Isolation and Characterization of Neurospora crassa Plasma Membranes*

Gene A. Scarborough
From the Department of Biochemistry, University of Colorado School of Medicine, Denver, Colorado 80220

SUMMARY
The isolation and characterization of plasma membranes from a cell wall-less mutant of Neurospora crassa are described. The plasma membranes are stabilized against fragmentation and vesiculation by treatment of intact cells with concanavalin A just prior to lysis. After lysis, the concanavalin A stabilized plasma membrane ghosts are isolated by low speed centrifugation techniques and the purified ghosts subsequently converted to vesicles by removal of the bulk of the concanavalin A. The yield of ghosts is about 50% whereas the yield of vesicles is about 20%. The isolated plasma membrane vesicles have a characteristically high sterol to phospholipid ratio, Mg2+-dependent ATPase activity and (Na+ + K+-)stimulated Mg2+-ATPase activity. Only traces of succinate dehydrogenase and 5'-nucleotidase are present in the plasma membrane preparations.

Earlier reports from this laboratory and others have described two glucose transport systems in wild type cells of Neurospora crassa (1-10). One, a facilitated diffusion system which is present in cells grown in a medium containing high levels of glucose and another, an active transport system which is derepressed in cells grown in a medium containing little or no glucose. With these two systems as models for eukaryote membrane transport, the primary goal in this laboratory is an understanding of the molecular events underlying the two fundamental aspects of transport, translocation and energy-coupling. Remarkable progress toward this goal has been realized by bacterial transport workers utilizing isolated bacterial plasma membrane vesicles (11). That progress made obvious the need for a similar preparation of Neurospora plasma membrane vesicles.

The isolation of plasma membranes from free living cells other than erythrocytes, isolated fat cells, and bacteria, in high yield and purity, is a task replete with difficulties. The specific problems which arose in our early attempts to isolate N. crassa plasma membranes were the unavailability of a suitable plasma membrane marker, the presence of a rigid cell wall which made gentle lysis impossible, the tendency for the Neurospora plasma membranes to fragment and vesiculate immediately upon lysis trapping other cellular constituents, and the variable densities of the plasma membrane particles which lead to extensive smearing in standard isopycnic centrifugation procedures. Most of these difficulties have been experienced by others with a variety of cell types (12-14). This communication describes procedures which eliminate all of these problems in the isolation of Neurospora plasma membranes. The chemical and enzymatic properties of the plasma membranes obtained are also presented.

Although the methods described here were designed specifically for the isolation of Neurospora plasma membranes, the principles of the isolation procedure should be applicable to a variety of eukaryotic cells.

EXPERIMENTAL PROCEDURE
Materials
Mannitol, deoxyribonuclease (DN25), a-methylmannoside, p-iodonitrotetrazolium violet, and yeast RNA were obtained from Sigma. Calf thymus DNA was from Worthington. Sulfanilic acid was from Baker. 3H-Sulfanilic acid was from American-Searle. 3H-Labeled algal hydrolysate was from Schwarz-Mann. Concanavalin A (three times crystallized) was from Miles-Yeda. Yeast extract and nutrient broth were from Difco. The cell wall-less mutant of N. crassa, designated sl (15), was obtained from the Fungal Genetics Stock Center, Arecata, Calif.

Growth of sl Cells
sl cells were grown in Vogel's N medium (16) supplemented with 2% (w/v) mannitol, 0.75% (w/v) yeast extract, and 0.75% (w/v) nutrient broth. Cultures were maintained by daily transfer of 1 ml of an overnight culture into 50 ml of fresh medium followed by rotary shaking (150 rpm) at 30° overnight. Five hundred-milliliter cultures were obtained by inoculating 450 ml of fresh medium with 50 ml of an overnight culture and shaking overnight as above. Tritiated cultures were obtained by overnight growth of cells in the above mentioned medium containing 10 μCi of 3H-labeled algal hydrolysate per 50 ml of medium.

Preparation of Diazotized [3H]Sulfanilic Acid
Diazotized sulfanilic acid was prepared immediately before use by a method similar to the method of Berg. 3Radioactive sulfanilic acid (200 μg) was dissolved in 2 ml of 2.5% (w/v) Na2CO3 followed by the addition of 80 mg of NaNO2. Sixty microliters of this solution were added immediately to 10 μl of radioactive sulfanilic acid solution (1.9 mCi per ml, 5.0 Ci per mmol) and mixed. This was followed by the addition of 20 μl of 3.5 M HCl and constant mixing until the diazonium salt precipitated out. The mixture was then chilled, centrifuged, and the supernatant fluid carefully removed. Without disrupting the pellet, the walls of the tube were washed with a small amount of ice-cold water. The pellet was then dissolved in 20 ml of Buffer

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1 H. Berg, personal communication.

