where $f(x, y)$ is the density distribution in the image; x, y are coordinates relative to its center of gravity; and p, q are integers; $p + q$ is the order of the moment.

By combining these moments, certain invariants can be formed which may be used to describe the image with increasing accuracy as the order of moments included increases (14). Instead of the images themselves, the profiles formed by its moment-invariants ϕ_i, $i = 1 \ldots n$ may therefore be used for the identification of projections in a micrograph of randomly oriented particles.

To explore the feasibility of this approach, we have computed general (ϕ,θ) projections of the 30S ribosomal subunit previously reconstructed by Verschoor et al. (2), and rotated these in the image plane by various azimuths. The set of projections so created (Fig. 2 *a*) simulates the appearance of some of the particle views in the micrograph, assuming that the three-dimensional model is correct and provided that no deformations of the particle take place. The seven-moment invariants $\phi_1 \ldots \phi_7$ associated with $p + q = 3$ that were given by Hawkes (11) were computed for each of the 40 ($= 8 \times 5$ azimuths) projections. Because of quantization errors due to image rotation or due to sampling of differently oriented images, the calculated ϕ values undergo fluctuations (see reference 12). At this stage of the investigation we would like to establish whether or not different projections of an asymmetric particle such as the ribosomal subunit give rise to recognizably different invariant-profiles, and also whether or not the quantization errors would tend to obscure divisions among classes found.

The invariant-profiles formed by $\phi_1 \ldots \phi_7$ were subjected to correspondence analysis (6, 7). What is apparent from the map of factor 1 vs. 3 (Fig. 2 *b*) is that the projections are recognized as different, irrespective of their in-plane orientation. (Similar grouping occurs on the map of factor 1 vs. 2 but with some ambiguities.) The model computation suggests that it may be possible, at least crudely, to identify molecular projections in noisy micrographs.

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SYNTHETIC OLIGODEOXYNUCLEOTIDES AS PROBES OF RNA CONFORMATION WITHIN THE RIBOSOME

D. G. GLITZ, H. M. OLSON, AND L. S. LASATER
Department of Biological Chemistry, University of California at Los Angeles School of Medicine, Los Angeles, California 90024

The prokaryotic ribosome is composed of over fifty distinct proteins plus three different RNA molecules in two dissimilar subunits. Electron microscopy has been of great value in the determination of ribosome and ribosomal subunit structure, and antibodies directed against specific ribosomal proteins have allowed placement of several proteins within the structure of each subparticle. A reasonable consensus on protein localizations has begun to emerge (1).

Much less is known about placement of the ribosomal RNA. About two-thirds of the mass of the *Escherichia coli*

ribosome is contributed by the RNA, and it is the RNA that appears to generate the basic size and shape of each subunit. Many ribosomal RNA sequences have been determined, and two-dimensional models that describe short range interactions as stem and loop structures have been proposed (2). But direct placement of elements of RNA structure has been limited to the chemically unique termini and a few naturally methylated nucleotides. We are now attempting to localize additional segments of RNA structure through the use of oligodeoxynucleotide probes that are complementary to specific 16S RNA sequences.

RESULTS

Oligodeoxynucleotides with chain lengths of 8–15 have been prepared using Vega Biotechnologies model 280 and model 300 automated synthesizers (Vega Biochemical Div., Newbury Energy Corp., Tuscon, AZ). Both the phosphotriester and phosphoramidite approaches have been used (3) with emphasis on the latter. Potential antibody-recognizable markers have been incorporated at the 3′-end (N^6-isopentenyladenosine) or the 5′-end (dimethoxytrityl group) or have been added to the oligomer after chemical synthesis using terminal transferase (1,N^6-ethenoadenosine). The oligonucleotides were purified by high performance liquid chromatography on anion exchange or C_{18}-reversed phase columns. Experiments reported here used oligonucleotides complementary to the 3′-end of *E. coli* 16S ribosomal RNA (Fig. 1).

