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PREPARATION AND IDENTIFICATION OF YEAST PLASMA MEMBRANE VESICLES

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SUMMARY

Vesicles isolated from ground yeast cells were separated on a sucrose density gradient into two fractions. From electron micrographs the vesicles of the denser fraction were identified as pure plasma membrane vesicles. The vesicles of the lighter fraction consist mainly of mitochondrial vesicles with some contamination by small plasma membrane vesicles. Biochemical findings support morphological characterization of the described vesicles. Respiration was absent from the plasma membrane fraction whereas the mitochondrial vesicles demonstrated respiration which could be almost completely inhibited by antimycin or KCN. Differences were also found in carbohydrate and DNA content and in protein composition. Two different ATPases, stimulated by various divalent cations, were observed in the two vesicle preparations. That in the mitochondrial fraction was highly sensitive to oligomycin, with a pH optimum between pH 8 and 9. The plasma membrane ATPase was not sensitive to oligomycin and maximal values were found around pH 7. The plasma membrane ATPase appears to be related to transport of divalent cations in intact yeast cells. This is indicated by the same order of affinity for transport as for ATPase activation by divalent cations and sensitivity to ATPase inhibitors. Mn^{2+} transport and Mn^{2+}-activated ATPase are insensitive to oligomycin and ouabain whereas both are inhibited by lanthanum and carbodiimide.

INTRODUCTION

Many transport functions have been attributed to the plasma membrane of the yeast cell, but this knowledge comes mainly from measurements on intact cells [1–5]. The aim of this study was to derive a preparation of plasma membranes, suitable for transport experiments. Several authors have recently described isolation and identification of yeast plasma membranes [6–9]. Yeast plasma membranes were identified by a characteristic particulate structure in electron micrographs and by so-called "plasma membrane markers". Markers are a mannan containing glycoprotein and the enzymes invertase and Mg^{2+}-dependent ATPase, for example. The

Abbreviation: EDAC, *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide.

Mg^{2+}-dependent ATPase differs from mitochondrial ATPase in being oligomycin insensitive. Also, the absence of oxidoreductase has been demonstrated. In a recent paper, Christensen and Cirillo [8] reported isolation of yeast plasma membrane vesicles, which showed some transport characteristics. In contrast to the above findings their vesicle preparation displayed respiration functions, especially NADH oxidase activity and sensitivity of the Mg^{2+}-dependent ATPase to oligomycin. We modified their preparation procedure and added a sucrose density gradient, which separated the vesicle preparation into two bands. This paper describes the morphological and biochemical characteristics of these two fractions.

MATERIALS AND METHODS

Vesicle preparation

Washed, fresh commercial bakers yeast (*Saccharomyces cerevisiae*, Hefefabriken Hindelbank AG, Bern) was starved, to utilize surplus substrate, under aeration for 15 h and washed again three times with distilled water. The yeast cells were checked for bacterial contamination by microscopic examination. They were then suspended and washed in an ice-cold solution of osmotic stabilizer (0.4 M KCl and 20 mM triethanolamine, pH 7) and packed for 20 min at $5000 \times g$.

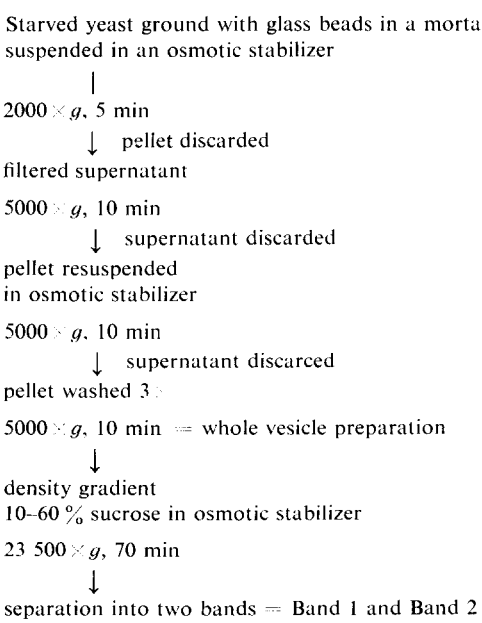


Fig. 1. Scheme of vesicle preparation.

The preparation of vesicles is shown in a scheme in Fig. 1. The method of Christensen and Cirillo [8] was modified. 30 g of the ice-cold packed yeast was mixed into a paste with 75 g of glass beads (0.25–0.30 mm diameter, Braun Melsungen, G. F. R.) in a mortar on ice. The mixture was ground by gentle rotation

for 5–10 min until it became slightly moist. After grinding, 70 ml ice-cold osmotic stabilizer solution was added and the suspension left to stand until the glass beads had settled. The overlying fluid was decanted into two 50-ml plastic tubes and the vesicles were separated by differential centrifugation in a Sorvall RC2-B centrifuge. At first the fluid was spun down at $2000 \times g$ for 5 min and the sediment, consisting of whole cells and heavy debris, discarded. The $2000 \times g$ supernatant was filtered using a glass fiber filter (Sartorius, 13 400, Membranfilter GmbH, (G.F.R.)) to remove remaining cells and spun at $5000 \times g$. Thereafter the supernatant, containing microsomes and other light particles, was discarded. The pellet was resuspended in osmotic stabilizer and washed three times at $5000 \times g$ for 10 min. In an additional step to the method of Christensen and Cirillo [8] the vesicle preparation was submitted to a density gradient (continuous or discontinuous) from 10–60 % sucrose in osmotic stabilizer solution at $23\,500 \times g$ for 70 min (Sorvall, Swinging Bucket Rotor HB-4). This resulted in the separation of the vesicles into mainly two bands (Fig. 7). After removal from the gradient, sucrose was washed from the two bands three times in osmotic stabilizer solution.

Analytical methods

Protein was determined by a modified Biuret method, taking into account turbidity [10].

