

## RAPID COMMUNICATIONS

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### RIBOSOME BINDING SITES VISUALIZED ON FREEZE-FRACTURED MEMBRANES OF THE ROUGH ENDOPLASMIC RETICULUM

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#### ABSTRACT

Freeze-fracture micrographs of cells of the green alga *Micrasterias denticulata* stabilized by ultrarapid freezing reveal imprints of polysomes on the rough endoplasmic reticulum membranes. The imprints appear as broad, spiral ridges on the P faces and as corresponding wide grooves on the E faces of the membranes. Distinct 110-Å particles with a spacing of  $270 \pm 45$  Å are associated with the P-face ridges. Where imprints of individual ribosomes can be discerned, it is seen that there is a 1:1 relationship between the ribosomes and the 110-Å particles, and that the 110-Å particles are located in a peripheral position with respect to the polysome spirals. We propose that the 110-Å particles could be structural equivalents of ribosome-binding sites, consisting of a molecule each of ribophorins I and II and a nascent polypeptide chain. These observations suggest that the spiral form of polysomes could result from the forces generated by the extrusion of the growing polypeptide chains to one side of the polysome.

In recent years, considerable interest has been focused on the attachment of ribosomes to the membranes of the rough endoplasmic reticulum (RER) (2, 11, 17, 19). It is now generally accepted that this binding involves both the attachment of the large ribosomal subunit to two specific, integral membrane proteins, ribophorins I and II (11, 12, 13), and the linkage brought about by the nascent membrane-penetrating polypeptide (2). Because the polypeptides produced by the membrane-bound ribosomes are often discharged into the cisternal lumen of the endoplasmic reticulum and thus have a transmembrane disposition (8), and because both ribophorins also span the RER membrane (12, 13), it can be reasoned that these ribosome-binding structures should be visible on freeze-fractured RER membranes and RER microsomes. However, all past attempts to relate a particular subgroup of intramembrane particles with ribosome-binding structures have produced

negative results (5, 7, 14, 17, 18, 22). The most careful and well-documented study of this kind has been reported by Ojakian et al. (17), who were able to show that certain treatments leading to a clustering of ribosomes on RER microsomes also induce a concomitant aggregation of intramembrane particles. Nevertheless, no positive correlation between ribosome-binding sites and a specific category of intramembrane particles was demonstrated.

In this communication, we demonstrate that after ultrarapid freezing of cells of the green alga *Micrasterias denticulata* distinct, spiral rows of intramembrane particles associated with imprints of polysomes on RER membranes can be recognized, an observation supporting the hypothesis that some of the intramembrane particles of RER membranes represent structural components involved in the binding of ribosomes to these membranes.

## MATERIALS AND METHODS

Cultures of *M. denticulata* (Cat. No. LB558) were obtained from the Culture Collection of Algae, Indiana University, Bloomington, Ind. (The collection is now located at the University of Texas, Austin, Tex.). Cells were grown in Waris medium MS (21) at 18°C on a 15 h light/9 h dark cycle. For each experiment, cells about halfway through the dark phase of the cycle were placed in fresh medium in continuous light which stimulated many cells to divide about 25 h later (9). Dividing cells were selected under a dissecting microscope and placed in a separate petri dish containing growth medium. Gold double-replica supports (Balzers High Vacuum Corp., Santa Ana, Calif.), which had been hollowed out to give "hat"-shaped supports, with a metal thickness of 0.005 inch in the central region, were coated with a thin layer of yeast paste. A thin (~40- $\mu$ m-deep) copper slot grid was placed on one support. Cells in which the young semicell had recently completed expansion and gross morphogenesis were selected under a dissecting microscope and placed on the gold support in the center of the slot grid. Another gold support was placed on top, forming a sandwich. The samples were frozen using a propane-jet device of the type described by Muller et al. (16) and were quickly transferred to liquid nitrogen. They were then fractured at -110°C and replicated in a Balzers BA360 freeze-etch unit fitted with a double-replica device. The yeast paste allowed recovery of large intact replicas, which were cleaned in commercial bleach and 70% sulfuric acid at 60°C, picked up on Formvar-coated slot grids, and examined in a Jeol EM 100C.

The terminology of Branton et al. (3) is used for designating the fracture faces. Statistical determinations are expressed as mean  $\pm$  standard deviation.