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A (0.05 M Tris-HCl, pH 7.5, containing 0.01 M MgSO₄ and 0.25 mM mannitol) at 25° and used immediately.

Isolation of Plasma Membrane Ghosts

One 500-ml culture of eels is combined with three 50-ml cultures of tritiated cells and chilled. The optical density at 650 nm of the combined cultures is variable from day to day but in the range of 0.6 to 0.9. The cells (approximately 250 mg of cell protein) are harvested by centrifugation at 700 × g for 10 min, resuspended in 80 ml of ice-cold Buffer A, divided into four 20-ml aliquots, and washed five times with 80 ml of ice-cold Buffer A (20 ml each) by alternate centrifugation and resuspension (50-ml glass tubes in a swinging bucket clinical centrifuge at 140 × g for 5 min). Prior to the final centrifugation, 5 ml of the 80-ml cell suspension are withdrawn, pelleted by centrifugation, and resuspended in 5 ml of ice-cold 0.01 M Tris-HCl, pH 7.5. This is the "cells" preparation referred to at various points in the text. If the washed cells are to be surface-labeled with diazotized [³⁸S]sulfanilic acid (experiments described in Fig. 1 and Table I only) they are resuspended in a total of 20 ml of diazotized sulfanilic acid in Buffer A prepared as described above and incubated at 25° for 9 min. After surface-labeling, the cells are then washed five more times in 80 ml of Buffer A as described above. Plasma membranes derived from surface-labeled cells behave the same as plasma membranes derived from nonsurface-labeled cells in all steps of the isolation procedure. The washed cells are then resuspended in a total of 20 ml of buffered cold Buffer A (25°), mixed with 20 ml of 0.5 mg per ml of concanavalin A in Buffer A, and incubated with occasional agitation for 30 min at 25°. The concanavalin A agglutinates the cells during this period. The concanavalin A-agglutinated cells are then chilled, centrifuged at 140 × g for 1 min, resuspended gently in 40 ml of cold ice-cold Buffer A, and centrifuged at 140 × g for 6 min. The resulting cell pellet is then resuspended in 50 ml of ice-cold 0.01 M Tris-HCl, pH 7.5, containing 5 mM MgSO₄ and 50 mg of DNase, and homogenized in a glass-Teflon tissue homogenizer (50 passes over a 10-min period; clearance approximately 0.008 inch). This is the lysate fraction referred to at various points in the text. Twelve-milliliter portions of the lysate are layered over 35 ml of ice-cold Buffer B (0.1 M Tris-HCl, pH 7.5, containing 0.5 mM mannitol) and the resulting two-phase systems are centrifuged at 140 × g for 30 min in a swinging bucket clinical centrifuge (4°). The supernatant fluids containing most of the cell contents are removed by aspiration and the plasma membrane pellets are resuspended in a total of 20 ml of ice-cold 0.01 M Tris-HCl, pH 7.5, and again homogenized in a glass-Teflon tissue homogenizer (20 passes, 4°). Ten-milliliter aliquots of the resulting suspension are layered over 30 ml of Buffer C resulting two-phase systems are centrifuged at 250 × g for 30 min in a swinging bucket clinical centrifuge (4°). The supernatant fluids are removed by aspiration and the pellets containing the plasma membrane ghosts are resuspended in a small volume of 0.01 M Tris-HCl, pH 7.5. This is the plasma membrane ghost fraction referred to at various points in the text. The plasma membrane ghost fraction is contaminated with significant amounts of a nonmembrane carbohydrate material and small amounts of succinate dehydrogenase, 5'-nucleotidase, and RNA, but the high yield, near-purity, and ease of preparation make it a suitable starting material for a variety of applications.

