RIBOSOME SUBSTRUCTURE IN INTACT MOUSE LIVER CELLS

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INTRODUCTION

The existence of large and small ribosomal subunits has been demonstrated in shadowed (7) and negatively stained (9, 17, 18) ribosomal preparations. Positively stained preparations have failed to demonstrate this subunit structure but instead reveal a 20–40 A substructure in ribosomes (9, 12). Although the evidence in some studies (13) on intact cells has indicated ribosomal subunits, ribosomes in intact cells generally do not demonstrate a subunit structure. On the bases of the large subunit attachment of ribosomes to endoplasmic reticulum membranes (13, 17, 18) and of the unidirectional transport of protein into endoplasmic reticulum cisternae (15), it has been suggested that nascent polypeptide chains might pass into the intracisternal space through a channel in the large subunit (15). In the present study, observations on positively stained, intact liver cells reveal structural details in ribosomes identical to those previously observed, or suggested, in studies of ribosomal preparations and in a few studies of intact cells. Micrographs detailing these structures were chosen from regions of hepatocytes containing predominantly granular endoplasmic reticulum.

MATERIALS AND METHODS

Livers of 1- to 3-day-old albino mice were fixed for 1 hr in 4% OsO_4 at 0–5°C (0.1 M *s*-collidine buffer, pH 7.4) (3), or for 1 hr in 4% glutaraldehyde (0.1 M phosphate buffer, pH 7.6) (16) followed by a 1-hr wash in 0.1 M phosphate buffer and a 1-hr postfixation in 2% OsO_4 (0.1 M phosphate buffer, pH 7.6). Blocks were subsequently dehydrated in cold ethanol and embedded in Epon 812 (5). Sections with "invisible" to dark gray interference colors were cut with diamond knives on a Porter-Blum MT-1 microtome, mounted on 2000-mesh nickel screening, and stained with uranyl acetate and lead citrate (21). Micrographs were taken on Cronar Ortho Litho type A film (E. I. du Pont de Nemours & Co. Inc., Wilmington, Del.) on a Hitachi HU 11A electron microscope operating at 50 kV with initial magnifications of 30–50,000 ×. Negatives were enlarged from 2 to 23 times with a Durst S-45 enlarger equipped with a 200-w mercury vapor point source (Durst [U.S.A.] Inc., Long Island City, N.Y.).

OBSERVATIONS AND COMMENTS

Ribosomes in the neonatal mouse hepatocyte are predominantly attached to membranes of the endoplasmic reticulum (Fig. 1). In these cells, 15–20% of the ribosomes are seen to be composed of large and small subunits (Figs. 2, 3, 5, 7, 8). These ribosomes have a subunit structure similar to that observed by Palade (13) in the ribosomes of rat pancreatic acinar cells. Surface views of endoplasmic reticulum membranes (Fig. 2) show whorl-like patterns of ribosomes similar to those observed by Watson (22) on nuclear envelopes of rat hepatocytes. In the section shown in Fig. 2 the membrane surface and ribosomal subunits are visible, suggesting that both large and small subunits are closely apposed to the underlying membrane. However, sections oriented slightly obliquely to the membrane plane (Figs. 3, 4, 7, 8) show ribosomes apposed to membranes only by large subunits, which indicates that ribosomes are oriented at an angle with respect to the membrane surface. These observations suggest that the small subunit–large subunit axes

of ribosomes arranged in whorls are disposed in three dimensions on the surface of a truncated cone. The orientation of small subunits toward the center of the whorl and large subunits toward the periphery appears similar to that observed by Shelton and Kuff (18) in rosette patterns of small polyribosomes. Since the above patterns have been shown to disappear during protein synthesis inhibition and to reappear when the inhibition is removed (1, 14), these patterns probably indicate active protein synthesis.

The small subunits are 75–100 A in height and 100–200 A in width: 70–75% of them appear circular to elliptical (Figs. 5, 7, 8) and 20–25% of them appear bipartite (Fig. 3). The latter shape is similar to that observed by Miller et al. (11) in small subunits of tobacco leaf ribosomes. Shelton and Kuff (18) observed bipartite structure only in small subunits of monomeric ribosomes and suggested that it may represent an inactive form of ribosomes. However, in this study bipartite small subunits occur in membrane-apposed ribosomes. If the ribosomes observed in this study are active in protein synthesis (1, 14), the bipartite structure observed may be a normal structural variation of small subunits.

