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Posttranslational Insertion of a Membrane Protein on *Caenorhabditis elegans* Sperm Occurs Without De Novo Protein Synthesis

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We have examined the mechanism of membrane protein insertion in the ameboid spermatozoa of *Caenorhabditis elegans* using two monoclonal antibodies which recognize the same set of eight sperm-specific polypeptides. Previous electron microscopic studies demonstrated that these antibodies label surface and cytoplasmic populations of antigen. Cells whose surface antigen had been removed by proteolysis were able to localize new membrane protein insertion at the tips of pseudopodial projections. *C. elegans* sperm do not contain the protein synthesizing machinery needed for delivery of new membrane to the cell surface. It has, therefore, been of interest to determine how localized membrane assembly occurs. Here we have determined the subcellular location of each of these eight polypeptides. A closely positioned doublet of bands around 97 kD (comprising 40% of the total antigen in sperm) represents surface (larger member of doublet) and cytoplasmic (lower member) forms of protein. Proteolysis of live cells eliminated this surface form from immunoblots but did not affect the cytoplasmic protein. When cells were allowed to reinsert new protein following removal of the enzyme, this surface form was regenerated. Since sperm are unable to synthesize new protein, this higher molecular weight species may arise from a posttranslational modification of proteins in the cytoplasmic pool. We present evidence suggesting that the surface protein is generated from this cytoplasmic pool by addition of fatty acid. Fatty acid acylation would account for both the observed decrease in electrophoretic mobility of the surface form and provide increased hydrophobicity to the protein which may allow for its insertion into the lipid bilayer.

Key words: nematode, motility, lipid, protein recycling, monoclonal antibody

Caenorhabditis elegans sperm are non-flagellated cells that do not contain actin yet are able to crawl rapidly over solid surfaces. The propulsive force for motility is generated by a single pseudopod that extends from a round cell body [1]. The continual insertion of new membrane proteins onto the pseudopod surface at the front of the cell and their movement rearward toward the cell body when cross-linked by external ligands

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[2,3] establishes unidirectional membrane flow. We have proposed that the force that drives membrane flow, coupled with insertion of new membrane proteins onto the advancing margin of the cell and their subsequent adhesion to the substrate, propels sperm locomotion [4].

Localized membrane assembly occurs at the front of most crawling cells [5,6, reviewed in 7], including the ameboid sperm of *C. elegans* [4]. Unlike most other cells, however, sperm do not contain the protein synthesizing machinery needed to generate new proteins for surface assembly [8–10]. In order to study the mechanism of membrane assembly in *C. elegans* sperm, we used monoclonal antibodies that label proteins exposed on the surface of the cell. These same antibodies also detect a pool of antigen in the pseudopod cytoplasm that is not associated with membrane-bound vesicles. These antibodies compete with each other for binding sites and therefore recognize identical or nearby epitopes. Immunoblots of total sperm proteins using these monoclonal antibodies have revealed that multiple sperm-specific polypeptides that range in molecular weight from 29 to 215 kD are recognized [11]. We suspect that this set of polypeptides may arise from modifications of a common precursor protein and that these modifications are part of a mechanism which localizes each protein to its correct cellular compartment in mature sperm [11,12]. We have investigated the possibility that the cytoplasmic protein may represent a precursor pool available for insertion onto the cell surface.

In this study the subcellular locations of the multiple polypeptides recognized on immunoblots have been established. Several biochemical properties of the two most abundant antigenic proteins suggest how assembly of new surface proteins may occur in the absence of membrane-vesicle fusion. We found that the lower molecular weight member of a closely positioned doublet around 97,000 molecular weight, may represent the soluble cytoplasmic precursor of a slightly larger surface form of the protein. The sensitivity of the surface form, but not the cytoplasmic form, to phospholipase C suggests that the upward mobility shift of the surface form on SDS gels is due to the posttranslational attachment of lipid. Thus, addition of fatty acid to the soluble cytoplasmic precursor protein may increase the hydrophobicity sufficiently to facilitate its insertion into the lipid bilayer.

MATERIALS AND METHODS

Worm Growth and Sperm Isolation

C. elegans strain CB1490:*him-5* (e1490) was grown in liquid culture as described by Nelson et al. [13] and used as the source of sperm which were isolated by squashing male worms between two plexiglass plates then passing the material through two 10 μm Nitex filters (Tetko Inc., Elmsford, NY) to remove the larger worm carcasses. Sperm that passed through the filters were collected by centrifugation at 2,500*g;* sperm not used immediately were stored at −80°C.

Monoclonal Antibody Production and Purification

Methods of production for the hybridoma cell lines secreting monoclonal antibodies (ABY's) TR11 and SP56 used in this study have been described previously [11]. Ascites tumors were induced in pristane-primed BALB/c mice by injection of approximately 5×10^6 hybridoma cells. Antibody was purified from ascites fluid by ammonium sulfate precipitation followed by affinity chromatography on a Sepharose-goat anti-mouse Ig column (Cooper Biomedical, Inc., West Chester, PA). Purified antibody was concentrated to 1 mg/ml and dialyzed into PBS.

Electron Microscopy

Preparation of colloidal gold particle-antibody conjugates, labelling of cells embedded in Lowicryl K4M, and electron microscopy were performed as described previously [2].