Adenosine triphosphatase (EC 3.6.1.4) activity was measured by enzymatic determinations of ATP or by the release of inorganic phosphate from ATP. ATPase reaction was stopped with $HClO_4$ and ATP or inorganic phosphate analysed in the supernatant after centrifugation. ATP was measured using Biochemica Test Combination No. 15979, Boehringer Mannheim GmbH, (G.F.R.) and inorganic phosphate according to Fiske and SubbaRow [11]. Corrections for inorganic phosphate from vesicles and ATP source were made.

Invertase (EC 3.2.1.26) activity was tested by measuring glucose released when vesicle preparations were incubated in osmotic stabilizer solution at pH 6, 28 °C with 2 mM sucrose. Glucose was analysed enzymatically [12] in the supernatant after centrifugation.

O_2 uptake was measured with a Clark oxygen electrode (Yellow Springs Instruments, Ohio, U.S.A.) in a closed chamber at 30 °C. The vesicle preparation was suspended in osmotic stabilizer solution with 10 mM KH_2PO_4 at pH 7.

Total carbohydrate, mannan and alkali soluble glycogen were determined using the anthrone reagent and fractionation scheme described by Trevelyan and Harrison [13].

Nucleic acids, fractionated according to Trevelyan and Harrison [13], were extracted with 0.5 M $HClO_4$ for 16 min at 90 °C. RNA was measured with orcinol reagent after Lusena [14] and DNA with diphenylamine reagent after Burton [15].

Sodium dodecylsulphate polyacrylamide gel electrophoresis of the proteins in the whole preparation, Band 1 and Band 2 was carried out according to the method of Fairbanks et al. [16].

Electron microscopy

For thin sectioning, pellets of the vesicle preparation were fixed in a mixture of 3 % glutaraldehyde and 3 % acrolein in 0.1 M cacodylate buffer, pH 7.2, washed in

0.1 M cacodylate buffer and postfixed in 1 % OsO_4 in 0.1 M cacodylate buffer, pH 7.2. This material was blockstained in a 0.5 % solution of uranyl acetate, dehydrated and embedded in a mixture of epon-araldite [17]. The thin sections were poststained with lead citrate [18].

For freeze-fracturing, the vesicle preparations were pelleted in the osmotic stabilizer solution containing 30 % glycerol and frozen in liquid freon 22. Freeze-fracturing was performed in a Balzers BA 360 apparatus after a slightly modified procedure of Moor [19]. Fracturing was done at minus 110 °C, immediately followed by shadowing.

Chemicals

ATP disodium salt, Boehringer Mannheim GmbH, G.F.R.; oligomycin, Serva Heidelberg, G.F.R.; *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDAC), Fluka AG Buchs SG, Switzerland. All other chemicals were also A.R. reagents. The chloride salts of divalent cations and lanthanum were used.

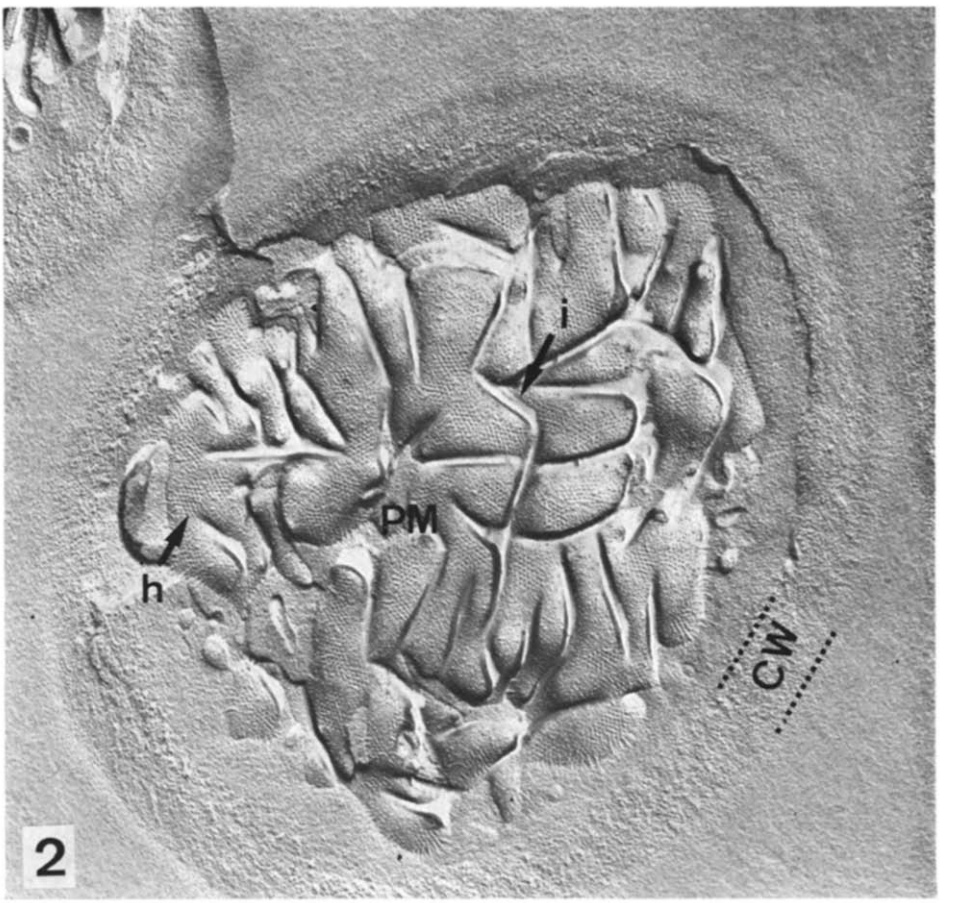
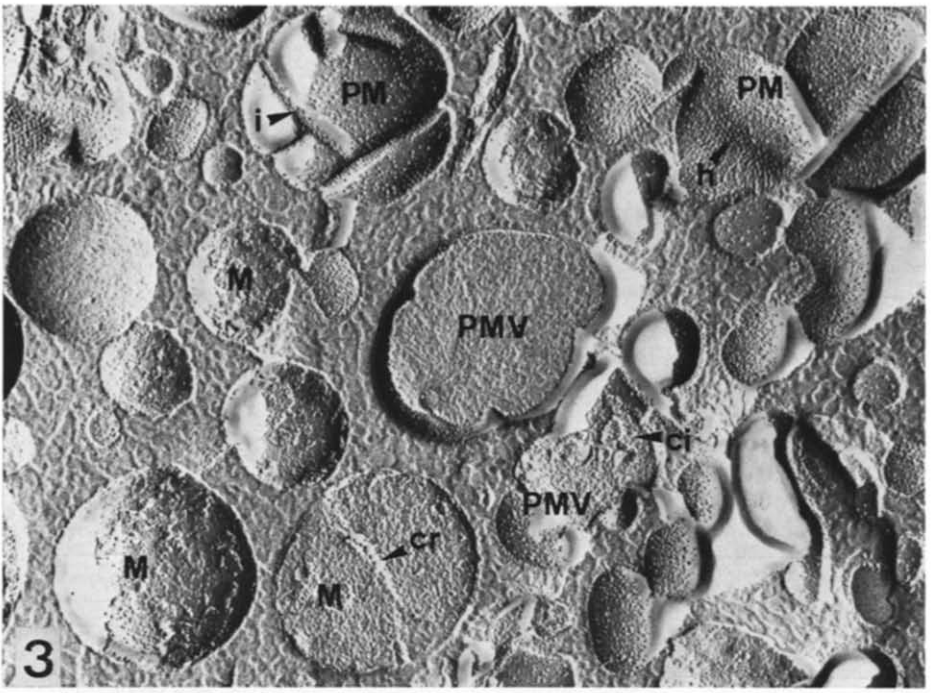


