THE CONFIGURATION OF ISOLATED POLYSOMES

PETER PFUDERER and D. C. SWARTZENDRUBER. From the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

INTRODUCTION

Behnke's demonstration (1) of helical polysomes in ultrathin sections of fetal rat gut epithelium has raised questions regarding the extent and significance of such structures. Since Behnke's report, helical polysomes have been found in differentiating muscle (2), differentiating pollen mother cells (3), and in the pleuropneumonialike organism A5969 (4). Thus polysome helices have been found in both plant and animal cells, but are seen only infrequently, usually in rapidly growing and differentiating tissue containing little endoplasmic reticulum.

It has recently been shown that the properties of polysomes in solution have one feature compatible with the helix described by Behnke, namely that the rate of increase of the polysome frictional coefficients with increasing size agrees very well with a helix of about 20° pitch and 3 or 4 ribosomes per turn (5, 6). This would suggest that all free polysomes could be helical in solution, or in a rodlike configuration resembling a helix. The present study was initiated to study this problem. Since it was not feasible to look at solutions with the electron microscope, sections of fixed ribosome pellets were examined, assuming that structures present in solution would be preserved in the pellet.

EXPERIMENTAL

Deoxycholate-treated Wettstein C ribosomes (7), a partially fractionated polysome preparation, were prepared from normal rat liver as described previously (6). The polysome pellet was fixed in glutaraldehyde, embedded in Epon 812, and observed by thin-section electron microscopy as described by Maniloff et al. (4).

Sections of these C-ribosome pellets disclosed very few configurations resembling helical polysomes, of the order of one ribosome in a helical configuration per 1,000 or so ribosomes. These configurations were rodlike structures, two ribosomes wide, which seemed to have a certain rigidity and were similar in appearance to sections through a helix. The term helical structure or configuration, in the context of this paper, will be taken to mean these rodlike structures, since it is not certain from our data that they are true helices. When we further fractionated these C ribosomes for large polysomes by spinning them through two layers of sucrose (1 ml of dissolved and clarified pellet in 0.5 M sucrose was layered on 1 ml of 2 M and 0.5 ml of 3 M sucrose, the rest of the tube being filled with buffer) at 105,000 *g* for 3 hr, so that only the heaviest polysomes pelleted, evidence was obtained of considerable structure in the pellet, as is shown in Fig. 1. This picture is characteristic of this enriched preparation. Sections from this preparation showed about 20 to 30 ribosomes in a helical configuration per thousand ribosomes. These structures presented two different appearances, a narrow structure of considerable pitch (labeled *1* in Fig. 1) and a wider structure of very little pitch (labeled *2*). There should also be approximately as many cross-sectional views of these structures as there are rods parallel to the section if the orientation is random, and it is possible that the structures labeled *E* in Fig. 1 are such cross-sections. They are similar to the cross-sections of helical ribosome cylinders described by Maniloff et al. (4).

A pelleted ribonuclease digest of C ribosomes was also examined to determine whether the structures were resistant to ribonuclease action, since single-stranded RNA should not be rigid enough to support such a structure, and, as a control, to determine how much apparent structure could be found in pelleted single ribosomes. Approximately 6 mg of the C ribosomes was digested with 0.3 μg of bovine pancreatic ribonuclease at pH 7.6, 0.005 M Mg, at 37°C for 5 min, then pelleted and examined as described above. An ul-

