FORMATION OF HELICAL POLYRIBOSOMES IN POLIOVIRUS-INFECTED CELLS OF THE 37 RC LINE

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A rapid disaggregation of host cell polyribosomes takes place after infection with picornavirus (5, 6). Later, when virus-modified protein synthesis begins, larger polyribosomes appear in the cytoplasm of infected cells. Ribosomes in these new aggregates are linked by longer messenger RNA, in this case viral RNA molecules (2, 3, 6, 7, 9) and usually have a conventional arrangement (1). Normal cells of the 37 RC line contain a fairly well-developed system of granular endoplasmic reticulum (GER) and free ribosomes organized in relatively small polysomes unpublished results). After inoculation of these cells with poliovirus, the profiles of GER become distended and finally disappear. Concomitantly, the number of free ribosomes augments and larger polyribosomal aggregates are formed. An appreciable fraction of these aggregates has a typical form of helical polyribosomes. Data concerning the appearance of helical polyribosomes in poliovirus-infected 37 RC cells are presented in this note and discussed on the basis of the kinetics of virus growth and virus-dependent decline of protein synthesis.

Throughout this study, confluent monolayers of 37 RC cells (*Cercopithecus* kidney cells; G. Rita, personal communication) obtained in 30-ml plastic Falcon Culture Flasks # 3012 were used (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.). The monolayers were propagated and maintained in Eagle's medium (MEM) supplemented with 10% inactivated calf serum. Poliovirus type 1 (Mahoney strain) was propagated in monkey kidney (MK) cells in MEM with 10% inactivated calf serum. Infected MK cells were collected 7 hr after inoculation, frozen-thawed three times, and centrifuged. The supernatant was used as a crude stock virus in all experiments. The supernatant obtained in the same way from uninfected MK cells was used for mock infections. Monolayers of 37 RC cells were infected with the crude stock of poliovirus at a multiplicity of about 10 PFU/cell. The mock-infected cultures served as controls. The infected and mock-infected cells were harvested at 0, 1, 2, 6, 8, 12, 24, 26 hr after inoculation. A set of aliquots was fixed for electron microscopy with phosphate-buffered (pH 7.2; 0.1 M) glutaraldehyde solutions ranging from 2 to 6.25% and post-fixed with 2–4% unbuffered osmium tetroxide solutions (pH 6.0). The observations on helical polyribosomes were facilitated when prefixation with glutaraldehyde was omitted. For microscopy, a Siemens Elmiskop IA electron microscope was used. Other sets of aliquots of infected and mock-infected cells were used to study the kinetics of protein synthesis by following the incorporation of radioactive leucine into trichloroacetic acid-precipitable material (for technical details, see legend to Fig. 2).

Poliovirus multiplication in the cells of the 37 RC line is presented in Fig. 1, which shows that the exponential viral multiplication occurs between 3 and 6 hr after inoculation. Between 6 and 8 hr after inoculation, the growth curve of the poliovirus begins to level off. Fig. 2 shows the kinetics of protein synthesis in cells synchronously infected with poliovirus at a multiplicity of about 10 PFU/cell. After an initial rapid decline, there is an evident rise in amino acid incorporation at about 2 hr after inoculation, followed by a gradual but constant decrease. At 6 and 8 hr after inoculation, the rate of incorporation of radioactive leucine into proteins determined for infected cells and

