AN ELECTRON MICROSCOPIC STUDY OF LARGE BACTERIAL POLYRIBOSOMES

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INTRODUCTION

Protein synthesis occurs on polyribosomes (polysomes). There have been many electron microscopic studies of these structures in tissue sections, but comparatively few studies have been reported on polysomes after they have been isolated from cells (21, 19). Studies of protein synthesis are frequently carried out in *Escherichia coli* but, although electron microscopic studies have been done on ribosomes (6, 8) and small polysomes (5), there have been no reports on the morphology of large polysomes of *E. coli*. In the course of studying the appearance of the inducible enzyme β-galactosidase on the polysomes of *E. coli*, gentle methods of cell lysis were developed which made it possible for us to prepare what appeared to be largely undegraded polysomes (11). The polysome-bound β-galactosidase is found associated mainly with a class of larger polysomes. The bound enzymatic activity can thus be used to identify a particular class of polysomes. Two other enzymes are induced together with β-galactosidase (9, 23), and it has been shown that the system is polycistronic by studying both the RNA (7, 1) and the polysomes formed subsequent to induction (12). Thus, these large polysomes are directing the synthesis of three enzymes. Here we report on an electron microscopic study of the large polysomes associated with the synthesis of β-galactosidase. These structures contain approximately 50 ribosomes which can be visualized in a more or less extended linear array. Examination of the ribosomal subunits shows that they frequently lie in an alternating configuration along the extended array.

MATERIALS AND METHODS

The preparation of polysomes from *E. coli* K-12 (Strain Hfr 3000) has been described previously (11). Cells were harvested in early log phase and then converted to spheroplasts by the addition of penicillin. Induction of β-galactosidase was carried out through

the use of 5×10^{-4} M methyl-β-thiogalactoside. The spheroplasts were lysed by the addition of a nonionic detergent (Brij 58) to a concentration of 0.5%. After the centrifugation at 10,000 *g* for 15 min, the cold supernatant was carefully collected with a large-bore pipette and then sedimented in a 15–30% sucrose gradient containing 0.01 M $MgSO_4$, 0.005 M Tris buffer (pH 7.4), and 0.06 M KCl. After centrifugation for 150 min in a Spinco Model L at 25,000 rpm in the SW 25 head, samples were collected through the bottom of the density gradient tube and pumped through a Gilford spectrophotometer for measurement of absorbance at 260 mμ. Samples were also analyzed for β-galactosidase activity by the method of Pardee, Jacob, and Monod (16).

The fractions containing the peak of polysomal-bound enzymatic activity were prepared for electron microscopy with techniques similar to those described previously (21). A droplet of a fraction from the sucrose gradient, with an optical density at 260 mμ of approximately 0.5, was deposited on a carbon-coated electron microscope grid. We gradually washed the droplet with buffer (0.1 M KCl, 0.01 M $MgCl_2$, 0.001 M Tris, pH 7.4) to remove the sucrose. While the grid was still moist, a droplet of 0.1% uranyl acetate (pH 4.5) was added and allowed to stain for 10–30 min. In all of the operations, the material was kept near 2–3°C. Uranyl acetate was used as a contrasting medium, since it acts as both a positive and negative stain for polysomes. We tried two preparative techniques in order to increase the proportion of intact large polysomes which were spread out and isolated. One method involved treating the carbon substrate with a protein solution (0.5% cytochrome *c*) prior to depositing the polysome solution. Another method involved centrifuging a dilute solution of polysomes onto a substrate film which had been placed on a platform at the bottom of a Spinco SW 25.2 swinging bucket. The addition of the cytochrome *c* seemed to improve spreading, and it reduced polysomal clumping. However, the centrifuging method resulted in the deposition of no polysomes even though flagella, used as test particles, were deposited. It was concluded that the necessarily large dilution factor resulted in dissociation of the ribosomes from the messenger RNA in spite of attempts to prevent this by increasing the magnesium concentration.

