The Localization of Protein L19 on the Surface of 50 S Subunits of Escherichia coli Aided by the Use of Mutants Lacking Protein L19*

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Three independently isolated mutants of Escherichia coli which apparently lacked protein L19 on their ribosomes, as judged by two-dimensional gels, were analyzed by a range of immunological tests to determine if the protein was indeed lacking. In two of the three, all the tests indicated that protein L19 was absent from both ribosome and supernatant. In the third, a drastically altered form of protein L19 was present on the ribosome.

Electron micrographs of ribosomes obtained from the mutants were indistinguishable from those of wild type strains. The location of ribosomal protein L19 on the surface of the large subunit was determined. It was situated at the base of the 50 S particle facing the small subunit, on the side where the rod like appendage originates.

The ribosome of a procaryote such as Escherichia coli comprises over 50 different proteins, in addition to three RNA moieties. The conservation of these proteins in evolution, even when considering such diverse organisms as Bacillus subtilis and E. coli, suggests that they all have an important function in the organelle. However, the role that most of these proteins might play in the structure, function, and assembly of the ribosome has not yet been accurately determined. One way is to investigate and characterize mutants with a particular protein missing from the ribosome. A considerable number of such mutants have now been described (Dabbs, 1979; Dabbs et al., 1983). The absence of a protein was initially determined on the basis of two-dimensional polyacrylamide gels of ribosomal proteins. There was the possibility in mutants that a protein was altered so that its spot coincided with that of another protein or that a much smaller remnant of the protein was present but not detected. It was also possible that the protein did not firmly assemble into the ribosome and was thus present in the ribosome-free supernatant, as has been found for a mutant whose ribosomes lacked protein S1 (Dabbs et al., 1983).

Since it was necessary to be certain a protein really was missing from the mutant cell, a battery of immunological techniques has been employed; these have been most comprehensive in the case of mutants lacking ribosomal protein L1 and L11 (Stöffler et al., 1980; Dabbs et al., 1981). Furthermore, ribosomes from mutants lacking a single ribosomal protein are ideal controls for the demonstration of antibody specificity in immuno-electron microscopic experiments (Dabbs et al., 1981). Most of the mutants have been isolated as spontaneous antibiotic-independent revertants from strains dependent for growth in the presence of the antibiotic erythromycin in the medium. We here describe for the first time two mutants with large ribosomal subunit protein L19 lacking from the ribosome. They were detected among further revertants of erythromycin-dependent strain AM.1

MATERIALS AND METHODS AND RESULTS

Immunoelectron Microscopic Localization of Protein L19—

The location of the epitopes of protein L19 on the 50 S subunit could now be determined by immunoelectron microscopy. Fig. 7a shows a general field of 50 S particles reacted with anti-L19, as observed in the electron microscope. About two-thirds of the subunits were present in dimeric immunocomplexes connected by either one or by a pair of IgG molecules; 12% of the 50 S particles had no IgG molecule bound. Typical ribosome-antibody complexes are indicated by arrows (Fig. 7a).

Altogether, 211 50 S-IgG and 616 50 S-IgG:50 S complexes were evaluated. The IgG molecule was best seen in monomeric immunocomplexes and thus allowed also an accurate positioning of the binding site on the ribosome (Fig. 7b). From the dimeric complexes an accurate positioning was only possible in about one-half of them. This might result from the frequent occurrence of dimeric immunocomplexes which are simultaneously connected by antibody pairs. This result was, however, important since it meant that at least two L19 epitopes existed on the large subunit surface.

