

ORDERLY ARRANGEMENT OF RIBOSOMES IN THE EMBRYOGENIC CALLUS TISSUE OF *CORYLUS AVELLANA* L.

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ABSTRACT

Cultured callus tissue of hazel (*Corylus avellana* L.), which has the potency of somatic embryogenesis, was used for the study of cell ultrastructure in the course of callus growth and embryoid formation. The meristematic cells of this tissue exhibit a specific organization of rough endoplasmic reticulum (RER), stacked into extensive parallel sheets. The membranes of the aggregated RER are associated with orderly arrays of bound ribosomes. The high regularity of the alignment of the attached ribosomes seems to be influenced by the distance between the two neighbouring membranes in the RER aggregate. The RER aggregates with orderly attached ribosomes are more frequently found in callus cells and in early embryogenesis than in the advanced stages of embryo development.

It is a common observation that cytoplasmic ribosomes are found either free in the cytoplasm or bound to cytomembranes. The free ribosomes, believed to be polysomes, have often been found to be aggregated in an unusual manner. Helical arrays of polysomes have been observed in various plant (4, 6) and animal (1) cells. Kingsbury and Voelz (7) have reported the induction of ribosomal helical arrays by vinblastine sulfate in *Escherichia coli*, and high degree of order was also found in the hypothermic chick embryo, where the ribosomes grouped into tetramer units formed a crystalline lattice (2, 8). There are very few reports of orderly arrays of bound ribosomes, especially in plant cells (3). In the course of ultrastructural studies of somatic embryogenesis in cultured callus tissue of *Corylus avellana*, we have come across a rather rare and interesting form of disposition of bound ribosomes, which will be discussed in the present study.

MATERIALS AND METHODS

The cultured callus tissue of hazel (*Corylus avellana* L.) was obtained through the proliferation of isolated imma-

ture embryos and cultivated in a manner previously reported (9). For electron microscopy, the tissue blocks were fixed for 2 h at 0°–4°C with 3% glutaraldehyde buffered with a 0.1 M phosphate buffer, pH 7.2. After thorough washing in cold buffer solution, the material was postfixed for 1 h in 2% osmium tetroxide in a 0.1 M phosphate buffer. The blocks were then rinsed in cold water and left overnight in a 0.5% aqueous uranyl acetate solution. The material was dehydrated in graded ethanols and embedded in Araldite. Silver sections were cut on an LKB ultramicrotome (LKB Instruments, Inc., Rockville, Md.), collected on uncoated copper grids, and double-stained with uranyl acetate and lead citrate. Sections were examined under a Siemens Elmiskop 101 electron microscope.

RESULTS

The meristematic cells of the embryogenic callus consist of dense cytoplasm with numerous organelles and a rather small vacuole. The abundant ribosomes occur either free or bound to the endoplasmic reticulum (ER). The ER, in turn, is dispersed throughout the cytoplasm in the form of individual cisternae. Besides these individual ER cisternae, the meristematic cells contain large