SDS-PAGE and Immunoblotting

SDS-PAGE was performed using 5–15% linear gradient gels as described by Laemmli [14]. Samples were prepared by lysing cell pellets in sample buffer containing 5% 2-mercaptoethanol. Two-dimensional gel electrophoresis was carried out essentially as described by O'Farrell [15]. Gels were stained with either 1% Coomassie brilliant blue or nickel-stained using the Kodavue Visualization Kit (Eastman Kodak Co., Rochester, NY). Gels for immunoblotting were transferred to nitrocellulose as described by Towbin et al. [16]. After blocking unbound sites on the nitrocellulose with 3% BSA (Sigma Chemical Co., St. Louis, MO), 3% fetal calf serum (Gibco Laboratories, Grand Island, NY) in Tris-buffered saline (TBS), pH 7.4, containing 0.005% Tween-20 (Sigma), the nitrocellulose replica was probed with primary monoclonal antibody at 5 μg/ml in TBS with Tween-20 and 1% BSA and washed for 2 h. Labelled bands were visualized by treatment with goat-anti-mouse Ig conjugated to horseradish peroxidase (Jackson Immunoresearch Laboratories, Avondale, PA) followed by a solution of 0.05% 4-choloro-1-napthol plus 0.003% hydrogen peroxide. Densitometry of stained gels and immunoblots was performed on a Bio-Rad Scanning Densitometer.

Surface Antigen Proteolysis and Regeneration

Live sperm were treated with the proteolytic enzyme, pronase (Calbiochem), at 0.66 mg/ml in sperm medium (SM) containing 10 mM sodium azide to digest the population of surface antigen without causing significant cell lysis as monitored by light microscopy. Following a 15 min digestion the cells were collected by centrifugation at 10,000*g* and immediately boiled in sample buffer containing 5% 2-mercaptoethanol to inactivate any residual enzyme.

To determine which of the digested surface bands could be replenished by the insertion of protein previously inside the cell, enzyme-treated sperm were washed 2× with SM that did not contain azide. This allowed greater than 90% of the cells to reactivate (defined by ruffling of the pseudopod as monitored by light microscopy) within 2 min. After 5 min the cells were pelleted and processed for electrophoresis and immunoblotted with ABY TR11.

Hypotonic Lysis of Sperm

To release antigen from the cytoplasm, spermatozoa were treated with a hypotonic lysis buffer (1 mM HEPES, 1 mM NaCl, 1 mM KCl, a mM $MgSO_4$, 1 mM $CaCl_2$, pH 7.6). Lysed cells were spun at 100,000*g*. Antigen in the lyophilized supernatant and pellet were compared by SDS-PAGE and immunoblotting using ABY TR11.

2-D CNBr Peptide Mapping of Isolated Sperm Antigens

Individual antigen bands were identified on nickel-stained polyacrylamide gels by lining the gels up with immunoblots. Antigen bands were excised using a razor blade and the protein was retrieved from the gel slice by electroelution for 12–24 h using a ISCO (Lincoln, NE) electroeluter in TBS buffer (pH 8.3) containing 0.1% SDS. Following electrolution the protein was dialyzed overnight against 0.05 M ammonium bicarbonate

buffer, pH 7.6, and lyophilized. Protein iodination was performed using the lactoperoxidase method [17]. Free iodine was removed by extensive dialysis against PBS containing protease inhibitors; greater than 95% of the remaining radioactivity could routinely be precipitated by TCA. To assure that the proper bands had been cut from the gel and to eliminate any non-antibody-recognized protein that may have co-migrated with the antigen, iodinated samples were immunoprecipitated using ABY TR11 and protein-A-sepharose (Calbiochem).

Cyanogen bromide cleavage of individual labelled proteins was performed by adding 25 μl of 100 mg/ml cyanogen bromide in 88% folic acid to approximately 500,000 cpm of ^{125}I-labelled protein in 70% folic acid. Following a 24 h incubation at room temperature, the samples were dried and made neutral with ammonium hydroxide vapors. The dried sample was resuspended in lysis buffer for isoelectric focussing, followed by second dimension separation. Radiolabelled peptides were detected by autoradiography of dried gels with Kodak XAR-5 film (Eastman Kodak Co.) using an intensifying screen at −70°C.

Phospholipase C Digestion of Live Sperm

Bacillus cereus phospholipase C (100 U, Sigma Chemical Co., St. Louis, MO) was incubated with 10^7 spermatozoa in 0.5 ml potassium phosphate buffer (ph 7.0) in the presence of 0.4 mM Zn^{2+}, 1 mM 2-mercaptoethanol, and 0.02% Triton X-100 at 37°C for 1 h. Following treatment, the cells were centrifuged at 10,000 *g* for 5 min; the pellet and lyophilized supernatant were boiled in sample buffer. Control sperm were incubated in the same buffer at 37°C for 1 hr except the enzyme was omitted. Proteins were analyzed by SDS-PAGE and immunoblotting using ABY TR11.

RESULTS

The intracellular distribution of antigen recognized by ABY's TR11 and SP56, described in detail previously [11], is summarized in Figure 1. Antigen is located in 1) the cell surface, 2) the membranes of the membranous organelles (MO's) in the cell body, 3) the pseudopod cytoplasm, and 4) the heads of the MO's. Despite several methods of fixation, vesicles in the pseudopod cytoplasm have never been observed by conventional EM or by HVEM of thick sections or critical-point-dried whole mounts [1,2,12] (our unpublished observations), although a variety of membrane-bound structures are preserved in the cell body. This does not, however, entirely exclude the possibility of vesicle involvement in the pseudopod cytoplasm.

As shown earlier [11], the antibodies used in this study recognize multiple sperm proteins on immunoblots (Fig. 2a). The most prominent of these, p97, can be resolved into two closely positioned bands by scanning densitometry (Fig. 2b) and on immunoblots containing less transferred protein (not shown). These two bands (designated p97a, upper, and p97b, lower) compose 40% of the multiple antigen (Fig. 2c) but represent only 0.5–1% of the total sperm protein (not shown). The percentage of ABY's TR11 and SP56 binding to each band, determined by labelling intensity, is summarized in Figure 2c along with the designations given to each.

