Vesicular membrane transfer between endoplasmic reticulum and Golgi apparatus of a green alga, *Micrasterias americana*

A 16 °C block and reconstitution in a cell-free system

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Summary. Transfer of membranes between endoplasmic reticulum and Golgi apparatus of the unicellular green alga, *Micrasterias americana*, is facilitated by 50-70 nm vesicles that form from part-rough, part-smooth transitional regions of the endoplasmic reticulum. In growing cells, the vesicles are present at the normal growth temperature of 23 °C. However, at 16 °C, vesicle accumulations occur. Golgi apparatus of non-growing cells exhibited both larger numbers of vesicles and larger dictyosomes at all temperatures. In non-growing cells, vesicle numbers also were increased at 16 °C. The 16 °C block was reconstituted in a cell-free system using Golgi apparatus- and endoplasmic reticulum-enriched fractions prepared from suspension cultures. When incubated in the presence of ATP and cytosol, transitional endoplasmic reticulum fragments of *Micrasterias* responded by formation of membrane blebs and vesicles resembling those seen in situ. When prepared from cells metabolically labeled with [3H]leucine, the isolated transition elements supported the transfer of radioactivity to Golgi apparatus preparations immobilized on nitrocellulose strips. The transfer was time- and temperature-dependent and stimulated by ATP. The ATP-dependent component of transfer expressed at 23 °C was reduced or absent at temperatures of 16 °C or below. This suggested that membrane transfer mediated by transition vesicles was the same rate-limiting step in endoplasmic reticulum to Golgi apparatus membrane trafficking both in situ and in the cell-free system. Growth, as evidenced by a progressively altered *Micrasterias* morphology, was slowed at low temperatures but showed no abrupt temperature transition as seen with the vesicular traffic between the endoplasmic reticulum and the Golgi apparatus.

Keywords: Algae; *Micrasterias*; Endoplasmic reticulum; Golgi apparatus (dictyosome); Membrane traffic.

Introduction

Opportunities to utilize completely cell-free systems to study membrane trafficking (Balch and Keller 1986, Morré et al. 1986, Nowack et al. 1987, Rothman 1987, Paulik et al. 1988) have considerable potential in the study of processes of membrane biogenesis and renewal. The algae, despite their inherent potential value as indices of response to environmental quality, have been traditionally difficult to investigate because of their slow growth rates and the associated techniques for application to algae and other aerobic organisms that would permit the facile determination of critical control points in endomembrane trafficking.

In this report, we focus on the step of membrane transfer between the endoplasmic reticulum and the Golgi apparatus. The findings provide new evidence for the involvement of 50 to 70 nm vesicles and for a low temperature block. Here, at 16 °C and below, ATP-dependent transfer in vitro is prevented and in situ transition vesicles from endoplasmic reticulum are observed to accumulate.

Materials and methods

Algal culture

*Micrasterias americana* was isolated from a pond in Nara, Japan. Cells were cultured in Volvox medium (Provasoli and Pintner 1959) at 23 °C with 13 h of illumination under fluorescent light and 11 h of darkness per day.
Temperature studies

Cells at an early developmental stage were selected and transferred individually into petri dishes with a thin Pasteur pipette under a binocular microscope. The dishes were transferred to incubators at 4, 8, 12, 16, 18, 20, 23, 28, or 37 °C with temperatures controlled thermostatically (±0.5 °C). The cells were cultured at the new growth temperature for 1 h and then fixed with glutaraldehyde. After rinses with phosphate buffer, the new developmental stages of the cells attained by growth at the different temperatures were determined with the light microscope.

Electron microscopy

Whole cells were fixed in a 2.0% glutaraldehyde buffered with 0.1 M sodium phosphate at pH 7.2 starting at the appropriate growth temperature followed by 2 h at 4 °C. Post fixation was in 1% osmium tetroxide overnight in the same buffer. After washing with water, cells were stained with 0.7% uranyl acetate and dehydrated through a graded acetone series and embedded in Epon (Luft 1961). Membrane fractions and nitrocellulose acceptor strips were fixed with 2% glutaraldehyde overnight and postfixed with 1% osmium tetroxide for 3 h. They were rinsed in water, dehydrated, and embedded. Thin sections were post stained with alkaline lead citrate and examined and photographed with a Philips EM 400 or EM 200 electron microscope.