In dark gray thin sections of osmium tetroxide-fixed hepatocytes (Figs. 4, 6) the large subunits measure 150–175 A in height and 175–200 A in width. Electron-transparent cores appear in 15–20% of these subunits. In similar dark gray sections of glutaraldehyde-fixed hepatocytes (Figs. 5, 7), the large subunits appear homogeneous. This difference in core electron opacity between glutaraldehyde fixation and osmium tetroxide fixation may reflect the greater amount of protein preserved by glutaraldehyde than by *s*-collidine-buffered OsO_4 fixation (23). The limitation of protein mainly to the ribosome core is suggested by biochemical studies of dye-ribosome (acridine orange) complexes (4), although some studies (24) suggest that the distribution of RNA and protein may be reversed. If the electron-transparent region observed in this study represents a channel in the large subunit whose long axis is parallel to the long axis of the ribosome, it may represent a passageway for nascent polypeptide chains, as suggested by Redman and Sabatini (15) and by Malkin and Rich (10). However, the exact orientation of the electron-transparent central region of large subunits cannot be determined by this study. A high resolution study of tilted specimens would help define the exact orientation of this region.

Extremely thin sections recorded at high magnification (Fig. 8) reveal that both large and small subunits are composed of 20–40 A electron opacities, which stand out above under- and over-focus background phase effects (6, 19). These opacities may correspond to the substructure observed by Nanninga (12) in *B. subtilis* 50S subunits, or to a 30–40 A filament observed by Hart (8) in surface features of tungsten-shadowed *E. coli* 50S subunits. Slayter et al. (20), observing a similar pattern in positively contrasted large bacterial polyribosomes, suggest that this pattern may arise from spurious stain exclusion or stain deposition. The reality of the electron image observed in this study must await further studies on the interaction of stain and biological material.

Observations at high resolution on the region of the junction between the ribosome and the endoplasmic reticulum membrane (Fig. 8) fail to reveal any stemlike connections of the type Benedetti et al. observed (2) between ribosomes and microsome membranes from rat liver. The observations in this study support the concept that ribosomes are attached to membranes by