To identify membranous objects which were found adhering to some polysomes, we made thin sections of the bacterial cells. The cells were prepared for sectioning by being washed in 0.1 M KCl, 0.01 M $MgCl_2$, and 0.001 M Tris, (pH 7.4), and then pelleting and resuspending for 10 min in 2% buffered glutaraldehyde (pH 7.4). The cells were then pelleted again and left for 1 hr in buffered glutaraldehyde. The pellet was washed overnight in buffer and then fixed with Dalton's chrome-osmium (3). Blocks were embedded in Epon-Araldite (14), and sections were counterstained with 0.5 M lead acetate.

All specimens were examined in a Siemens 1 electron microscope at instrumental magnification ranging from 40,000–200,000 using double condenser illumination and a 50-μ objective aperture. Most micrographs were recorded about 1 μ under focus to increase contrast. High resolution photographs were taken within 0.1 μ of focus.

RESULTS AND DISCUSSION

A sucrose gradient analysis of the polysomal material from fully induced *E. coli* Hfr 3000 is shown in Fig. 1. The peak labeled 70S represents single ribosomes, and the polysomal optical density peak is seen to the left of it. This material was prepared 30 min after induction with thiomethyl galactoside, and a pronounced peak of enzymatic activity and specific activity is seen in the region of fractions 4–6. If the polysome preparation were extensively degraded, the enzymatic activity would

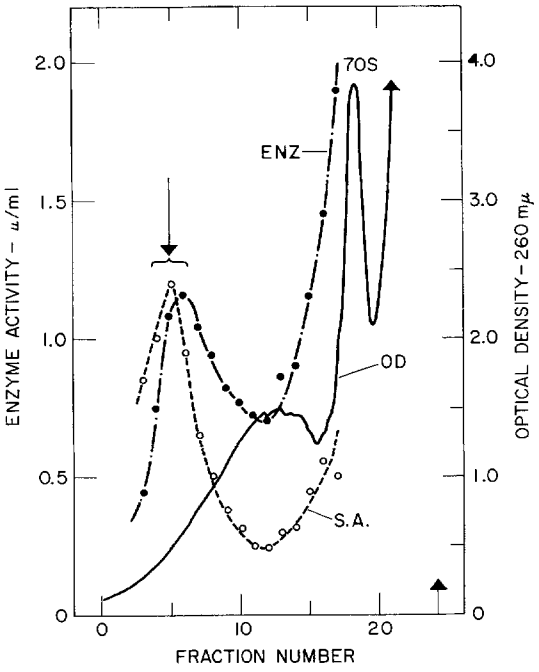

FIGURE 1 A sucrose gradient of a lysate of *E. coli* Hfr 3000 fully induced for β-galactosidase. Direction of sedimentation is to the left, and the arrow on the base line shows the position of the last fraction. Solid line = optical density at 260 mμ. β-galactosidase activity is shown with solid circles, specific activity, *S.A.*, enzymatic activity divided by optical density is represented by open circles. The bracketed region was examined in the electron microscope.

not appear as a peak in this region, but would be displaced to the right where it would be associated with smaller polysomes or with the large amount of soluble β-galactosidase activity which is seen at the top of the gradient. Samples for electron microscopy were obtained from the region of the gradient enclosed by the bracket.

In an electron microscopic study of HeLa cell polysomes (18), it has been pointed out that although the visualization of complete fields of undegraded smaller polysomes is possible, the microscopic fields of large polysomes appeared somewhat fragmented. The same has been found with polysomes from *E. coli*. The tendency for these large polysomes to aggregate and to fragment has made it difficult for us to obtain good statistical data for the number of ribosomes per polysome in the β-galactosidase fraction. However, we collected statistical data by counting the number of ribosomes in each polysomal cluster over several electron microscopic fields. The histogram plotted from these data had a flat maximum in the region of polysomes containing 20–30 ribosomes, but it was clear that the specimens had fragmented during sample preparation, since there were many smaller polysomes as well as larger ones. The largest polysomes in these fields contained approximately 50 ribosomes in each cluster, and it is likely that the maximum observed in the region of 20–30 ribosomes resulted from a single scission of the larger polysomal structure. It is probable that shearing forces generated during manipulation and sample preparation are responsible for the observed fragmentation. This is likely since the smaller polysomes would not have sedimented that far down the gradient. Electron microscopic observations were made through all the regions of the gradient. The region near fraction 10 contains polysomes with 20–30 ribosomes, but does not contain units with 50 ribosomes. The presence of polysomes with 50 ribosomes as well as polysomes with 20–30 ribosomes in fraction 5 is, therefore, most readily ascribable to fragmentation during sample preparation.