Phosphotungstic acid at low pH to stain plasma membrane

Thin sections of fractions, acceptor strips or whole cells prepared for electron microscopy as above were collected from a water surface with nickel grids. To stain the plasma membrane, sections were first incubated in 1% aqueous periodic acid for 1 h to completely destain the sections. This was followed by five changes of distilled water for 10 min each, treatment for 5 min with 1% phosphotungstic acid prepared in 10% chromic acid (Roland et al. 1972) and 5 washes of 10 min each with distilled water.

Electron microscope morphometry

The number of transfer vesicles between ER and the stack of cisternae of the Golgi apparatus (dictyosomes) were counted on paraffin sections from negatives photographed at a primary magnification of 16,000 and enlarged to a final magnification of 45,000 times. One to three dictyosomes from each of three different cells, which had been fixed in a near median cross section perpendicular to the flat surface of cisternae, were analyzed for each determination.

Metabolic labeling

Micrasterias cultures containing active growing cells were labeled with 500 μCi [4,5-3H(H)]leucine (New England Nuclear) for 1 h and rinsed once with culture medium.

Preparation of homogenate and microsomal pellet

Cells were centrifuged and sonicated in 50 mM HEPES, 10 mM KCl, 1 mM EDTA, 10 mM ascorbic acid, 5 mM dithiothreitol and 0.1% bovine serum albumin, pH 7.5 (homogenization medium), containing 0.4 M sucrose, for 15 sec with a Sonifier cell disrupter (Ultrasonics, Inc.) at 4 °C. The homogenates were centrifuged for 30 sec at 1,500 rpm and the supernatant was removed. The unbroken cell pellet then was sonicated an additional 15 sec. This process was repeated a total of four times. The homogenates were centrifuged for 10 min at 2,400 rpm to remove unbroken cells, mitochondria, and nuclei.

Endoplasmic reticulum and Golgi apparatus

The 2,400 rpm (10 min) supernatant was layered onto a discontinuous sucrose gradient consisting of 5 ml of 37% (w/v) sucrose and 10 ml of 21.5% (v/v) sucrose both prepared in homogenization medium and in 35 ml tubes. After centrifugation for 30 min at 22,000 rpm (Spinco SW-28 rotor), the material at the homogenate/21.5% sucrose (for endoplasmic reticulum) and at the 21.5%/37% sucrose (for Golgi apparatus) interfaces were collected, resuspended in the clear yellow supernatant from the top of the gradient and collected by centrifugation for 30 min at 25,000 rpm (Spinco SW-28 rotor). To remove contaminating plastids and plasma membranes, the pellets were resuspended in 0.3 ml of 5 mM potassium phosphate buffer containing 0.25 M sucrose, pH 6.8 and applied to 4 g aqueous two-phase systems consisting of equal volumes of 5.9% (w/w) Polyethylene Glycol 3350 (Fisher) and 5.9% (w/w) Dextran T-500 (Pharmacia) as described (Morré et al. 1991). The phases were mixed in the cold, and resolved by low speed centrifugation (5 min, 1,000 rpm). The upper phases (plus the interfaces) and lower phases were collected separately, diluted with homogenization medium and the membranes in each phase were collected by centrifugation for 30 min at 25,000 rpm. The lower phase pellets contained the purified endoplasmic reticulum (homogenate/21.5% sucrose interface) or Golgi apparatus (21.5%/37% sucrose interface).

Plasma membranes

The material passing through the 37% sucrose layer of the first gradient centrifugation was resuspended in 0.6 ml of 5 mM potassium phosphate buffer containing 0.3 M sucrose and 2 mM KCl, pH 7.8, and applied to a 4 g aqueous two-phase system consisting of 6.4% (w/w) polyethylene glycol and 6.4% (w/w) dextran according to Kjellbom and Larsson (1984). The tubes were mixed, separated into phases, diluted and centrifuged exactly as described for endoplasmic reticulum and Golgi apparatus above. The plasma membranes were collected from the upper phase.