Isolation of Plasma Membrane Vesicles

The procedure for the isolation of plasma membrane vesicles is identical with the procedure for isolation of plasma membrane ghosts up to the final centrifugation through Buffer B. Instead of layering over Buffer B, 10-ml aliquots of the plasma membrane ghosts suspension are each layered over a two-phase system consisting of 30 ml of Buffer B layered over 5 ml of Buffer C (0.01 M Tris-HCl, pH 7.5, containing 20% (w/v) sucrose) and the resulting three-phase discontinuous gradients are centrifuged at 250 × g for 30 min. The supernatant fluids are removed by aspiration and the pellets resuspended in a total of 4 ml of 1 M α-methylmannoside in 0.01 M Tris-HCl, pH 7.5. The resulting plasma membrane suspension is incubated with shaking for 30 min at 30°, chilled, diluted with 16 ml of ice-cold 0.01 M Tris, pH 7.5, and homogenized in a glass-Teflon tissue homogenizer (100 passes, 4°). The mixture is then carefully layered over 30 ml of ice-cold Buffer C and centrifuged at 250 × g for 30 min. The plasma membrane vesicles, which remain on top of the Buffer C, are removed with a Pasteur pipette, diluted to 50 ml with ice-cold 0.01 M Tris-HCl, pH 7.5, and centrifuged at 12,000 × g for 30 min (4°). The supernatant fluid is decanted and the pellet containing the plasma membrane vesicles is resuspended in a small volume of 0.01 M Tris-HCl, pH 7.5. This is the plasma membrane vesicle preparation referred to at various points in the text.

Chemical Analyses

Protein and carbohydrate determinations were carried out on "cells," and plasma membrane vesicle preparations which had been dialyzed against 1 liter of 0.01 M Tris-HCl, pH 7.5, overnight (4°). Lipid and nucleic acid determinations were carried out on undialyzed material.

Protein—Since the plasma membrane vesicles contain significant amounts of concanavalin A, direct determination of plasma membrane protein was not possible. For this reason, the protein content of the plasma membrane vesicles was determined from the tritium content. The amount of protein in the "cells" by the method of Lowry et al. (18) and, in addition, the tritium content of the cells was determined by liquid scintillation counting (in PG). From the ratio of counts per minute per milligram of protein in the "cells" to the milligrams of protein in the plasma membrane vesicle preparation was calculated. This method relies upon the assumption that the total cellular proteins and the plasma membrane proteins are equally labeled, and, in an overnight culture, this is a reasonable assumption.

Lipid—The total lipids present in the "cells" and plasma membrane vesicle preparations were extracted by the method of Bligh and Dyer (19) as modified by Gibson et al. (20), with a single 0.5% KCl in 50% aqueous methanol wash. The sterol content (as ergosterol equivalents) of the lipid extracts was determined by the method of Moore and Baumann (21). The phospholipid phosphorous content of the lipid extracts was determined by the method of Bartlett and Dyer (19). The phospholipid phosphorous content of the lipid extracts was determined by the method of Bartlett and Dyer (19).

Carbohydrate—The total carbohydrate content (as glucose equivalents) of the "cells" and plasma membrane vesicle preparations was determined by the anthrone method of Roe (23) as described by Spiro (24).

Nucleic Acids—RNA and DNA were determined by the orcinol and diphenylamino methods described by Schneider (25) with yeast tRNA and calf thymus DNA as standards.

Enzyme Analyses

Enzyme analyses were carried out on "cells," ghosts, and vesicles which had been dialyzed against 1 liter of 0.01 M Tris-HCl, pH 7.5, overnight (4°) and frozen once.

Mg²⁺-ATPase—Manganese-dependent ATPase activity was measured by the estimation of inorganic phosphate liberated from ATP in the presence or absence of Mg²⁺. The assay mixtures contained suitable amounts of "cells," ghosts, or vesicles, di-sodium ATP (10 mM), Tris-HCl, pH 7.5 (20 mM), and MgSO₄ (10 mM) when added, in a final volume of 0.5 ml. Incubations were carried out for 30 min at 30°. The reactions were stopped by the addition of 2 ml of ice-cold 3.75% perchloric acid, centrifuged, and the inorganic phosphate in the supernatant fluids estimated essentially by the method of Stonton (26).

(Na⁺ + K⁺)-stimulated Mg²⁺-ATPase—Assay conditions were identical with those described for Mg²⁺-dependent ATPase activity with the addition of 47 mM NaCl and 3.3 mM KCl.

β-Nucleotidase—The β-nucleotidase assay was identical with the Mg²⁺-dependent ATPase assay with AMP (Na⁺ salt) replacing ATP in the incubations.

