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Identity of the Ribosomal Proteins Involved in the Interaction with Elongation Factor G*

(*E. coli*/ribosomal protein antibodies/translocation/GTP/fusidic acid)

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ABSTRACT **Rabbit antibodies produced against 50 of the 55 individually purified ribosomal proteins of *Escherichia coli* were tested for their ability to interfere with the formation of the ribosome·EF-G·GDP complex. Only antibodies produced against proteins L7 and L12 inhibited complex formation, and they did so completely. These two proteins were previously shown to be immunologically indistinguishable and necessary for the interaction between ribosomes and EF-G. The present data are consistent with the view that the interaction between ribosomes and EF-G that results in GTP hydrolysis occurs on, and is limited to, proteins L7 and L12 on the surface of the 50S ribosomal subunit.**

Establishment of the structure–function relationships of ribosomes and the mechanisms of GTP involvement in ribosome function are two of the major goals in defining the process of translation of the genetic code (for recent reviews, see refs. 3 and 4).

Elucidation of ribosomal structure–function relationships has been thwarted by the complexity and cooperativity of ribosomal structure (the ribosome is composed of more than 50 distinct macromolecules), as well as the complexities of its functions. The availability of antibodies produced against individually purified ribosomal proteins now provides a potentially powerful tool with which to investigate the functional topology of ribosomes. Already, experiments involving the use of antibodies have successfully defined the specific ribosomal proteins involved in streptomycin and spectinomycin binding (5)**,††, yielded information on the identity of the ribosomal proteins that bind ribosomal RNA (6, 7), and implicated proteins L7 and L12 in the interaction between ribosomes and EF-G (8).

Similarly, elucidation of the roles of GTP in ribosome function has been hampered by the complexities of the reactions and their participants. An experimental approach to this problem is provided by the "single step" hydrolysis of GTP by EF-G and the 50S ribosomal subunit in the presence of fusidic acid (9, 10). This system is one of the simplest manifestations of GTP participation in protein synthesis, and recent results suggest that it may involve a ribosomal site in common with the other GTP-requiring elongation step of protein synthesis, aminoacyl-tRNA binding (2, 11–14).

In the experiments to be reported here, we have simply asked, which antibodies to ribosomal protein (when reacted with the ribosome) will prevent the formation of the ribosome·EF-G·GDP complex? The results are surprisingly simple; only antibodies prepared against proteins L7 and L12 inhibit this reaction, suggesting that they are the only proteins physically involved in this interaction. To our knowledge these results provide the first direct definition of a ribosomal site of interaction with a factor, and support earlier work that suggested this relationship (8, 15, 16).

MATERIALS AND METHODS

Preparation of Materials. The preparation of antisera against individual ribosomal proteins, and the purification and characterization of IgGs and their corresponding Fab-fragments from these sera were as described (17, 18)‡‡. The methods of preparation of *E. coli* EF-G and ribosomes were also described (19). [^{3}H]GTP was obtained from New England Nuclear Corp. and had a specific activity of 5.7 Ci/mmol. Miss Barbara Stearns of E.R. Squibb kindly provided the fusidic acid.

Reaction of Antibodies with the Ribosome. Ribosomes (30 μg) were reacted for 3 min at 0° with the appropriate IgG or Fab preparation in the amounts indicated. The reaction (final volume, 20 μl) contained, in addition to ribosomes and antiserum: 10 mM Tris·HCl (pH 7.4), 10 mM magnesium acetate, 60 mM NH_4Cl, and 1 mM dithiothreitol.

Formation of the Ribosome·EF-G·[^{3}H]GDP Complex. 15 μl of the ribosome–antibody reaction mixture described above was added to 35 μl of a solution that contained 10 mM Tris·HCl (pH 7.4), 10 mM magnesium acetate, 28 mM

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NH_4Cl, 1 mM dithiothreitol, 11 units of EF-G (19), 3 mM fusidic acid, and 10 pmol of [^{3}H]GTP. Under these conditions the antigen–antibody complex remained in solution. The mixture was incubated for 5 min at 0°. The ribosome·EF-G·[^{3}H]GDP complex was measured by a Millipore filtration method (20). Under these conditions, the binding of [^{3}H]GDP to Millipore filters was limited by ribosomes; in the absence of IgG, about 2 pmol was bound.

RESULTS

As summarized in Table 1, only two types of results characterized the effect of antibodies prepared against individual 50S ribosomal proteins on the ability of 70S ribosomes to participate in the subsequent formation of the ribosome·EF-G·[^{3}H]GDP complex. All but two IgG preparations either were without effect or stimulated the interaction somewhat. By contrast, the IgGs prepared in response to proteins L7 and L12 suppressed this interaction by more than 90%. In similar experiments with IgGs prepared in response to proteins of the 30S subunit (Table 2), only the neutral or stimulatory effect was observed. These results should be compared to earlier findings††,‡‡ (also ref. 5 and G. S., unpublished data) that the majority of IgG preparations inhibited the ability of ribosomes to participate in overall protein synthesis, i.e., polyphenylalanine formation.

