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*

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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).

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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

TABLE I
COORDINATES AND RADII OF GYRATION OF 30S RIBOSOMAL PROTEINS

	x	σ_x	y	σ_y	z	σ_z	R_g	σ_{R_g}
S1	−7.0	20.3	47.0	25.7	21.7	15.8	55.7	20.2
S3	0	—	0	—	0	—	17.3	15.2
S4	61.0	4.3	0	—	0	—	29.9	4.4
S5	51.6	5.3	39.7	3.2	0	—	13.4*	7.8
S6	75.0	14.3	67.9	10.8	90.5	6.9	13.1*	1.0
S7	−10.7	12.7	22.7	8.6	85.4	7.2	13.6*	0.9
S8	70.9	7.8	55.3	5.4	17.1	5.5	21.8	3.9
S9	−18.9	9.6	14.9	6.0	53.0	8.0	12.7*	0.5
S10	−9.3	10.3	−23.0	5.6	42.0	9.9	12.5*	5.0
S11	22.6	13.0	83.8	6.7	69.5	8.0	12.5*	1.0
S12	85.9	7.2	4.0	7.7	31.8	4.9	12.4*	0.6
S13	−62.5	12.7	38.8	11.9	79.7	14.6	12.5*	3.0
S14	−51.0	8.6	−17.5	9.3	23.5	17.1	12.1	5.0
S15	61.2	13.5	82.8	5.5	28.8	9.8	26.8*	1.6
S16	47.7	6.3	12.8	8.1	39.0	6.9	24.5	9.2
S17	103.5	8.3	47.8	9.1	33.7	7.7	11.2*	1.7
S18	74.4	17.1	37.4	9.8	77.3	5.3	10.9*	1.1
S19	−34.6	12.8	−36.4	6.7	50.6	13.6	11.9*	5.5
S20	25.9	10.0	−20.2	8.1	49.1	8.8	19.8	14.7

Coordinates, radii of gyration and standard errors are given in Å. Radii worked with an asterisk are set by constraints introduced during the mapping calculation which keep radii within physically reasonable bounds. Standard errors assigned these radii are unrealistically small.

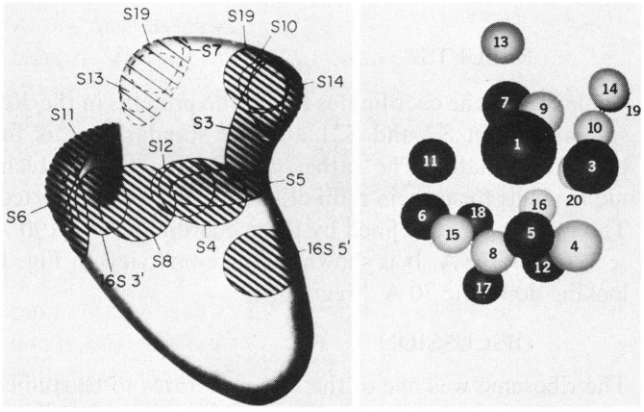

FIGURE 1 The 19 Protein Neutron Map and the Immune Electron Microscopic Image. The right-hand image in this figure is the protein array of the 30S subunit. Proteins are depicted as spheres whose volumes are to scale. The center-to-center distance from S13 to S17 is 173 Å. The left-hand panel shows the electron microscopic view of the 30S subunit with sites of antigenic determinants indicated following the work of Lake and his colleagues (6).

structure of the ribosome, a result that suggests the need for caution in interpreting crosslinking data from other systems.

The functional correlations range from the interesting to the perplexing. An example of an interesting correlation involves in vitro assembly where it is known that strong functional interrelationships between proteins exist (4). For example, it is known that the addition of S3 to an assembling ribosome is strongly facilitated by the prior presence of S10, which in turn depends on S9; and S9 depends on S7. Thus there is an arc of interdependencies running across the "top" of the particle from one nearest neighbor to the next (see Fig. 1). All strong interactions in assembly turn out to be interactions between nearest neighbors in the mature particle.

A large number of experiments have been done linking specific proteins with ribosomal activities in protein synthesis. The tendency has been that the longer a particular function has been analyzed to identify contributing components, the longer the list of suspects has grown, and it is natural to take the view that the longer that list, the less one believes any of it. For example, various short mRNA analogues have been used to affinity label the site (sites?) where mRNA is decoded by tRNA. By 1980 (see reference 5 for review) the list of components included: S1, S3, S4, S5, S12, S18, and S21. The proteins listed spread across the lower part of the protein array for 100 Å (see Fig. 1). Either much of these data is wrong or we don't understand in any way what goes on during the decoding interaction.