The experiments described below were designed to correlate individual antigen bands detectable on immunoblots with either surface or internal cellular compartments and to make a biochemical comparison of these two protein populations.

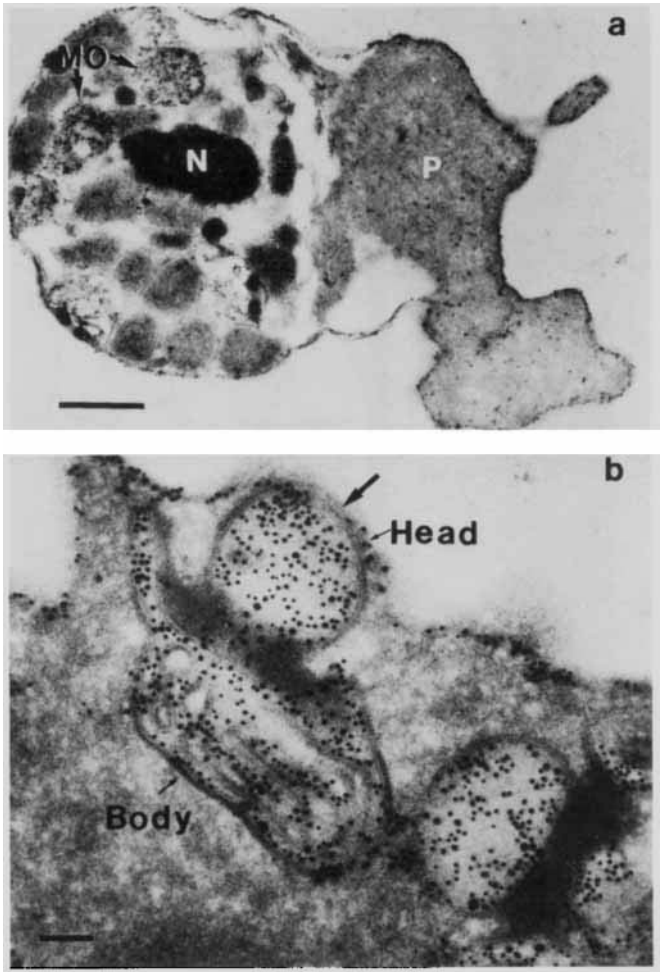
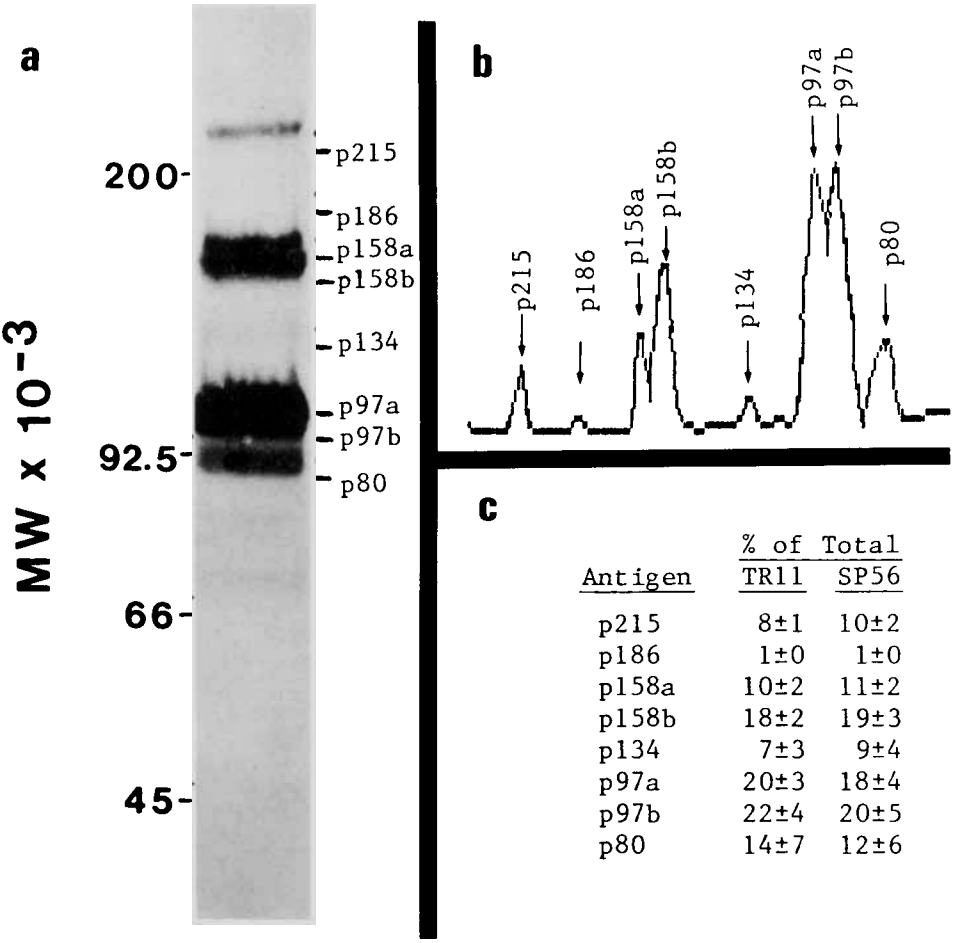


Fig. 1. Total cellular antigen distribution detected by post-sectioning labelling. Thin sections of spermatozoa embedded in Lowicryl K4M were probed with CGP-ABY SP56. **a:** The antibody binds to the cell surface, the pseudopod cytoplasm (P). **b:** The internal membranes of the body of membranous organelles (MO) and the luminal contents of the head of the MO. Bar a = 1 μm; in b = 10 nm.