## RESULTS

Fig. 1 is a survey micrograph illustrating the appearance and general preservation of a *Micrasterias* cell stabilized by ultrarapid freezing. The thickness of these fan-shaped cells (~25  $\mu$ m in diameter) prevents vitrification of the cellular water throughout the entire depth of the cytoplasm, but the structural preservation, even in their centers (where the largest ice crystals are formed), is still sufficient to allow positive identification of virtually all types of membranes and organelles. Furthermore, the small amount of ice crystal formation (indicated by the slightly lumpy texture of organelle membranes, such as the chloroplast envelope) within the cytoplasm of these ultrarapidly frozen cells may be essential for obtaining imprints in the freeze-fractured RER membranes (Figs. 1-4), whose spiral morphology and dimensions strongly suggest that they are derived from polysomes. These imprints, in turn, have proven invaluable for determining which intramembrane particles are related to ribosome attachment sites. Although the described conditions for visualizing polysomal imprints may seem fortuitous, it should be emphasized that we have observed such im-

prints in nearly all of the *Micrasterias* cells examined.

Two complementary fracture faces of the boxed membrane area in Fig. 1 are shown at higher magnification in Fig. 2 *a* and *b*. On the P face (Fig. 2 *a*), the imprints of the polysomes appear as broad (200-300- $\text{\AA}$ -wide), spiral ridges decorated by distinct, and fairly evenly spaced large particles. The E-face image (Fig. 2 *b*), on the other hand, reveals the polysomal imprints as broad, spiral grooves with scattered particles around their margins and, only very rarely, a larger particle within the groove. The density of the particles on the P face is  $1,274 \pm 188$  per  $\mu\text{m}^2$ , and, on the E face,  $596 \pm 95$  per  $\mu\text{m}^2$ .

Figs. 3 and 4 depict P-face views of ribosome attachment sites at higher magnifications. Where imprints of individual ribosomes are discernible, they appear to have a roughly square outline. The distinct, fairly evenly spaced particles located on top of the imprint ridges (one over each ribosome imprint) have a diameter of  $110 \pm 17$   $\text{\AA}$  and a repeat of  $270 \pm 47$   $\text{\AA}$ . The number of such particles associated with a polysomal imprint varies from 2 to 20. Analysis of double replicas reveals that only very few of these distinct, polysome-associated membrane particles seem to fracture with the E face. In some instances, small particles are also located on top of the broad imprint ridges, but only in terminal positions are they seen in a fairly consistent relationship with the larger and more distinct main particles. Thus, they are probably not directly related to the ribosome attachment regions. One striking and potentially important feature of the evenly spaced 110- $\text{\AA}$  particles seen in Figs. 2 *b*, 3, and 4 is their peripheral location with respect to the spiral imprints of the polysomes. Fig. 4, which reveals generally less distinct polysomal imprints than Fig. 3, shows a clear row (arrow) of evenly spaced (repeating distance ~260  $\text{\AA}$ ) particles with a diameter of ~110  $\text{\AA}$ . The similarities between this particle row and the particle rows located on top of the polysome imprint ridges suggest that it could be related to a polysome that was not pressed against the RER membrane during the freezing process.

## DISCUSSION

As Kiermayer has (10) pointed out, the only extensive, sheetlike membrane system in the ground plasm of *M. denticulata* is the RER. Thus, we can be reasonably sure that the large membranous