Fig. 2 shows some of the larger polysomes. Individual ribosomes are clearly visualized, and the polysomal structure seems to be organized in a more or less extended linear array. Thin strands are not seen uniformly between ribosomes, owing partly to incomplete staining and partly to the fact that the ribosomes appear to be closely packed along the messenger RNA strand, with only occasional gaps. Figs. 3 and 4 show other specimens at different magnifications. In Fig. 4 *a*, a region of exposed messenger almost 400 A long is shown at high resolution. It is clear that there is no gap between ribosomes to the left of the strand. Ribosomal substructure can be visualized by the uranyl stain in Fig. 4 *a* at the 25 A level, which is quite distinct from the substrate noise in the background which occurs at the resolution limit of approximately 6A. This stained substructure is not apparent in the lower resolution photographs shown in Fig. 2, owing to confusion with the image of the substructure of the carbon film (20).

Any conclusion concerning the relationship of the pattern of stain deposition to the structure of the ribosome would have to be based upon an understanding of the contrasting process. In the case of positive contrasting of ribosomes, it has been our experience that much of the uranyl salt is removed by thorough washing after staining. It is quite possible that contrast is partly, if not largely, due to stain exclusion by the ribosomal structure. In any event, striations 25 × 100 A or more are not infrequently seen within the 70S unit in photographs such as Fig. 4 *a*. This pattern of stain deposition could arise from a ribosome built out of a large number of protein chains on a ribosomal RNA matrix.

Negatively contrasted polysomes often appear to have adjacent ribosomes lining up on alternate sides of the polysomal axis, as shown in Fig. 4 *b–d*. Under these circumstances, the smaller of the two ribosomal subunits, when it can be distinguished, is observed to lie closer to the polysomal axis than the larger one. Double rows of ribosomes are often seen in tissue sections (22, 2, 4, 13, 17), and this arrangement with the smaller subunit in the center may be associated with a very efficient packing of asymmetric ribosome units along a linear axis. On the other hand, in positively contrasted preparations, a more extended array is found, possibly because of decreased support from the staining medium. Even these extended arrays are frequently marked by regions in which at least two ribosomes lie abreast of each other instead of alternating (See Fig. 2 *d* and Fig. 3 *a*, *c*, and *d*).

The maximum number of ribosomes per polysome in the region of peak polysome-bound β-galactosidase activity is near 50 (Figs. 2 and 3). This value is the same whether staining or shadowcasting is used as the method of contrast enhancement. However, clumps of polysomes are