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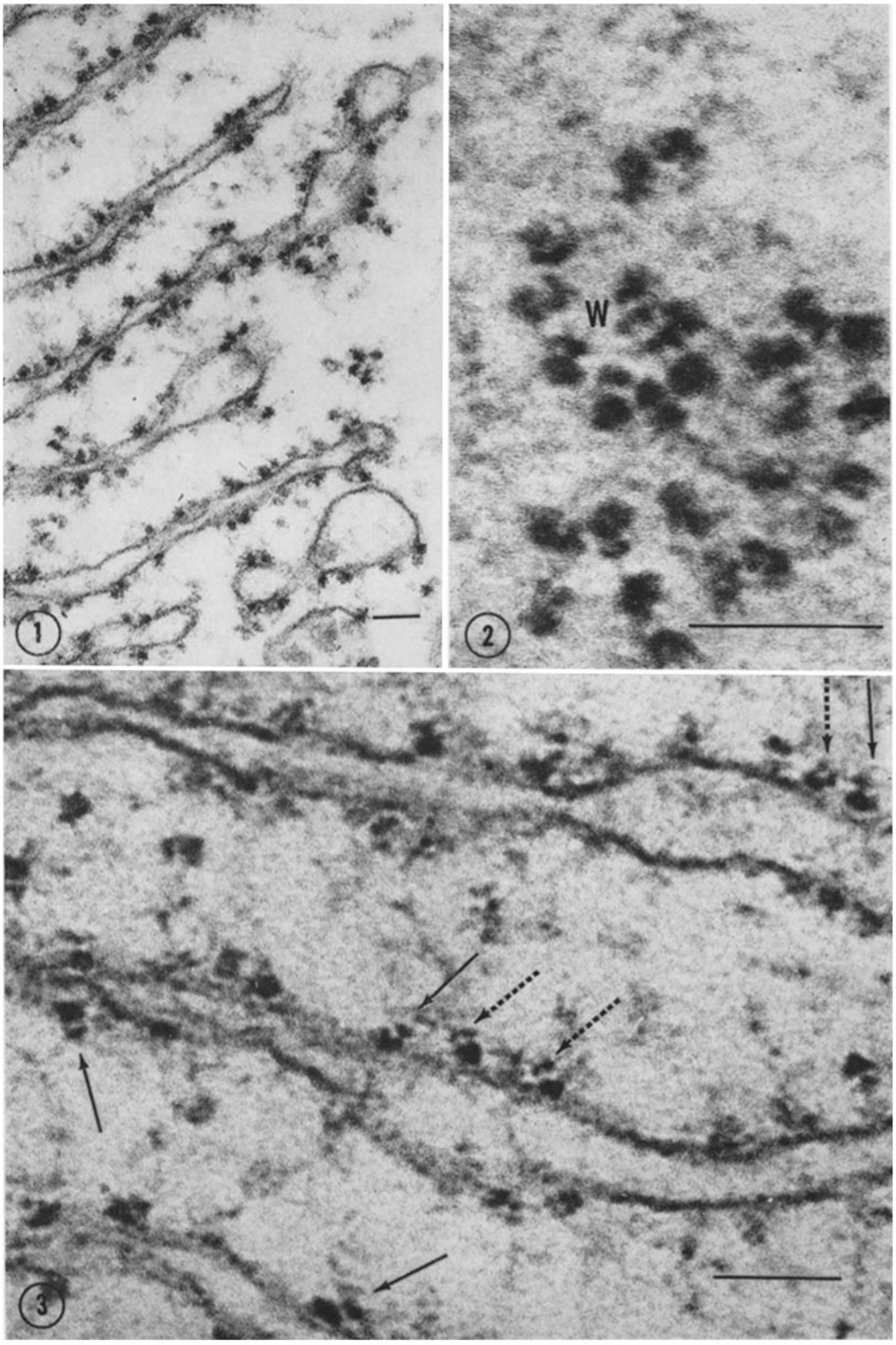
The scale in each figure indicates 0.1 μ.

FIGURE 1 Low magnification micrograph of granular endoplasmic reticulum of mouse hepatocyte fixed with collidine-buffered OsO_4. × 70,000.

FIGURE 2 In surface views of endoplasmic reticulum membranes, ribosomal aggregates appear to be arranged in whorls (*W*). The small subunits are oriented toward the center and the large subunits are oriented toward the periphery of the whorl. OsO_4 fixation. × 300,000.

FIGURE 3 Normal or slightly oblique sections of endoplasmic reticulum membranes show ribosomes attached to membranes by large subunits (solid arrows). Note bipartite substructure in some small subunits (dotted arrows). OsO_4 fixation. × 200,000.



large subunits, as described by Sabatini et al. (17) in guinea pig hepatic microsomes and by Shelton and Kuff (18) in rabbit reticulocyte and neoplastic mouse plasma cell microsomes. However, no membrane discontinuities were observed underlying large subunits, as Redman and Sabatini postulated (15).

SUMMARY

Observations on granular endoplasmic reticulum in intact mouse liver cells clearly reveal ribosomal subunit structure and the large subunit attachment of ribosomes to endoplasmic reticulum membranes. Ribosomes fixed with osmium tetroxide alone show large subunits with occasional electron-transparent cores, whereas ribosomes fixed with glutaraldehyde-OsO_4 show homogeneous large subunits. In addition, bipartite substructure appears to be a relatively frequent structural variation of small subunits.

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Figures 4 and 6 Favorably oriented sections of OsO_4-fixed ribosomes show large subunits with occasional electron-lucent cores. × 300,000.

Figures 5 and 7 Similarly oriented sections of glutaraldehyde-fixed ribosomes showing homogeneous large subunits. × 300,000.

Figure 8 Extremely thin section taken at high magnification. Note 20–40 A electron opacities in both large and small subunits (arrow) and underlying endoplasmic reticulum membrane. OsO_4 fixation. × 410,000.