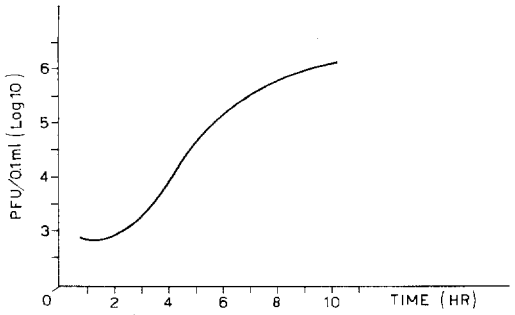

FIGURE 1 Growth curve of poliovirus in the 37 RC cell line. Replicate monlayers of 37 RC cells were inoculated at a virus:cell multiplicity of 10:1. Virus absorption was carried out for 1 hr at 37°C. Then the monolayers were washed three times with Hanks' solution and incubated in MEM with 10% calf serum at the same temperature. At hourly intervals, replicate cultures were collected and stored at −20°C. After thawing, the adhering infected cells were scraped off the plastic bottles with rubber policeman, and the cell suspensions were transferred to test tubes and frozen-thawed three times. After spinning down the cell debris, the supernatants were titrated by plaque assays performed on MK monolayers in Falcon plastic bottles #3012. After 1 hr of absorption at 37°C, the inoculum was removed and the cultures were covered with 7 ml of an overlay mixture consisting of equal volumes of 3% agar noble (Difco Laboratories Inc., Detroit, Mich.) and two times concentrated MEM with 10% calf serum without phenol red. After 52 hr of incubation at 37°C, the cultures were stained for 3 hr with 1:12,000 dilution of neutral red in 1.5% agar noble. Then the plaques were counted.

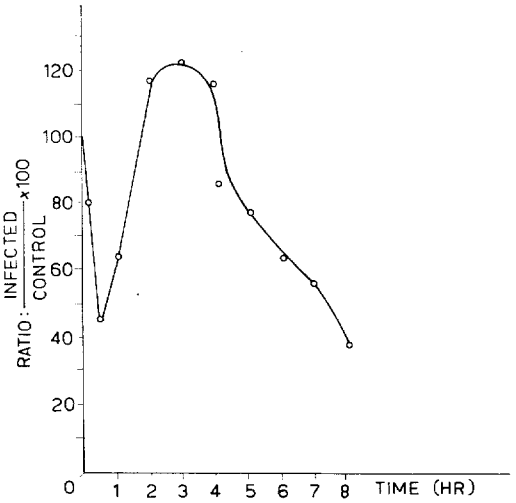

FIGURE 2 Rate of protein synthesis in cells of the 37 RC line during the growth cycle of poliovirus. Confluent monolayers of 37 RC cells grown in plastic bottles were infected with poliovirus containing 1 mM guanidine-HCl (Koch-Light Laboratories, Colnbrook, Bucks, England) in the inoculum to synchronize the infective process (8). After 1 hr of absorption at 37°C, the inoculum was removed and the monolayers were washed twice with cold phosphate buffer saline (PBS) (4) without Ca^{++} and Mg^{++}. 2 ml of the prewarmed MEM with 10% calf serum were added to each culture. At determined intervals the medium was removed from infected and control cultures and 1 μCi of leucine-^{3}H (specific activity 22,200 mCi/mmole, The Radiochemical Centre, Amersham, England) in 0.5 ml of leucine-free MEM was added to the monolayers for a period of 10 min. The incorporation was stopped by the addition of 1 ml of 1 N KOH in which the cells were incubated at room temperature for 30 min. Then 0.4 ml aliquots were taken from each bottle, and 0.4 ml of 20% trichloroacetic acid added. The precipitated radioactivity was collected on Millipore filters (Millipore Corp., Bedford, Mass.) and measured with a Beckman LS 133 scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.).

expressed as a percentage of the value found for uninfected cells is 60 and 40%, respectively. (The details will be reported elsewhere). Electron microscopy of mock-infected cells revealed that their GER cisternae, which are often arranged parallel to one another, had an average width (membrane to membrane) of ~480 ± 20 A. The free ribosomes of these cells were scattered throughout the cytoplasm and showed a low degree of aggregation. Occasionally short, single, helical polyribosomes could be observed in mock-infected cells. The GER cisternae of poliovirus-infected cells as observed at 6 hr after inoculation were greatly distended (Fig. 3). Their mean diameter was 1.1 ± 0.1 μ. These cisternae disappeared between 6 and 8 hr after inoculation. Concomitantly, large free polyribosomal aggregates appeared in the cytoplasm of infected cells (Fig. 3). Among these aggregates helical polyribosomes were constantly encountered, and their number was augmented significantly in the period between 6 and 8 hr after inoculation.

The structure of helical polyribosomes in our material resembled greatly that of polyribosomes with a zigzag configuration described by Weiss and Grover (10), except for minor morphological differences such as right-handed helices, incomplete helices, and helices composed of both right-handed and left-handed segments (Fig. 4). In some helices found in inoculated cells, structures resembling a central core could be seen (Fig. 4). The size of ribosomes seemed to augment, during the formation of a helix, from 200 ± 20 A, typical