Succinate Dehydrogenase—Succinate dehydrogenase activity was estimated by the method of Pennington (27). Assay mixtures (1 ml) contained suitable amounts of "cells," ghosts, or vesicles, potassium phosphate buffer pH 7.4 (100 mM), and isodinotetrazolium violet (0.2 mg) in the presence or absence of succinate (100 mM). Incubations were carried out for 20 min at 30°. The reactions were stopped by the addition of 0.1 ml of 50% (w/v) trichloroacetic acid and the mixture was extracted with 4 ml of ethyl acetate. The formazan produced was estimated

² The abbreviation used is: PG, liquid scintillation counting.
by measuring the absorbance (490 nm) of the resulting ethyl acetate extracts.

**Electron Microscopy**

Small aliquots of intact cells were withdrawn immediately before and after the concanavalin A treatment and pelleted by centrifugation at 250 × g for 5 min (4°). The cell pellets were then fixed in 3% glutaraldehyde in Buffer A for 2 hours at 4°, and postfixed in 1% OsO4 in three-quarters strength Buffer A for 1 hour at 4°. The pellets were then washed once with cold Buffer A, dehydrated in a graded acetone series, and embedded in Spurr's resin (28). Thin sections, cut on a Porter-Blum MT-2B ultramicrotome fitted with a diamond knife, were picked up on copper grids, double stained in uranyl acetate and lead citrate, and photographed with a Philips EM300 electron microscope. Plasma membrane ghosts and vesicles prepared as described above were pelleted by centrifugation at 12,000 × g for 30 min (4°), treated with Karnovsky's formaldehyde-glutaraldehyde fixative (29) for 2 hours at 4°, rinsed overnight in 0.2 M sodium cacodylate buffer, pH 7.2, postfixed for 1 hour in 1% OsO4 in 0.2 M sodium cacodylate buffer, pH 7.2, and subsequently treated as described above for the intact cells.

**RESULTS**

**Identification of sl Plasma Membranes**—The experiment presented in Fig. 1 shows the behavior of surface-labeled plasma membranes during the first step in the isolation procedure. A lysate of 3H-surface-labeled, tritiated cells was obtained essentially as described under “Experimental Procedure” (one-tenth scale), and 3.5 ml were layered over a two-phase system containing 10 ml of Buffer D over 1 ml of 80% (w/v) sucrose in an 18-ml cellulose nitrate centrifuge tube. The 80% sucrose cushion was included to stop the plasma membrane fraction from pelleting, thus facilitating the collection of all the material in the tube. The resulting discontinuous gradient was centrifuged at 140 × g for 30 min in a swinging bucket clinical centrifuge (4°), fractionated from the top in an ISCO density gradient fractionator, and the fractions obtained were dialyzed and counted in PG. It can be seen that the majority of the 3H-surface label sediments to the bottom of the Buffer B whereas most of the intracellular constituents (3H) remain at the top. A second centrifugation of the sedimented material through Buffer B results in a similar distribution of surface label and further purification.

**Yield**—Unlike the marker enzyme approach for plasma membrane identification, the surface-labeling approach permits estimation of the yield of plasma membranes obtained in a given procedure. Table I indicates the yields of plasma membrane ghosts and vesicles obtained by our procedure. The lysate, plasma membrane ghost, and plasma membrane vesicle fractions were obtained from 35S-surface-labeled, tritiated cells as described under “Experimental Procedure,” dialyzed, and counted for 35S and 3H content. The amounts of the total 3H surface label recovered in the ghost and vesicle preparations indicate a yield greater than 50% for the plasma membrane ghosts and a yield greater than 20% for the plasma membrane vesicles.

**Electron Microscopy**—Presented in Fig. 2 are electron micrographs of samples taken at various points in the plasma membrane isolation procedure. Fig. 2A represents the intact cell surface just prior to the concanavalin A treatment, and Fig. 2B represents the cell surface just after the concanavalin A step. The effect of the accumulation of concanavalin A on the cell surface can be clearly seen. The size of the particles suggests that they are aggregates rather than individual concanavalin A molecules. Fig. 2, C and D, are low and high magnification pictures of the plasma membrane ghost preparation. The presence of concanavalin A particles (on one side only) is quite evident and plasma membranes at this stage exist primarily as nonvesiculated sheets. Fig. 2, E and F, are low and high magnification pictures of the plasma membrane vesicle preparation. Most of the concanavalin A aggregates are removed and the plasma membranes exist predominantly as small vesicles.