External Proteolysis and Regeneration of Surface Proteins

Live, intact spermatozoa were treated with pronase to digest the extracellular portions of surface exposed proteins. Following digestion, the predominant antigen bands that remain detectable by immunoblotting are p97b and p158b (Fig. 3). Washing surface digested cells with SM and allowing 10 min for insertion of new surface proteins to occur resulted in the reappearance of two major bands, p97a and p158a (Fig. 3). The other major bands, p80 and p215, appeared to represent surface forms of antigen which were not regenerated upon removal of enzyme. In some experiments, p215 appeared in washed cells. This was occasionally seen as a faint band in lane 3 of Figure 3; however, this result was not reproducible. Earlier immunofluorescence and EM studies [2] showed that surface labelling was abolished by protease treatment and that removal of the



Antigen	% of Total TR11	SP56
p215	8±1	10±2
p186	1±0	1±0
p158a	10±2	11±2
p158b	18±2	19±3
p134	7±3	9±4
p97a	20±3	18±4
p97b	22±4	20±5
p80	14±7	12±6

Fig. 2. **a:** Immunoblot with ABY TR11 against total sperm proteins. A comparison of the labelling pattern of TR11 and SP56 is shown **c**. **b:** A typical densitometric tracing of a blot using either TR11 or SP56 is illustrated. c: The summary of antigen labelling represents the average percent area under the peaks generated from scanning 8 TR11 blots and 5 SP56 blots and expressed ± the standard deviation of the mean.

enzyme resulted in reappearance of antigen on the surface. Thus, regeneration of p97a and p158a detected here correlate with those immunolabeling studies. When cells were intentionally lysed prior to pronase treatment no antigenic bands were detectable by immunoblotting (data not shown), demonstrating that the cytoplasmic antigen is not merely resistent to proteolysis.

Hypotonic Lysis of Sperm to Release Soluble Antigen

Immunoblot analysis of membrane and soluble fractions of spermatozoa (Fig. 4) showed that a single band, corresponding to p97b, was released into the supernatant from sperm lysed in hypotonic buffer. This suggested that p97b may represent the soluble form of antigen in the pseudopod cytoplasm and agreed with the results of surface preteolysis experiments which demonstrated that p97b is not exposed at the cell surface. Despite repeated efforts, however, p158b could not be detected in the soluble

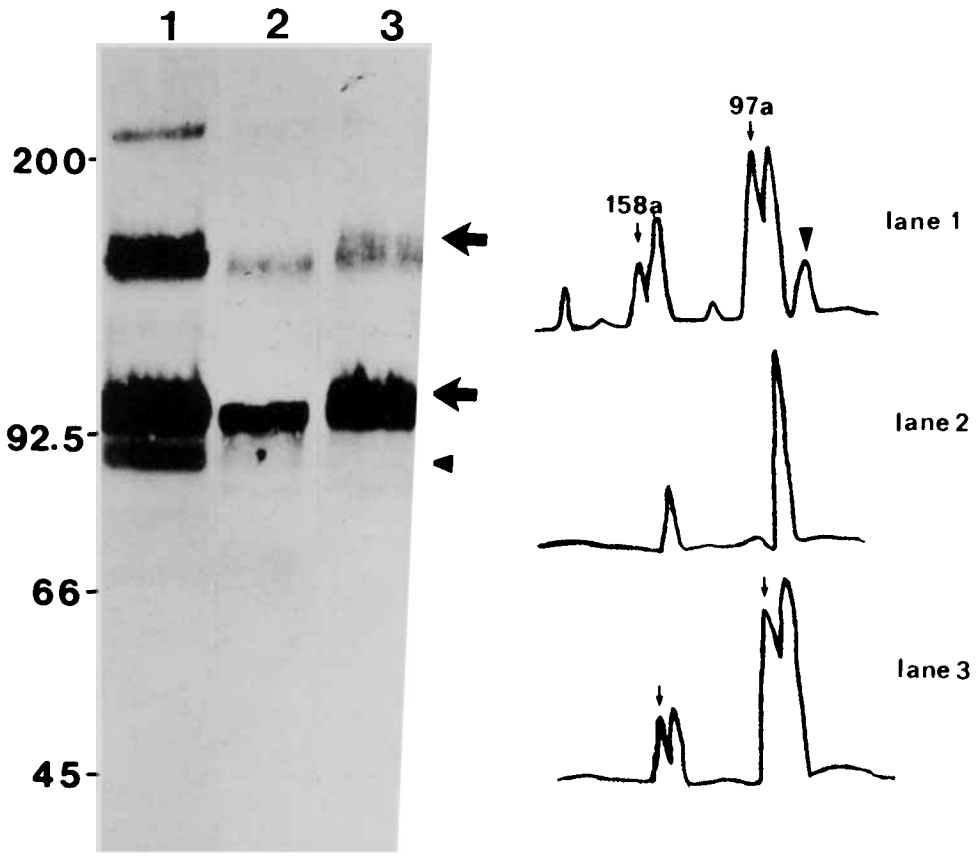


Fig. 3. Proteolytic surface digestion and regeneration of sperm surface antigens. 10^7 sperm were activated using monensin and processed immediately for SDS-PAGE to demonstrate the initial pattern of antigen bands (**lane 1**). 2×10^7 cells were treated for 15 min with a mixture of 0.66 mg/ml pronase and 10 mM sodium azide to remove the existing surface population of antigen. Half of these cells were processed for SDS-PAGE (**lane 2**), while the other half were washed three times in SM to allow reactivation of the cells and the insertion of new protein onto the cell surface (**lane 3**). Following SDS-PAGE and transfer to nitrocellulose, the blot was probed with ABY TR11. The large arrows indicate the positions of P97a and P158a. The arrowhead indicates the position of p80. A densitometric tracing of these lanes is shown.

fraction. A large fraction of the soluble proteins remained unextracted, however, as evidenced by the presence of 15.5 kD major sperm protein (MSP), a known soluble protein, in the pellet.