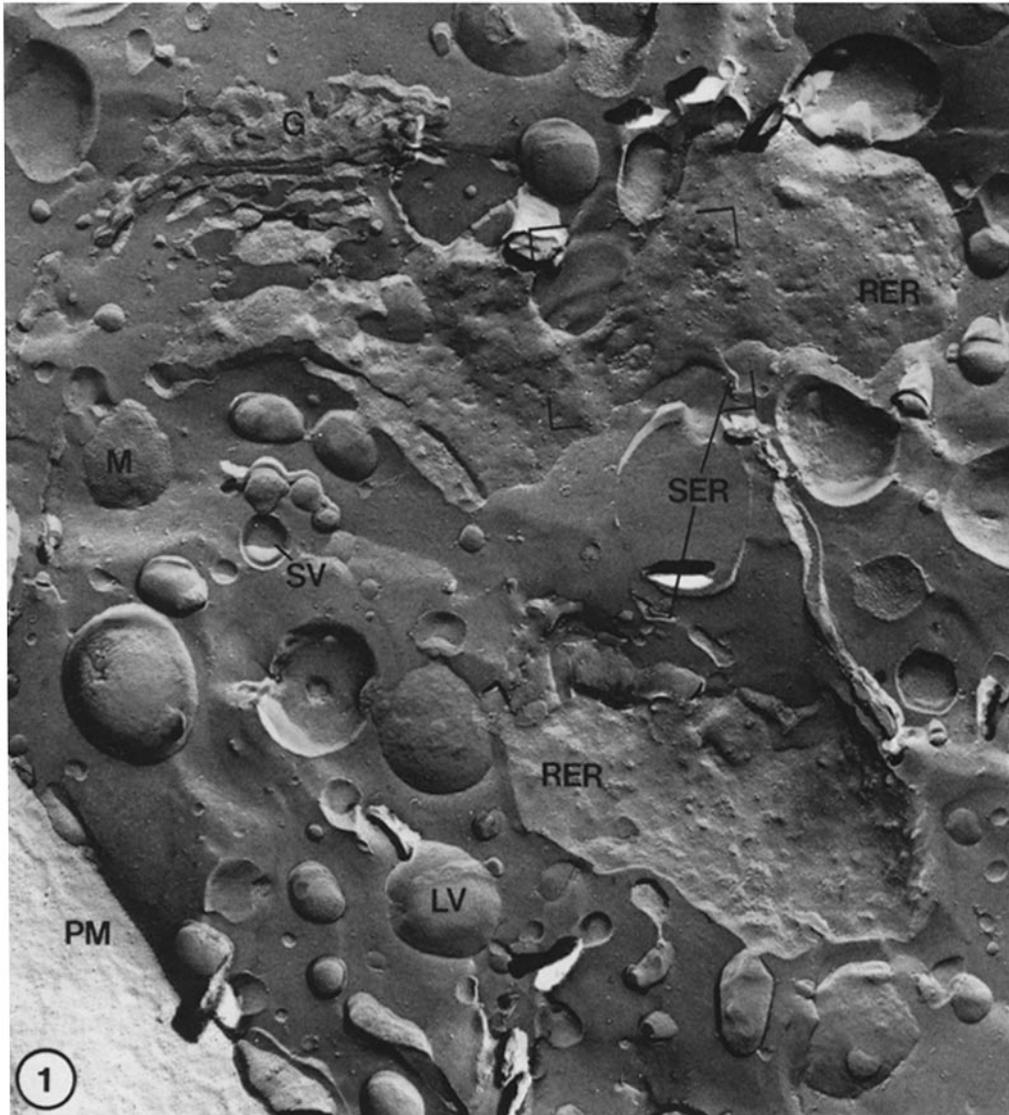
