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RIBOSOME STRUCTURE: THREE-DIMENSIONAL DISTRIBUTION OF PROTEINS S14 AND S4

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The smaller ribosomal subunit of E. coli is composed of a single molecule of 16S RNA and about 20 different proteins. The general outlines of its chemistry and function are understood fairly well, as are the major aspects of its self-assembly. Traub and Nomura (1), for example, first demonstrated that functional subunits may be reconstituted from a mixture of small subunit proteins and 16S RNA under the appropriate ionic and temperature conditions without any requirements for additional factors. Small subunit self-assembly is also known to proceed stepwise (2), first requiring the interaction between RNA and specific ribosomal proteins before subsequent proteins can be incorporated. By omitting individual ribosomal proteins and studying the effects of this omission on the sedimentation coefficients and on the functional properties of reconstituted small subunits, several classes of proteins have been discerned. Some proteins are clearly necessary for the assembly of subunits of the correct structure. Among these "assembly" proteins is protein S4, which is perhaps the most prominent member of this group, in addition to S7, S8, S9, and S17. Omission of other "functional" proteins, such as S14, while producing only minor changes in the sedimentation value of reconstituted subunits, results in particles which are inactive in some or all functional tests.

In an effort to decipher the spatial relationships among proteins, a number of biochemical studies have used reagents to crosslink neighboring proteins or have examined the binding of ribosomal proteins to specific fragments of 16S RNA. These studies, although very useful, have supplied information which is primarily two dimensional or which relates nearest neighbors, but which is difficult to extrapolate to next nearest

neighbors without making arbitrary assumptions such as assuming that all ribosomal proteins are spherical. However, traditional methods of x-ray crystallographic analysis cannot be applied to study prokaryotic ribosomes since they have not yet been successfully crystallized. Nevertheless, complete three-dimensional structural studies are needed to bring together information from these diverse sources and to understand in the organization of ribosomal proteins and RNA.

Fortunately, other methods of structure analysis can be applied to ribosome structure. Electron microscopy is particularly well suited for this. Using electron micrographs of the in vivo ribosome crystals from Entamoeba invadens, complete three-dimensional density maps have recently been calculated to a Fourier resolution of 100 Å (3). Because it is possible to reconstitute E. coli small subunits and because more is known, in general, about their function we have chosen to study them in detail.

Seen in the electron microscope, the E. coli small subunit is characterized by a partition which divides its profile into an upper "one third" and a lower "two thirds" fraction. This feature, which is characteristic of small subunits, was first recognized by Nonomura et al. (4) in small subunits from rat liver ribosomes and subsequently by them in E. coli. Since it is found in both eukaryotes and prokaryotes it appears to be an evolutionally conserved feature of small subunits [for a review see (5)]. The two views of E. coli small subunits which correspond to approximately orthogonal orientations of the subunits and which are characteristically seen in electron micrographs of fields of purified subunits are shown schematically in Figs. 3 and 5 (electron micrographs of single subunits with antibodies attached can be seen in row D of Figs. 2 and 4). The symmetrical view is characterized by an approximate line of mirror symmetry which coincides with the long axis of the subunit, while the asymmetric view shows both a concave profile and a convex profile. In the asymmetric view, the upper "one third," or "head," region is tilted forward toward the concave side and a "hump" is present just below the partition on the convex side, or "back." A model constructed from these two characteristic views will be neither right handed nor left handed because of the approximate mirror symmetry seen in the symmetrical view. In practice, of course, small subunits have a definite handedness at the resolution of protein subunits, provided that the specific proteins can be identified. Antibodies fortunately offer a direct approach for mapping and identifying three dimensional protein locations in conjunction with electron microscopy. With the availability of IgG antibodies specific for individual proteins (6, 7) it has become possible to map protein distributions in the ribosome. In general the approach is to react bivalent IgG antibodies specific for a single ribosomal protein with small subunits in conditions of subunit excess, to purify subunit dimers which are connected by antibodies using sucrose gradients or column chromatography, and to subsequently observe them by electron microscopy.