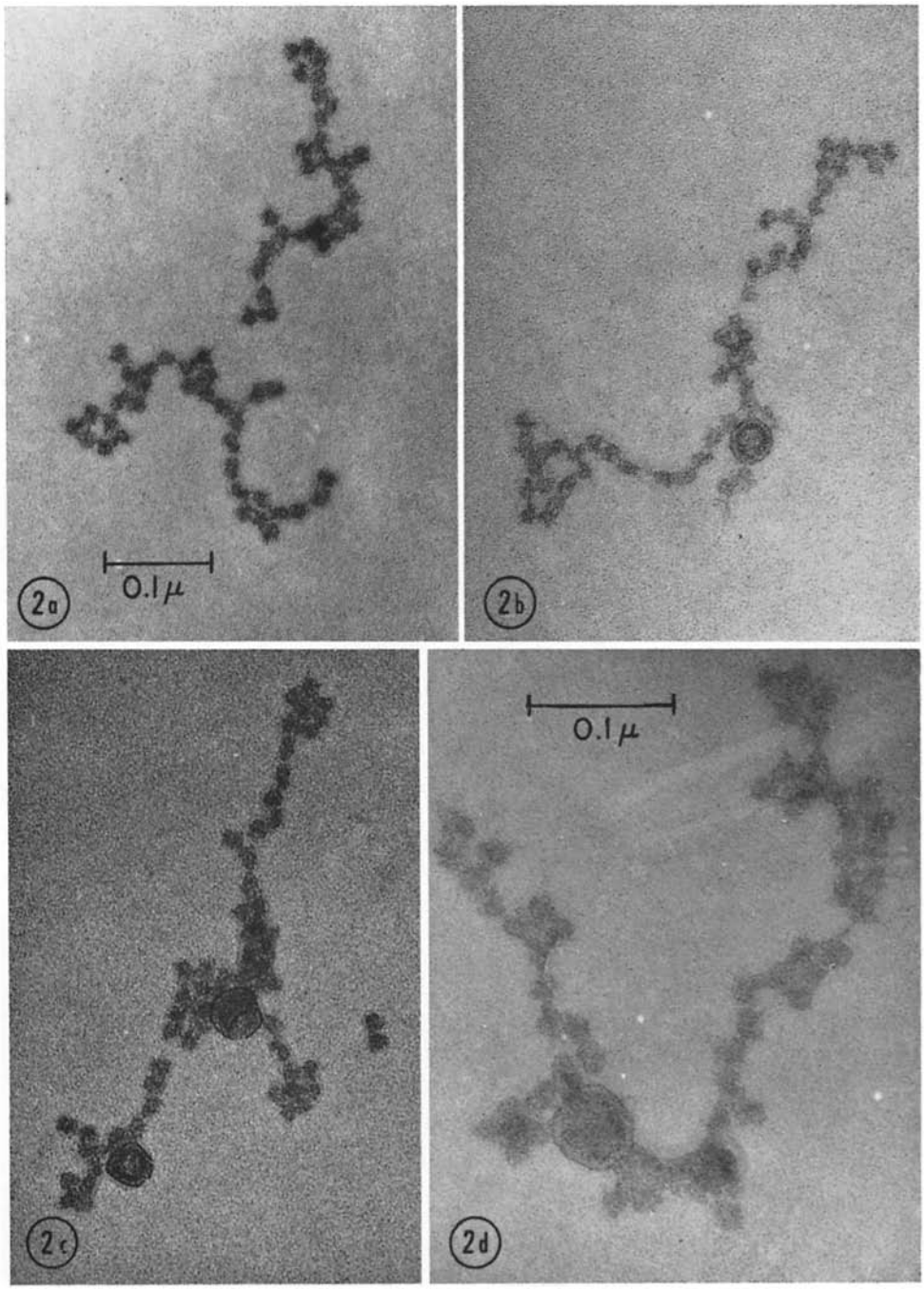

FIGURE 2 *a–d* Polyribosomes positively contrasted with uranyl acetate. *a–c*, × 150,000. *d*, × 200,000.

occasionally found which are larger than this maximal size, by a factor of two or three (see Fig. 3 *c*).