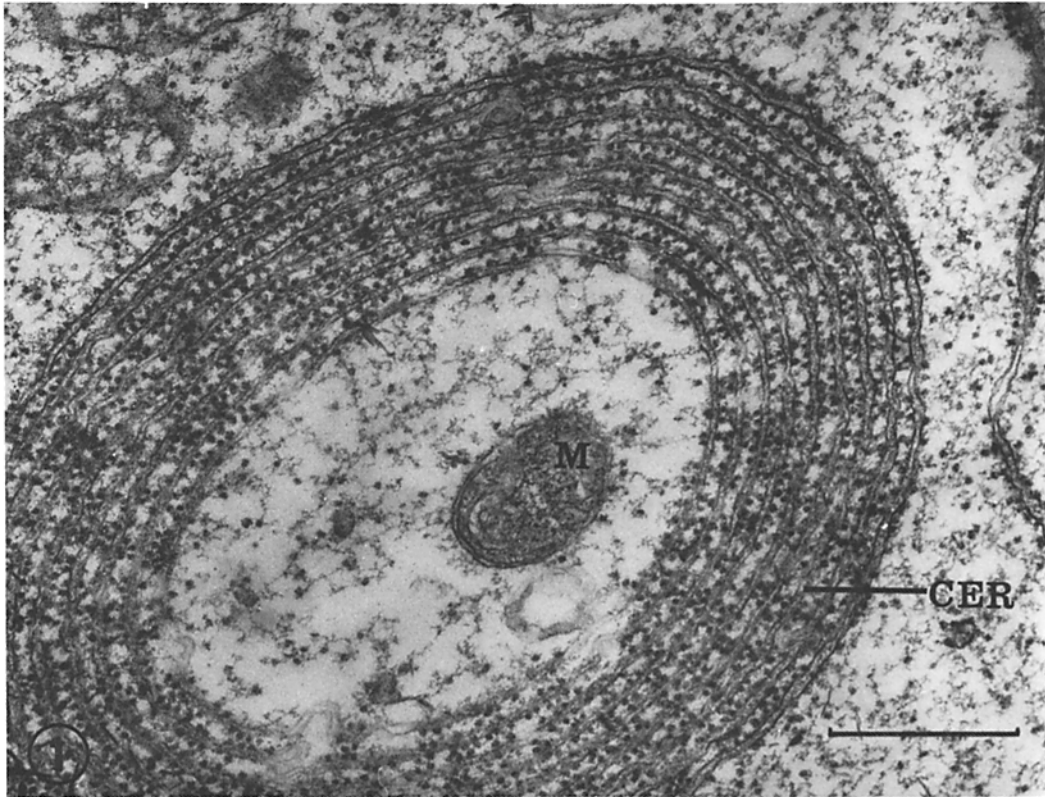


FIGURE 1 Concentric whorl made up of stacked parallel cisternae of rough endoplasmic reticulum (CER). In the center of the whorl are a part of the cytoplasm and a mitochondrion (M). Scale bar = $0.5 \mu\text{m}$. $\times 50,000$.

aggregates of stacked ER. The aggregates are comprised of a number of parallel, flattened cisternae of rough endoplasmic reticulum (RER), and the number of cisternae varies from 2 to 12 within an aggregate.

The RER aggregate is frequently found in the form of concentric whorls (Fig. 1), which encircle a portion of the cytoplasm together with an organelle, such as a plastid or a mitochondrion. The aggregated stacks can also be flat (Fig. 2) or curved (Fig. 3).

The ribosomes which are bound to the aggregated RER were found to be either randomly attached to the membranes (Figs. 1 and 2) or lined up in an orderly manner (Figs. 3 and 4). The orderly distribution of bound ribosomes becomes strikingly regular when the distance between two adjacent membranes in an aggregate does not exceed 50 nm (Fig. 4). In one type of section perpendicular to the plane of the membrane, a pair of ribosomes bound to one membrane seems to come into close contact with pairs of ribosomes on

the neighboring membrane. In favorable views of this type, the ribosomal arrangement produces a distinct pattern in which groups of four closely packed ribosomal profiles are evenly spaced between parallel cisternae. Frequently, groups of four profiles are also aligned in a direction perpendicular to the membrane plane throughout stacks of cisternae (Fig. 4).

In another type of section perpendicular to the membrane and probably normal to the previous one, the rows of ribosomes on two adjacent membranes seem to interdigitate (Fig. 3). In sections tangential to the membrane surface, paired rows of ribosomes are seen (Figs. 3 and 4). The orderly ribosomal arrangement becomes disaggregated at the free cisternal ends which diverge into the cytoplasm (Figs. 3 and 4). This also applies to the outermost surface of the ER membranes in the stacks which are in juxtaposition with the cytoplasm and are lined with ribosomes without any apparent regularity.