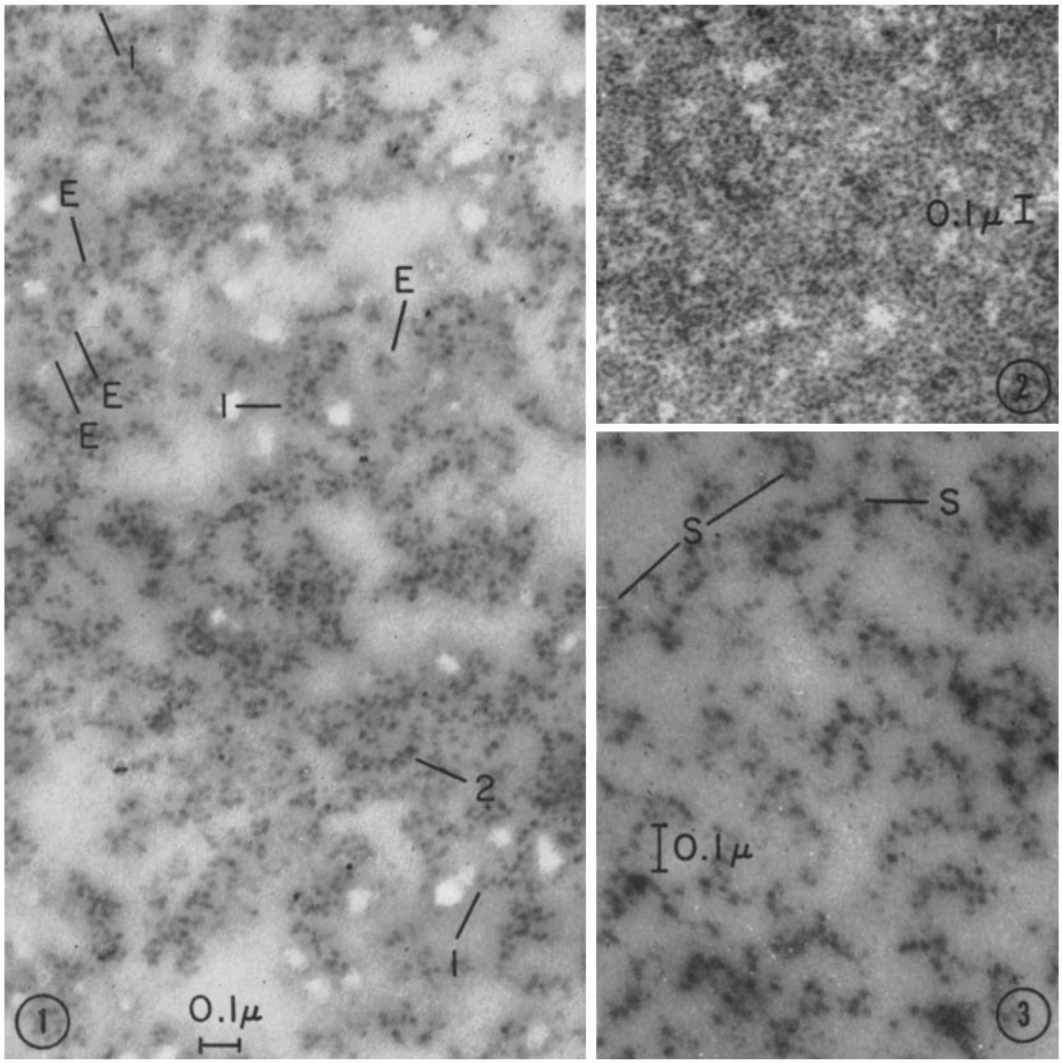

FIGURE 1 Thin-section electron micrograph of an enriched polysome pellet. The light areas are holes in the section. Some of the helixlike structures are indicated by arrows. Much of this pellet is laced with structures two ribosomes in cross-section. Two examples of narrow helices with a large pitch are indicated by *1*, and two examples of the wider helix are indicated by *2*. *E* indicates possible cross-sections of the helical structures.

FIGURE 2 Thin-section electron micrograph of ribonuclease-digested polysomes. The small electron-opaque particles are ferritin.

FIGURE 3 Thin-section electron micrograph of ribonuclease-digested polysomes, from the same pellet shown in Fig. 4. A few single-stranded polysome chains are indicated by *S*.

tracentrifuge run showed that the polysomes were broken down to monomers and dimers, with only a trace of polysomes of three units and higher. Fig. 2 shows that these ribosomes packed together much closer than the polysomes shown in Fig. 1. No evidence of a helical structure can be seen in Fig. 2, although the dense packing makes it difficult to distinguish structure in this pellet.