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COMPLEMENTARY OLIGONUCLEOTIDE PROBE OF VESICULAR STOMATITIS VIRUS MATRIX PROTEIN mRNA TRANSLATION

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We decided to predict the secondary structure of a messenger ribonucleic acid (mRNA), then pick a single-stranded region as a target for hybridization arrest by complementary oligonucleotides (1), and test the predictions in vitro. Translation of Rous sarcoma virus RNA was inhibited in vitro by a tridecanucleotide complementary to a site near its 5′ end, with a 50% inhibition dose of ~100 nM (1). Translation of all five vesicular stomatitis virus (VSV) mRNA was inhibited in mouse L cells by a methylphosphonate octanucleotide complementary to the ribosome binding site of the N protein mRNA, with a 50% inhibition dose of ~150 μM (2).

VSVmatrix protein (M) is a 26,024 d polypeptide coded by an mRNA with 831 nucleotides (nt) (3). The mRNA is translated on free polyribosomes in the cytoplasm (4), so it may be more accessible than in membrane-bound polyribosomes. Its prominence and separation from other VSV proteins on polyacrylamide gels (5), as well the modest size of its mRNA, identified it as a plausible model system for complementary oligonucleotide probes.

MATERIALS AND METHODS

RNA secondary structures were predicted with the program RNAFLD (6). Equilibrium constants for the association of oligonucleotides with predicted single-stranded regions of VSV M mRNA were calculated at 37°C from nearest-neighbor enthalpies and entropies of base pairing (7). No correction of the free energies was made for RNA-DNA hybridization, instead of RNA-RNA hybridization, nor was any correction made for topological constraints on RNA loops or bulges.

Oligonucleotides were synthesized (8) on an Applied Biosystems (Foster City, CA) oligonucleotide synthesizer, purified by gel electrophoresis, and sequenced chemically (9). The full length M gene was isolated as a Pst I fragment of the plasmid pM309 (3).

VSV mRNAs were isolated from infected baby hamster kidney (BHK) cells as described (5). VSV mRNAs were translated in rabbit reticulocyte lysate (Promega Biotec, Madison, WI), in the presence of [^{35}S]Met, 1064 Ci/mol (New England Nuclear, Boston, MA), with or without added oligonucleotides. The oligonucleotides and mRNA were annealed for 3 min at 52°C, then cooled for 10 min at room temperature prior to translation. Labeled translation products were analyzed by hot 5% trichloroacetic acid precipitation and by 10% polyacrylamide gel electrophoresis (10) followed by fluorography.

RESULTS

A potential secondary structure for the 5′ half of the VSV M mRNA was calculated (Fig. 1), and then a structure was calculated for the entire 831 nt message (Fig. 2). In Fig. 1, nucleotides 17–31 appear to form a single-stranded bulge, a likely target for a complementary oligonucleotide; this site is midway between the cap and the initiation codon. In Fig. 2, nucleotides 37–46 form a somewhat smaller single-stranded bulge, which is thus a less favorable target for a complementary oligonucleotide. However, this site includes the initiation codon.

Thermodynamic calculations (7) at 37°C for the association of 5′-TTGGGATAACACTTA-3′ with nucleotides 17–31 of the structure in Fig. 1 yield an association constant of 2.0×10^{15}/M. The calculated association constant drops to 6.2×10^{6}/M if one assumes the secondary structure calculated for the entire sequence (Fig. 2).

When total VSV mRNA was translated in rabbit reticulocyte lysate with added 5′-TTGGGATAACACTTA-3′, we observed inhibition of overall VSV mRNA translation (Table I) with 50% inhibition at ~14 μM. Polyacrylamide gel electrophoresis revealed that production of all VSV proteins was inhibited equally (not shown). Brome mosaic virus mRNA translation was also inhibited nonspecifically, with 50% inhibition at 45μM observed with a pentadecamer complementary to nucleotides 37–46 (unpublished results). Hybridization arrest of M mRNA translation occurred after annealing with the full length M gene, the Pst I fragment of pM309. The M band on a gel fluorograph was reduced by 83%, relative to the N band, compared with a control lane of labeled VSV proteins.

DISCUSSION

Coordinate suppression of all VSV mRNA translation by the complement of M mRNA nucleotides 17–31 correlates with a similar observation for N mRNA (2), and suggests