**Chemical Composition**—The chemical composition of the “cells” and plasma membrane vesicle preparations is presented in Table II. Since the plasma membrane ghost preparation is contaminated with significant amounts of a nonmembrane carbohydrate material, and small amounts of RNA, succinate dehydrogenase, and 5’-nucleotidase, the chemical analysis of the ghost preparation is not presented. The plasma membrane vesicles contain large amounts of sterol and the molar ratio of sterol to phospholipid is around 1.3. A high sterol to phospholipid ratio is characteristic of all eukaryote plasma membranes studied so far (12, 30). From the yield data in Table I it can be calculated that the plasma membrane of intact cells contain approximately 60% of all the cellular sterol, which is clearly not the case for any other chemical constituent of the plasma membranes measured. This observation is suggestive of a sine qua non relationship between eukaryote plasma membranes and sterols. The carbohydrate content of the plasma membrane vesicle preparation represents about 14% of the mass of the plasma membrane vesicles. Only very small amounts of RNA and DNA are present in the plasma membrane vesicle preparation.

**Enzyme Activities**—Table III summarizes the enzyme activities thus far detected in the plasma membrane ghost and plasma membrane vesicle preparations. The enzyme activities present in the “cells” preparation are included for comparison. The plasma membrane ghosts and vesicles have a Mg++ATPase ac-

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**Table I**

|                      | Preparation |          |          |          |          |          |
|----------------------|-------------|----------|----------|----------|----------|----------|
|                      | Lyzate      | Plasma membranes |          |          |          |          |
|                      | Ghosts      | Vesicles  |          |          |          |          |
| I                    | II          | I        | II       | I        | II       |
| 3H (cpm)             | 185,593     | 262,488  | 7,333    | 12,033   | 3,001    | 5,709    |
| 35S (cpm)            | 18,853      | 25,578   | 9,084    | 13,663   | 3,615    | 6,365    |
| 3H:35S               | 9.84        | 10.26    | 0.81     | 0.88     | 0.83     | 0.90     |
| 35S yield (%)        | 100         | 100      | 48       | 53       | 19       | 25       |
FIG. 2. Electron photomicrographs of the intact cell surface ghosts, and plasma membrane vesicles. See text for details.

Activity and a (Na\(^+\) + K\(^+\))-stimulated Mg\(^{2+}\)-ATPase activity. When corrected for yield the plasma membrane vesicles have about 15\% of the total cellular Mg\(^{2+}\)-ATPase activity and about 14\% of the total cellular (Na\(^+\) + K\(^+\))-stimulated Mg\(^{2+}\)-ATPase activity. It should be pointed out that the activities expressed in Table III are only approximate since optimum assay conditions have not yet been established. The plasma membrane ghosts have small amounts of succinate dehydrogenase and 5'-nucleotidase activities and, unlike the ATPase activities, these activities are reduced to trace levels in the plasma membrane vesicle prep-
Purity of Plasma Membrane Preparations—As mentioned above, the plasma membrane ghost preparation is contaminated by significant amounts of succinate dehydrogenase and 5'-nucleotidase. There is little contamination of the ghost and vesicle preparations by nuclei as indicated by the extremely low DNA content of these preparations. Contamination by endoplasmic reticulum is unlikely due to the extremely low g forces employed in the isolation procedure and the low RNA content of the preparations. The electron photomicrograph shown in Fig. 2 support the above conclusions. In addition, another criterion of purity arises from the yield experiment described in Table I. Since the amount of 3H-labeled protein and 32S surface label are lost at a constant ratio in the conversion of ghosts to vesicles it can be concluded that there is little 3H-labeled protein in the ghost preparation which is not plasma membrane in origin.

**DISCUSSION**

As pointed out in the Introduction, the isolation of plasma membranes from most eu-karyotic cells in high yield and purity is a difficult task. The major problems which impeded our efforts to isolate *Neurospora* plasma membranes were the unavailability of a suitable plasma membrane marker, the presence of a rigid cell wall which makes gentle lysis impossible, the tendency of the *Neurospora* plasma membranes to fragment and vesiculate immediately upon lysis trapping other cellular constituents, and the variable densities of the plasma membrane particles which lead to extensive smearing in standard isopycnic centrifugation procedures.