Fig. 3 shows a small region of the same ribonuclease-treated pellet, presumably the bottom, contain-

ing polysome aggregates not present in a high enough concentration to show in the ultracentrifuge run. These polysomes present a picture very different from that of the polysomes shown in Fig. 1, in that the predominant structure here seems to be an apparently flexible polysome chain resembling the groups of membrane-bound ribosomes seen in tissue sections. Other workers have recently reported finding ribonuclease-resistant polysomes (8–10), and those observed in Fig. 3 may be such polysomes. This section of the

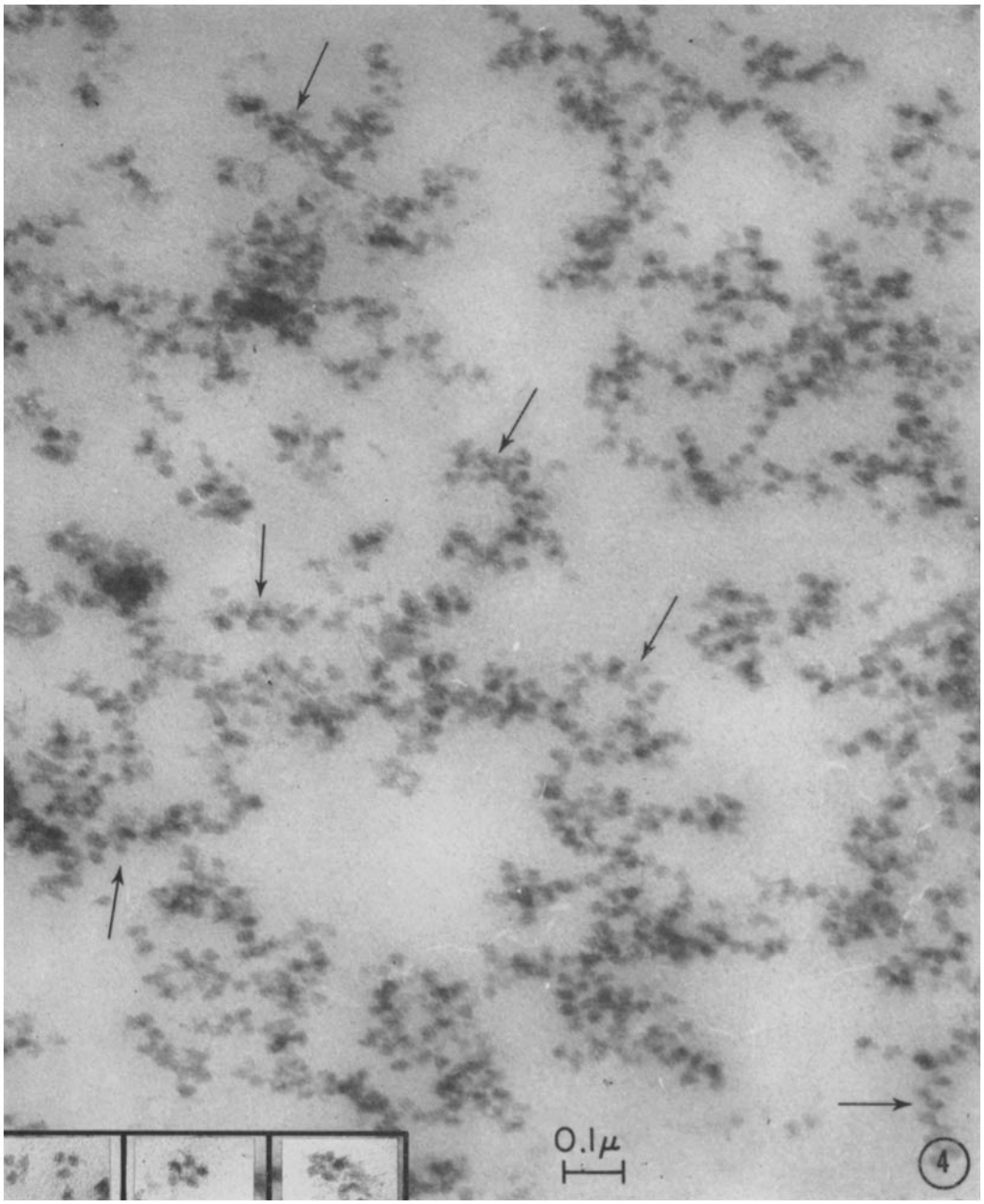

FIGURE 4 Thin-section electron micrograph of a pellet from tube No. 39 in Fig. 5. Helical structures are indicated by arrows; the inserts at the bottom show possible cross-sections.

pellet also shows the large spacings between polysomes that were seen in Fig. 1, and were not seen in Fig. 2. It is in this region of the pellet that one would expect to find any helical structures present in the sample. No clear-cut examples of helices were found here, however. The rodlike structures found in our preparations would require either interribosome contact or a rigid backbone for support. None of the micrographs show interribosome contact, yet most of the rodlike structures seem to be susceptible to ribonuclease. One model which would fit these data would be a stiff RNA complex (probably with protein) containing occasional breaks such that primarily monosomes are released by ribonuclease action. A similar model involving basic protein has been proposed by Aepinus (8) to explain his data.

Fig. 4 shows a micrograph of a pellet taken from tube No. 39 in the zonal ultracentrifuge run (11) shown in Fig. 5, containing polysomes of 10 units or higher. The gradient used was a linear 10 to 30% sucrose gradient with a cushion of 55% sucrose. Several helical structures are indicated by arrows.