Absence of Protein Synthesis in Spermatozoa

Regeneration of p97a and p158a must be due either to 1) cleavage of a higher molecular weight antigen, 2) posttranslational modification of a lower molecular weight antigen, or 3) new protein synthesis. Because all protein synthesizing machinery is segregated to the residual body of late secondary spermatocytes [8], it is unlikely that new protein synthesis occurs in spermatozoa. This was confirmed by labelling live cells with ^{3}H-leucine. As shown in Figure 5, several proteins ranging in size from 85 kD to 30 kD were synthesized, however none of these correspond to any of the antigens recognized

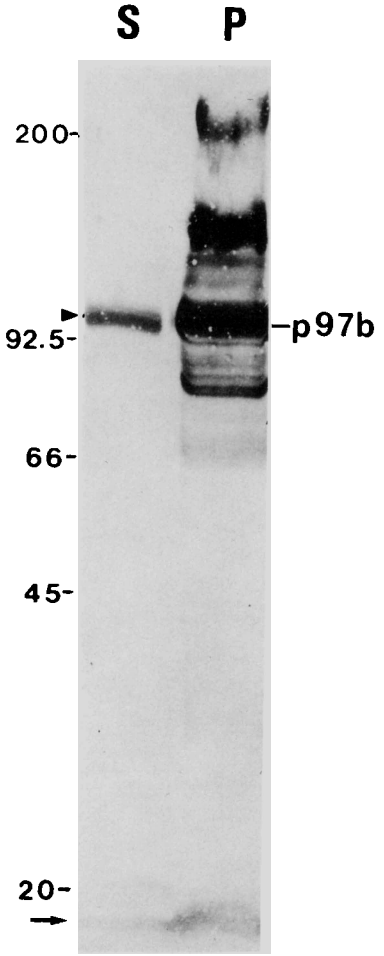


Fig. 4. Hypotonic lysis of spermatozoa. 10^7 spermatozoa were lysed in hypotonic buffer and centrifuged at 50,000*g* for 30 min. The supernatant was lyophilized. Proteins in the pellet (**P**) and supernatant (**S**) were electrophoresed, transferred to nitrocellulose, and probed with ABY TR11. The only detectable antigen in the supernatant corresponds to the lower member of the p97 doublet (p97b). The nitrocellulose was then probed with CGP-ABY-TR20 to visualize a known soluble protein, MSP. These bands, which appear reddish-brown, are at 15.5 kD (MSP).

by our antibodies. Note that lane 3 was overexposed in order to reveal the less intense bands in lanes 4 and 5. This labelling was inhibited by increasing concentrations of the mitochondrial protein synthesis inhibitors chloramphenicol and tetracyline and by sodium azide (lane 6). Thus, the only detectable protein synthesis in spermatozoa occurs in the mitochondria. Several of the proteins labelled under these conditions may be synthesized by bacteria which are used as a food source for worms and are retained during the isolation of sperm (S. Ward, personal communication). Also, when ^{3}H-leucine-labelled sperm were subjected to immunoprecipitation using ABY TR11, no newly synthesized antigen was detected (lane 7) although this antibody could immunoprecipitate antigen that had been eluted from gels and labelled with ^{125}I (lane 8).

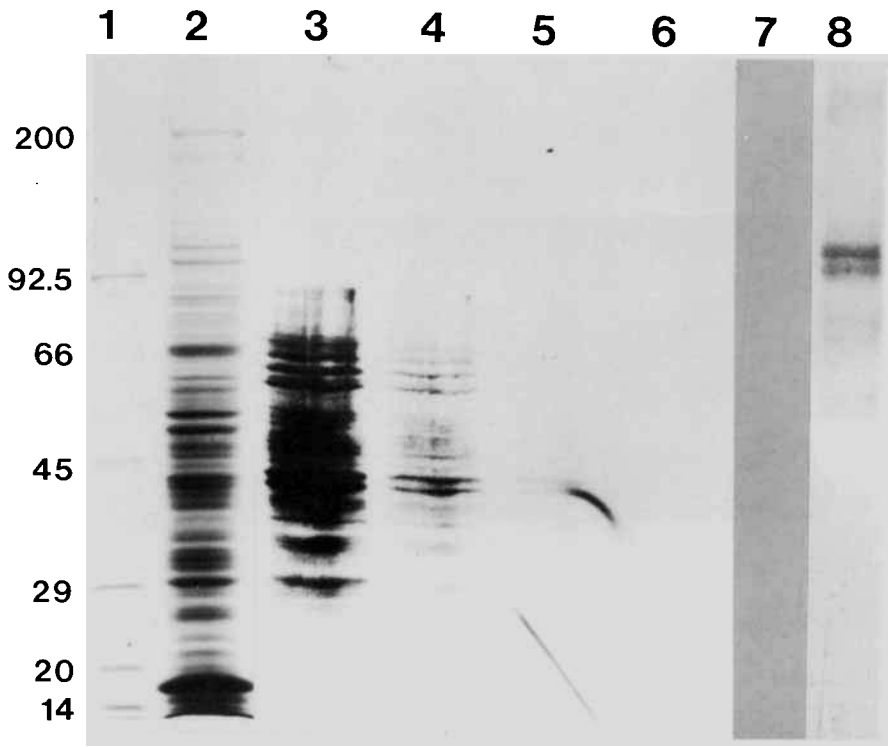


Fig. 5. A total of 10^7 live spermatozoa were incubated in ^{3}H-leucine in the absence (**lane 3**) or presence of either 10 μg/ml (**lane 4**) or 100 μg/ml (**lane 5**) chloramphenicol/tetracycline or 10 mM sodium azide (**lane 6**). Following SDS-PAGE and fluorography, lanes 3–6 show the result of a 7 day exposure to X-ray film. **Lane 7:** Immunoprecipitate of a ^{3}H-leucine-labelled sperm extract prepared as shown in lane 3 using ABY TR11. The autoradiogram was exposed for 14 days. **Lane 8:** Immunoprecipitate of iodinated p97a/b using ABY TR11. **Lanes 1,2:** Molecular weight standards and total sperm proteins, respectively, stained with Coomassie blue.