Fig. 2. Starved yeast cell after freeze-fracturing. Plasma membrane (PM) with invaginations (i) and hexagonal particle arrrangements (h). The cell wall (CW) is indicated. · 40 000.



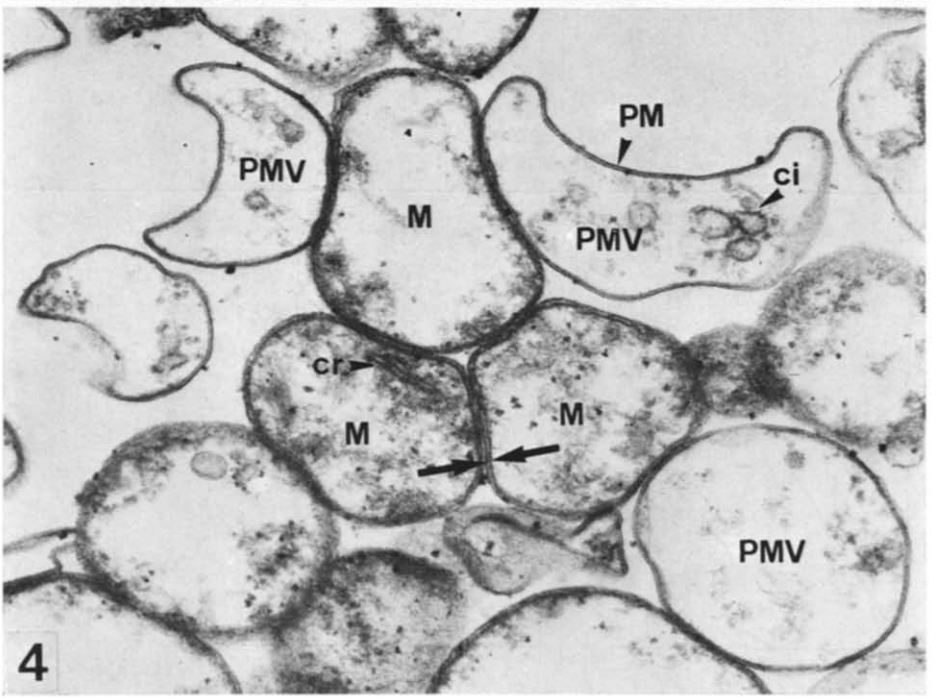


Fig. 3. Freeze-fracture replica of a whole vesicle preparation. Plasma membrane vesicles (PMV) are shown in cross section. They contain cytoplasmic inclusions (ci). Fracture faces of the plasma membrane (PM) show invaginations (i) and hexagonal particle arrangements (h). Mitochondrial vesicles (M) in cross section show residual cristae (cr). × 40000.

Fig. 4. Thin section of the same preparation seen in Fig. 3. Labelled as above. The two arrows indicate the outer and inner mitochondrial membranes. × 50000.

RESULTS

In Fig. 2 a typical view of the plasma membrane fracture face of a starved yeast cell is seen. The plasma membrane is characterized by its numerous invaginations and regions with hexagonal arrangements of membrane particles. These structures are the morphological markers for identification of the yeast plasma membrane. Due to the hypertonic medium the protoplast is detached from the surrounding cell wall.

The next electron micrograph (Fig. 3) shows a freeze-fracturing of the whole vesicle preparation before its separation on the density gradient. It consists of approximately equal amounts of vesicles with invaginations and hexagonal arrangements of membrane particles and spherical vesicles without such surface structures. The first kind of vesicle can be morphologically identified as plasma membrane vesicles frag-

TABLE I

RESPIRATION OF THE WHOLE VESICLE PREPARATION OF BAND 1 AND BAND 2

Data are in natoms/mg protein per min. Experimental conditions are given in Methods. Substrate concentration was 1 mM. Number of experiments in brackets, results expressed as mean ± S.E.