A low-power field of dimers linked by IgG antibodies specific for protein S14 is shown in Fig. 1. The field contains many dimers and a few of them have been indicated by arrows. Close inspection shows that the subunits are linked by antibodies attached to the head region. The attachment can be seen more clearly in the field shown in the upper panel of Fig. 2. There the IgG antibodies can be seen to form a "Y" with the Fab regions attached to the head of the subunits. Usually a single antibody is attached to each dimer

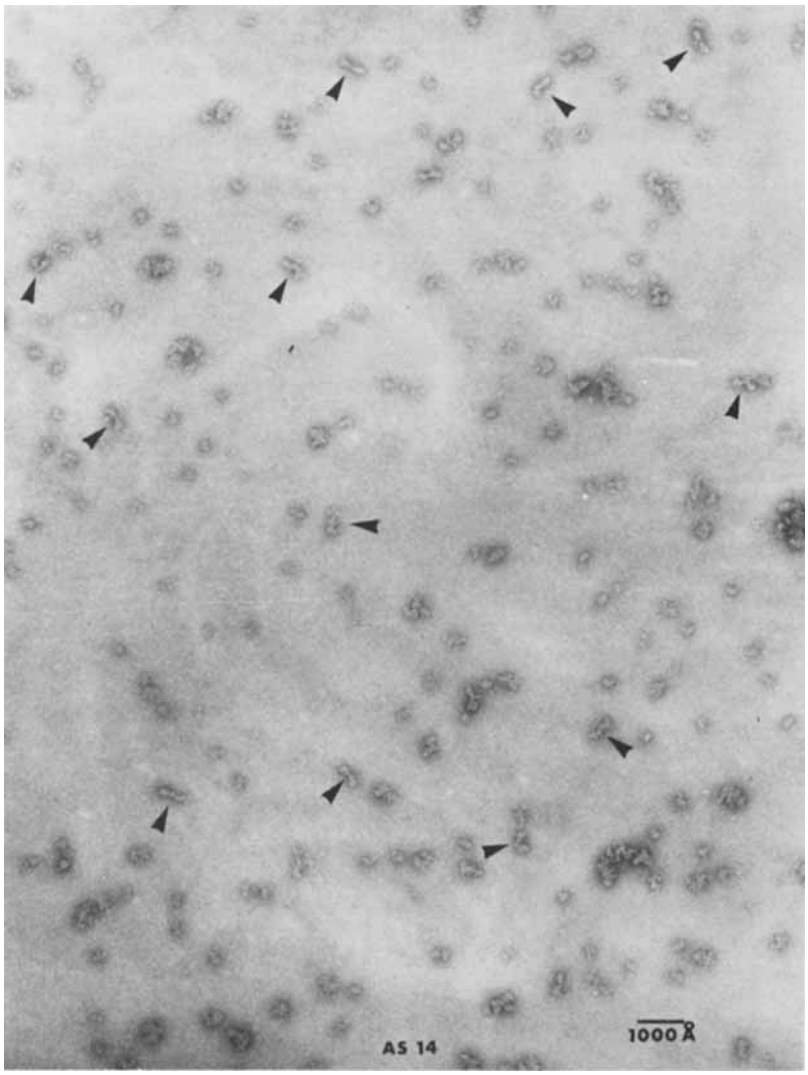

Fig. 1. A low-power electron micrograph of E. coli ribosomal small subunits which have been reacted with IgG antibodies directed against protein S14. Dimers of subunits are joined by antibodies attached to the head region.

pair; however, in about 30% of the S14 dimers two antibodies join a dimer. A double antibody can be seen in the dimer pair at the left of the field. This double binding indicates that a moderately large surface area of S14 is exposed. The simultaneous binding of three antibodies to protein S14 is never observed, however. Subunits with single antibodies attached can be seen in Fig. 2D in the symmetrical view (the three panels at the left) and