The gaps between ribosomes are found to be small. The center-to-center spacing between ribosomes, where they were extended linearly, ranged from 192 to 232 A; the average was 214 A. However, where the ribosomes are seen as double

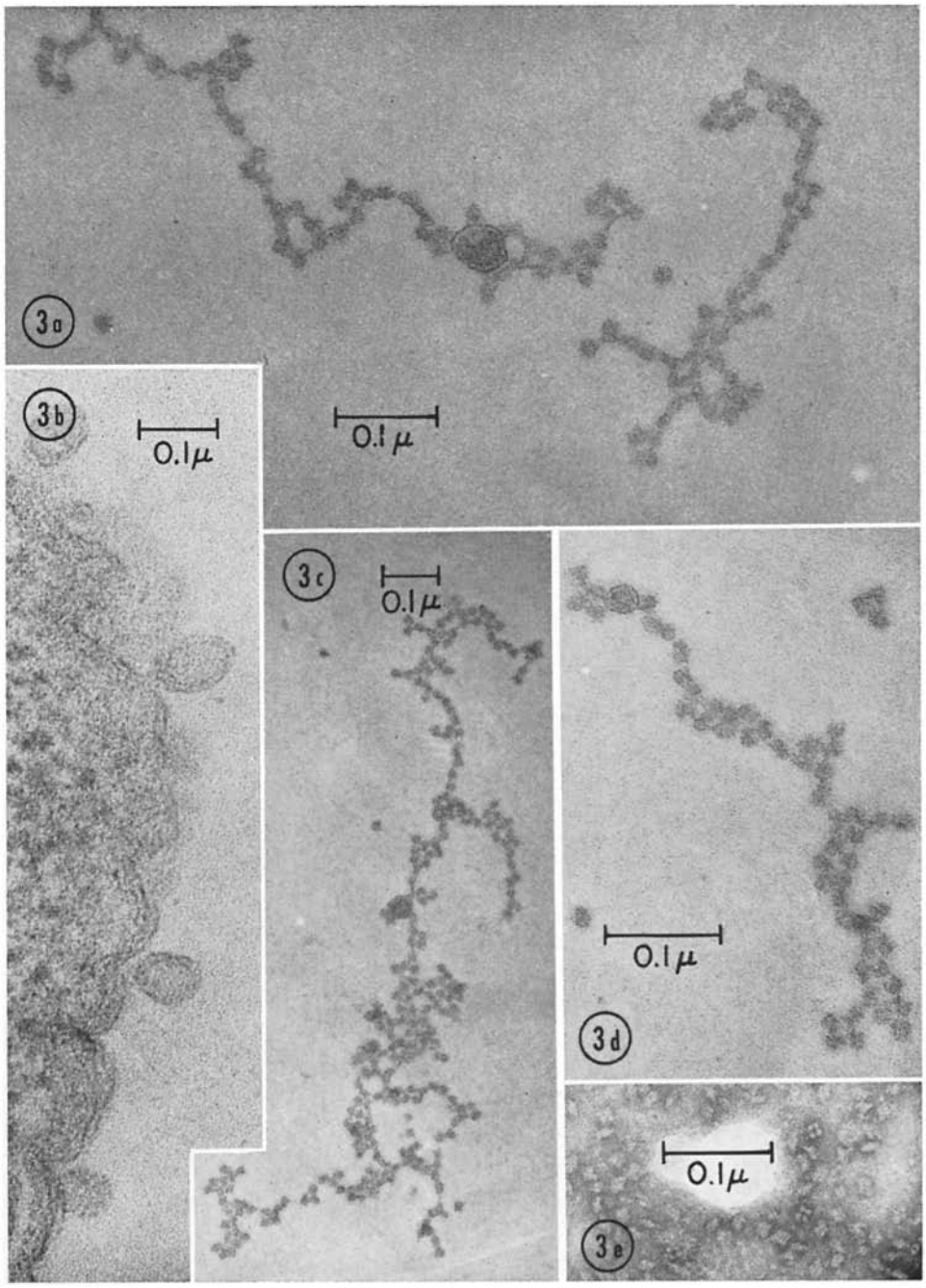

FIGURE 3 *a*, *c*, *d* Polyribosomes positively contrasted with uranyl acetate. × 138,000, 75,600, and 161,000, respectively.

FIGURE 3 *b* A thin section of *E. coli* Hfr 3000 fixed in glutaraldehyde-OsO_4, embedded in Epon-Araldite, and counterstained with lead citrate. It shows the particles which are found outside the cell. Particles of the same size range and morphology are frequently found associated with large polyribosomes, as shown in *a*, *c*, and *d*. × 110,000.