It was observed that regular ribosome packing

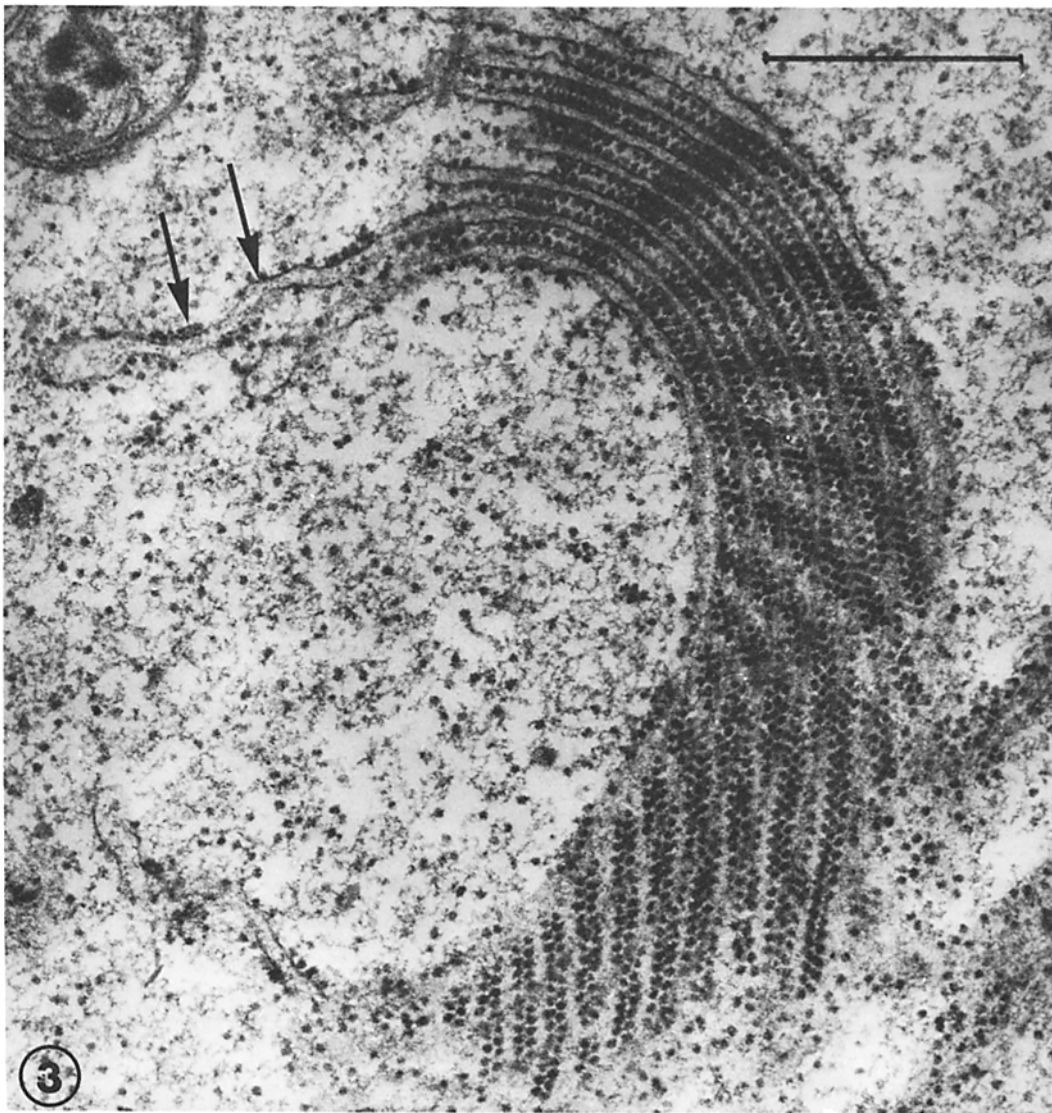
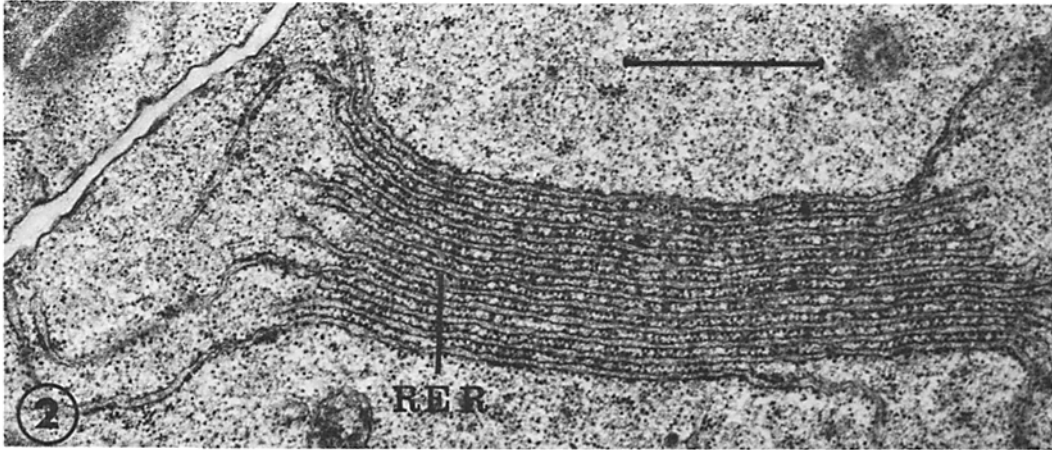


FIGURE 2 Flat aggregate of 12 parallel cisternae of rough endoplasmic reticulum (*RER*) with dispersed ribosomes. Scale bar = 1 μm . \times 26,000.