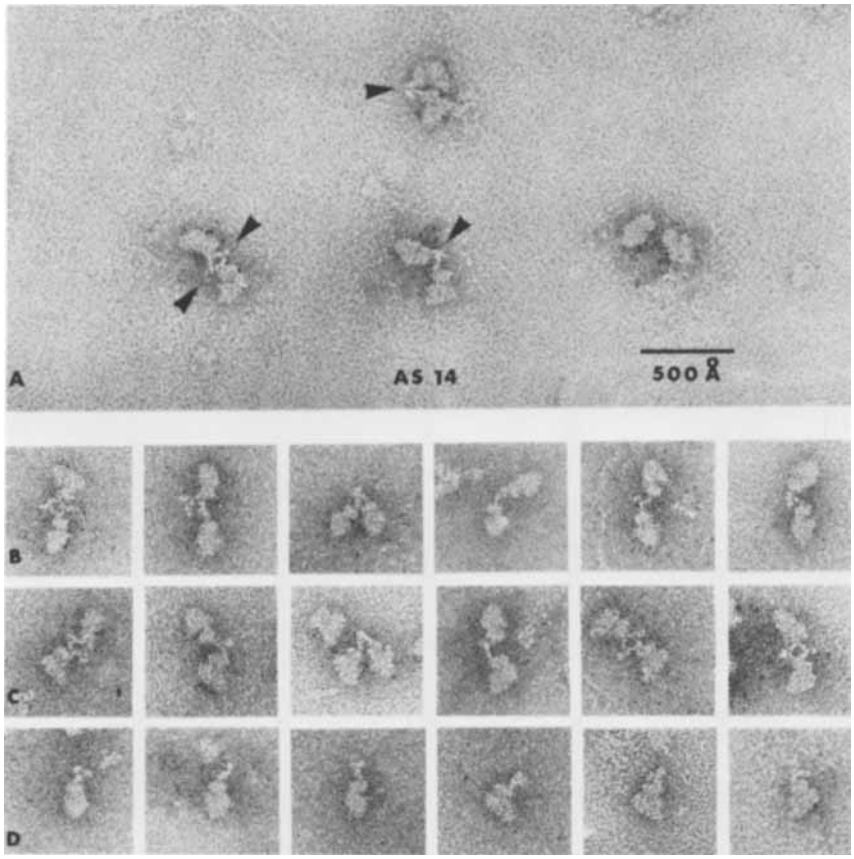

Fig. 2. Electron micrographs of small subunits reacted with AS14 antibodies. The antibodies attach only to a single region located on the head of the subunit. The Fab regions and the Fc regions of the antibodies are both visible. (A) A field showing dimers of subunits linked by one or two antibodies (indicated by arrows); (B) A gallery of dimers in the symmetrical projection; (C) A gallery of dimers in the asymmetrical projection; (D) A gallery of monomers in the symmetrical projection (first three squares at the left) and in the asymmetrical projection (last three squares).

in the asymmetrical view (three right panels), and views of dimers in the symmetrical and in the asymmetrical view can be seen in Fig. 2B and C, respectively, of the gallery. Examining these images, as well as hundreds of others, makes it clear that the exposed antigenic determinants of protein S14 are located on the concave side of the head in the asymmetrical view and near the center line of the head in the symmetrical view. The extent of the site is shown diagrammatically by stippling in Fig. 3.

The precise function of S14 is not known. Evidence has been presented both for its forming part of the tRNA A binding site. Nevertheless, it can be seen that its location near the "one third, two thirds" partition is consistent with tRNA binding and with the finding of Nonomura et al. (4), who concluded in their analysis of electron micrographs

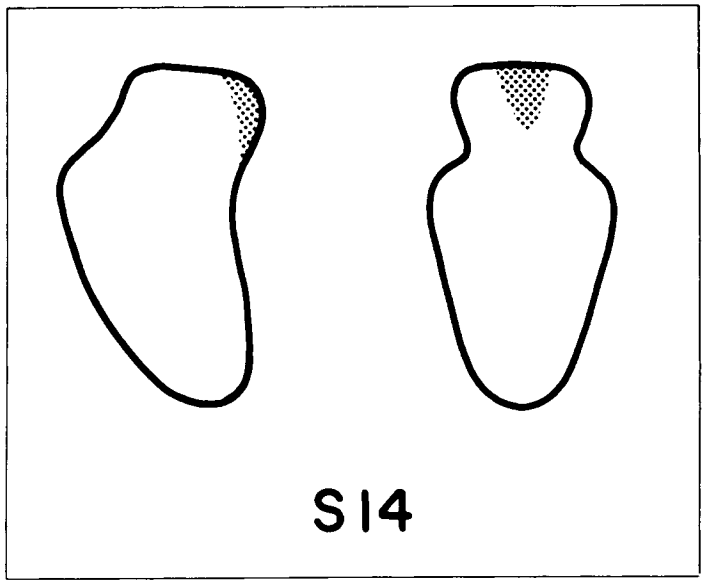

Fig. 3. A diagrammatic representation of the region of attachment of antibodies to protein S14. The site is represented by stippling in the symmetrical view (right) and in the asymmetrical view (left).

of rat liver polysomes that mRNA passes near the partition.

