150000. Most of the protein of these minor bands is present in two bands of mol.wt. 45000 and 50000. The 50000-mol.wt. band has the same mobility as the protein in fraction S-IV (Scheme 1), which is probably tubulin (Gibbons, 1965).

Gels stained with periodate–Schiff stain show a broad carbohydrate-containing band of mol.wt. 10000 or less, in addition to the 250000-mol.wt. band. This low-molecular-weight band is perhaps glycolipid, since it stains weakly, if at all, for protein. No other carbohydrate-containing bands were visible on gels containing up to 0.3mg of total membrane protein.

Immobilization antigens

The major membrane protein (mol.wt. 250000–300000; Plate 2*b*) has the same molecular weight as the immobilization antigens (i-antigens) that have been extensively studied by Reisner *et al.* (1969*a*,*b*), Preer (1959), Steers (1961, 1962) and Jones (1965) (reviewed by Preer, 1969).

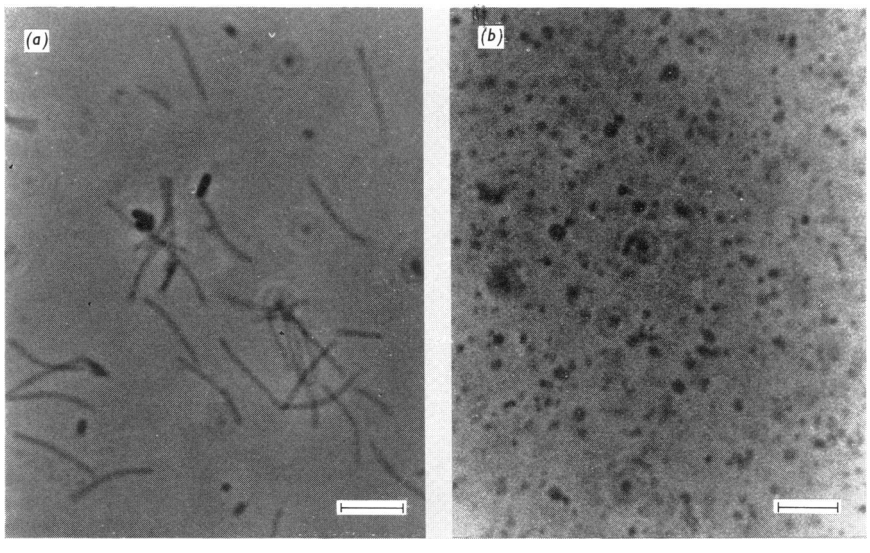
The i-antigens are a class of soluble proteins located on the outer surface of *Paramecium*. A cell normally produces only one chemically and immunologically distinct type of i-antigen at a time. Various changes in environmental conditions such as temperature cause a cell to produce a different i-antigen. In particular, when cultures are grown at 32°C, all cells express only i-antigen A, which has mol.wt. 300000. These cells are called 'serotype A'. When cultures are grown at 17°C, all cells express only i-antigen B, which has mol.wt. 270000 (Preer, 1969). These molecular weights were obtained through sedimentation equilibrium (Reisner *et al.*, 1969*a*; Steers, 1962). We obtained similar molecular weights with sodium dodecyl sulphate–polyacrylamide-gel electrophoresis (see Plate 3).

Similarities of the i-antigen and the major membrane protein

The i-antigen and the major protein of the ciliary membrane are isolated from *Paramecium* by very different techniques. Nonetheless, both proteins are large, single polypeptide chains of mol.wt. 250000–300000, since their molecular weights do not decrease with the addition of mercaptoethanol (Reisner *et al.*, 1969*a*; Hansma, 1974, 1975).

To investigate further the similarity between the i-antigen and the major membrane protein, we isolated ciliary membranes and i-antigens from single cultures of cells of different, known serotypes. These solubilized membranes and i-antigens were electrophoresed on sodium dodecyl sulphate–polyacrylamide gels of 3.4% acrylamide as shown in Plate 3. In paramecia of serotype A, both the i-antigens and the major membrane proteins had molecular weights of 280000–320000, and in paramecia of serotype B the molecular weights of both the i-antigens and the major membrane proteins were 230000–270000 (Plate 3*a*). Some cultures contained both serotype-A cells and serotype-B cells. The electrophoretic patterns of i-antigens and ciliary membranes from such cultures showed bands with the mobilities of both i-antigen A and i-antigen B (Plates 3*b* and 3*c*). These experiments show that the i-antigen and the major membrane protein from a given population of paramecia are very similar. They are either identical, closely related, or under co-ordinated control such that any cell producing a certain i-antigen is also producing a membrane protein of the same molecular weight.