The synthetic oligodeoxynucleotide dAAGGAGGT was labeled with ^{32}P at the 5′-end using polynucleotide kinase. Interaction with 30S ribosomal subunits was studied using a nitrocellulose membrane filter-binding assay (described in the legend to Fig. 2). Functional activation of 30S subunits by pretreatment at 37°C or 50°C (4) for 15 min had no effect on specific binding of the octanucleotide, although nonspecific binding (to 50S subunits) was increased by 50°C treatment. Interaction of octanucleotide with 30S subunits for 1 h at 28°C, 37°C, or 50°C prior to overnight incubation on ice similarly showed little or no effect on the extent of specific binding. In contrast, concentrations of monovalent and divalent cations affected binding significantly; at $MgCl_2$ levels below 3 mM, results were not reproducible, while optimal binding was seen at 5–10

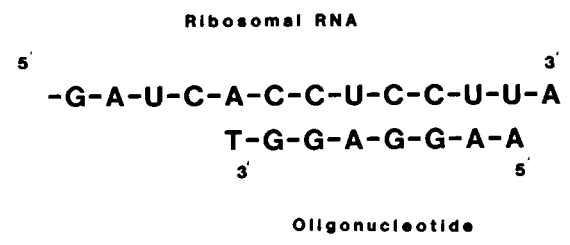

FIGURE 1 Interaction of a synthetic oligodeoxynucleotide with the 3′-terminal sequence of *E. coli* 16S RNA.

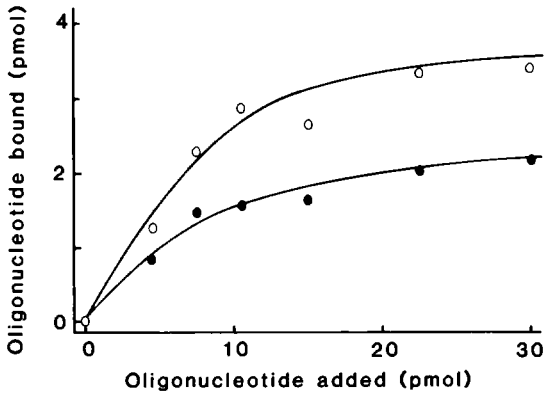

FIGURE 2 The interaction of the octanucleotide dAAGGAGGT with *E. coli* 30S ribosomal subunits as measured in a membrane filter binding assay. The oligomer was labeled with ^{32}P at the 5′-end using polynucleotide kinase. Varying amounts of oligonucleotide were incubated on ice for 15 h with 10 pmol (●) or 15 pmol (○) of activated 30S subunits in 50 μl of a buffer composed of 10 mM Tris-HCl, pH 7.5, 150 mM NH_4Cl, 10 mM $MgCl_2$, and 36 μM 2-mercaptoethanol. Reaction mixtures were diluted with 1 ml of cold buffer and rapidly filtered through pre-wet Millipore type HA nitrocellulose membranes (Millipore Corp., Ashby Fld., Bedford, MA). The filters were washed with three 2-ml portions of buffer, and bound ^{32}P was measured in a liquid scintillation counter.

mM. Ammonium chloride concentrations were varied from 60–500 mM; maximal specific interaction was seen at 150 mM.

Fig. 2 shows the effects of subunit and oligonucleotide concentration on complex formation. Oligonucleotide binding approaches its maximum at oligonucleotide:subunit ratios of ~1.5; at this point ~20% of the subunits have bound the octamer. Binding of the 13-mer dTAAGGAGGTGATT in similar experiments approximated 60%. The latter result is shown in Fig. 3, in which size exclusion

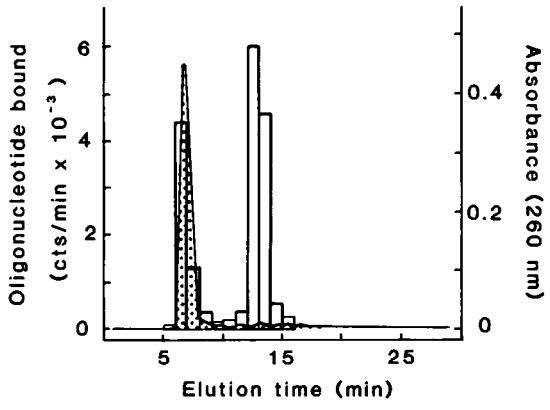

FIGURE 3 The interaction of the oligonucleotide dTAAGGAGGTGATT with *E. coli* 30S subunits as measured by size-exclusion HPLC. Oligonucleotide (218 pmol), labeled at the 5′-end with ^{32}P, and 30S subunits (138 pmol) were incubated on ice for 24 h in the buffer of Fig. 2. Five μl were chromatographed on a 7.5 × 300 mm Spherogel TSK-3000 column (Beckman Instruments Inc., Fullerton, CA) at a flow rate of 1 ml/min. Absorbance at 260 nm was monitored (shaded area) and ^{32}P was determined in aliquots of 1 ml fractions (bars).

