Analysis of the Helical Ribosome Structures of *Mycoplasma gallisepticum*

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**ABSTRACT**  The helical ribosome structures of *Mycoplasma gallisepticum* have been analyzed by optical diffraction and the rotational symmetry technique. The helix repeat (510 Å) is composed of 10 ribosomes in 3 turns. The rotational studies show some ribosome fine structure.

Harvesting of the microorganism *Mycoplasma gallisepticum* by centrifugation causes cytological changes that lead to the appearance of rounded cells, showing a loss of intracytoplasmic material and condensation of nuclear material. However, accompanying these destructive events, 70S ribosomes of the cells appear to aggregate into cylindrical or helical structures (1).

Since these organized arrangements of ribosomes promise to be useful for investigations of ribosome fine structure, we studied the packing of ribosome particles in these ordered structures. The studies have involved the analysis of electron micrographs of thin-sections or the structures by optical diffraction and the rotational technique of Markham, Frey, and Hills (2).

**MATERIALS AND METHODS**

**Sample preparation.**

Cultures of *Mycoplasma gallisepticum*, strain A5969, were grown in a tryptose broth medium (3). Cells were harvested by centrifugation for 10 min at 14,000 × g, at room temperature, to produce the intracytoplasmic ribosomal structures. The pellets were then fixed in cold 6.25% glutaraldehyde (in 0.1 M cacodylate buffer, pH 7.2) (3), or in 2% osmium tetroxide (in 0.1 M s-collidine buffer, pH 7.4) (4). The fixed pellets were dehydrated in ethanol and embedded in Epon (3). Thin-sections were cut, stained with saturated uranyl acetate (in 50% ethanol), and/or lead citrate (5), and examined with a Siemens Elmiskop I (converted to 1A) electron microscope. Micrographs were recorded on Kodak Electron Image Plates at initial magnifications of ×40–54,000.

**Optical diffraction**

A light microscope was used as the optical diffractometer, as described by Gall (6). For these experiments, a Reichert Zetopan microscope, equipped with a mercury-arc source, was used. The diffraction mask was made by taking a 35-mm picture of the electron micrograph on Kodak High Contrast Copy Film. The mask was cut into a circle (diameter about 1 cm) and positioned in a sliding holder above a ×10 objective lens.

A 30-μm electron-microscope aperture, placed between the light source and the condenser, served as a point source. A Kodak Wratten Filter (no. 74), which principally transmits the 546-nm mercury line, was also placed in the light path. Diffraction patterns were recorded on Kodak Tri-X Pan film.

**FIG. 1.** Electron micrograph of glutaraldehyde-fixed, uranyl-stained, cluster of ribosome helices of *M. gallisepticum*. ×150,000.

**FIG. 2.** Optical transform of Fig. 1. Left side is a short exposure and shows larger spacings. Right side is a longer exposure, necessary to see the smaller spacings.
Biochemistry: N9,

FIG. = 10 = X594,000. (a), (b), pictures, respectively. The structures shown in Figs. 1 and 3 are 403 Å in diameter, with a hollow 67-Å core, and are composed of 188-Å ribosomes. These measurements could be made most accurately on rotated micrographs, such as those in Fig. 3.

Fig. 1 is one of the largest clusters of ribosome structures observed thus far and gave a good diffraction pattern. The optical transform of Fig. 1, shown in Fig. 2, is the transform of a helix; it was analyzed by the theoretical methods described by Cochran, Crick, and Vand (7). The seven spacings measured are listed in Table 1, with their layer-line assignments. The helix repeat is, therefore, 510 Å—the spacings calculated for this repeat distance are also in Table 1 and are close to the observed values. Since the third layer-line has a strong near-meridional reflection (and is the only near-meridional reflection), the helix must have three turns per 510-Å repeat. The 170-Å spacing of the third layer-line is the helix pitch (the axial distance per turn) and gives the size of the scattering particle.