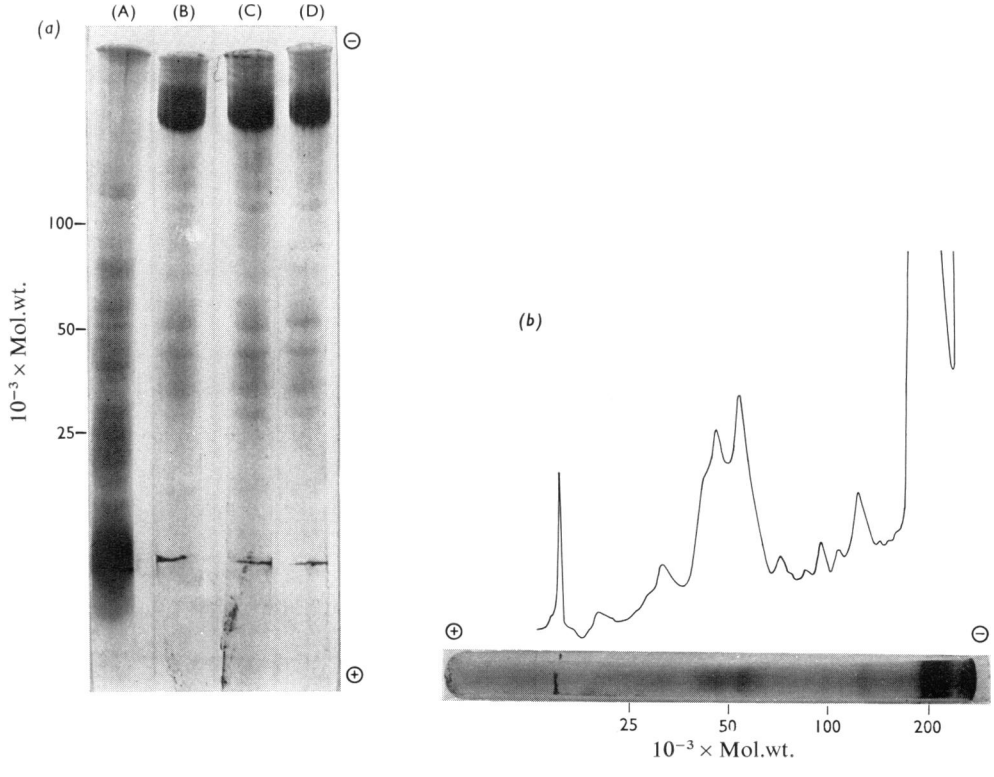
Further, the i-antigen and the major membrane protein are indistinguishable by immunodiffusion (Plate 4*a*). Antiserum against *Paramecium* of serotype A was made to react against serotype-A membranes and against i-antigen A. The precipitin arcs of membranes and i-antigens are completely fused, and no difference can be seen between the fusion of arcs from adjacent i-antigen wells and the fusion of i-antigen arcs with membrane arcs (Plate 4*a*). The i-antigen and the major membrane protein are thus closely related, if not identical, molecules.



EXPLANATION OF PLATE 1

Detached cilia from Paramecium (*a*) *and ciliary membrane vesicles* (*b*)