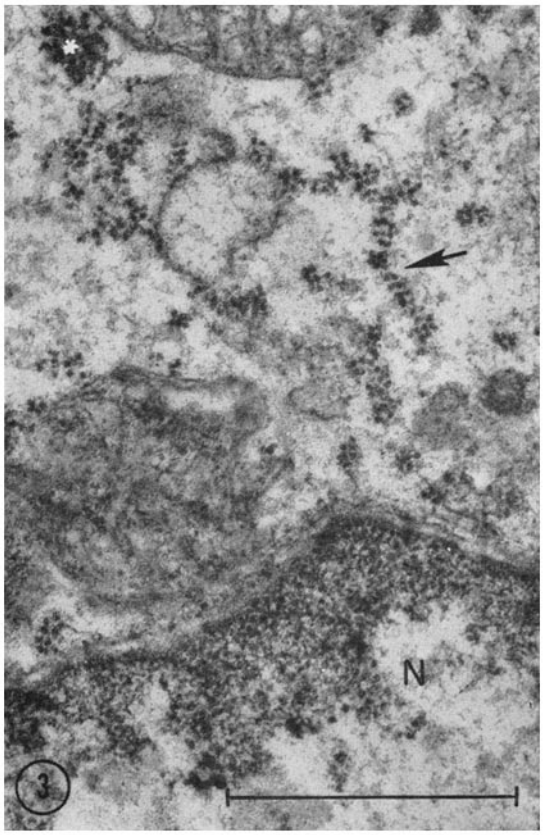

FIGURE 3 Part of a poliovirus-infected 37 RC cell fixed at 6 hr after inoculation. Large clumplike polyribosome is marked with an asterisk. Arrow indicates conventional polyribosome with chainlike arrangement of ribosomes. No helical polyribosomes are visible in this area. Nucleus is marked *N*. Fixed in 4% unbuffered osmium tetroxide. Magnification mark corresponds to 1 μ. × 38,000.

for the small ribosomes of conventional polyribosomes, to 300 ± 30 A in the helical polyribosomes, but this point requires further investigation before being considered as firmly established. The distribution of helical polyribosomes in infected cells is irregular. In some cells they are scarce whereas in others they constitute the predominant type of polyribosomes present. An estimate of the frequency of occurrence of helical polyribosomes in mock-infected and poliovirus-infected cells was carried out by counting all polyribosomal aggregates visible on electron micrographs of appropriate cells. Each experimental group, i.e. mock-infected cells and poliovirus-infected cells taken at 6 and 8 hr after inoculation, was represented by 50 electron micrographs taken from different cells. It was found that helical polyribosomes constituted about 0.1% of free polyribosomal aggregates in mock-infected cells, about 3% in infected cells observed at 6 hr after inoculation, and about 7% in infected cells examined at 8 hr after inoculation. Helical polyribosomes which were not well defined morphologically, i.e. those sectioned transversely or obliquely, were excluded from the counting. Thus the number of helical polyribosomes especially in infected cells was underestimated. Beginning at 12 hr after inoculation, the cytoplasmic regions occupied previously by large polyribosomal aggregates were replaced gradually by vacuolar formations filled with dense granules. These granules, however, were never seen to form regular crystals at any stage of the poliovirus development in cells of the 37 RC line.

The decline in virus multiplication at 6–8 hr after inoculation coincides with the formation of a majority of helical polyribosomes in poliovirus-infected cells of the 37 RC line. This finding suggests that the formation of helical polyribosomes in these cells does not depend directly on the process of viral multiplication. Moreover, the kinetics of protein synthesis in the same cells show that a progressive reduction in the rate of incorporation of radioactive leucine into the trichloroacetic-precipitable material coincides with the appearance of most helical polyribosomes in the investigated cells. These results favor the hypothesis proposed by Wooding (11) in his recent paper on the formation of helical polyribosomes under certain experimental conditions in mature plant cells. He states . . . "it seems more reasonable to regard the conventional chain of ribosomes connected by mRNA as the actual model for protein synthesis and the tighter aggregates as storage forms." The drop in protein synthesis concomitant with the appearance of helical polyribosomes in poliovirus-infected 37 RC cells furnishes experimental support for this hypothesis. The problem of the nature of the binding among ribosomes in the helix is an interesting one, and its solution should help in understanding the functional role of these structures. So far, we are not able to furnish any data on this aspect.

Our thanks are given to Prof. G. Rita for supplying the cells of the 37 RC line used in this study. For the photographic prints we are indebted to Mr. Mario Mari, and for excellent technical assistance to Mr. Bruno Pasquetti.