FIGURE 3 Curved *RER* aggregates with an orderly alignment of ribosomes. Note the irregular ribosome attachment at the free ends of cisternae (arrows). Scale bar = 0.5 μm . \times 68,000.

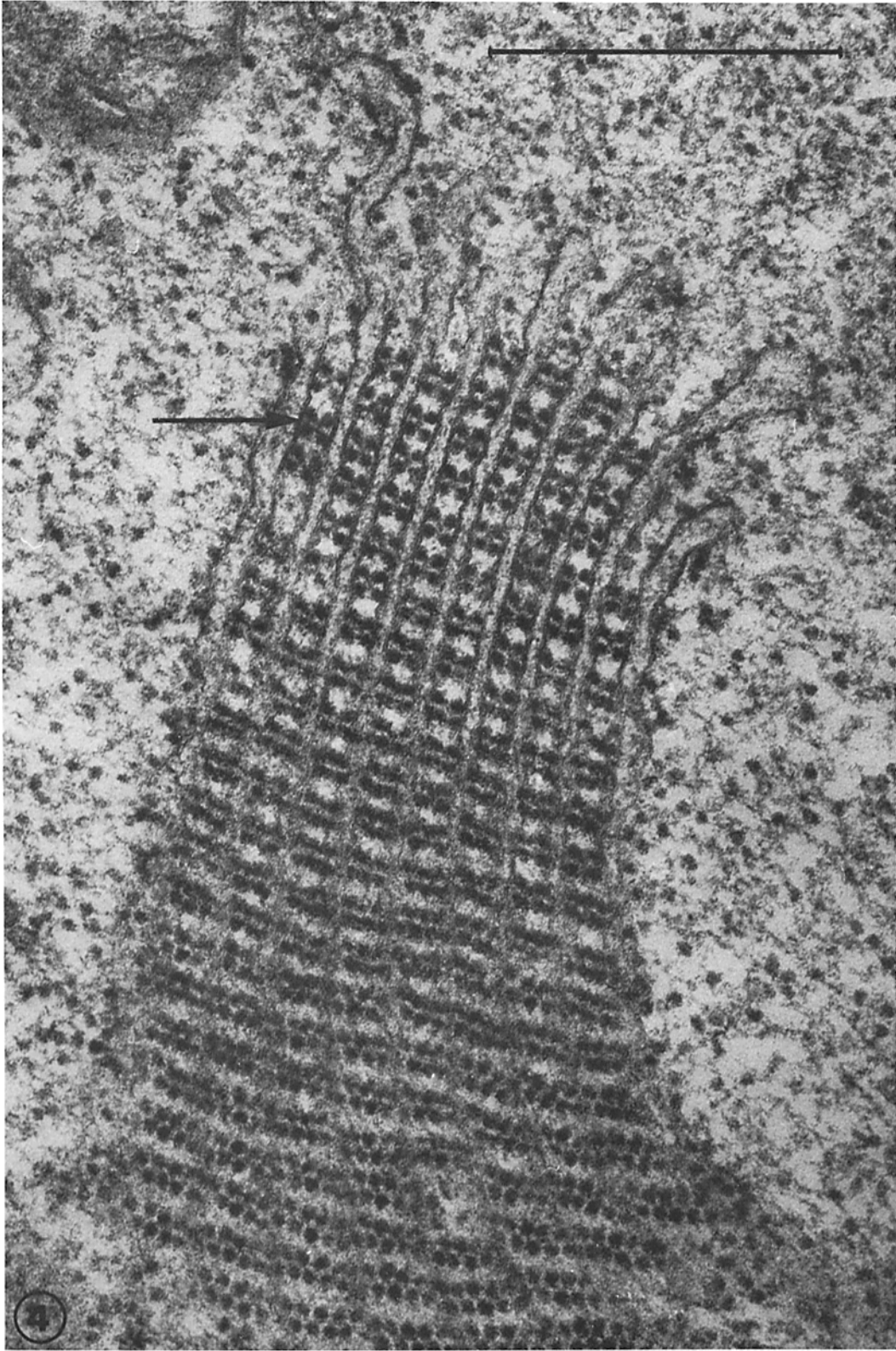


FIGURE 4 RER aggregate with regular pattern of four ribosomes in a square (arrow). Scale bar = $0.5 \mu\text{m}$. $\times 110,000$.