Reconstituted membrane transfer

Incubations were in 8 ml glass shell vials at 23 °C (room temperature), 16 °C, 12 °C, or 4 °C (ice bath temperature) with or without ATP plus cytosol. The ATP/ATP regenerating system (Balch et al. 1984, Dunphy and Rothman 1985) contained 30 mM HEPES, 30 mM KCl, 2.5 mM Mg acetate, 50 μM ATP, 300 μM UTP, 2 mM creatine phosphate and 10 IU/ml creatine phosphokinase (rabbit muscle), pH 7.0. For the cytosol, the clear yellow supernatant from the top of the gradient (see above, Endoplasmic reticulum and Golgi apparatus) was used. The final transfer medium was freshly constituted by combining 125 μl of 30 mM HEPES containing 30 mM KCl and 2.5 mM Mg acetate (HEPES/Mg(OAc)2/KCl), 125 μl of radiolabeled donor, 125 μl cytosol and either 125 μl ATP + ATP regenerating system (+ ATP) or 125 μl HEPES/Mg(OAc)2/KCl (− ATP). The reactions were started immediately after the addition of ATP + ATP regenerating system by introducing the immobilized acceptors.

Preparation of radiolabeled donors

Radiolabeled donors were held as pellets on ice. For each preparation a very small wedge was removed from the center of the pellet and transferred to fixative for electron microscopy. Just prior to reconstituting as the transfer system, the pellets were resuspended in the required volume of HEPES/Mg(OAc)2/KCl, usually 300 μl for two assay vials. A 5 μl sample was removed for determination of radio-
activity and samples of 2.5, 5, and 10 µl were removed for determination of protein. Sufficient membranes were resuspended to give final protein concentration of between 2 and 6 mg/ml.

Preparation of immobilized unlabeled acceptor

Preparation of immobilized acceptor was begun 1 to 2 h in advance of reconstitution of the assay as described (Nowack et al. 1987). Pellets were resuspended in HEPES/Mg(OAc)$_2$/KCl at a final concentration of 1 to 10 mg/ml depending on membrane source. Strips 0.5 x 1 cm were cut from nitrocellulose sheets (S & S Scientific, Keene, NY). The strips were added simultaneously to ensure uniform contact to the resuspended acceptor fractions and incubated for 1 h at 4 °C. One strip was removed for electron microscopy and another was removed and rinsed through four changes of cold HEPES/Mg(OAc)$_2$/KCl for determination of protein. The remaining strips were then transferred with forceps to 5% bovine serum albumin prepared in HEPES/Mg(OAc)$_2$/KCl and incubated an additional 1 h at 4 °C with shaking to block sites on the nitrocellulose surface which were unoccupied by membranes. The strips, each loaded with 6 to 8 µg acceptor membrane and blocked with albumin, were then rinsed through four changes of cold HEPES/Mg(OAc)$_2$/KCl, edge blotted to remove excess solution and added to the transfer system, folded slightly in the center, four per vial. Following incubation with acceptor for 0, 15, 30, and 60 min, the strips were removed, washed through four changes of cold HEPES/Mg(OAc)$_2$/KCl, edge blotted to remove excess solution and transferred to scintillation vials. Radioactivity was determined by scintillation methods. Proteins were estimated by the BCA procedure (Smith et al. 1985) using bovine serum albumin as standard.

Results

In actively growing cells, the Golgi apparatus (dictyosomes) were smaller in diameter than those in non-growing cells and produced large vesicles (Noguchi 1978) at the peripheries of the trans cisternae (Fig. 1 A). The region of endoplasmic reticulum-dictyosome interface of growing cells contained 3 to 5 of the 50–

Fig. 1. Golgi apparatus regions of Micrasterias americana illustrating the Golgi apparatus-endoplasmic reticulum interface at the normal growth temperature of 23 °C. The transitional endoplasmic reticulum exhibited numerous budding profiles (large arrows). Transition vesicles (small arrows) occurred in the space between the transitional endoplasmic reticulum and the Golgi apparatus. A Growing cell with smaller dictyosomes. B Non-growing cell in which the dictyosome was approximately twice the diameter of those of growing cells. Bar: 0.5 µm
70 nm vesicles per cross-sectional area of the correct size and location to be transition vesicles. These vesicles appeared to bleb from the part-rough, part-smooth transitional regions of the endoplasmic reticulum and migrate to and fuse with peripheral membranes of cisternae at the cis face of the dictyosomes.