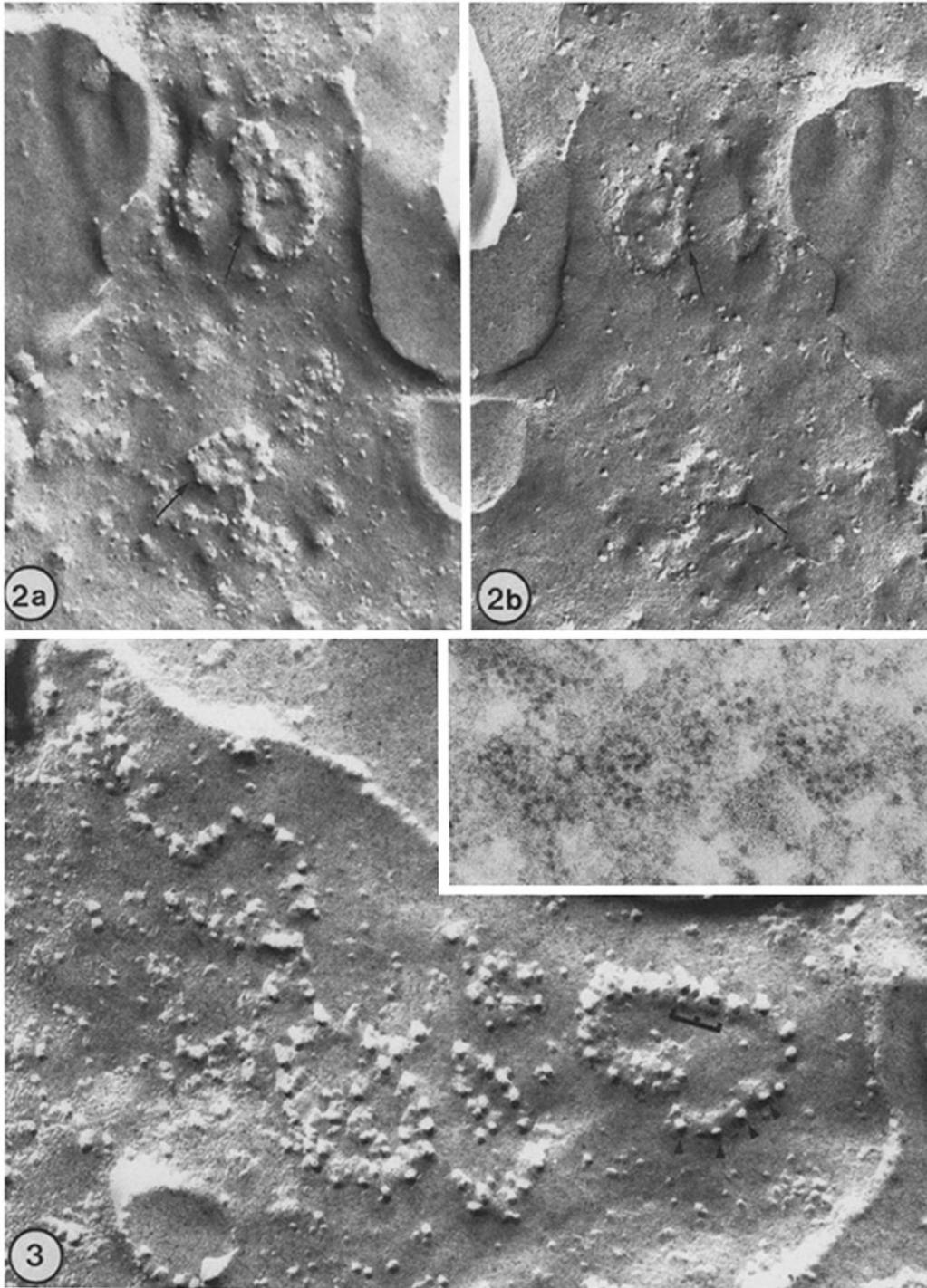