Evidence That p97b May Be the Cytoplasmic Precursor of Surface p97a

Indirect evidence, based on 1) the subcellular location of p97a (surface) and p97b (internal and soluble), 2) the ability of p97a to be regenerated following proteolytic surface digestion, and 3) the absence of new, non-mitochondrial protein synthesis, suggested that p97b may be the cytoplasmic precursor of the surface protein p97a. To test this hypothesis further, live cells were used to examine the temporal relationship between these proteins. Live spermatozoa incubated in a low concentration of pronase in the absence of sodium azide continued to ruffle their pseudopods. If p97b is inserted continuously onto the surface of sperm, then long-term exposure to protease should have resulted in gradual depletion of the p97b pool. This was the case. Sperm were incubated in pronase for 15, 30, or 45 min. The enzyme was removed; the cells were incubated in buffer without enzyme for an additional 10 min to allow for insertion of new surface proteins, pellected, and boiled in sample buffer. Total proteins were separated by gel electrophoresis and nickel-stained (Fig. 6). The amount of p97a on these cells did not

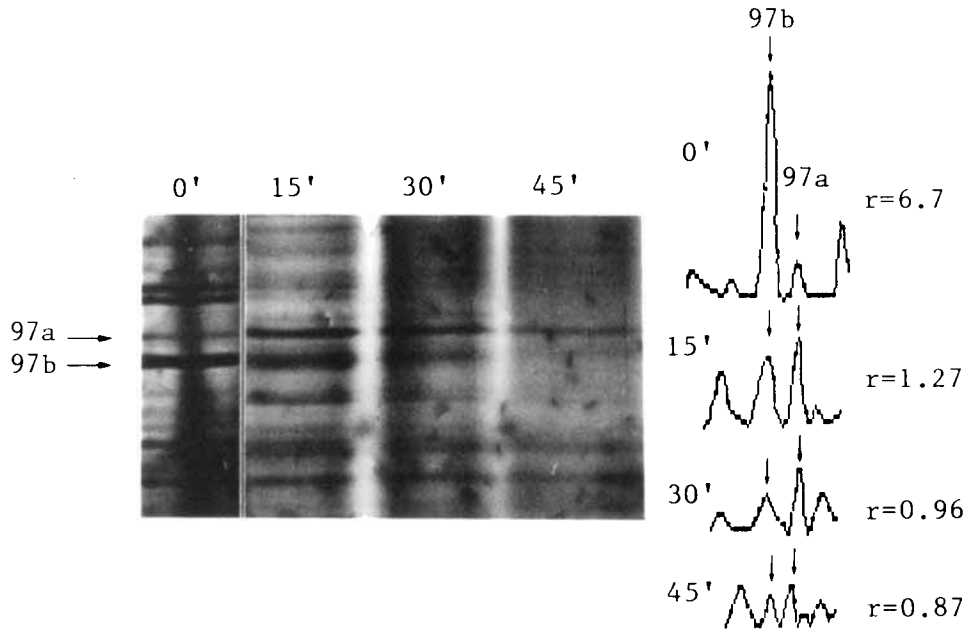


Fig. 6. A total of 3×10^7 spermatozoa were incubated in SM containing 0.1 mg/ml pronase. At 15, 30, and 45 min intervals, 10^7 cells were removed, washed 2× in SM alone, and incubated for a total of 10 min to allow insertion of new surface proteins in the absence of pronase before being boiled in sample buffer. Then, 10^7 spermatozoa were processed for SDS-PAGE without pronase to demonstrate the initial quantities of p97a and p97b (0'). Following electrophoresis, the gel was nickel-stained and analyzed by scanning densitometry. The densitometric tracing is illustrated at the right. r = ratio of the area under the peaks of p97b:p97a. Note that the quantity of p97a increases after activation (compare 0 min and 15 min) before slowly decreasing after 30 and 45 min; p97b decreases steadily and rapidly.

change significantly with increased exposure to pronase but the quantity of p97b dropped dramatically over time. Quantitation of these proteins on nickel-stained gels by densitometry revealed a seven-fold decrease in the ratio of p97b:p97a during the 45 min incubation in the enzyme. Although these data do not establish a precursor-product relationship between the gradual depletion of p97b during exposure of live cells to pronase, they suggest that cytoplasmic p97b may represent a precursor pool for surface p97a. Because the p158 doublet represented such minor sperm proteins, it could not be accurately identified when lined up with a parallel immunoblot.

Peptide Mapping of p97a and p97b

If surface p97a is generated from cytoplasmic p97b, then the peptide maps of these proteins should be similar except for any modifications which account for the mobility shift observed by SDS-gel electrophoresis. Figure 7 illustrates that these proteins generate similar two-dimensional (2-D) peptide maps following cleavage by cyanogen bromide (CNBr). No spots of differing molecular weight between the two proteins are visible. Several spots of identical pI are, however, present on p97a that are not seen on p97b. Thus, these proteins appeared to share extensive regions of primary amino acid sequence homology.