In the immunocomplexes, the 50 S subunits were seen in both the crown and the kidney projection (Fig. 7, b-f). Kidneys were observed relatively more frequently in anti-L19 immunocomplexes than in standard 50 S preparations. In the crown projection, the antibody bound to the base of the subunit. Close inspection of the immunocomplexes revealed that the antibody attachment site was between the central point of the base and the region from where the stalk originates (see schematic drawing in Fig. 7d). In the crown projections, the Fab arm was not visible in its whole length. This was especially obvious with monomeric immunocomplexes (Fig. 7b). Inspection of these allowed us to conclude that the antibody-

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1 E. R. Dabbs, unpublished results.
2 Portions of this paper (including "Materials and Methods," part of "Results," and Figs. 1-6) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-2185, cite the authors, and include a check or money order for $4.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
FIG. 7. Electron micrographs and schematic drawings of 50 S subunits reacted with L19-specific antibody. a, general field. Subunits with antibody attached are arrowed. Selected 50 S-IgG (b) and 50 S-IgG-50 S (c and d) complexes, in which 50 S subunits are seen in the crown projection. 50 S-antibody complexes with subunits seen in the kidney projection (e) and dimeric immunocomplexes (f) in which a kidney and a crown projection is joined by an antibody molecule. Each interpretative scheme illustrates the electron micrograph immediately to its left. The scale is 50 nm.
Protein L19 at the Ribosomal Interface

FIG. 8. The location of protein L19 (shaded area) and its relationship to locations of large subunit proteins mapped in our previous studies (Dabbs et al., 1981; Kastner et al., 1981; Noah, 1982; Stoffler-Meilicke et al., 1983a, 1983b).

binding site did not lay on the contour line when the subunit was in the crown projection.

When the 50 S subunit was seen in the kidney projection, antibody binding was observed to the notched side of the subunit (Fig. 7e). From complexes in which a crown form was connected with a kidney projection (Fig. 7f) we concluded that it was the same epitope we were pinpointing on both projectional forms. Only 2% of the immunocomplexes showed aberrant antibody binding. The position of protein L19 in the three-dimensional model of the 50 S subunit is shown in Fig. 8.

The localization of protein L19 was additionally undertaken using 50 S particles of mutant AM149. 50 S particles of mutant AM149 were indistinguishable in electron micrographs from particles of wild type strains. After reconstitution of ribosomes from AM149 with protein L19, the protein could be localized at the same site as in wild type ribosomes.

Accessibility of the Epitope of Protein L19 in the 70 S Ribosome—Electron microscopic investigations led to the conclusion that protein L19 was on the surface of the large subunit facing the small subunit (Fig. 8). The three-dimensional model of the ribosome (Kastner et al., 1982) indicated that the L19 epitope is not exposed in the 70 S particle (Fig. 9). Therefore, L19-specific antibodies should not bind to 70 S ribosomes. Various anti-L19 IgG preparations were tested for their reactivity with 70 S particles, and in no case 70 S-IgG-70 S complexes were observed (data not shown). Therefore, the L19 epitope is covered when the small subunit associates with the large subunit.

DISCUSSION

Protein L19 is located on a unique region of the 50S subunit which is, in the 70S ribosome, at the subunit interface (Fig. 9). Results indicating that protein L19 was located at the interface region have previously been reported by Zeichardt (1976). Furthermore, it has been found (Noll et al., 1976) that binding of L19-specific antibodies to 50 S particles prevents those particles associating with 30 S subunits. Of all the protein-specific antisera which do not react with the 70 S monosome (see Fig. 9 and Stoffler and Stoffler-Meilicke, 1983), this same effect has only been found for anti-S11 (Noll et al., 1976). Since 70 S particles derived from L19-lacking mutant AM149 were stable, it was, however, likely that protein L19 played no essential role in subunit association. However, it could not be excluded that other compensatory mutations were present in the mutant which countered an adverse effect of absence of protein L19 on subunit association. A more detailed study of the various parameters involved in dissociation and reassociation of ribosomal particles should be interesting.

No other ribosomal proteins have so far been mapped in vicinity of the protein L19 epitopes. Protein L19 has also not been cross-linked to any other large subunit ribosomal protein (Traut et al., 1979). Since protein L19 is located at the ribosomal interface, cross-linking data between components of the two ribosomal subunits have to be considered. Among the many protein-protein cross-links between 30 and 50 S proteins, there are two cross-linked pairs with protein L19, viz. S4-L19 and S13-L19 (Cover et al., 1981). The large region of the 30-S subunit on which epitopes of protein S4 have been mapped could definitely allow a cross-link between these two proteins (see Fig. 9). The cross-link between protein S13 and L19 is, however, incompatible with any of the four proposed 70-S models (Lake, 1976; Boublik et al., 1977; Kastner et al., 1982; Vasiliev et al., 1983).