FIGURE 1 Low-power micrograph illustrating the general preservation of the cytoplasm in a *Micrasterias* cell stabilized by ultrarapid freezing. The large, sheetlike membranes of the rough endoplasmic reticulum (RER) stand out against the other, small organelles. Note the relationship of the RER membrane to the Golgi complex (G). Mitochondria (M), smooth endoplasmic reticulum (SER), large, mucus-containing vesicle (LV), small vesicle containing a "rosette complex" of cellulose fibril producing enzymes (SV), plasma membrane (PM). Boxed area is shown at higher magnification in Fig. 2a.  $\times 32,000$ .

sheets identified as RER in our freeze-fracture replicas are indeed RER membranes. Furthermore, both the RER membranes seen in thin sections and the corresponding membranes seen in our replicas appear to be most highly developed during the formation of semicells. Lobes of the RER are also found adjacent to the forming face

of the Golgi apparatus (Fig. 1; reference 10). The correlation of the spiral indentations of the freeze-fractured RER membranes (Figs. 2-4) with attachment sites of polysomes is based on the following findings: only RER and outer nuclear membranes exhibit these structures, and the shape, length, and width of the imprints are essentially the same as



those of the membrane-associated polysomes seen in thin sections (Fig. 3, inset).

In the past, many researchers (5, 7, 14, 18, 22) have examined RER membranes and RER microsomes for the presence of intramembrane particles that could be related to the attachment sites of polysomes, but to no avail. The success of our present study can be traced to three factors: the freezing conditions that produced the polysomal

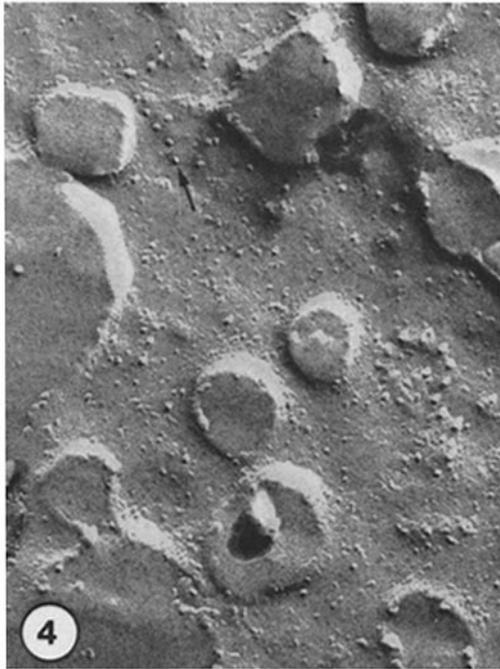


FIGURE 4 The imprint ridges are less distinct than in Fig. 3, and in one area (arrow) a row of 110-Å particles with a repeat of  $\sim 270$  Å can be detected, without a clear, underlying polysome imprint.  $\times 100,000$ .

imprints, the lower than usual density of P-face particles on the RER membranes of *Micrasterias* ( $\sim 1,300/\mu\text{m}^2$  in contrast to  $\sim 3,100/\mu\text{m}^2$  in liver RER; see reference 17), and the larger than usual size of the polysome-associated particles. To date, we have not been able to preserve *Micrasterias* cells well enough by conventional means (glutaraldehyde fixation followed by glycerination) to obtain clear micrographs of the RER that would enable us to determine whether the same particles can be visualized following these treatments.

Measurements on micrographs of thin-sectioned *Micrasterias* cells reveal a center-to-center spacing of the ribosomes in membrane-bound polysomes of 210–260 Å, whereas our freeze-fracture micrographs show  $270 \pm 45$  Å as the repeat distance of the polysome-associated 110-Å particles. These values are considerably lower than those reported for liver RER polysomes (17) but are internally consistent, the values for the fixed, dehydrated, and thin-sectioned *Micrasterias* cells being slightly smaller than those of the freeze-fractured specimens preserved by rapid freezing alone.

The nature of the 110-Å particles associated with the polysome attachment sites is unknown. However, in analogy to other systems (1, 4, 6, 15, 20), we can assume that they represent complexes of integral membrane proteins. Based on our current understanding of ribosome binding to the RER, the most likely constituents would be a molecule each of ribophorins I and II and a nascent polypeptide chain. Further studies are needed to clarify this point.

As seen most clearly in Fig. 3, the polysome-associated particles tend to be located in a peripheral position with respect to the polysome spirals, which suggests that the attachment site of the large ribosomal subunit to the ribophorins, as well as

FIGURE 2 Complementary replica images of a membrane of the RER, revealing distinct imprints of associated polysomes (arrows), which appear as broad ridges on the P face (a) and as corresponding wide grooves on the E face (b). A few scattered particles are visible at the margins of the polysomal imprints. Details of the P-face ridges are illustrated more clearly in Figs. 3 and 4.  $\times 94,000$ .

FIGURE 3 P face of RER membranes depicting several spiral imprints of polysomes. Note the regularly spaced 110-Å particles, located in a peripheral position with respect to the imprints of the polysome spirals (arrowheads). The substructure of the imprint ridge presumably reflects imprints of the individual ribosomes (two are indicated by brackets). Generally, there appears to be one  $\sim 100$ -Å particle for each ribosomal imprint, although occasionally there are indications of additional particles.  $\times 145,000$ . Inset: thin-section view of spiral polysomes in a section tangential to the RER surface in *Micrasterias*. Note the similarity between the shape and size of these polysomes with those of the imprints observed in the freeze-fractured membranes (micrograph by D. Brower).  $\times 77,000$ .

the extrusion site of the nascent polypeptide chain, could be located toward one side of the large subunit, and not in the center of the ribosomal base. If this is the case, one could explain the spiral architecture of the polysomes as resulting from the forces generated by the extrusion of the growing polypeptide chains by the ribosomes. If all ribosomes of a polysome extrude their nascent polypeptides to one side of the polysome, the polysome can be expected to be bent into a spiral configuration.

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