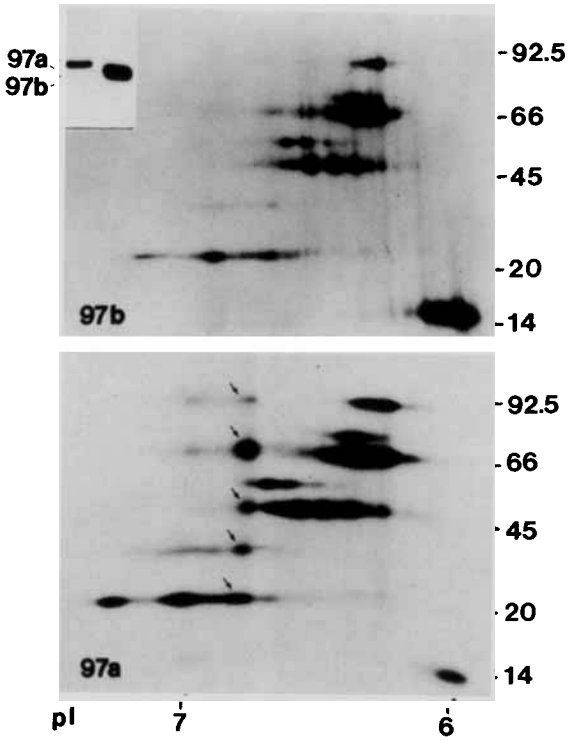


Fig. 7. 2-D CNBr peptide maps of p97b and p97a. Individual iodinated antigen bands, obtained as described under Materials and Methods, were re-electrophoresed on one-dimensional gels and are shown at the **upper left.** These proteins were CNBr-digested and separated in the first dimension using a combination of pH 3–10 and 5–7 ampholines and in the second dimension by SDS-PAGE on a 10% gel. Molecular weight markers are in kilodaltons shown at the right; pI is shown along the bottom of the lower gel; note that only a portion of the gel contains protein and is shown. Arrows indicated major differences between the two peptide maps.

Phospholipase C Treatment of Sperm

Treatment of sperm with phospholipase C resulted in removal of p97a from the cell surface and a drop in the apparent molecular weight of the protein released into the supernatant. Cytoplasmic p97b was not, however, sensitive to phospholipase C. Figure 8 shows an immunoblot of sperm treated with the enzyme compared to untreated controls. p97a is not present on the blot following treatment suggesting that 1) bound lipid is necessary to maintain association of the protein with the membrane during centrifugation and/or 2) lipid removal results in a downward mobility shift of p97a. Scanning densitometry of this blot (Fig. 8) did not detect an increase in the amount of p97b present in the pellet following phospholipase treatment suggesting that lipid removal causes p97a to be released into the supernatant. Two major and several minor antigenic bands were found in the supernatant. One of these major bands co-migrates with p97b; however, controls omitting the enzyme also contain a small amount of this band in the supernatant suggesting that some leakage from the cell may occur, possibly due to the presence of 0.02% Triton X-100 in the reaction mixture. Without a specific probe for p97a we are

