ROUGH THYLAKOIDS: POLYSOMES ATTACHED TO CHLOROPLAST MEMBRANES

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INTRODUCTION

Increasing evidence has accumulated during the last few years that cytoplasmic membranes, particularly the endoplasmic reticulum (ER) and the plasma membrane, can play an important role in initiating formation of polysomes (14, 16) and stabilizing them (43, 30, 5); however, it is not known whether such membrane-bound polysomes may also occur in other cell compartments such as mitochondria and chloroplasts which are known to be at least partially autonomous in that they possess a separate system for protein synthesis. In

the case of plastidal protein synthesis, the question arises whether chloroplast membranes could act in initiating and stabilizing polysomes in a similar manner as the membranes of the rough ER act in the cytoplasmic protein synthesis.

For cytoplasmic protein synthesis it seems to be well established that at least two distinct categories of polysomes exist: (*a*) membrane-attached polysomes which are arranged in characteristic whorls, spirals or rosettes as first described for animal cells by Palade (26) and for plant cells by Falk (12), and (*b*) polysomes occurring free in the cytoplasm which are predominantly arranged in a helical configuration (3, 41, 42; for plant cells, 11, 19, 20, 36). Since Bartels and Weier (2) were able to show helically arranged polysomes lying free in the chloroplast matrix (compare also reference 23), the question is whether whorl-like polysomes also occur in chloroplasts and, specifically, whether such a whorl-like configuration is attached to membranes. Observations which might give a clue to these questions are reported in the present article.

MATERIALS AND METHODS

In order to achieve an optimal preservation of polysomes during the procedures of the preparation for electron microscopy, use was made in this study of a special kind of fixation, namely a simultaneous glutaraldehyde-OsO_4 fixation combined with postosmication. This fixation method which had been developed and modified from the simultaneous fixation procedures reported by Trump and Bulger (40) and Hirsch and Fedorko (18) was found in our laboratory to be advantageous in preserving nucleoprotein-containing structures and to reveal high ratios of polysomes to total ribosomes in sections through various plant and animal cells (13).

1–4 mm^2 pieces of leaves from young bean plants (*Phaseolus vulgaris* L., 1–3 wk old, grown under greenhouse conditions) were fixed in a mixture freshly prepared from buffered stock solutions in a 1:1 ratio to a final concentration of 2% glutaraldehyde and 2% OsO_4, buffered with 0.05 M cacodylate to pH 7.0. Fixation was carried out at 0°–2°C for 30 min. After repeated washing with ice-cold cacodylate buffer the pieces were postfixed with a 2% OsO_4 solution (cacodylate-buffered to pH 7.0) for 3 hr at 5°C, then washed several times with cold distilled water, and dehydrated either stepwise through a series of ethanol or continuously by use of the acetone vapor method (33). After embedding in Araldite, the material was sectioned on a Reichert ultramicrotome OmU2, double-stained with uranyl acetate and lead citrate, and observed with a Siemens Elmiskop 101.

The electron-opaque particles interpreted in this study as representing ribosomes were identified in morphological analogy only, not by biochemical or cytochemical methods such as enzymatic RNA digestion.

RESULTS AND DISCUSSION

That cytoplasmic and plastidal polysomes would have similar spiral configurations could not have been expected from the outset, since the ribosomes of the cytoplasm and the chloroplasts are significantly different with respect to size and composition (35, 7, 21, 37, 8, 22, 29). Fig. 1, however, shows the arrangement of ribosomes into whorls or spirals attached to chloroplast membranes (arrows). The arrangement is identical to that generally observed for the ER-membranes within the cytoplasm (10, 12). Many thylakoids in the material studied appear to be "rough." Especially in sections tangential to the thylakoidal membranes, it is obvious that ribosomes (up to 10) are arranged into whorl-like polysomes (Figs. 1–6). Besides the more frequent excentric spiral whorls (Figs. 1, 2, 4), circular whorls often with one ribosome located in the center (Fig. 6) and rather linear assemblies of ribosomes can also be detected (Figs. 3, 7). Thus, the spiral configuration of the polysomes attached to thylakoid membranes is so similar to that of the ER-membranes in cytoplasm that both kinds of polysomes can hardly be distinguished. The center-to-center distance, as determined from the original micrographs of the ribosomes within such a plastidal polysome, is in the range of 170–240 A. These values are slightly less than those obtained for cytoplasmic polysomes of eucaryotic cells (3, 10, 31, 42). In plant root and leaf cells the corresponding distances were 200–260 A (Falk, unpublished observations). The center-to-center distances within the plastidal polysome spirals, however, are nearly identical to the values reported by Slayter et al. (34) for a special sort of polysomes in *E. coli* (194–232 A) and noticeably greater than the values of about 130 A communicated for a helical type of polysome in *Rhodopseudomonas palustris* (38). At higher magnification an electron-opaque strand, 10–15 A broad, can be occasionally encountered connecting the ribosomes into the spiral polysome, thus suggesting its interpretation as the messenger-strand (Figs. 3, 4, 5, arrows). Its appearance in such sections resembles that of the positively stained preparations of isolated polysomes presented by