In the non-growing cells, most of the stacked cisternae of dictyosomes were located near the surface of the chloroplast. The dictyosomes consisted typically of stacks of eleven or twelve cisternae, about 3.7 μm in diameter (Fig. 1B). A lamella of endoplasmic reticulum was usually located between each dictyosome and the chloroplast. The region of endoplasmic reticulum-dictyosome interface contained, on average, 8 to 13 of the 50–70 nm transition vesicles per cross-sectional area. In cells which had been cultured at 8°C to 37°C for 1 h, the morphology of the dictyosome was similar at all temperatures to that observed at the normal culture temperature of 23°C (Fig. 1). However, after culture at 4°C, most dictyosomes were distorted. Normal appearing cisternae of dictyosomes of growing cells cultured at 4°C were few. In their place were large swollen vesicles (Fig. 2A). The swollen cisternae were visible at 8°C but were less frequent than at 4°C. In non-growing cells at 4°C, the distortion was represented by a characteristic curvature of the cisternae (Fig. 2B). About 4–6 trans cisternae were curved outward toward the trans pole and the remaining cis cisternae were curved in the opposite direction toward the cis side. The trans curvature may occur first, since dictyosomes which consisted of flattened cis cisternae sometimes were observed only with curved trans cisternae. The peripheries of curved trans cisternae were characterized by many small, 80–90 nm, electron dense buds. At

![Fig. 2. Abnormal dictyosome morphologies encountered at growth temperature extremes.](image-url)
37°C, secretory vesicles attached to the trans pole of the dictyosome were represented by a population smaller (0.1–0.2 μm) than were those at other growth temperatures (0.3–0.5 μm) (Fig. 2C). The endoplasmic reticulum was well developed with numerous ribosomes at all temperatures.

When cells were incubated at different temperatures, the number of transition vesicles per dictyosome increased with increasing temperature to an optimum at 16°C (Fig. 3). At temperatures greater than 16°C, the numbers of vesicles declined abruptly to a constant value near that observed at the normal growth temperature of 23°C in growing cells. The decline of the number of transitional vesicles above 16°C was observed in non-growing cells as well. Although there were approximately twice the number of transition vesicles per median dictyosome cross section for non-growing cells, the dictyosomes were of twice the diameter so that the average number of transition vesicles per unit cisternal diameter was very similar in growing and non-growing cells.

To confirm that the temperature dependence of transition vesicle accumulations was related to membrane transfer capacity, a cell-free system was developed and tested for temperature responsiveness. For this purpose, transitional endoplasmic reticulum- and Golgi apparatus-enriched fractions were prepared as donor, and Golgi apparatus- and plasma membrane-enriched fractions were prepared as acceptors bound to nitrocellulose strips.

The donor fractions, obtained by sucrose gradient centrifugation followed by aqueous two-phase partition, consisted of small fragments of the endoplasmic reticulum (Fig. 4A and B) or Golgi apparatus (Fig. 4C and D). When incubated with ATP, ATP regenerating system containing UTP, and Microasterias cytosol, the endoplasmic reticulum fractions responded by the formation of 50–70 nm blebs and vesicles resembling the transition vesicles seen in situ (Fig. 4B). Golgi apparatus, as either acceptor for transfer from endoplasmic reticulum or donor for transfer to plasma membrane, consisted of homogeneous preparations of stacked cisternae (dictyosomes) with attached secretory vesicles (Fig. 4C). As donor, the Golgi apparatus were incubated in suspension. When incubated with HEPES/Mg(OAc)$_2$/KCl in the absence of added sucrose the Golgi apparatus tended to swell with an attendant distortion of the stacked morphology (Fig. 4D). The Golgi apparatus immobilized on nitrocellulose as acceptor exhibited this same type of morphology.

Plasma membrane vesicles were separated from the pellet fraction of the sucrose gradients using aqueous two-phase partition (Fig. 5A). The plasma membranes were > 90% vesicles that reacted cytochemically with phosphotungstic acid at low pH (Fig. 5B) as is characteristic of the plasma membrane of Microasterias (Fig.
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5 C). When immobilized on nitrocellulose, the plasma membrane vesicles formed a thin layer (Fig. 5 D) corresponding to about 20 μg protein/cm² of nitrocellulose.

With transitional endoplasmic reticulum membranes prepared from cells radiolabeled with [3H]leucine for 1 h at 23 °C, transfer of the radioactivity to unlabeled Golgi apparatus was time- and temperature-dependent (Fig. 6). At 23 °C, a small but highly reproducible ATP-dependent component of transfer was observed with both endoplasmic reticulum (Figs. 6 A and 7 A) and Golgi apparatus (Figs. 6 E and 7 B) as donor and either Golgi apparatus (Fig. 6 A) or plasma membrane (Fig. 6 E) as acceptor, respectively. However, at incubation temperatures of 16 °C or below, no ATP-dependent transfer was observed within the error of the determination (Fig. 6 B–D and F–H).