Inserts of some 3- and 5-ribosome ring structures also found in this pellet are shown on the bottom of Fig. 4. Since the polysomes in this pellet should be 10 ribosomes long or greater, assuming no degradation, it is possible that these are cross-sections of helices.

The number of helices found in this pellet varied from 2% to 5% of the ribosomes, and averaged about 3%.

Pellets from polysomes 1 to 6 in this run were also examined and proved similar in appearance to Fig. 2, i.e., very densely packed with no obvious helices.

DISCUSSION

The probability of discerning a helical rod in a sectioned pellet of randomly oriented polysomes would be proportional to the angle the rod could be rotated from the plane of the section and still be recognizable as a helix, and also proportional to the concentration of polysomes in the preparation large enough to form a recognizable helix. The pictures show that one would need to see three turns of a helix in a section in order to clearly identify it as such. Since we are dealing with helices containing an integral number of ribosomes per turn, probably 3 or 4, this would require either nine ribosomes and about 1,200 A of length along the rod, or 12 ribosomes and 1,500 A of length (6), assuming the rod is centered in the section. More length is required if it is not. For a section 600 to 900 A thick, the maximum angular deviation a rod could have from the plane of the section and still contain three turns in the section would be 23° to 49° (sin θ = 900/1200 or 600/1500). Therefore, if all orientations are equally probable, the probability of a long rod being correctly oriented will be of the order of 0.1 (P = 23°/360°).

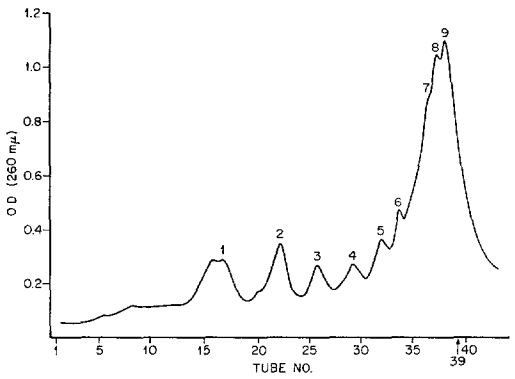

FIGURE 5 Zonal ultracentrifuge separation of liver polysomes. Speed 40,000 RPM; time 100 min; 10 to 30% linear sucrose gradient with a 55% sucrose cushion. The direction of sedimentation is from Tube 1 (top) to Tube 40.