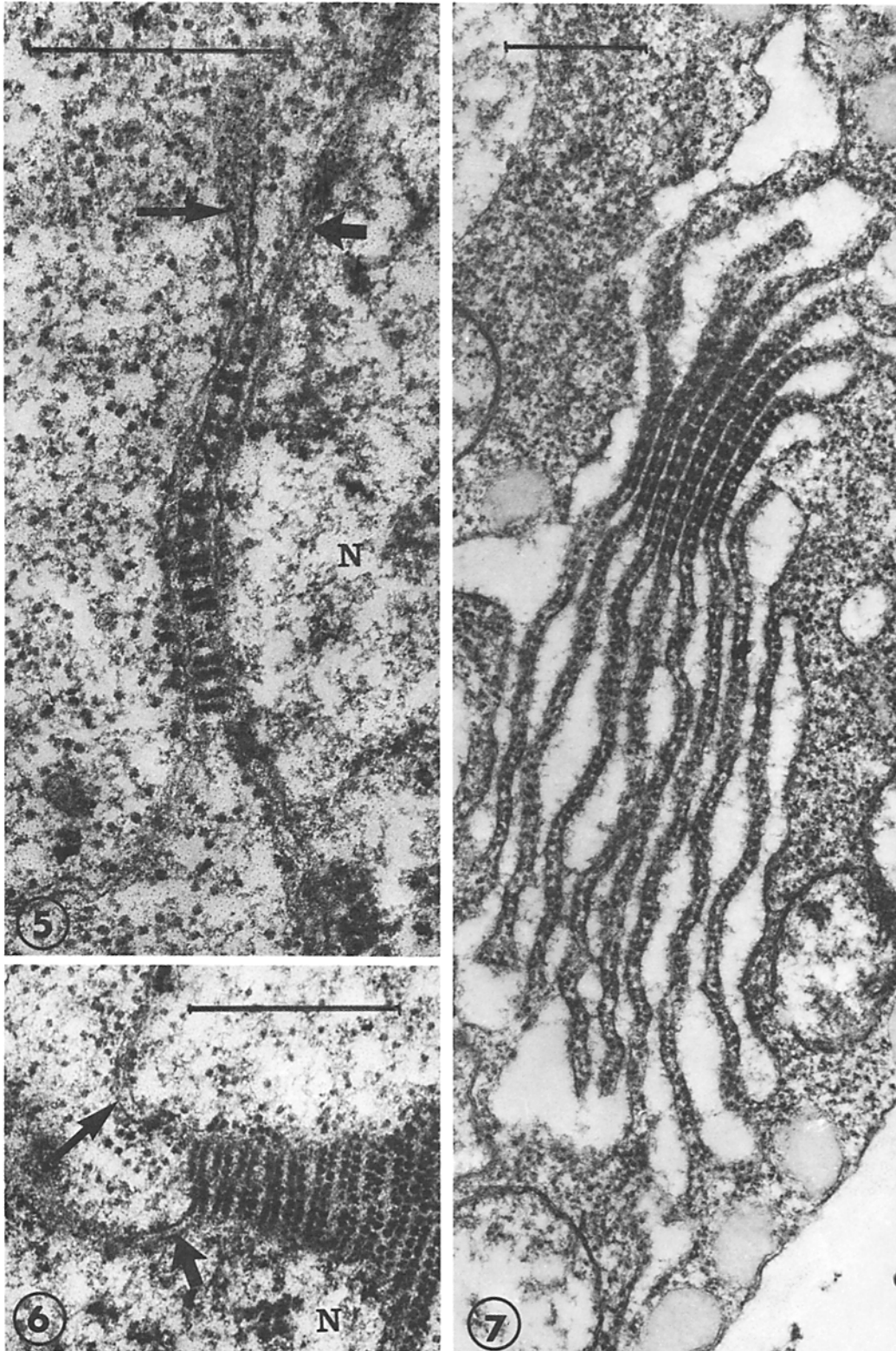


FIGURE 5 and 6 Pairing of ribosomes bound to ER membrane (long arrow) and ribosomes bound to the nuclear membrane (short arrow). Figure 6: Tangential section of nucleus (N), the ER membrane (long arrow), and the nuclear membrane (short arrow). Scale bar = $0.5 \mu\text{m}$. $\times 80,000$ and $64,000$, respectively.