Protein S4 is an assembly protein and could be predicted to have structural properties quite different from those of protein S14. This is in fact borne out by antibody labeling studies which show that antibodies to S4 attach at three distinct regions of the small subunit, indicating that it is apparently a quite extended protein. The three sites can be seen in the field of subunits linked by AS4 antibodies in Fig. 4. A number of antibodies are indicated by arrows. Two of the sites are in the head region and the third is located below the partition in the vicinity of the "hump." These sites are shown diagrammatically in Fig. 5 in addition to a fourth site which is labeled vla (very low affinity) and which seems to be of sufficiently low affinity that it is incapable of holding subunits together, since antibodies are seen attached to only single subunits at the vla site. The specific attachment of antibodies to these three sites indicates that parts of protein S4 are probably exposed there; nevertheless, it should be emphasized that this is a preliminary result. The evidence concerning the specificity of AS4 comes from experiments done in different buffer conditions from the ones in these experiments and using relatively unconcentrated antisera. In those experiments, antibody was shown not to cross-react with any of the other purified ribosomal proteins (7). It is perhaps possible that the antigenicity of non-S4 proteins could be altered in the assembled subunit or that a low amount of non-AS4 antibody could be present. The possibility of such an occurrence is low, but nevertheless the results presented here should be viewed as preliminary until the necessary controls are completed (Lake, Kahan, and Nomura, work in progress).

A gallery of AS4 antibodies attached to site 3 is also shown in Fig. 5. Double

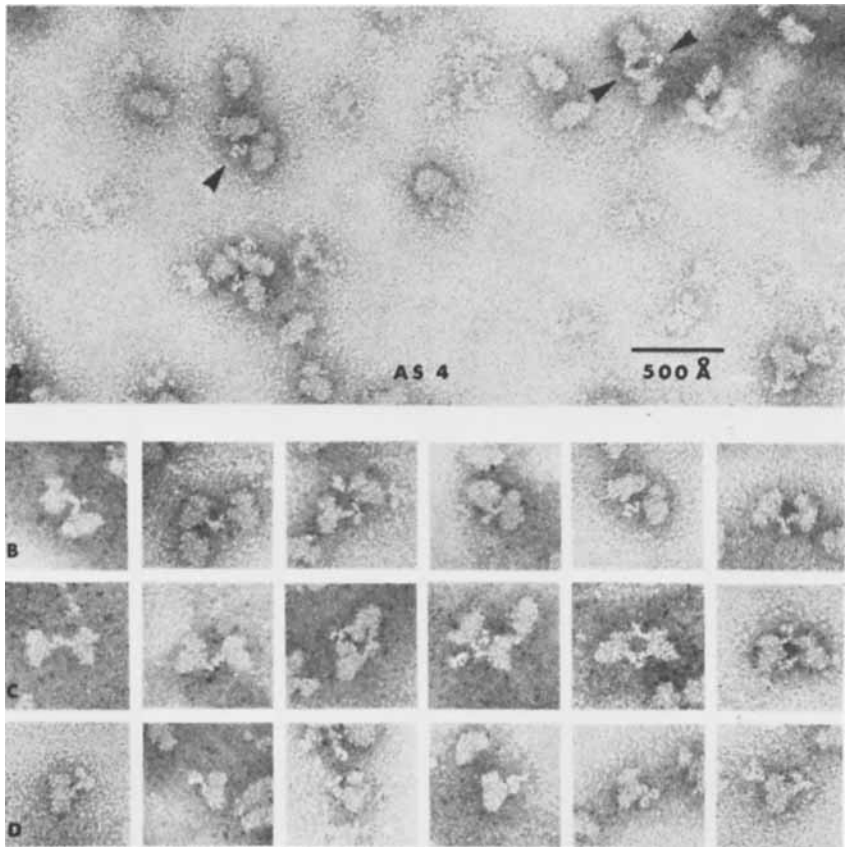

Fig. 4. Electron micrographs of small subunits reacted with AS4 antibodies. The antibodies attach to three regions of the subunit. (A) A field showing the attachment of antibodies at all three regions (arrows); (B) A gallery of dimers seen in the symmetrical projection and linked by antibodies attached to region 3; (C) A gallery of dimers seen in the asymmetrical projection and linked by antibodies attached to region 3; (D) A gallery of monomers seen in the symmetrical projection (first three squares at the left) and in the asymmetrical projection (last three squares) with antibodies attached to region 3.

dimers can be seen joining all combinations of the three sites. The views provided by the double dimers (as well as other views not shown) facilitated the site determinations shown in Fig. 5 and they were particularly useful in showing that sites 2 and 3 are located on the same side of the subunit in the symmetrical profile.