Polysomes 9 ribosomes and larger comprised roughly 15% of the weight of the C ribosome fraction, and polysomes 12 ribosomes and larger, 5%. This was measured by the area under the Schlieren peaks, corrected only for radial dilution. A preparation of enriched polysomes similar to that used in Fig. 2 was found to contain roughly double these amounts of large polysomes.

Assuming that all the polysomes in the preparation are helical, the probability that a given ribosome in the C ribosome preparation will be recognized as part of a helical array is then of the order of 0.1 × 0.1, or 1%.

The three pellets described should then have shown 10, 20, and 100 ribosomes in helices per thousand ribosomes, assuming all the polysomes were helical; the numbers found were roughly 1–2, 4–10, and 10–50, respectively.

The per cent found over that predicted for the three pellets would then be 15, 30, and 33%, respectively.

The difference between the results for the three pellets could be explained by a partial orientation in the pellet, or by either a minimum size necessary for helix formation or a number of ribosomes per turn larger than four in the helix. Since there is probably some selection in the picking of samples to photograph, these are probably upper estimates.

Liver polysomes in vivo are normally bound to the endoplasmic reticulum, and liver cells have few free polysomes (12). They are grouped in large loops and spirals on the reticulum, and are probably not helical. However, we have demonstrated that, when these polysomes are freed from their reticulum with deoxycholate, sections from the polysome pellet show helical structures similar to the free polysomes of rapidly growing and differentiating tissue. These helical structures were found in the large polysome fractions with $n \geq 10$.

We would like to thank Dr. Jack Maniloff for making his manuscript available to us, Dr. Marialuisa Melli for help in preparing the rat liver ribosomes, Dr. Piero Cammarano under whose impetus this project was started, and Dr. W. D. Fisher for making the zonal run.

This research was sponsored by the United States Atomic Energy Commission under contract with the Union Carbide Corporation.

Received for publication 8 November 1965.

REFERENCES

1. BEHNKE, O., *Exp. Cell Research*, 1963, **30**, 597.
2. WADDINGTON, C. H., and PERRY, M. M., *Exp. Cell Research*, 1963, **30**, 599.
3. ECHLIN, P., *J. Cell Biol.*, 1965, **24**, 150.
4. MANILOFF, J., MOROWITZ, H. J., and BARRNETT, R. J., *J. Cell Biol.*, 1965, **25**, No. 1, pt. 1, 139.
5. PFUDERER, P., CAMMARANO, P., HOLLADAY, D. R., and NOVELLI, G. D., *Biochem. and Biophysic. Research Commun.*, 1965, **18**, 355.
6. PFUDERER, P., CAMMARANO, P., HOLLADAY, D. R., and NOVELLI, G. D., *Biochim. et Biophysica Acta*, 1965, **109**, 595.
7. WETTSTEIN, F. O., STAEHELIN, T., and NOLL, H., *Nature*, 1963, **197**, 430.
8. AEPINUS, K. F., *Biochem. Z.*, 1965, **341**, 139.
9. RABINOWITZ, M., ZAK, R., BELLER, B., RAMPERSAD, O., and WOOL, I. G., *Proc. Nat. Acad. Sc.*, 1964, **52**, 1353.
10. MANNER, G., GOULD, B. S., and SLAYTER, H. S., *Biochim. et Biophysica Acta*, 1965, **108**, 659.
11. ANDERSON, N. G., BARRINGER, H. P., BABELAY, E. F., and FISHER, W. D., *Life Sc.*, 1964, **3**, 667.
12. PALADE, G. E., and SIEKEVITZ, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 171.