There should be a meridional reflection on the layer-line corresponding to the number of particles per repeat. Since reflections at such spacings (expected to be smaller than the 75 Å thus far observed) have not been seen, rotational symmetry, as described in the next section, was used to determine

RESULTS

Optical diffraction

The micrograph in Fig. 1 shows a typical cluster of the ribosome structures. Sections perpendicular to the z axis (or "fiber axis") are shown in Fig. 3. The structures shown in Figs. 1 and 3 are 403 Å in diameter, with a hollow 67-Å core, and are composed of 188-Å ribosomes. These measurements could be made most accurately on rotated micrographs, such as those in Fig. 3.

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Professional Film and developed in Diafine (to give an ASA rating of 2400). This diffraction system has an effective specimen-to-camera film distance of about 86.4 cm—100 and 150 mesh electron microscope grids, whose spacings were easily measured, were used for the calibration.

Rotational symmetry

The enhancement of rotational symmetry was performed by the method of Markham et al. (2). For this technique, the electron micrograph negative was enlarged ×11 and the image recorded on Kodabromide F-5 photographic paper. The paper was exposed N times, but was rotated 2π/N between exposures. For an N-fold rotational symmetry axis, this should reduce the background noise and enhance the images of the N repeating units.

FIG. 3. Helix cross-sections analyzed for rotational symmetry. The samples were fixed with osmium and stained with uranyl and lead. ×594,000. (a), (b), (c), and (d) Original, and N = 9, N = 10, and N = 11 rotated figures, respectively. Only the N = 10 picture shows an enhanced image. (e) and (f), (g) and (h), (i) and (j), (k) and (l). Other sections showing the original and N = 10 pictures, respectively.

FIG. 4. Model of ribosome helix with repeat of 10 particles per 3 turns. (a) View of model where pitch appears quite steep. (b) View of model where ribosome packing appears almost cubic.
that the helix z axis is a 10-fold rotational symmetry axis. Hence, there must be 10 ribosome particles per 510-Å repeat.

Therefore, the selection rule for the orders, n, of the possible Bessel functions \( J_n \) on each layer-line, \( l \), is given by:

\[
l = 3n + 10m,
\]

where \( n \) and \( m \) can be any positive or negative integer. The reflection intensities are what is expected from the selection rule. The reflections on the equator are the second and third maxima of \( J_n \) (the first is at the origin) and correspond to a radial spacing of 435 Å, which is the helix-to-helix distance. Considering the strong reflection as the first maximum of a \( J_n \), a radial spacing of 130 Å is obtained for the radial distance to the centers of the scattering ribosome particles.

**Rotational symmetry**

These studies were carried out to determine whether the helix z axis is an \( N \)-fold rotational symmetry axis; if so, it would be reasonable to conclude that the helix has \( N \) ribosomes per repeat. An upper bound for the value of \( N \) was determined from the optical diffraction studies, as follows: Consider a cylinder coaxial to the helix z axis, and with a radius equal to the radial distance from the z axis to the centers of the ribosomes (130 Å). This cylinder can be cut, parallel to the z axis, opened, and laid flat. This is the helix net, or radial projection, described by Klug, Crick, and Wyckoff (8).

A line joining the ribosome centers, on the helix net, must make three turns, at a pitch of 170 Å. From this data, the length of the line is calculated to be 2502 Å. If the ribosomes are approximately spherical (with a 170-Å diameter), then 14 particles would fill the repeat distance. Since ribosomes are actually slightly prolate ellipsoids of revolution (9), the azimuthal distance covered by a ribosome (which would be expected to be the major axis of the ellipse) is probably greater than 170 Å and the number of ribosomes per repeat would be expected to be less than 14.

Electron micrographs of thin-sections perpendicular to the helix z axis were examined for rotational symmetry, through \( N = 14 \). Of the 20 helices examined, 16 showed a 10-fold rotational symmetry axis (Fig. 3). No enhancement was seen at \( N = 9 \) or \( N = 11 \) (Figs. 3b and d). Since not all of the helices examined would be expected to be strictly perpendicular to the plane of section, it is not surprising that some did not show a symmetry axis and that there is some variation in the enhanced images seen.

It seems reasonable to conclude that the 10-fold symmetry reflects the packing of 10 ribosomes per helix repeat. The ten enhanced regions seen in the \( N = 10 \) micrographs (Fig. 3) are due to the heavy-metal staining of the ribosomes. Therefore, this technique has enhanced the appearance of ribosome parts that have bound uranyl ions. If one looked down the helix axis, as in Fig. 3, a single ribosome would cover an azimuthal distance extending over three of the ten enhanced regions. Hence, each region can give information only on a small sector of the ribosome.