Vesicle preparation	Q_0 (natoms/mg of protein per min)				
	Endogenous respiration	NADH	Succinate	Ethanol	Pyruvate
Whole preparation	0 (1)	263.36 (1)	30.94 (1)	19.04 (1)	7.14 (1)
Band 1	2.23±1.71 (6)	195.05±45.13 (6)	33.72±12.31 (5)	8.81 (2)	9.67 (2)
Band 2	0 (6)	0.28±0.28 (6)	0 (4)	0 (2)	0 (2)

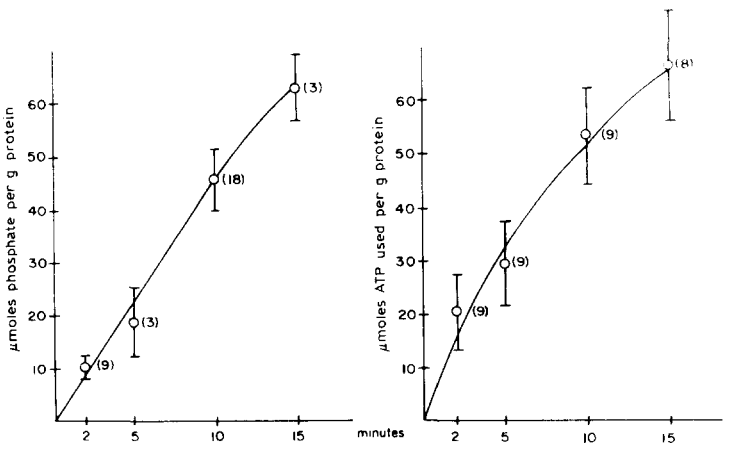


Fig. 5. Comparison between inorganic phosphate released and ATP used by Mg^{2+}-activated ATPase in whole vesicle preparation. Vesicles were incubated with 1 mM $MgCl_2$ and 1 mM ATP at pH 7 and 22 °C. The number of experiments is given in brackets, results as mean ± S.E.

mented from the yeast protoplast. Some small vesicular inclusions are seen in cross fractures. The second kind of vesicles has double membranes and sometimes residual cristae. From a morphological standpoint, they are therefore of mitochondrial origin and designated mitochondrial vesicles.

In a thin section of the same preparation (Fig. 4) the double membranes of the

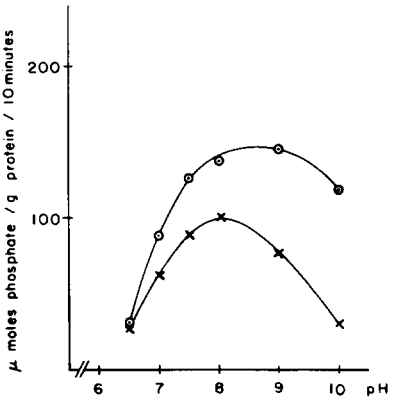


Fig. 6. pH-dependence of Mg^{2+}-activated ATPase in whole vesicle preparation with (×–×) and without (○–○) oligomycin (34 µg/ml). Experimental conditions as in Fig. 5.

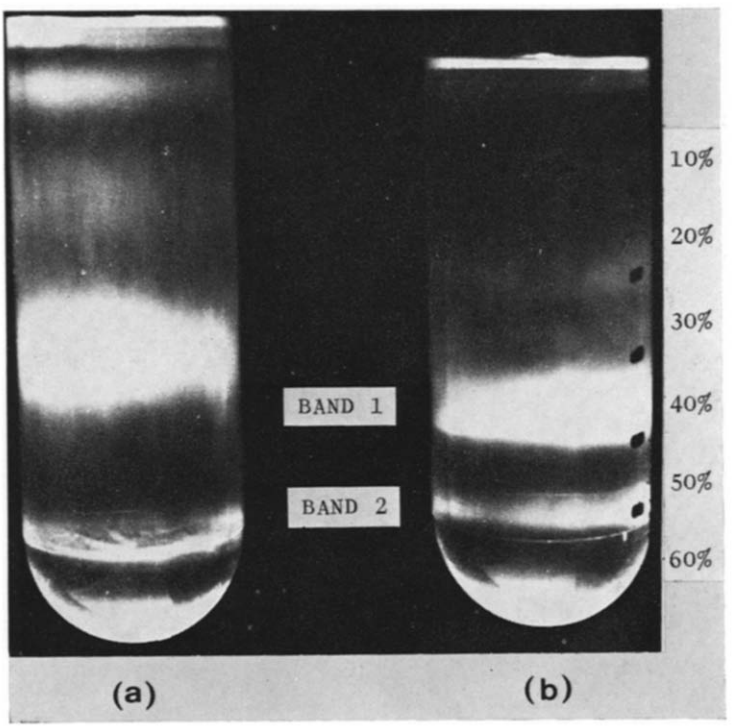


Fig. 7. Separation of whole vesicle preparation on sucrose density gradients: a, continuous sucrose gradient from 10–60 % and b, discontinuous sucrose gradient from 10–60 %.