Two research groups have identified proteins cross-linked
to the heterologous RNA (Baumert et al., 1978; Sköld, 1981; Chiam and Wagner, 1983); some of them has found protein L19 cross-linked to 16 S RNA. It seems in general that most of the "interface cross-links" are found between components which are around the edges of the interface and not at that part of the subunit interface where there is the most intimate contact. Cross-linking molecules may not have access to this area of most intimate contact, which probably is also the region where protein L19 is located.

We have found no evidence for any assembly defect of 50 S subunits lacking protein L19. This is in agreement with the 50 S assembly map, in which protein L19 has only weak interactions with protein L4 and L17 (Nierhaus, 1982). We did not find any evidence that these proteins are present in reduced amounts on 50 S ribosomes nor could elevated levels be detected in the supernatant fraction. However, the mutants grow slowly and ribosomes from the mutants employed in an in vitro system programmed with poly(U) synthesized artificial made, this kind of experiment is generally applied with a high frequency, and common penta- and hexapeptides have also been found. Protein L19 has pentapeptides in common with proteins L15 and L16 and tetrapeptides with proteins L1, L3, L6, L10, L12, and L24 (Wittman-Liebold, 1980).

The absorption experiments with TP70 from the mutant lacking protein L19 and the observation that anti-L19 does not react with mutant ribosomes unless they are reconstituted with a 21,000 polypeptide (rpsP).

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Protein L19 at the Ribosomal Interface

Supplementary Material to
The Localization of Protein L19 on the Surface of 50S Subunits of Saccharomyces cerevisiae by the Use of Mutants Lacking Protein L19
Georg Stiefler, Michael Noth, Martin Stöffler-Nolte, Eric R. Samba

MATERIALS AND METHODS
Isolation of mutants was as previously described (Samba et al. 1979).

Growth conditions: Cells were grown to late log to early stationary phase in rich medium. Medium was described by Samba (1978).

Preparation of ribosomes, chromosome and chromosome preparations (1981). Ribosomes were isolated as described (Samba et al. 1977, 1979) and 50S subunits were prepared by sonication (Stöffler-Nolte et al. 1978). 50S subunits from wildtype were left active in 25-25% glycerol complex formation (Doyley et al. 1978). Ribosomal proteins were extracted from ribosomes with acetic acid. Two-dimensional gel electrophoresis was performed according to Hazeldine and Wittmann (1978); cellular auto gel electrophoresis was performed according to Samba et al. (1977). Origin was from Chemicon, Melbourne, Australia. Proteins from the 50S were prepared as described (Stöffler-Nolte et al. 1973) and also by chromatography on protein A sepharose.

Results

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Figure 1. Two-dimensional polyacrylamide gel electrophoresis. The 70S subunit of strain AK (lanuginoosa) a and b (2) were isolated from the wildtype strain and were compared. The band corresponding to protein L19 is resolved on the left band of the two-dimensional gel. In Fig. 1a, the protein L19 is indistinguishable from protein L19. Similar results were obtained in two-dimensional gel electrophoresis with protein L19, which is also indistinguishable from protein L19. In this experiment, the protein L19 is shown as a spot. The protein L19 is shown as a spot, but the protein L19 is shown as a spot, and the protein L19 is shown as a spot. The protein L19 is shown as a spot, and the protein L19 is shown as a spot.

Table 1. Two-dimensional gel electrophoresis of 70S proteins from mutants AK10, AK10, AK10, and AK10. AK10 is from the wildtype strain and was compared. The band corresponding to protein L19 is resolved on the left band of the two-dimensional gel. In Fig. 1a, the protein L19 is indistinguishable from protein L19. Similar results were obtained in two-dimensional gel electrophoresis with protein L19, which is also indistinguishable from protein L19. In this experiment, the protein L19 is shown as a spot. The protein L19 is shown as a spot, but the protein L19 is shown as a spot, and the protein L19 is shown as a spot. The protein L19 is shown as a spot, and the protein L19 is shown as a spot.