The extended nature of S4 is not inconsistent with its physicochemical properties. In particular it may explain how S4 attaches to such large regions of 16S RNA and how it facilitates the subsequent attachment of additional proteins in the process of small subunit self-assembly. It will be particularly interesting to ascertain if other assembly proteins are also extended, since extended proteins could conceivably interact in a more

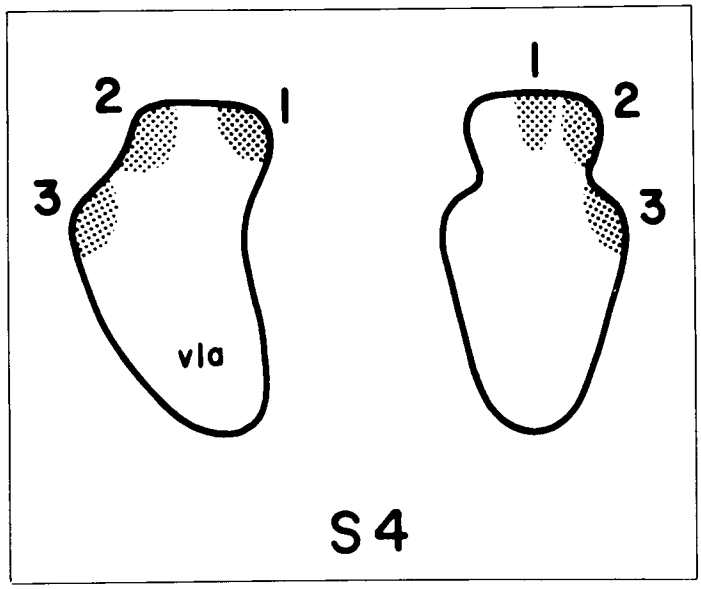

Fig. 5. A diagrammatic representation of the three regions of attachment of antibodies to protein S4. The regions are indicated by stippling in the symmetrical view (right) and in the asymmetrical view (left) of the subunit and are numbered.

advantageous manner with multiple RNA and protein binding sites. In any event the property of having multiple antigenic regions which is displayed by protein S4 is, so far, unique to it. The single exposed region of S14, however, seems to be typical of functional proteins, judging from the four other members of this class which we have so far studied. With the availability of antibodies to 20 ribosomal proteins, we have now started to map the three-dimensional distributions of all proteins within the small subunit and we anticipate learning more about their roles in small subunit assembly and function.

ACKNOWLEDGMENTS

This is a preliminary account of a lecture presented by one of us (J.A.L.) in March at the 1974 Squaw Valley Conference on Assembly Mechanisms. A complete report will be published elsewhere (Lake, Pendergast, Kahan and Nomura, manuscript in preparation).

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REFERENCES

1. Traub, P., and Nomura, M., Proc. Nat. Acad. Sci. U.S. 59:777 (1968).
2. Mizushima, S., and Nomura, M., Nature 226:1214 (1970).
3. Lake, J. A., and Slayter, H. S., J. Mol. Biol. 66:271 (1972).
4. Nonomura, Y., Blobel, G., and Sabatini, D., J. Mol. Biol. 60:303 (1971).
5. Lake, J. A., Nonomura, Y., and Sabatin, D., "Ribosomes," ed. Nomura, M., Tissières, A., and Lengyl, P. (Cold Spring Harbor Laboratory, N.Y., 1974, in press).
6. Stöffler, G., and Wittmann, H. G., Proc. Nat. Acad. Sci. U.S. 68:2283 (1971).
7. Higo, K., Held, W., Kahan, L., and Nomura, M., Proc. Nat. Acad. Sci. U.S. 70:944 (1973).