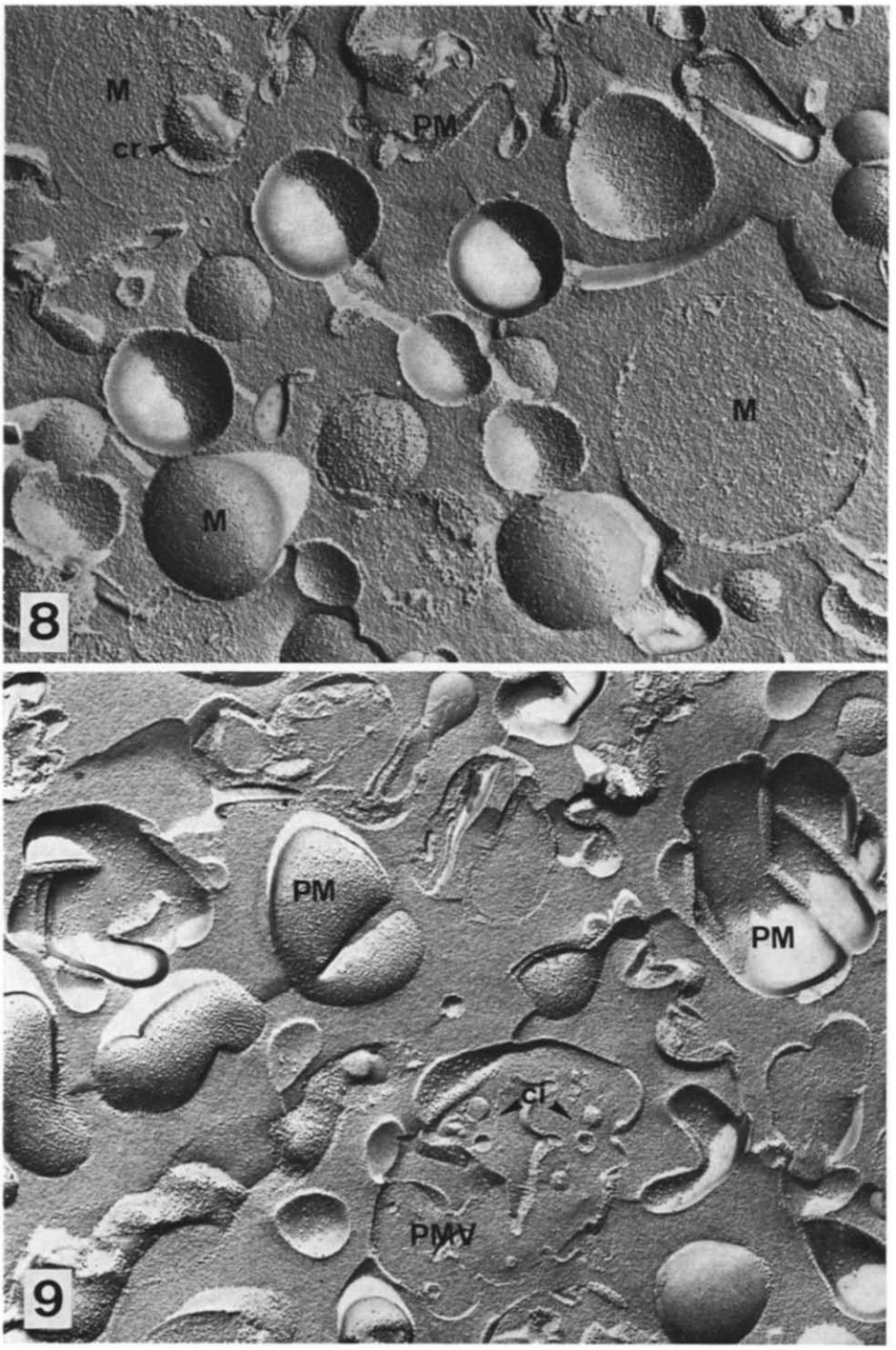


Fig. 8. Freeze-fractured pellet of Band 1. It consists mainly of mitochondrial vesicles (M) and some plasma membranes (PM).

Fig. 9. Freeze-fractured pellet of Band 2. It is a purified preparation of plasma membranes (PM). The plasma membrane vesicles (PMV) show cytoplasmic inclusions (ci).

mitochondrial vesicles are clearly visible. The central distance of the two dark lines of their unit membranes measures only 40 Å. The plasma membrane vesicles, however, have a much thicker unit membrane of 70 Å central distance. They have vesicular inclusions and amorphous material as in mitochondrial vesicles.

Biochemical results on the above preparation are in accordance with the data reported by Christensen and Cirillo [8]. The preparation shows respiration functions as summarized in Table I. On addition of NADH respiration is highest whereas succinate, pyruvate or ethanol only slightly stimulate O_2 consumption. We also find invertase and Mg^{2+}-dependent ATPase activity which splits added ATP into ADP and inorganic phosphate at pH 7. Fig. 5 shows this by comparing phosphate released with ATP used. The ATPase activity has a pH dependence comparable with the findings of the above authors. There is an increased activity towards the alkaline region with an optimum above pH 8 (Fig. 6) and inhibition by oligomycin.

Fig. 7 shows that the vesicle preparation described above separates into two distinct bands on a sucrose density gradient. This was possible on a continuous as well as a discontinuous density gradient. A brownish pellet is obtained from the lighter fraction, designated Band 1 and a white one from the denser fraction, Band 2.

In Figs 8 and 9, the freeze fracturings of Bands 1 and 2 are seen. Band 1 (Fig. 8) consists mainly of spherical mitochondrial vesicles, similar to those in the whole vesicle preparation (Fig. 3). There is, however, slight contamination by small plasma membrane vesicles. Band 2 (Fig. 9) consists almost entirely of pure plasma membrane vesicles, which contain cytoplasmic inclusions. The plasma membrane vesicles are all right-side-out oriented.

Not only morphologically is there a clear-cut distinction between Band 1 and Band 2, but also in biochemical tests. Table I shows respiration measurements on the whole vesicle preparation in comparison with Bands 1 and 2. Endogenous respiration was not measurable except in Band 1 on a very low level. After addition of substrates such as NADH, succinate, ethanol or pyruvate, comparable rates of respiration could be measured in the whole vesicle preparation and in Band 1. The same order of stimulation was obtained, the highest respiration being recorded with NADH. Respiration could be completely inhibited by 0.3 μg/ml antimycin or 2 mM KCN. No significant respiration was measurable after addition of substrates in Band 2.

In Table II the vesicle composition of Band 1 and Band 2 is given in respect to total carbohydrate, mannan, alkali soluble glycogen, RNA and DNA. The carbohydrates, except glycogen, are significantly higher in Band 2 than in Band 1. The mannan content is high, approximately 1/3 of the total carbohydrate. In both preparations DNA was measurable, that in Band 2 significantly lower than in Band 1. On a weight to weight basis, with protein assumed to be 50 %, the total carbohydrate content of Band 1 is 8.0 % compared with 14.4 % in Band 2. RNA content is in the region of 0.9 % for both bands and DNA 0.21 % in Band 1 and 0.13 % in Band 2.