The experiment was repeated a total of three times. To correct for differences in total radioactivity of the donor fractions among the different experiments, percent transfer was calculated. Percent transfer is given as the ratio of radioactivity transferred per cm² of nitrocellulose to the amount of radioactivity present in the donor for each transfer assay x 100 (Fig. 7). Approximately 0.8% of the radioactivity was transferred per cm² of nitrocellulose with transfer from endoplasmic reticulum to Golgi apparatus (Fig. 6 A) being somewhat greater than from Golgi apparatus to plasma membrane (Fig. 7 B). The results were highly reproducible at all temperatures except at 16 °C (Fig. 8) which was very near the temperature of transition. Total transfer after 30 min as a function of incubation temperature showed, on the average, an increase of 1.3

![Fig. 5](image-url) Acceptor plasma membrane of *Micrasterias americana* prepared by aqueous two-phase partition and in situ. A Isolated fraction after conventional lead staining. The preparations consisted of homogenous fractions of vesicles with thick (ca. 9 nm) membranes. B Fractions after destaining with sodium periodate and restaining with phosphotungstic acid at low pH to identify vesicles of plasma membrane derivation. C Portion of a cell of *Micrasterias americana* reacted with phosphotungstic acid at low pH to illustrate the specificity of staining. Only the plasma membrane (PM) and secretory vesicles (arrows) were intensely stained. Endomembranes and organelles were unstained. Cp Chloroplast; CW cell wall. D Plasma membrane vesicles immobilized on nitrocellulose and stained with phosphotungstic acid at low pH. The vesicles attached as a monolayer on the nitrocellulose surface. Arrow denotes the location of the nitrocellulose strip. Bar: 0.5 μM
between 4 °C and 13 °C and an increase of 2 between 14 °C and 23 °C (Fig. 8). This suggests that at or below 16 °C, transfer was largely a physical process whereas above 16 °C transfer was an active process with a $Q_{10}$ of about 2. The sharp temperature transition at 16 °C was illustrated most clearly by the effect of temperature on ATP-dependent transfer for the three experiments (Fig. 9). Here, essentially no ATP-dependent transfer was observed for temperatures of 16 °C and below, whereas above 16 °C, ATP-dependent transfer was observed.

The response of ATP-dependent transfer to temperature, with either endoplasmic reticulum or Golgi apparatus as donor, showed the same response as formation of transition vesicles (Fig. 3). At temperatures of 16 °C and below, ATP-dependent transfer was reduced or absent whereas transfer in the absence of ATP showed the more typical temperature dependence of a chemical reaction with a $Q_{10}$ of approximately 2. In three repetitions of this experiment, no ATP-dependent transfer was observed at either 4 °C or at 12 °C, whereas transfer was observed at 23 °C in all three experiments. At 16 °C, no transfer was observed in two of the three experiments but was equivalent to 23 °C in a third experiment suggesting that 16 °C was very near the temperature of transition as deduced from morphological studies.

A temperature transition similar to that observed for transition vesicle formation and cell-free transfer was sought for in the pattern of growth and development of the daughter half-cells. After mitosis, the cells were divided by a septum across the isthmus resulting in the formation of two daughter cells (Fig. 10 A). A daughter cell began to protrude first as a hemispherical bulge (Fig. 10 B), followed by a three lobed stage (Fig. 10 C and D) and finally by a five lobed stage (Fig. 10 E, F, and G). At 23 °C, the daughter half-cells developed to a size as large as the mother half-cells within 2.5 h after mitosis. When cells were incubated just after cell division (Fig. 10 initial) at different growth temperatures for 1 h, the daughter half-cells passed steadily through each developmental stage without induction of abnormal outer shapes between 16 °C and 23 °C (Fig. 10).
Fig. 10. Effect of temperature on the development of cells of *Micrasterias americana*. Cells at an early developmental stage were collected individually with a thin Pasteur pipette and transferred to dishes at the different growth temperatures incubated for 1 h and then fixed. Cells were assigned to the various developmental stages illustrated at the top of the figure (A–G) and the percentage of cells in each stage calculated. These percentages are given in the figure with the full scale in each square being equal to 100%. The number of cells analyzed is given in parenthesis below the growth temperature in the right hand column. Unlike ATP-dependent endoplasmic reticulum to Golgi apparatus transfer which is blocked at temperatures of 16°C and below, cell development in *Micrasterias* continues in a temperature dependent fashion over the entire range of growth temperatures with only a slight indication of an inflection between 12 and 18°C. Bar: A–F, 100 μm; G, 200 μm