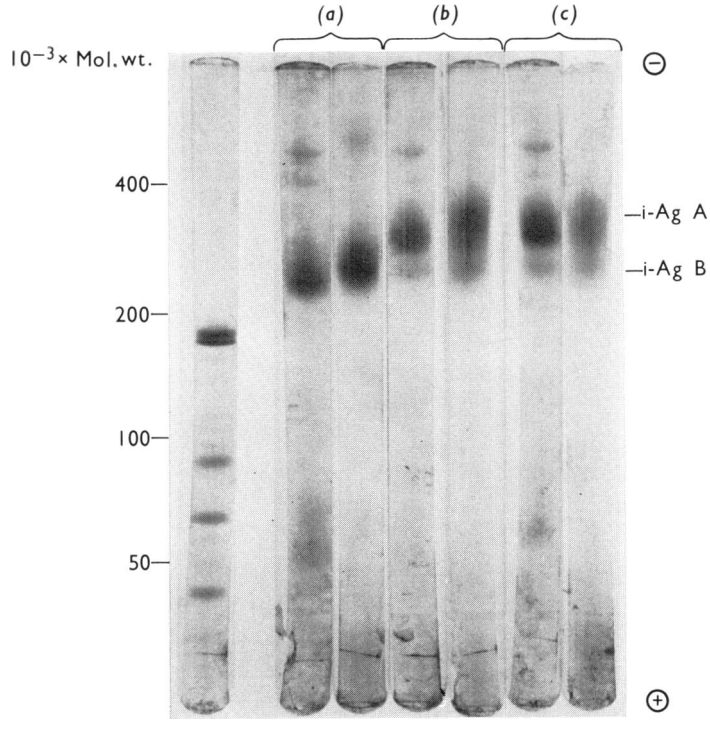
Some bacteria are present in (*a*). Bar = 5 μm. Photographed through a Zeiss phase-contrast microscope at 800× magnification.



EXPLANATION OF PLATE 2

Gel electrophoresis of ciliary membrane fractions from sucrose gradients and cell bodies of wild-type Paramecium (*a*) *and ciliary membrane protein of wild-type Paramecium* (*b*)

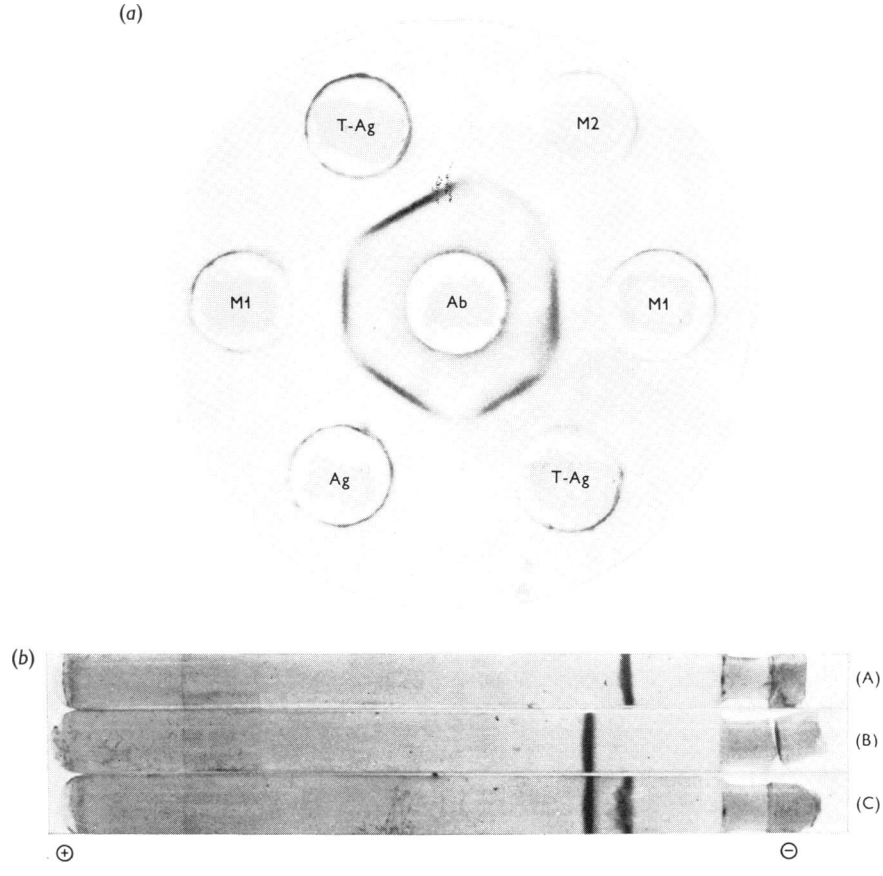
(*a*) The 5.6% acrylamide gels containing 0.1% sodium dodecyl sulphate were stained with Coomassie Blue. (A) Cell bodies (100 μg of protein); (B) ciliary membranes banding on 40% sucrose (60 μg of protein); (C) ciliary membranes banding on 50% sucrose (70 μg of protein); (D) ciliary membranes banding on 60% sucrose (65 μg of protein; gel is broken at approx. 90000 daltons). (*b*) Ciliary membrane protein (70 μg) was electrophoresed on a 5.6% acrylamide gel containing 0.1% sodium dodecyl sulphate. The gel was stained with Coomassie Blue (below) and scanned in a densitometer at 550 nm (above).