Differences in protein composition between Band 1 and 2 are also seen in the densitograms from sodium dodecylsulphate gel electrophoresis (Fig. 10). In the whole vesicle preparation (Fig. 10A) there are 9 prominent peaks. After separation of the whole vesicle preparation into Band 1 and Band 2 the size distribution of the peaks is changed (Fig. 10B). Band 1 shows a similar pattern to the whole vesicle preparation, whereas in Band 2 some peaks are absent or reduced so that mainly 4

TABLE II

THE COMPOSITION OF BAND 1 AND BAND 2 IN RESPECT TO TOTAL CARBOHYDRATE, MANNAN+GLYCOGEN, MANNAN, GLYCOGEN, RNA AND DNA

Data are mmoles hexose or pentose per g protein. Number of experiments in brackets, results given as mean ±S.E. Statistical differences between Band 1 and Band 2 shown by *** ($P = 0.001$), ** ($P = 0.01$) and *+ ($P = 0.025$).

Vesicle preparation	Total carbohydrate	Mannan + glycogen	Mannan	Glycogen	RNA	DNA
Band 1	0.444*** ±0.061 (6)	0.198** ±0.042 (6)	0.166*+ ±0.057 (6)	0.039 ±0.021 (6)	0.0225 ±0.0027 (8)	0.0053** ±0.0012 (8)
Band 2	0.798*** ±0.109 (6)	0.344** ±0.062 (6)	0.275*+ ±0.037 (6)	0.068 ±0.027 (6)	0.0239 ±0.0028 (8)	0.0032** ±0.0013 (8)

peaks can be seen.

Invertase activity could be detected in both bands. In Band 2 about twice that in Band 1. The mean activity of three experiments was 1.9 μmoles glucose per mg protein per min for Band 1 and 3.9 μmoles glucose per mg protein per min for Band 2.

In Table III the Mg^{2+}-dependent ATPase activity was measured at pH 7 in Band 1 and Band 2. Also the sensitivity of the ATPase of the two bands to oligomycin was tested. The preparation of Band 1 showed an ATPase activity of 85.05±22.62 μmoles phosphate per g protein in 10 min, which was strongly inhibited by 62 %

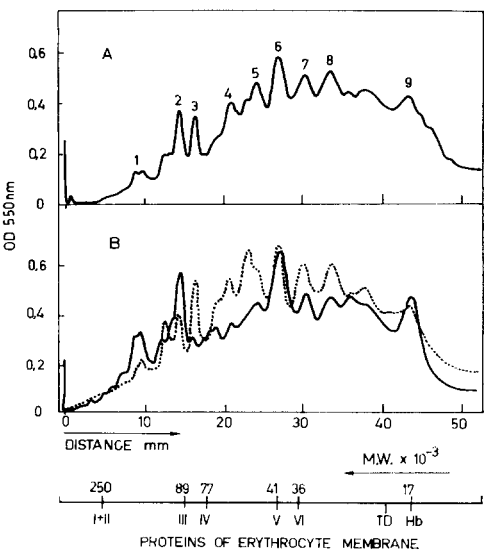


Fig. 10. Density profiles of sodium dodecylsulphate gel electrophoresis on the whole vesicle preparation (A) and both bands (B). Band 1 (· · ·) and Band 2 (———). Membrane proteins of known molecular weights (components I–VI, see ref. 16) were run in parallel to establish an approximative molecular weight scale. TD = tracking dye; Hb = hemoglobin.

TABLE III

Mg^{2+}-ACTIVATED ATPase OF BAND 1 AND BAND 2 WITH AND WITHOUT OLIGOMYCIN

Results are given in μmoles phosphate released per g protein in 10 min. The vesicles were incubated with 1 mM $MgCl_2$ and 1 mM ATP at pH 7 and 22 °C. The number of experiments is given in brackets, results expressed as mean $\pm$S.E. Statistical differences between the related values with and without oligomycin is marked by ** ($P = 0.01$) and * ($P = 0.05$).

Vesicle preparation	No oligomycin	Oligomycin (17 μg/ml)	Oligomycin (34 μg/ml)
Band 1	85.05 $\pm$ 22.62	32.66 $\pm$ 5.96*	24.28 $\pm$ 4.48*
	(8)	(8)	(8)
	100 %	38.4 %	24.3 %
Band 2	50.57 $\pm$ 10.20	40.70 $\pm$ 6.61	34.78 $\pm$ 7.25**
	(8)	(8)	(8)
	100 %	80.5 %	68.8 %

with 17 μg oligomycin per ml and by 76 % with 34 μg oligomycin per ml. The ATPase activity in Band 2 was 50.57 $\pm$ 10.20 μmoles phosphate per g protein in 10 min, not significantly inhibited by the lower oligomycin concentration and by 31 % with the higher.

In Bands 1 and 2 the pH dependence of the ATPase activity with Mg^{2+} and Mn^{2+} was measured (Fig. 11). Both divalent cations stimulate the ATPase to about the same degree. ATPase activity of Band 1 increases with either cation in the alkaline region, maximal values were obtained between pH 8 and 9. The ATPase activity in Band 2 has its optimum between pH 7 and 8 with Mg^{2+} and pH 7–7.5 with Mn^{2+}. Fig. 12 shows, in a parallel experiment on both bands, the pH dependence of Mn^{2+}-activated ATPase with and without oligomycin. The ATPase of Band 1, in