The growth rate, in terms of the speed passing through each stage, decreased with decreasing temperature. For example, the growth rates were 65% at 16°C, 40% at 12°C, and 15% at 8°C compared to that at 23°C. At 4°C cells hardly grew and more than 50% of them did not appear to grow at all. With the elevation of the temperature above 28°C, various abnormal shapes were induced in the developed half cells. At 37°C, more than half the cells were abnormal in shape. Unexpectedly, however, there was no clear transition in growth and development at 16°C corresponding to the low temperature block. Rather, the growth and development characteristics exhibited the normal temperature response characteristic of biological processes.

**Discussion**

The Golgi apparatus and cell wall formation has been the subject of a number of studies involving *Micrasterias* species (reviewed by Kiermayer 1981). The occurrence of transition vesicles derived from endoplasmic reticulum and their fusion with the Golgi apparatus were noted early in the pioneering studies of Drawert and Mix (1962) and Kiermayer (1970). Our findings of a low temperature block in transition vesicle function offer an opportunity for investigation of the role of transition vesicles in membrane transfer between the endoplasmic reticulum and Golgi apparatus as with animal cells (Tooze et al. 1984, 1988; Tartakoff 1986) and tissues (Morré et al. 1989). Additional evidence for a low temperature compartment was provided from studies with virus-infected cells (Tooze et al. 1984, 1988). The accumulation of transition vesicles at temperatures of 16°C or below is interpreted as the production of vesicles at these low temperatures but their failure to migrate to and fuse with the Golgi apparatus.
Reduced temperature has been shown to affect post-translational processing and secretion in a number of cell systems. Evidence for a low temperature compartment was first provided for transport and secretion in pancreatic ascinar cells by Tartakoff (1986). Tartakoff (1986) demonstrated that at 10 °C secretory proteins of the exocrine pancreas accumulated in pre-Golgi apparatus transition vesicles, while at temperatures of 22 °C or greater, these proteins progressed through the Golgi apparatus and into condensing vacuoles. Histamine secretion from mast cells also decreased markedly at temperatures below 18 °C (Lagunoff and Wan 1974). Secretion of IgM and the appearance of HLA-1 on the cell surface decreased gradually in lymphoid cells as the temperature was reduced from 37 °C to 20 °C but no intracellular accumulation was observed (Brand et al. 1985). With hepatic cells, secretory proteins were blocked in a pre-Golgi apparatus compartment at 18 °C, but at 20 °C these proteins were exocytosed normally (Fries and Lindstrom 1986). This was similar to rat pancreatic cells where, at 16 °C, most of the labeled secretory proteins remained in the endoplasmic reticulum while at 20 °C the medial Golgi region was reached (Saraste et al. 1986). These and other data, especially with the intracellular transport and surface expression of proteins in virus-infected cells (Copeland et al. 1988; Griffiths et al. 1985; Holmes et al. 1981; Matlin and Simons 1983; Saraste and Kuismann 1984; Tooze et al. 1984, 1988) has led to the concept of a pre-Golgi apparatus compartment. This pre-Golgi apparatus compartment would differ from the bulk of the endoplasmic reticulum and would presumably be located in the secretory pathway between the transitional endoplasmic reticulum and the Golgi apparatus. Despite the considerable evidence for the existence of such a compartment based on the interruption of processing and transport of secretory and membrane proteins, the data on the morphological identification of such an intermediate compartment in uninfected cells are still limited and somewhat conflicting. Local increases in lamellar rough endoplasmic reticulum as reported by Meindl (1990) for *Micrasterias denticulata* treated at 7 °C for 2 h were observed as well in the present study but not quantitated.

The functional relationship between the low temperature block and reduced transfer from transitional endoplasmic reticulum to Golgi apparatus in *Micrasterias* was provided from a reconstituted cell-free system involving these cell compartments. In the reconstituted cell-free system, fragments of the transitional endoplasmic reticulum were induced to form vesicles by incubation with ATP, and ATP-regenerating system and cytosol. When prepared from cells radiolabeled with [3H]leucine, the transition elements supported the transfer of radiolabeled membrane constituents to acceptor Golgi apparatus immobilized on nitrocellulose. The ATP-dependent component of this cell-free transfer was blocked by low temperature, suggesting that the low temperature block seen in situ involve the endoplasmic reticulum to Golgi apparatus transfer step.

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