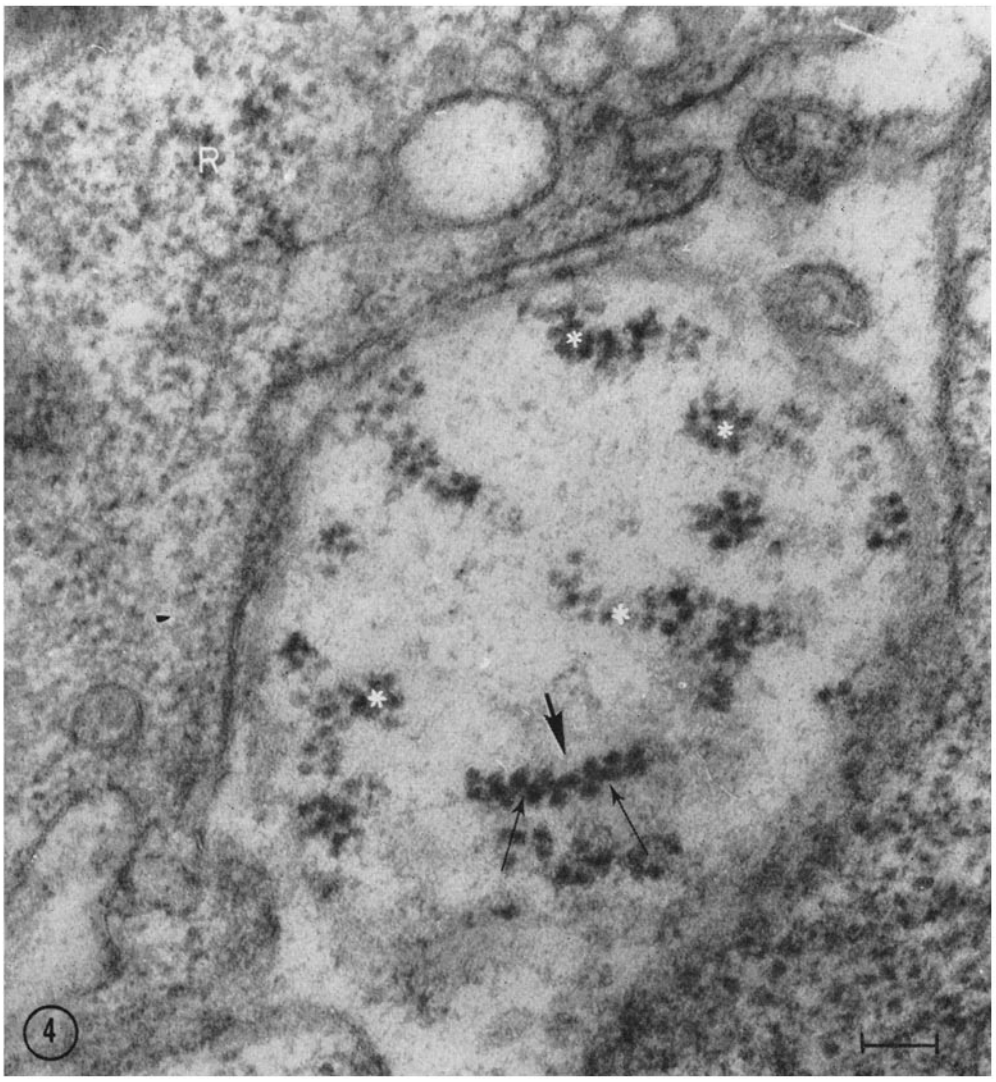

FIGURE 4 Electron micrograph of poliovirus-infected 37 RC cells fixed at 8 hr after inoculation. The helical polyribosome visible in the micrograph is composed of left-handed and incomplete right-handed segments. Thick arrow indicates the point of junction of these segments. A possible axial structure in the helix is pointed out by small arrows. Among the large conventional polyribosomes marked with asterisks are tangentially and obliquely cut helical polyribosomes. A cytoplasmic area occupied by single ribosomes and small conventional polyribosomes is visible at *R*. Note the difference in the sizes of the ribosomes present in the area and of those composing the helix. Fixed in 4% unbuffered osmium tetroxide. Magnification mark corresponds to 0.1 μ. × 92,000.

This study was supported in part by grant from C.N.R. Italy.

Received for publication 31 July 1969, and in revised form 24 November 1969.

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