HPLC was used to separate complex from free oligonucleotide.

The eventual aim of these experiments is localization of the complexed oligodeoxynucleotide by immunoelectron microscopy. Preliminary experiments using the octanucleotide, modified by addition of a 3'-terminal $1,N^6$-ethenoadenosine residue, resulted in a tentative placement on the subunit platform, close to the previously placed 3'-end and nearby dimethyladenosine residues (1, 2). But both the efficiency of modification and of binding of oligonucleotide in these experiments was low and the number of complexes observed was very small. We cannot thus yet be certain that these observations are both significant and specific.

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QUATERNARY ORGANIZATION OF THE 30S RIBOSOMAL SUBUNIT OF *ESCHERICHIA COLI*

P. B. MOORE,*‡ M. CAPEL,‡ M. KJELDGAARD,* AND D. M. ENGELMAN,‡

**Department of Chemistry and ‡Molecular Biophysics & Biochemistry, Yale University, New Haven, Connecticut 06511; and Biology Department, Brookhaven National Laboratory, Upton, New York, 11973*

The determination of the positions of proteins in the 30S ribosomal subunit of *Escherichia coli* by neutron scattering in solution undertaken in this laboratory over a decade ago is nearing completion. More than 80 interprotein distances have been measured positioning 19 of the 21 proteins in the structure. Many techniques used for exploring the structures of complex biological structures, such as crosslinking, and immune electron microscopy, have been applied to the 30S ribosomal subunit. Their reliability can be assessed using the neutron map, a point of interest to those struggling to understand less tractable structures. Further, the large body of existing data implicating the various ribosomal proteins in functions related to ribosome biogenesis and function in protein synthesis can now be evaluated in light of its three-dimensional organization.

METHODS

The neutron method for the analysis of quaternary structure depends on the fact that it is possible to alter the thermal neutron cross-section of a macromolecular aggregate by deuterium-substituting its constituents. In the case of the *E. coli* ribosome this is achieved by reconstituting particles from mixtures of constituents, one or two of which are heavily labeled with ^2H. The neutron scattering given by a solution of pairwise-labeled particles includes a measurable interference fringe whose angular periodicity is inversely related to the distance between the two constituents. The positions of the constituents may be obtained by triangulation based on these distances (for details see reference 1).

Dr. Kjeldgaard's current address is Division of Biostructural Chemistry, University of Aarhus, Kemisk Institute, DK-8000 Aarhus C, Denmark.

RESULTS

Table I gives the coordinates for all the proteins in the 30S subunit except S2 and S21 and the standard errors for these coordinates. The rather crude estimates the technique yields for protein radii of gyration are also reported. The protein array defined by these coordinates is ~190 Å × 120 Å × 70 Å. It is shown in "face-on" view in Fig. 1, looking down the 70 Å direction.

DISCUSSION

The ribosome was one of the first structures to be studied intensively by immune electron microscopy (IEM), a technique which takes advantage of the antigenic uniqueness of the ribosomal proteins to map their locations in the structure in the electron microscope (for review see reference 2). The correspondence between the IEM analysis and the neutron map is spectacular (Fig. 1). It appears that a carefully executed IEM (or neutron) study on a complex aggregate is capable of producing a reliable description of its quaternary structure.

Crosslinking is a less secure route to knowledge. In a recent article Lambert et al. (3) list 29 protein-protein crosslinks in the 30S subunit involving proteins we have mapped. Eight of the 29 involve proteins whose distance of closest approach measured by neutron methods exceeds 40 Å, a distance well out of reach of the crosslinking agents used. Furthermore, 10 of the 19 pairs of proteins in the neutron map whose center-to-center distances are <50 Å have never been crosslinked. It is clear that crosslinking data cannot provide a reliable model for the time-average