Several different patterns with 10-fold symmetry were observed; examples of these are in Fig. 3. In analyzing the rotated photographs, it was noted that, although the radius of the helix core is about 34 Å, no structure was noted at radial distances less than 59 Å, indicating that the 25 Å of the ribosome closest to the z axis is not visualized by this technique. Between radii of 59 and 88 Å, some micrographs showed 10 repeating structures (Fig. 3j), and the inner ring of particles in 3h), while others showed an unstained region (Fig. 3c and f). This annular region is 29-Å wide and, if considered with the 25-Å part of the ribosome closest to the core, would comprise a 54-Å part of the ribosome. Some micrographs showed ten repeating units, at radial distances between 88 and 138 Å, a 50-Å wide annular region (Figs. 3c, f, i, and the outer ring of particles in 3h). No repeating images were seen in the outer 64 Å of the ribosomes. These outer two regions, if considered together, make up a 114-Å part of the ribosome.

The particles in the inner 29-Å annulus appear as unstained or lightly stained areas (Fig. 3h), sometimes showing some fine structure (Fig. 3j). In the outer 50-Å annulus, two types of images are mainly seen; the repeating units either appear polygonal (Figs. 3a and f) or rounded with some fine structure (Fig. 3l). Variations in the angle of the helix axis relative to the plane of section may account for the differences in the appearances of the repeating units.

**DISCUSSION**

The various measurements of the ribosome helices, from the electron microscopic and optical diffraction studies, are summarized in Table 2. The values are in good agreement. The helix-to-helix distance (435 Å) is somewhat larger than the helix diameter (403 Å), indicating that there is about a 30-Å space between helices.

From the data, a model of the *M. gallisepticum* ribosome helix has been built and is shown in Fig. 4. The model has 10 ribosomes per 3 turns. Two possible solutions to this helical arrangement are to regard it as a single helix, of 10 particles per 3 turns, or as a double helix, with each helix having 5 particles per 2 turns. The latter possibility seems to be steri-

<table>
<thead>
<tr>
<th>Table 2. Ribosome helix measurements</th>
<th>Determined by</th>
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<tbody>
<tr>
<td></td>
<td>electron microcopy</td>
<td>optical diffraction</td>
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<tr>
<td>Ribosome diameter (Å)</td>
<td>168</td>
<td>170</td>
</tr>
<tr>
<td>Helix-core diameter (Å)</td>
<td>67</td>
<td>...</td>
</tr>
<tr>
<td>Helix diameter (Å)</td>
<td>403</td>
<td>...</td>
</tr>
<tr>
<td>Radial distance to center of ribosomes (Å)</td>
<td>118*</td>
<td>130</td>
</tr>
<tr>
<td>Helix-to-helix distance (Å)</td>
<td>...</td>
<td>435</td>
</tr>
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* Calculated as \( 1/4 \) (ribosome diameter + core diameter).
improbable, since adjacent ribosomes in each helix would not be in contact; hence, the structure must be a single helix. A ribosome can be carried into the next one by a rotation of 108° and a vertical translation of 51 Å. Depending on the orientation of the model, the helix appearance may be angular (Fig. 4a) or somewhat cubic (Fig. 4b). Both views have been observed in electron micrographs (3).

From these data, the cross-section of the volume occupied by the ribosome is defined by a sector of an annulus. The annulus is between radii of 34 and 202 Å (from the electron microscope data) and the sector is a 108° angle. The height of this structure is 170 Å (from the diffraction data). Regarding the ribosome in this volume as a slightly prolate ellipsoid, the axial ratio is calculated to be 1.2–1.4, depending on where the chord of the sector (the major axis of the ellipse) is chosen. Axial ratios of 1.2–1.4 have also been calculated from the data of Spirin and Gavrilova for Escherichia coli 70S ribosomes (9).