FIGURE 3 *e* Negatively stained preparation of β-galactosidase, contrasted with uranyl acetate. × 150,000.

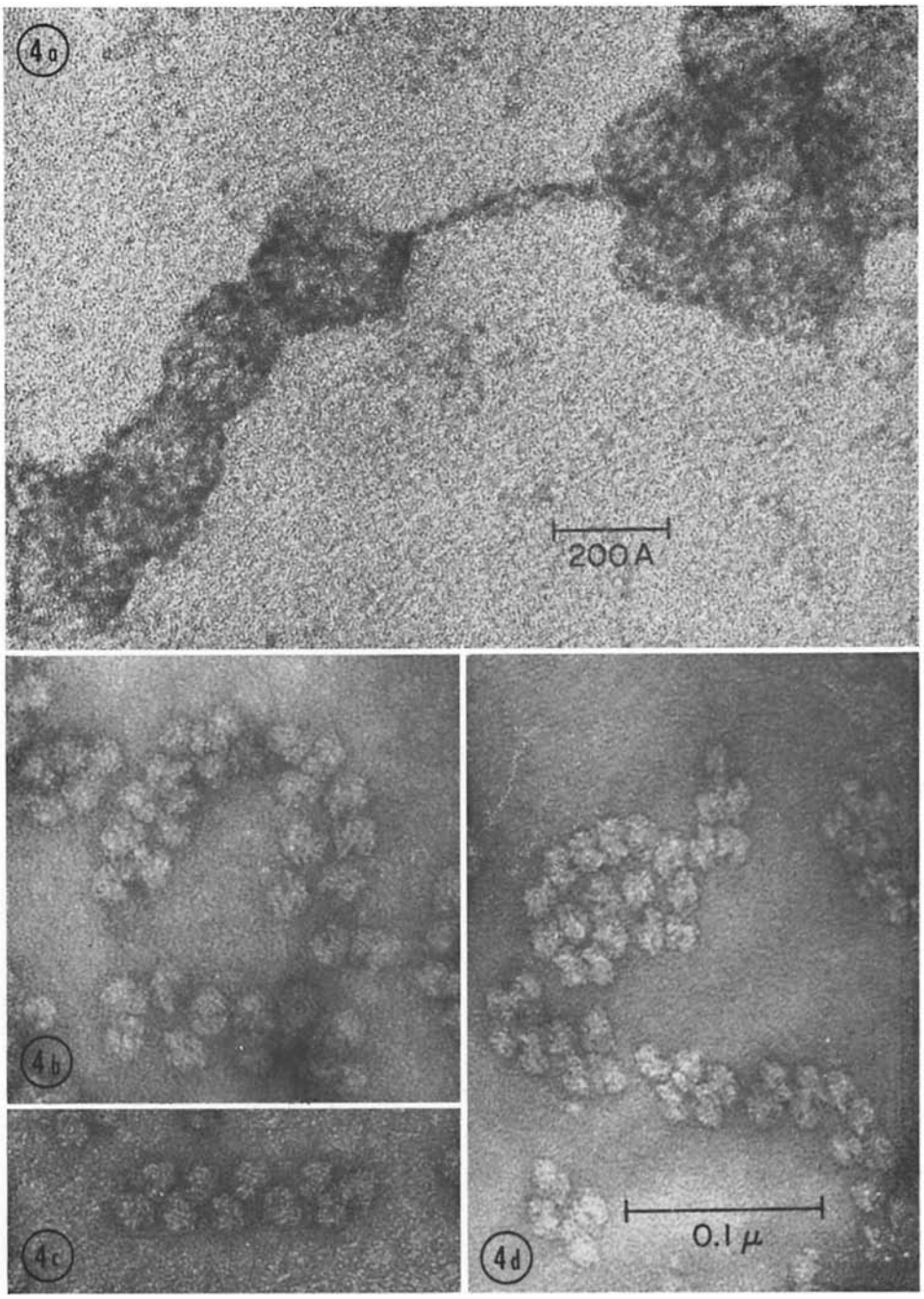

FIGURE 4 *a* Polyribosomes positively contrasted with uranyl acetate. × 800,000.