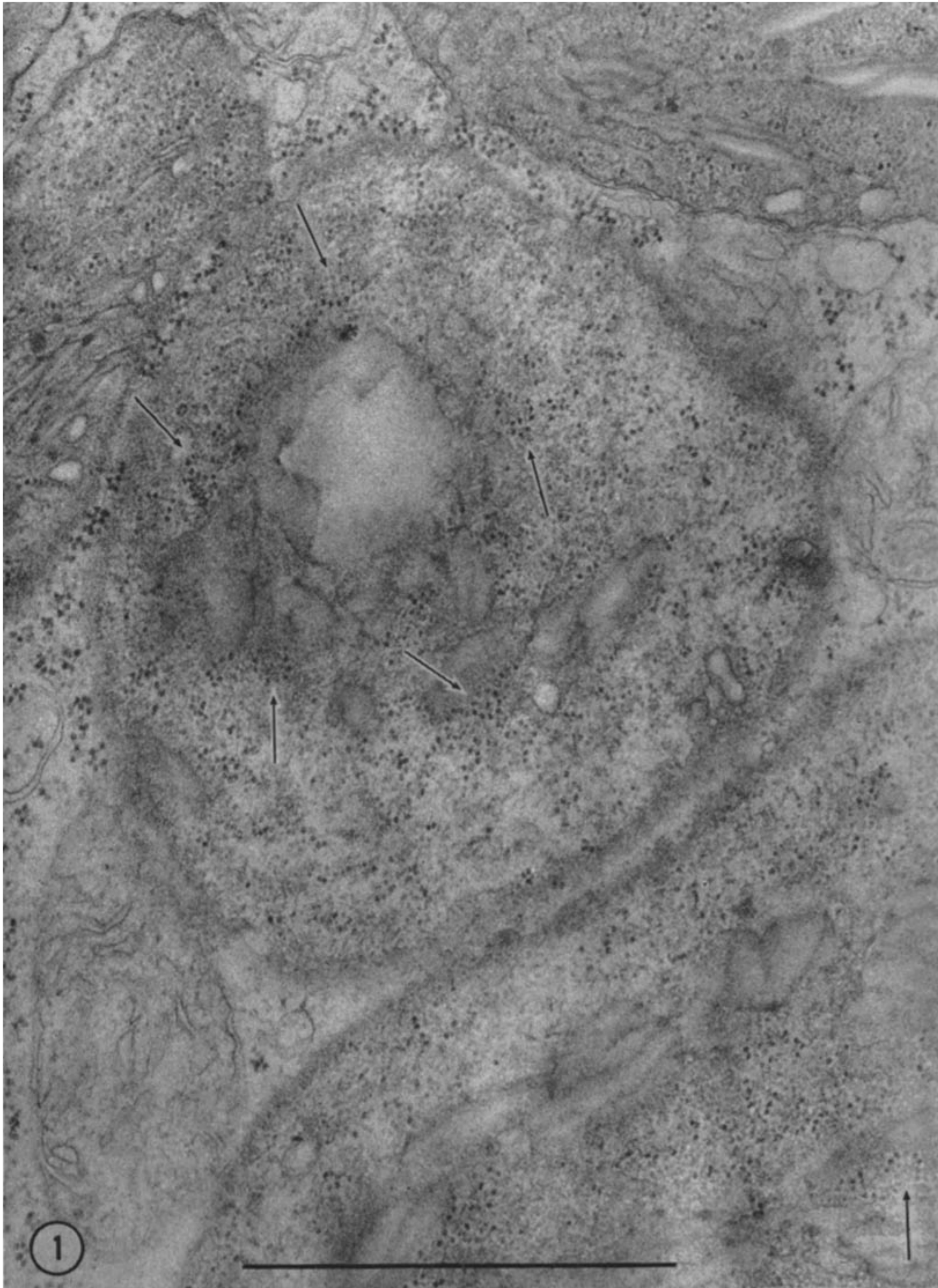

Figure 1 Chloroplasts of bean leaf cut tangential to the grana stacks. Many polyribosomes of the whorl-like type can be recognized within the chloroplasts as attached to the thylakoidal membranes (arrows). Scale indicates 1 μ. $\times$ 63,000.

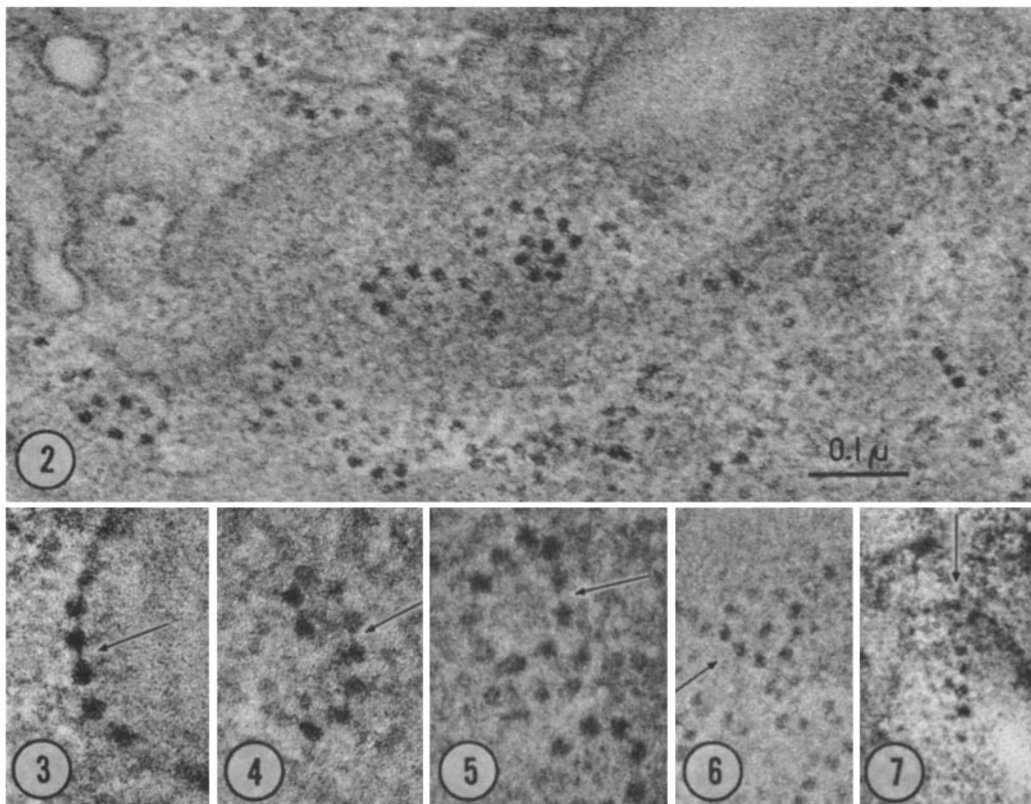

FIGURE 2 Excentric spiral whorls of chloroplast polysomes lying on a tangentially grazed thylakoid. × 135,000.

FIGURES 3, 4, and 5 Plastidal polysomes of different configuration at higher magnification. A thin electron-opaque strand can be revealed as connecting the ribosomes into the polysome (arrows). × 200,000.

FIGURE 6 A nearly circular polysome whorl showing a central ribosome (arrow). ×150,000.