EXPLANATION OF PLATE 3

Gel electrophoresis of ciliary membranes and i-antigens of Paramecium

The 3.4% acrylamide gels containing 0.1% sodium dodecyl sulphate were stained with Coomassie Blue. Left: molecular-weight standard (*E. coli* RNA polymerase). (*a*) Ciliary membranes (left) and i-antigens (right) from wild-type *Paramecium* of serotype B; (*b*) ciliary membranes (left) and i-antigens (right) from *Paramecium* mutant d4-133 of mixed serotypes A and B; (*c*) ciliary membranes (left) and i-antigens (right) from *Paramecium* mutant d4-94 of mixed serotypes A and B.



EXPLANATION OF PLATE 4

Immunodiffusion of ciliary membranes and i-antigens from wild-type Paramecium of serotype A (*a*) *and Triton X*-100–*polyacrylamide-gel electrophoresis of ciliary membranes and i-antigens of wild-type Paramecium* (*b*)

(*a*) Ab (centre well), antibody (rabbit serum against *Paramecium* of serotype A). M1, Membranes suspended in 0.7% Triton X-100. M2, Membranes (another preparation) suspended in 0.7% Triton X-100. Ag, i-antigen A (extracted from cells from the same population as M1). T-Ag, i-antigen A suspended in 0.7% Triton X-100. (*b*) (A) Ciliary membranes (17 μg of protein); (B) i-antigen (16 μg of protein); (C) ciliary membranes and i-antigens mixed together (16 μg of protein in each). Gels were stained with Coomassie Blue.

Differences between the i-antigen and the major membrane protein

There are two lines of evidence, however, which suggest that the i-antigen and the major membrane protein are not identical. First, they differ in solubility. The i-antigen is soluble in aqueous solutions without detergent, whereas the major membrane protein is not. In our experiments, the i-antigen is extracted in a dilute salt–ethanol solution. In preparing ciliary membranes, the cells are suspended in STEEP solution (Scheme 1, step 2), which is similar to the salt–ethanol solution (Preer, 1959) used for extracting i-antigens. The major membrane protein is not removed by this treatment.

The second difference between the i-antigen and the major membrane protein is their electrophoretic mobility on Triton X-100–polyacrylamide gels (Plate 4*b*). The mobility of the ciliary membrane protein (Plate 4*b*, gel A) is lower than that of the i-antigen (Plate 4*b*, gel B). On these Triton X-100–polyacrylamide gels, there is no difference in mobility between i-antigens A and B or between the major membrane proteins from cell cultures of serotype A and B. This is not surprising, since there is only about 10% difference in molecular weight between i-antigens A and B, and their isoelectric points are very close [4.0 and 3.9 respectively (Steers, 1961)].

It is possible that the mobility difference between the i-antigen and the major membrane protein is an artifact caused by the isolation procedures. Several experiments were done to test this possibility. First, the ciliary membranes and the i-antigens were extracted with chloroform–methanol (1:1, v/v) to remove non-covalently bound lipid. This did not alter the electrophoretic mobilities in Triton X-100 of either the i-antigen or the major membrane protein.

Secondly, the unfractionated i-antigen extract in the salt–ethanol solution was electrophoresed in Triton X-100. The major band of this fraction had the same mobility as the i-antigen. This experiment showed that the subsequent steps in the purification of the i-antigen did not alter its electrophoretic mobility.

Thirdly, some intermediate fractions in the membrane-isolation procedure were electrophoresed on Triton X-100–polyacrylamide gels. The detached cilia in STEEP solution (fraction S-I in Scheme 1) showed both the membrane band and the i-antigen band. The ciliary supernatant (the STEEP solution, fraction S-II in Scheme 1) showed mainly the i-antigen band, with a trace of the membrane band. The pelleted cilia (fraction P-II in Scheme 1) showed mainly the membrane band, with various amounts of the i-antigen band, which decreased if the cilia were washed.