FIGURE 4 *b–d* Negative contrast with uranyl acetate. × 268,000.

arrays, the average center-to-center spacing between adjacent ribosomes is closer to 240 A.

As shown in Fig. 2, particles about 300–500 A in diameter are frequently found attached to large polyribosomes from *E. coli*. These particles are very similar in size and morphology to membrane-bound particles which are found attached to the outside of *E. coli* K12 (Hfr 3000) cells (see Fig. 3 *b*). They are not found inside the cells. We conclude that these particles stick to polyribosomes after cell breakage. The large polyribosomes often show these particles as a contaminant, which may modify the sedimentation properties of these structures.

A striking feature of these large polyribosomes is that, rather than being fully extended axially, they are very often doubled up laterally. It is unlikely that these large polysomes are fully extended inside cells since their length may be as great as or greater than the diameter of the cell. It is not unlikely that the extended arrays shown here result from the spreading and drying which is associated with sample preparation. The finding that negatively contrasted large polysomes often occur as a staggered double array opens the question as to what configurational changes occur in the dehydration process. However, this finding does suggest a pattern for the juxtaposition of ribosomes which, one expects, would contribute to structural stability by virtue of ribosomal interactions. This pattern could have arisen by the dehydration of a helically wound polysome such as that inferred from electron micrographs of thinly sectioned cytoplasm from various tissues. Eiserling et al. (5) as well as Shelton and Kuff (19) showed that smaller polysomes often formed rosettes or circles, with the smaller ribosomal subunit in the center. This configuration would also maximize the interaction of adjacent ribosomal subunits.

Throughout the investigation, we kept in mind the possibility of visualizing attached nascent protein. The entire β-galactosidase molecule has a readily visualizable tetrameric structure with a diameter near 110 A (10). However, preliminary experiments indicated that optimal conditions for visualizing the polysome did not correspond to optimal conditions for visualizing the enzyme. We have been able to visualize the enzyme alone (Fig. 3 *e*) where it has a well defined, four-lobed configuration in an occasional favorable orientation. However, the usual cross-section presented in negatively contrasted preparations could easily be confused with a 30S ribosomal subunit. Furthermore, the calculated frequency with which an active enzyme would be expected to be found on a polyribosome, multiplied by the probability of finding an intact polysome extended for maximum visibility, results in a low probability for success unless a better marker than the enzyme itself can be found. A ferritin antibody experiment might be successful in this regard.

Some of the large polysomes in this region of the gradient are polycistronic and are involved in synthesizing the three proteins of the lactose operon. In examining these electron microscopic fields, we kept in mind the possibility of observing a nonuniform distribution of ribosomes in the polysomes. However, although some gaps were observed, as seen in Figs. 2 and 4, we could come to no consistent conclusion regarding their number and distribution. A more interesting sample for the study of unequal distributions of ribosomes in polysomes would be amber mutations in the β-galactosidase gene, since these are associated with a termination in the synthesis of this polypeptide chain but the continued production of the other proteins in the operon. There is a relation between the position of the amber mutation in the galactosidase gene and the amount of transacetylase produced (15). Unfortunately, the identification of this class of polysomes would be difficult since there is no polysome-bound β-galactosidase which can be used as a marker.

SUMMARY

Polyribosomes were isolated from *Escherichia coli* which had been induced for β-galactosidase production. The polyribosomes containing bound β-galactosidase were examined in the electron microscope after being stained with uranyl acetate. The largest polysomes contain approximately 50 ribosomes in a more or less extended array. A frequent configuration was a ribosomal double row in which the larger ribosomal subunit lay farther away from the axis of the double row than the smaller ribosomal subunit.

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