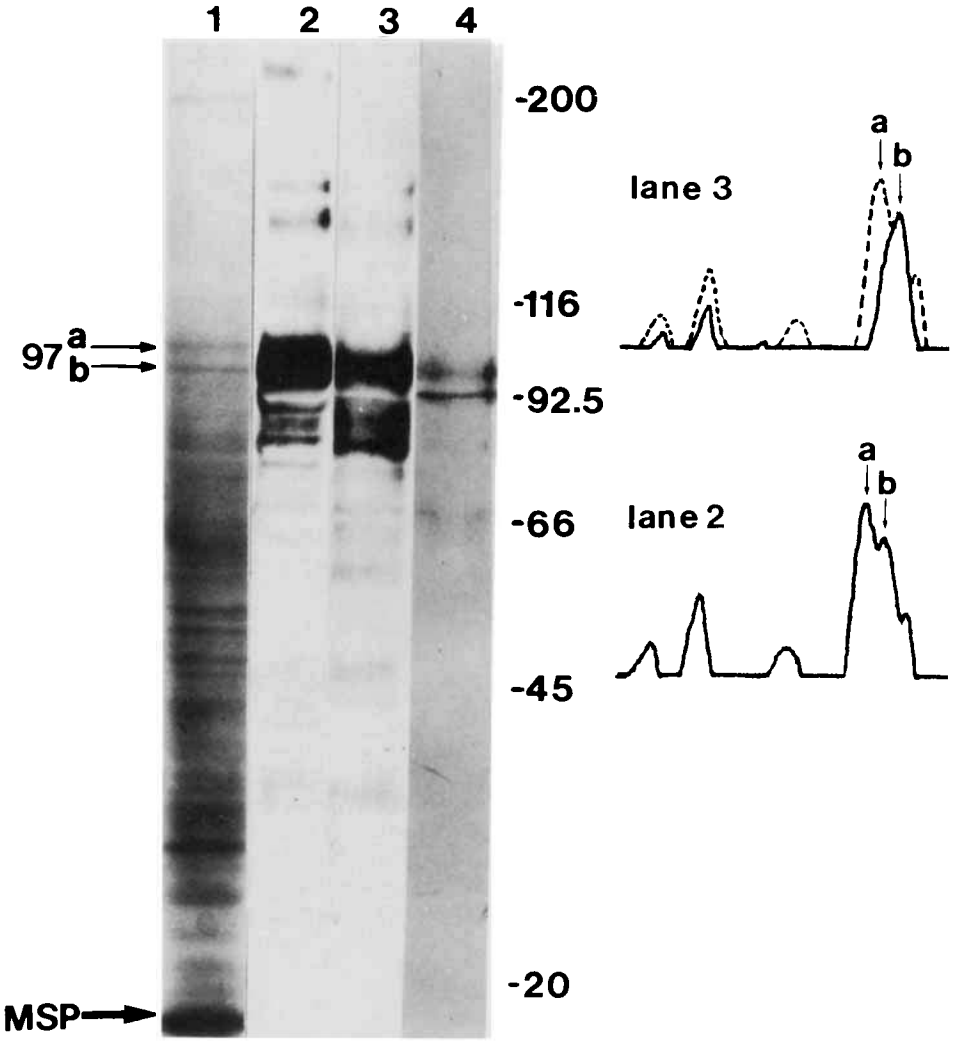


Fig. 8. Phospholipase C treatment of spermatozoa. **Lane 1:** Coomassie blue-stained total sperm proteins. **Lanes 2–4:** ABY TR11 immunoblot of control sperm incubated for 1 h at 37°C in buffer without phospholipase C (lane 2), the pellet of sperm treated with phospholipase C for 1 h (lane 3), and the supernatant remaining after phospholipase C treatment (lane 4). **To the right,** scanning densitometry of the relevant portions of lanes 2 and 3. Solid lines indicate the densitometric tracing of that lane; the dotted line represents the tracing of lane 2 superimposed over that of lane 3. The additional bands between 80 and 90 kD in lanes 2 and 3 appear to be degradation products generated during the 1 h at 37°C incubation. The lower molecular weight bands in lane 3 appeared only after phospholipase C treatment.

uncertain which of the bands represents the remnant of p97a removed by phospholipase treatment.

DISCUSSION

Continual insertion of new protein onto the pseudopod surface of *C. elegans* sperm implies that a cytoplasmic protein pool must exist which is available for surface

assembly. We detect multiple antigens by immunoblotting using either of two monoclonal antibodies. Cytologically, antigen is labelled by these antibodies both on the surface and in the cytoplasm [2,6]. We have shown previously that new antigen is rapidly inserted from the cytoplasm onto the cell surface [2]. This surface antigen is capable of adhering to antibodies that have been used to coat glass coverslips and cells are able to use these adhesions to promote crawling [4]. In most eukaryotic cells, plasma membrane expansion results from fusion of membrane-bound vesicles with the cell surface [18,19]. Several methods of fixation have been used to prepare *C. elegans* sperm for ultrastructural examination; none have revealed vesicles in the pseudopod cytoplasm, although other membranous structures in the cell body are preserved by these methods [2,9,10]. Instead, we detected a pool of apparently soluble protein in the pseudopod cytoplasm that was not associated with membranes [2].

The protein (p97b) released from hypotonically lysed cells appears to represent this cytoplasmic precursor pool for the surface protein (p97a). Several lines of evidence support this hypothesis. First, 2-D peptide maps of these two proteins were very similar suggesting that their primary amino acid sequences are closely related. Second, the surface form, p97a, was completely eliminated from immunoblots when intact cells were treated with the proteolytic enzyme, pronase. Third, this surface protein was replenished following extracellular digestion when live cells were allowed to recover for several minutes following enzyme treatment; the reappearance of this protein on blots correlated with the ability of antibodies to label the cell surface. Fourth, a dramatic decrease in the amount of the cytoplasmic protein was observed over time when cells were incubated in pronase and allowed to assemble new proteins onto the surface. At any point during this incubation the amount of detectable surface protein, however, remained approximately constant suggesting that as new protein was continually being added to the cell surface, the cytoplasmic pool was gradually depleted.

The insertion of proteins into the plasma membrane by non-vesicular mechanisms has been shown to occur in several experimental systems. Hydrophobic regions of certain cytoplasmic proteins are known to become exposed during protein folding which may facilitate their insertion [20,21], thus, uncoupling protein insertion into the lipid bilayer from protein synthesis. Bacterial mutants in which normal protein export is blocked by point mutations that place a single charged amino acid in an exposed hydophobic region support such as posttranslational, non-vesicular protein insertion mechanism [22]. Additionally, the export of proteins synthesized only on cytosolic ribosomes in yeast [23] and the posttranslational import of proteins into cellular organelles [24] provide further, although less direct, support for non-vesicular protein insertion into eukaryotic membranes.

An alternative mechanism for protein insertion involves the attachment of lipids to cellular proteins which has clearly been shown to enhance their association with membranes [25,26]. Protein acylation has been proposed as a modification which could account for protein assembly and/or anchorage of the protein into the membrane [27]. In support of this mechanism phospholipase C has recently been shown to release proteins attached to the membrane in over 30 cases through release of a phosphotidylinositol-protein linkage (reviewed in [28]). Phospholipase C treatment of whole *C. elegans* sperm caused the surface protein (p97a) to be released into the supernatant in the presence of mild detergent and decreased the molecular mass of the released protein as detected by SDS-gel electrophoresis. The cytoplasmic protein was apparently unaf-

fected. The presence of attached fatty acid to the surface form of the protein would account for both its increased size and enhanced membrane association relative to the cytoplasmic form. Preliminary results from our laboratory have shown that a subset of Triton X-100-soluble proteins are labelled with tritiated palmitic acid. Attempts at immunoprecipitating acylated 97a have been unsuccessful. Further studies are necessary to determine the nature of any lipid moiety attached to p97a and whether specific acyltransferases and phospholipases exist in sperm to facilitate acylation and deacylation of cytoplasmic proteins. Because the membrane of *C. elegans* sperm pseudopods exhibits such dynamic membrane activity, which appears to play a central role in motility, it will be particularly interesting to understand fully the mechanism of new membrane protein assembly.

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