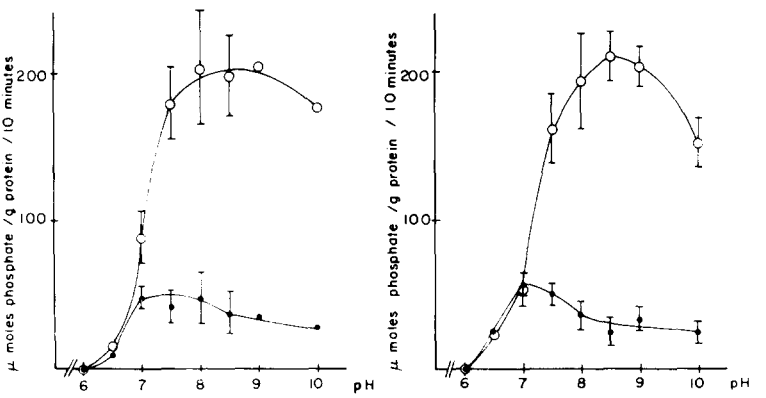


Fig. 11. pH-dependence of Mg^{2+}- (left side) and Mn^{2+}-activated ATPase (right side) in Band 1 (○–○) and Band 2 (●–●). Vesicles were incubated with 1 mM $MgCl_2$ or $MnCl_2$ and 1 mM ATP for 10 min at 22 °C. The number of experiments for Mg^{2+}-activated ATPase was 3 at pH 6.5, 7.5, 8.0 and 8.5, 2 at pH 9.0 and 10.0 and 10 at pH 7.0. For the Mn^{2+} experiment the number was 4 at pH 6.5, 7.5, and 8.0, 3 at pH 8.5, 9.0 and 10.0 and 10 at pH 7.0. Results expressed as mean $\pm$S.E.

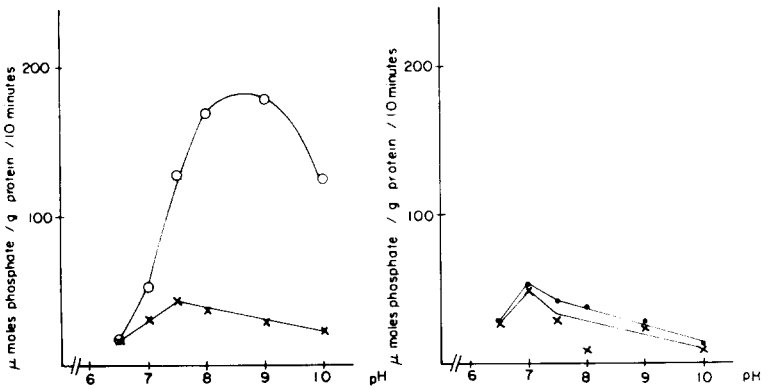


Fig. 12. pH-dependence of Mn^{2+}-activated ATPase in Band 1 (○-○) and Band 2 (●-●) with (· - - ·) and without oligomycin (34 µg/ml). Experimental conditions as in Fig. 11.

the tested region from pH 6.5–10.0 is strongly inhibited by oligomycin at all pH values. The remaining ATPase activity demonstrates a change in the optimum towards pH 7.0. In Band 2 only a slight inhibitory effect by oligomycin and no change of the pH optimum were observed.

The dependence of the ATPase activity on different divalent cations and the trivalent lanthanum at pH 7 is summarized in Table IV. The results in the different preparations vary only slightly. In respect to transport of divalent cations in intact yeast cells, the results on the plasma membrane vesicles in Band 2 are especially interesting, since the data are parallel to the transport affinities of the respective cations [4, 20]. La^{3+} inhibits the ATPase activity strongly, Sr^{2+} does not activate, Ca^{2+} and Ni^{2+} activate slightly whereas the other divalent cations highly activate when compared with controls without divalent cations.

TABLE IV

ACTIVATION OF ATPase IN THE WHOLE VESICLE PREPARATION, BAND 1 AND BAND 2 BY SEVERAL DIVALENT CATIONS AND La^{3+}

Preparations were incubated with 1 mM divalent cation or La^{3+} and a control without such cations, 1 mM ATP at pH 7 and 22 °C. The number of experiments is given in brackets, results expressed as µmoles phosphate released per g protein in 10 min, mean ±S.E.

	Control	La^{3+}	Sr^{2+}	Ca^{2+}	Ni^{2+}	Mn^{2+}	Zn^{2+}	Co^{2+}	Mg^{2+}
Whole preparation	11.74 ±2.26 (6)	3.00 ±1.32 (6)	10.01 ±2.32 (6)	19.07 ±6.24 (9)	21.89 ±6.05 (8)	54.30 ±5.80 (7)	62.79 ±8.35 (9)	81.45 ±6.33 (10)	45.63 ±5.66 (18)
Band 1	15.72 ±3.44 (6)	8.24 ±4.24 (5)	20.09 ±5.73 (7)	25.97 ±3.17 (8)	34.51 ±2.65 (7)	41.32 ±7.87 (8)	50.58 ±6.87 (8)	77.12 ±18.95 (8)	64.16 ±16.88 (11)
Band 2	8.76 ±2.70 (5)	0.19 ±0.18 (4)	7.56 ±3.75 (6)	14.79 ±5.16 (7)	14.98 ±3.99 (6)	61.34 ±8.74 (7)	45.93 ±6.68 (7)	92.25 ±8.74 (7)	47.34 ±8.93 (11)

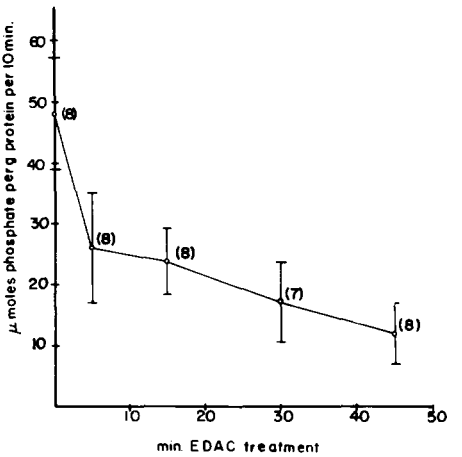


Fig. 13. Mn^{2+}-activated ATPase in plasma membrane vesicles (Band 2) after pretreatment with 5 mM EDAC. Vesicles were incubated with 1 mM $MnCl_2$ and 1 mM ATP for 10 min at pH 7 and 22 °C. Number of experiments is given in brackets and results are expressed as mean ± S.E.

Fig. 13 demonstrates the sensitivity of Mn^{2+}-activated ATPase from plasma membrane vesicles (Band 2) at pH 7 to EDAC, which is known to inhibit transport ATPase in several membrane preparations [21–23]. Ouabain, the well known inhibitor of the Na^+, K^+ and Mg^{2+}–ATPase, had no effect.