The unavailability of a suitable plasma membrane marker was the first problem to be surmounted. In a previously uncharacterized free living cell, the only predictable difference between the plasma membrane and other cellular membranes is that the plasma membrane is exposed to the external environment. Thus, surface labeling by membrane impermeable reagents (see Ref. 12 for a review) seems to be the most dependable method for identifying plasma membranes in any isolation procedure involving free living cells. In addition to providing a method for confidently identifying plasma membranes, the surface-labeling approach allows for an accurate estimation of the yield of plasma membranes in a given procedure. This is not possible with the marker enzyme approach. The surface-labeling approach to plasma membrane isolation has been applied recently in the isolation of a yeast plasma membrane fraction (31).

The rigid cell wall problem was obviated simply by switching from the wild type strain to a cell wall-less mutant of *Neurospora*.

The extreme problems which arise when plasma membranes fragment and vesiculate immediately upon lysis can be solved only by somehow stabilizing the plasma membranes prior to lysis so that they do not spontaneously fragment and vesiculate and may be isolated as sheets or "ghosts" which represent the intact plasma membrane and other cellular membranes is that the plasma membrane is exposed to the external environment. Thus, surface labeling by membrane impermeable reagents (see Ref. 12 for a review) seems to be the most dependable method for identifying plasma membranes in any isolation procedure involving free living cells. In addition to providing a method for confidently identifying plasma membranes, the surface-labeling approach allows for an accurate estimation of the yield of plasma membranes in a given procedure. This is not possible with the marker enzyme approach. The surface-labeling approach to plasma membrane isolation has been applied recently in the isolation of a yeast plasma membrane fraction (31).

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ghosts it was found that a considerable amount of nonmembrane carbohydrate material, perhaps glycogen granules, cosediments with the ghosts. This nonmembrane carbohydrate material is the only major contaminant in the ghost preparation thus far detected. It is removed in the final purification step. In this step, the marked change in the density of the plasma membranes which takes place when the bulk of the concanavalin A is removed (i.e. the ghost to vesicle transition) is exploited. Any material which is dense enough to cosediment with the concanavalin A coated membrane ghosts sediments again in the 20% sucrose floatation step whereas the plasma membranes which were dense only as a result of the concanavalin A coat can no longer enter the 20% sucrose layer after the bulk of the concanavalin A is removed by the α-methylmannoside treatment. Thus the nonmembrane carbohydrate material and any other cellular material which was not previously complexed with concanavalin A are removed in the 20% sucrose floatation step.

The advantages of Neurospora as an experimental organism for the investigation of membrane structure and function have been discussed (33). The isolation of N. crassa plasma membranes reported here makes feasible a great variety of biochemical approaches to plasma membrane structure and function which were heretofore impossible with Neurospora. The ability to isolate the st plasma membranes predominantly as open sheets and the ability to subsequently convert the sheets to vesicles offers unique advantages for studies designed to explore the nature of energy coupling in the glucose active transport system. During the sheet to vesicle transformation it should be possible to load the vesicles with ATP or other high energy compounds and in this manner gain insight as to the nature of the high energy compound which energizes the active transport system. This approach, of course, requires that a significant proportion of the plasma membrane vesicles be resed in a right-side-out orientation. If this is not the case, it should be possible to load the vesicles with glucose, and energize active export with externally added ATP or other high energy compounds. Determination of the sidedness of the plasma membrane vesicles may be possible with immobilized concanavalin A columns (34). The ATPase activity of the isolated Neurospora plasma membranes is also of interest. Recently a very strong case has been made for the identity between plasma membrane ATPase activity and the electrogenic pump which maintains the Neurospora membrane potential (35). Biochemical characterization of the st plasma membrane ATPases should provide additional support for this contention. An earlier report from this laboratory showed a role of inositol phosphatides in the function of the glucose active transport system (36), and, we have recently demonstrated the presence of polyphosphoinositides in Neurospora. But further progress on the nature of the role of inositol lipids in transport was impossible without a purified plasma membrane preparation. The availability of highly purified Neurospora plasma membranes now provides an experimental basis for studies designed to correlate some aspect of inositol lipid metabolism with the function of the glucose active transport system. The many-fold enrichment of transport system components afforded by prior isolation of the plasma membranes greatly increases the sensitivity of attempts to identify such transport components. Experiments designed to identify components of the derepressible glucose active transport system utilizing double labeling techniques are presently underway. Finally, the ability to isolate Neurospora plasma membranes in high yield and purity makes possible studies designed to probe the nature of plasma membrane biogenesis and development. Such studies are now in progress.

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