FIGURE 7 Dilated ER aggregate in the cell of the torpedo stage. Ribosomal regular arrays are still present. Scale bar = $0.5 \mu\text{m}$. $\times 42,000$.

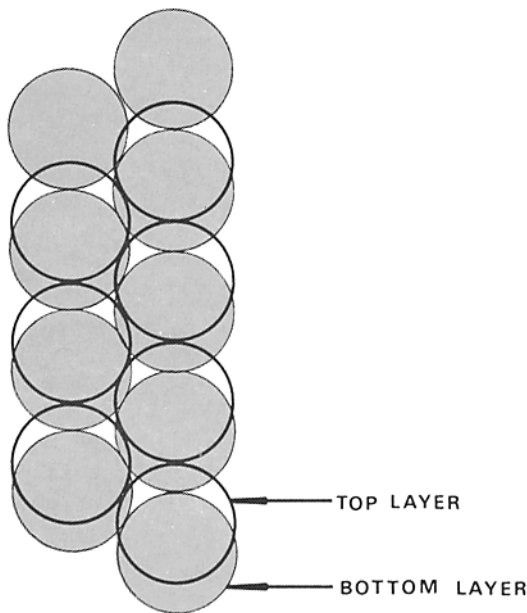


FIGURE 8 Schematic diagram showing how the membrane-bound ribosomes in *Corylus* cells are arranged in evenly spaced, double interdigitating rows which are paired with the corresponding rows on the adjacent cisterna.

also occurs between the ribosomes bound to the outer nuclear envelope and those attached to the ER membrane, when the latter is in the near vicinity of the nucleus (Fig. 5). In this case, the distance between the ER membrane and the nuclear external membrane was also about 50 nm. In some sections, the ribosomes between the two membranes appeared to be linearly distributed, i.e., two lines of ribosomes in a row (Fig. 6).

The embryogenic callus passes through all successive embryogenic stages, and the RER aggregates with orderly packed ribosomes persist even though the RER cisternae may become greatly dilated (Fig. 7). In the differentiated tissue of more advanced embryo development, such as the cotyledons of a young plant, we were not able to detect either RER aggregation or regularity of ribosome distribution.

DISCUSSION

The meristematic cells of callus tissue and somatic embryoids are characterized by a high degree of order in the distribution of membrane-bound ribosomes. The orderly packing of ribosomes has been more often reported for ribosomes which are free in the cytoplasm (1, 2, 4, 6, 7, 8) than for membrane-bound ones (3, 5). The slow cooling of

chick embryos (2, 8) induced the aggregation of free ribosomes into crystalline sheets, having four ribosomes in a square. Since the membrane-bound ribosomes of *Corylus* cells in some sections appeared as 4 U in a square, it was thought at the beginning (9) that the pairing of bound ribosomes might be a similar phenomenon.

From the data obtained in the present study, however, and especially after analysis of diverse sectioning planes, it was concluded that the orderly arrangement of the membrane-bound ribosomes is reminiscent of observations reported by Ito and Winchester (5) and Duckett (3).

Ito and Winchester (5) demonstrated the presence of locally differentiated ER in the bat gastric mucosa. The ER hexagonal tubes were associated with rows of ribosomes. The ribosomes of adjacent tubes were paired, thus regularizing the pattern in transverse sections. Duckett (3) has recently reported a similar observation made on fertilized fern eggs which were undergoing normal morphogenesis. Stacked ER was associated with pentagonal arrays of ribosomes, and it was suggested that the phenomenon was a manifestation of cellular reorganization.

The membrane-bound ribosomes of *Corylus* cells are arranged in evenly spaced, double interdigitating rows which are paired with the corresponding rows on the adjacent cisterna (Fig. 8). Depending on the plane of sectioning of the stacked ER membranes, the ribosomes appear as 4 U in a square or as a pair of interdigitating rows when cut tangentially. The pairing of the ribosomes attached to adjacent ER membranes seems to be influenced by the distance between the membranes, since it is apparent only when the distance is about 50 nm. The regularity of the distribution is lost when the cisternae are farther apart. The ribosome pairing is a phenomenon which seems to persist through early embryogenic stages, but it can no longer be seen in the young plant stage, which is in accordance with Duckett's findings that arrays of ribosomes were found in young tissue undergoing a profound cellular reorganization.

It is left to be determined whether or not this phenomenon of almost perfect regularity of ribosome distribution on the stacked ER membranes is an effect which is induced under the culture conditions or is an inherent feature of the *Corylus* embryo.

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