The 70S ribosomes of M. gallisepticum dissociate into 30 and 50S particles (1, 10). For the 70S ribosome of E. coli, the 30S particle accounts for one-third of the 70S ribosome and the 50S particle for two-thirds (9). Therefore, in the rotated micrographs discussed above, it is tempting to speculate that the inner 54 Å of the ribosome may be the 30S particle and the outer 114 Å may be the 50S. These are the only interfaces seen that would give the one-third-two-thirds ribosome particle. This would mean that the 30S part of the ribosome is oriented toward the helix core. Most of the area of interaction of adjacent ribosomes would then be between their 50S parts.

If this interpretation is true, then it is especially interesting that the enhanced rotational images are seen on either side of the 30S–50S interface. This would identify the most invariant parts of the ribosome structure with the area that must be most active in protein synthesis. Since rotated images of the outer parts of the ribosomes are not seen, the structure in these areas must be variable and, hence, lost as noise in the rotational processing.

The interaction of the 50S subunits may stabilize the helix, since it has been shown that antibiotics that bind to this subunit (chloramphenicol and lincomycin) do not allow the ribosomes to form helices, whereas antibiotics that bind to the 30S particles (streptomycin and tetracycline) do not affect helix formation (11). These studies also showed that inhibition of RNA synthesis (by actinomycin D) or protein synthesis (by puromycin) did not affect the ability of the ribosomes to form helices. This excludes the possibility that the helices are polysomes, and also the possibility that a small polysome might act as a “seed” for the condensation of the ribosomes. Therefore, the helices must be regarded as an example of self-assembly. However, the above studies also showed that inhibition of DNA synthesis (by mitomycin C) reduces helix formation, suggesting that DNA strands may stabilize the helical structures, perhaps by forming a stable “seed” for further helix formation.

Helical ribosomal structures have been reported in so many systems that no extensive review will be attempted here. In some cases the helices are believed to represent structures that play a role in the cellular physiology, either as polysomes (12, 13), or as ribosome storage bodies in Entamoeba (14, 15), or in some undetermined way in developing tissue (16, 17). The helices observed in most organisms apparently arise due to an environmental perturbation, such as chemical treatment (with a Vinca alkaloid) in the case of E. coli (18), or centrifugation in the case of M. gallisepticum.

The only ribosome helices that have been analyzed in detail, for comparison with the data presented here, are those making up the chromatoid bodies of Entamoeba invadens (19, 20); although some measurements of the helices in Rhodopseudomonas palustris have been reported (21), the helix parameters of these structures have not been determined. The comparison of the Entamoeba and Mycoplasma helix measurements are: repeat distance, 900 and 510 Å; pitch (ribosome size), 180 and 170 Å; helix diameter, 400 and 403 Å; and radial distance to the ribosome centers, 150 and 130 Å. The other differences are that the Mycoplasma helices have an empty core, the Entamoeba do not; and that the repeat in Mycoplasma is 10 ribosomes per 3 turns and in Entamoeba is 12 ribosomes per 5 turns (the equivalent structure is a double helix with 6 particles per turn and this is the most probable structure). In spite of the fact that the ribosomes of these two kinds of cells condense into different helical structures, it should be noted that the ribosomes from both cells have corrected sedimentation coefficients of 74–75S (10, 22) and similar antibiotic sensitivities (23, 24).

The ribosome helices present oriented arrangements of ribosomes and should allow studies of ribosomal fine structure. That fairly small clusters of Mycoplasma helices give diffraction patterns, and that subribosomal stained regions are enhanced by the rotational technique, indicate that there is a high degree of order in the packing of the ribosomes into the helices; this property should be useful in further structural studies.

NOTE ADDED IN PROOF

After this manuscript was submitted for publication, Lake and Slayter (Nature 227, 1032, 1970) showed that a better indexing of the Entamoeba helix diffraction pattern indicates that the repeat is 17 ribosomes in 5 turns. In addition, recent data (D. C. Barker, personal communication) indicate that these are 38S ribosomes, as had been reported earlier (14). A third helix structure has now been characterized. Kingsbury, Nauman, Morgan, and Voelz (Proc. 7th Intern. Congr. Electron Microscopy, Grenoble, 3, 69, 1970) have shown that the vinblastine-induced ribosome helices of Escherichia coli have a repeat of four particles in one turn.

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