Thus it appears that there is a real difference between the i-antigen and the major membrane protein with respect to their mobilities on Triton X-100–polyacrylamide gels. There is no evidence that this difference is an artifact of the isolation procedures, since there is no change in the electrophoretic mobility of either protein with purification, and both proteins appear to be present in the unfractionated extract, S-I (Scheme 1). Therefore the results are all consistent with the hypothesis that the i-antigen and the major membrane protein are closely related but not identical proteins.

Discussion

Ciliary membrane proteins

The surface membrane of *Paramecium* is easily studied, because one can grow at least 1×10^8 cells of known genotype and phenotype and because two-thirds of the surface membrane covers the cilia, which are easily detached. We have isolated the ciliary membrane of *Paramecium* and have analysed its protein composition. This protein composition is unusual in that a single protein accounts for approximately three-quarters of the total membrane protein. In erythrocyte membranes, for example, no single peptide accounts for more than 20–30% (by wt.) of the total membrane protein (Guidotti, 1972). The protein compositions of many other membranes have been studied, and there are few other membranes in which a single protein predominates so heavily.

There are, however, at least three other membranes in addition to the ciliary membranes of *Paramecium* in which one protein accounts for more than one-half of the total. These are the flagellar membrane of *Chlamydomonas*, the disc membrane from the outer segments of rod cells, and myelin.

The membrane of *Chlamydomonas* flagella shows only one protein of about 200000mol.wt. by electrophoresis on polyacrylamide gels (Witman *et al.*, 1972), although traces of other proteins are probably also present in this membrane (McLean & Bosmann, 1974). This raises the interesting possibility that *Chlamydomonas* flagella might have a protein similar to the i-antigen of *Paramecium*, although, as the authors point out, proteins of this size have also been found in erythrocyte membranes (Witman *et al.*, 1972).

The major protein of rod discs is rhodopsin, a protein of mol.wt. 40000 that mediates visual transduction (Heitzmann, 1972). Myelin contains only three different proteins (Guidotti, 1972). This membrane has a very high lipid/protein ratio and functions mainly as electrical insulation. Thus the unusual protein composition of these two membranes probably reflects their highly specialized functions.

It is possible that the ciliary membrane of

Paramecium also performs a single specialized function that is carried out by the major membrane protein. It is more likely, however, that the ciliary membrane of *Paramecium* performs a variety of functions, since it covers two-thirds of the surface of a non-specialized cell. The minor proteins are probably present in sufficient amounts to provide the various 'gates', 'pumps', 'channels' and transport systems. If these proteins are each present in approximately the same density as the sodium channels of nerve membranes [30–80 channels/μm^2 (Keynes, 1972)], then each type of protein accounts for only about 0.1% of the total ciliary membrane protein (Hansma, 1974).

The major membrane protein and the immobilization antigen

From the results presented in this paper, it appears that the major membrane protein is related to, but not identical with, the immobilization antigen (i-antigen), which is a soluble surface protein of *Paramecium*. The similarity of the i-antigen and the major membrane protein was shown by immunodiffusion (Plate 4*a*) and by the electrophoretic mobilities in sodium dodecyl sulphate–polyacrylamide gels of these two proteins from *Paramecium* of serotypes A and B (Plate 3). The major membrane protein and the i-antigen are not identical, however, as shown by their different electrophoretic mobilities on Triton X-100–polyacrylamide gels (Plate 4*b*) and their different solubilities.

It is improbable that the i-antigen and the major membrane protein are the products of two different genes, since they are immunologically related and co-ordinately controlled. A more likely hypothesis is that the i-antigen and the major membrane protein are the product of the same gene and bear a precursor–product relationship. One model for such a relationship is the following: the membrane protein is synthesized and incorporated into the membrane; it is then modified in some way so that it becomes the more hydrophilic i-antigen, which moves up out of the membrane and accumulates on the outside of the cell. Preer (1969) has estimated that the i-antigen layer is 20–30 nm thick, based on the amount of i-antigen in cilia. The outer layer of this thick i-antigen coat is perhaps sloughed off into the medium.

Since the i-antigen migrates ahead of the major membrane protein on Triton X-100–polyacrylamide gels, it is perhaps more anionic, and its solubility characteristics suggest that the i-antigen is more hydrophilic. Modifications of the major membrane protein that might convert it into the more hydrophilic i-antigen include the removal of a terminal peptide or the addition of prosthetic groups such as phosphate or carbohydrate.

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