Figure 2. Two-dimensional gel electrophoresis of 70S proteins from mutants AK10, AK10, AK10, and AK10. AK10 is from the wildtype strain and was compared. The band corresponding to protein L19 is resolved on the left band of the two-dimensional gel. In Fig. 1a, the protein L19 is indistinguishable from protein L19. Similar results were obtained in two-dimensional gel electrophoresis with protein L19, which is also indistinguishable from protein L19. In this experiment, the protein L19 is shown as a spot. The protein L19 is shown as a spot, but the protein L19 is shown as a spot, and the protein L19 is shown as a spot. The protein L19 is shown as a spot, and the protein L19 is shown as a spot.

Figure 3. Modified immunoelectronmicroscopy on cells acetate gel electrophoresis. (a) 50S subunit of strain AK (lanuginoosa) a and b (2) were isolated from the wildtype strain and were compared. The band corresponding to protein L19 is resolved on the left band of the two-dimensional gel. In Fig. 1a, the protein L19 is indistinguishable from protein L19. Similar results were obtained in two-dimensional gel electrophoresis with protein L19, which is also indistinguishable from protein L19. In this experiment, the protein L19 is shown as a spot. The protein L19 is shown as a spot, but the protein L19 is shown as a spot, and the protein L19 is shown as a spot. The protein L19 is shown as a spot, and the protein L19 is shown as a spot.

Figure 4. Two-dimensional gel electrophoresis of 70S proteins from mutants AK10, AK10, AK10, and AK10. AK10 is from the wildtype strain and was compared. The band corresponding to protein L19 is resolved on the left band of the two-dimensional gel. In Fig. 1a, the protein L19 is indistinguishable from protein L19. Similar results were obtained in two-dimensional gel electrophoresis with protein L19, which is also indistinguishable from protein L19. In this experiment, the protein L19 is shown as a spot. The protein L19 is shown as a spot, but the protein L19 is shown as a spot, and the protein L19 is shown as a spot. The protein L19 is shown as a spot, and the protein L19 is shown as a spot.

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Figure 5. Two-dimensional gel electrophoresis of 70S proteins from mutants AK10, AK10, AK10, and AK10. AK10 is from the wildtype strain and was compared. The band corresponding to protein L19 is resolved on the left band of the two-dimensional gel. In Fig. 1a, the protein L19 is indistinguishable from protein L19. Similar results were obtained in two-dimensional gel electrophoresis with protein L19, which is also indistinguishable from protein L19. In this experiment, the protein L19 is shown as a spot. The protein L19 is shown as a spot, but the protein L19 is shown as a spot, and the protein L19 is shown as a spot. The protein L19 is shown as a spot, and the protein L19 is shown as a spot.
It was essential to demonstrate that the reactive epitope in 50S subunits was provided by protein L19. We thus investigated the effect of preincubating antibody with protein L19 upon its ability to bind to 50S subunits. For this purpose an antibody concentration was chosen which would disperse half the number of subunits that could be dispersed. The result of this experiment is shown in Fig. 6a. The addition of 1 μg of protein L19 to anti-L19 IgG completely inhibited the formation of 50S-50S complexes in sucrose gradients. The experiment was repeated with several other sera and with anti-L19 IgG prepared against different mutant proteins and it was found that the results in Fig. 6a were obtained with sera from rabbits which were immunized with L19. As shown in Fig. 6a, the addition of protein L19 to anti-L19 IgG also inhibited the formation of 70S-70S complexes in the presence of 100 μg of T70. Although the addition of 20 mg of protein L19 had no effect on the precipitation of 70S-70S complexes, it was found that new 100S-100S complexes could be formed in the presence of 100 μg of T70 and 10 mg of L19. Thus, 10 mg of protein L19 might be sufficient to form new 100S-100S complexes even in the presence of 100 μg of T70. Similar results were obtained with sera from rabbits which were immunized with L19. The results of these experiments indicate that the reactive epitope in 50S subunits is provided by protein L19.
The localization of protein L19 on the surface of 50 S subunits of Escherichia coli aided by the use of mutants lacking protein L19.

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