FIGURE 7 Linear assembly of ribosomes (arrow). × 150,000.

Slayter et al. (34) and Benedetti et al. (4). The polysomal whorls occur on the outermost thylakoid membranes of the grana stacks (Fig. 8 *a–c*) as well as on the single thylakoids running through the chloroplast matrix (stroma thylakoids, Fig. 8 *a*, *d*). The alignment of ribosomes on the grana membranes can be detected in micrographs published by various other authors (24, 39). The distance of the ribosome center from the membrane, as can be determined in transverse sections, is generally below 100 A. This is equal to or even somewhat less than that usually found for ribosome attachment to the ER-membranes in the cytoplasm of the same leaf cells (Falk, unpublished).

On the basis of the findings of Bartels and Weier (2) and those of the present study, the conclusion seems to be justified that both types of polysomes known to exist in the cytoplasm occur also in the matrix of chloroplasts. These are (1) helically arranged polysomes not attached to membranes and (2) whorl-like polysomes attached to membranes. This indicates that, as far as the binding of the larger ribosomal subunit to the membrane is concerned, ER-membranes and thylakoids have at least this one feature in common. The observation of this relationship between two kinds of membranes so different with respect to other properties and functions leads to the

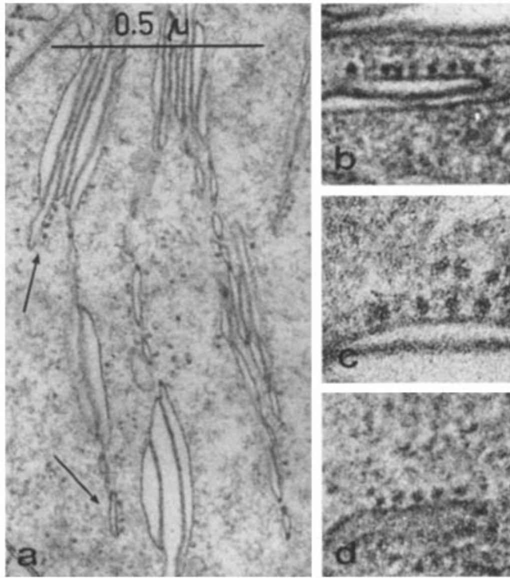

FIGURE 8 Rough thylakoids in transverse section. Membrane-attached ribosomes can be seen on the outermost membranes of grana (Figs. 8 *b* and *c*) as well as on stroma thylakoids (Fig. 8 *a*, arrows, and Fig. 8 *d*). *a*, × 56,000; *b* and *d*, × 120,000; *c*, × 180,000.

general concept of the importance of polysome-membrane association in the control of protein synthesis (e.g. reference 9) as well as in the specificity of the proteins synthesized either on free or on membrane-attached polysomes (17, 16, 1, 15, 25, 28, 27).

In connection with their remark on the occurrence of the helical, "not membrane-attached" polysomes in the proplastids of wheat seedlings, Bartels and Weier (2) have pointed out the necessity of polysome formation for the development of thylakoid membranes (see also references 6 and 32). Such hypotheses are all the more encouraged by the finding of whorl-like, membrane-attached polysomes as shown in this study in young leaves. Thus, the conclusion of Dallner et al. (10), that the membrane material of growing membranes is most likely synthesized by the polyribosomes which are attached to these membranes, seems to be not only conceivable for the cytoplasm but also for the chloroplasts.

In a recent publication, Ohad et al. (24) drew attention to a possible control mechanism by which the synthesis of chlorophyll could regulate the synthesis of thylakoidal structural protein at the translation level. The assumption, as suggested from the present observation, that the synthesis of thylakoidal membrane protein may be performed directly at the site of the thylakoid-attached polysomes sheds some light on this hypothesis. Mutual regulation of the syntheses of chlorophyll and membrane protein during the greening process could thus take place at the site of the thylakoidal membrane without the prerequisite of any further-reaching migration of molecules. The nascent ploypeptide chain of the membrane protein could be removed from the ribosomes by forming the chlorophyll-protein complex, and this gearing complex formation could be achieved by the lipid and pigment moiety of the growing membrane itself.

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