DISCUSSION

The paper of Christensen and Cirillo [8] describes the preparation of yeast plasma membrane vesicles, osmotically active with energy-independent transport functions such as amino acid, uracyl and sugar transport. By freeze-fracturing the vesicle preparation which was obtained by a slight modification of Christensen and Cirillo's method, we found plasma membrane vesicles and mitochondrial vesicles. The freeze-fractures of the mitochondrial vesicles are indistinguishable from freeze-fractures of yeast promitochondria, published by Packer et al. [28]. Apart from the two types of vesicles, occasionally lipid vesicles and vacuolar membranes were present (not shown). From our electron microscopic examinations the "unit membrane" of the starved yeast plasma membranes measures between 100 and 140 Å. This is somewhat more than 75 Å in mammalian membranes [27] and might be due to the high carbohydrate, especially mannan, content. The values for lipids of the yeast plasma membrane, by dry weight, are in the range of 30–46 % and for proteins 30–50 % [6, 7, 24–26].

Our biochemical data confirm Christensen and Cirillo's [8] findings. The rates of respiration on the above preparation were comparable with their values [8]. We too found Mg^{2+}-dependent ATPase activity, with a similar pH dependence, a pH optimum above pH 8 and inhibition with oligomycin. Finally, both their vesicle preparation and ours have invertase activity and a high mannan content. They, however, attribute the respiration functions to intravesicular mitochondria whereas in our preparation a mixture of mitochondrial and plasma membrane vesicles has been shown.

When the vesicle preparation is separated on a sucrose density gradient, the denser fraction consists almost entirely of pure plasma membrane vesicles. They often have small vesicular enclosures and amorphous material but enclosures of mitochondria are seldom and cell nuclei, or parts of them, could not be seen. The lighter fraction consists mainly of mitochondrial vesicles. Generally this fraction is the less pure of the two because it retains small plasma membrane vesicles.

Biochemical examinations of both fractions support these morphological findings. The plasma membrane fraction shows no significant respiration whereas in the lighter mitochondrial fraction respiration is increased by addition of substrates. Because yeast mitochondria oxidize added NADH [30], the respiration with NADH as substrate does not mean that the mitochondrial vesicles are damaged, as one would assume in mammalian mitochondria. From these results and almost complete inhibition with antimycin [31] or KCN, it can be concluded that a respiration chain is present in these vesicles.

The total carbohydrate content of the plasma membrane vesicles was 14.4 %, which lies in the range of 3–30 % reported by several authors [6, 7, 24–26]. This wide range could be due to differences in preparation methods. When using snail enzyme for isolating plasma membranes the carbohydrate content is usually low compared with that obtained by mechanical isolation procedures. The high mannan content and low invertase activity of our plasma membrane vesicles agree well with the data of Matile [7]. However, the DNA content of 0.13 % cannot be readily explained. From sodium dodecylsulphate gel electrophoresis on the plasma membrane fraction four main protein peaks, 1, 2, 6 and 9, with approximate molecular weights of 150 000, 90 000, 40 000, and 17 000 are seen. In yeast mitochondria there are usually more than six different-sized proteins [32]. Our results on the mitochondrial fraction demonstrate 11 peaks ranging between 17 000 and 150 000 Daltons. The coincidence with the four main peaks of the plasma membrane fraction points to the already mentioned contamination of the mitochondrial fraction. Peak 6 is common to both fractions in equal amounts.

Both fractions have Mg^{2+}-dependent ATPase which splits ATP into ADP and inorganic phosphate. This was demonstrated not only by parallel determinations of ATP used to phosphate released from ATP source, but also by thin-layer chromatography using labelled ATP [33]. The only nucleotide produced was ADP, no significant amounts of AMP or cyclic AMP could be detected. The pH dependence of the Mg^{2+}-activated ATPase in both fractions agree with the reported data [6, 7, 35–37]. In the plasma membrane fraction there is a broad pH optimum around pH 7, while in the mitochondrial fraction ATPase activity increases significantly in the alkaline region, with maximal values between pH 8 and 9. A similar curve was obtained with Mn^{2+} instead of Mg^{2+} as activating cation. Sharper pH optima were found, that in the plasma membrane fraction at pH 7 and that in the mitochondrial fraction at pH 8.5. The effect of oligomycin on the two fractions is in accordance with results published on mitochondrial ATPase and Mg^{2+}-dependent ATPase of purified yeast plasma membranes [6, 7, 32]. The slight inhibition of the Mg^{2+}-activated ATPase in our plasma membrane fraction with 34 μg/ml oligomycin could be due to an unspecific ATPase inhibition which is known to occur also in non-mitochondrial ATPases at high oligomycin concentrations [34] and possibly contamination by soluble ATPase from the mitochondrial fraction.

Finally we examined the properties of the divalent cation-activated ATPase in plasma membranes in relation to divalent cation transport in intact yeast cells more closely. Mg^{2+}, Co^{2+}, Zn^{2+} and Mn^{2+} have the highest affinity for transport [4, 20] and show highest activation of the ATPase in plasma membrane vesicles. Ni^{2+} and Ca^{2+} have a low affinity and activate only slightly, while Sr^{2+} is not transported nor does it activate. La^{3+} acts as a strong inhibitor in both systems. A similar activation by divalent cations on mitochondrial ATPase is known [36]. Further comparisons between the Mn^{2+}-activated ATPase in plasma membranes and Mn^{2+} transport in intact yeast cells gave parallel results regarding their reactions to ATPase inhibitors. There was no inhibitory effect by oligomycin or ouabain and inhibition by EDAC [38]. These results support the assumption that divalent cation-activated ATPase in plasma membranes of yeast cells is related to transport.

It is evident from morphological and biochemical findings that fragments of plasma membrane have been isolated. However, the intactness of these fragments in respect to transport functions has not yet been fully explored and is under investigation.

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