TABLE 1. *The effect of IgG binding (specific for 50S proteins) on the formation of the ribosome·EF-G·[^{3}H]GDP complex*

Ribosomal protein specificity of IgG preparation	% [^{3}H]GDP binding
L1	140
L2	126
L3	122
L4	120
L5	164
L6	126
L7	*4*
L8	116
L9	138
L10	130
L11	122
L12	*6*
L13	113
L14	96
L15	138
L16	138
L17	118
L18	115
L19	100
L20	140
L21	114
L22	144
L23	130
L24	116
L25	142
L26	N.D.
L27	140
L28	120
L29	135
L30	120
L31	N.D.
L32	N.D.
L33	108
L34	N.D.

The reaction conditions were as described in the text. 0.32 A_{280} units of IgG was present in the initial reaction with ribosomes, yielding an IgG/ribosome ratio of 135. The amount of [^{3}H]GDP binding observed in the absence of IgG is defined as 100%.

The concentration dependence of the inhibition of complex formation by anti-L7 and -L12 IgGs is compared in Fig. 1 with the response to a typical noninhibitory IgG. Since proteins L7 and L12 are immunologically identical (18), the difference in the ability of these two IgG preparations to inhibit complex formation presumably reflects only a difference in antibody titer. The significance of the stimulation of complex formation observed with low amounts of the IgG preparations is not clear. However, since it is known that only a portion of the ribosomes is capable of binding EF-G and GDP (20), it is likely that IgG binding (either specific or nonspecific) alters ribosome conformation so as to increase this fraction.

In order to eliminate the possibility that the observed inhibitions by antibodies to proteins L7 and L12 resulted from nonspecific precipitation of ribosomes, monovalent Fab-fragments were prepared from both antibodies. These Fab-fragments yielded inhibition curves essentially identical to those shown in Fig. 1 (data not shown).

The ribosome·EF-G·GDP complex was shown (10) to result from the direct interaction of ribosomes, EF-G, and GDP. Inhibition curves similar to those shown in Fig. 1 for IgG L7 were also obtained when this complex was formed starting with GDP rather than GTP (data not shown). These results are consistent with the view that all of the interactions between EF-G and ribosomes that are necessary for GTP hydrolysis occur on, and are limited to, proteins L7 and L12 on the surface of 50S ribosomal subunits.

DISCUSSION

Proteins L7 and L12 are acidic 50S ribosomal proteins that can be removed from ribosomes either with CsCl or with ethanol and NH_4Cl. Neither the resulting protein-deficient particles nor the separated proteins themselves are able, by various criteria, to interact with EF-G and GTP. However, this interaction can be readily restored by the readdition of proteins L7 and L12 to the protein-deficient particles (8, 15, 16). These results suggest, but by no means prove, that EF-G interacts with these proteins on the ribosome, for it is known that the conformation of ribosomes is important to this interaction (21), and these proteins may simply promote the required conformation.

The ribosome·EF-G·GDP complex forms on the 50S subunit (9); the present experiments demonstrate that of antibodies prepared against 30 individual purified 50S ribosomal proteins (thus far, preparation of the remaining four antibodies has not succeeded for technical reasons), only antibodies produced in response to proteins L7 and L12 prevent formation of this complex. In addition, we have tested antibodies prepared against 20 of the 21 30S proteins, and found all of them to be without inhibitory activity. These results, taken together with the fact, as mentioned above, that the presence of proteins L7 and L12 on ribosomes is required for this interaction, lead us to conclude that these proteins, and probably only these proteins, comprise the physical region of contact between elongation factor G and ribosomes. However, we have no information with respect to the possible involvement of the four 50S proteins for which we are presently unable to obtain antisera.

TABLE 2. *The effect of IgG binding (specific for 30S proteins) on the formation of the ribosome·EF-G·[^{3}H]GDP complex*

Ribosomal protein specificity of IgG preparation	% [^{3}H]GDP binding
S1	116
S2	123
S3	122
S4	115
S5	104
S6	150
S7	97
S8	96
S9	119
S10	125
S11	128
S12	116
S13	149
S14	124
S15	108
S16	119
S17	N.D.
S18	132
S19	101
S20	99
S21	118

The reaction conditions were as described in Table 1.

Proteins L7 and L12 are, in several respects, unique among ribosomal proteins. (*i*) They are acidic, while the majority of ribosomal proteins are basic (22–24). (*ii*) Their amino-acid compositions are distinctive: each contains ϵ-*N*-monomethyllysine and about 25 mol % alanine (22–24). (*iii*) Compared to other ribosomal proteins, they have a high α-helix content (about 50–60%) (24, 25). (*iv*) Proteins L7 and L12 are immunologically identical (18) and, except for an *N*-acetyl-blocked terminal serine in L7, they appear to be identical in sequence (26, 27)¶¶. Such extensive structural homologies between other ribosomal proteins have not been found. (*v*) Together, proteins L7 and L12 appear to be present in at least two, but probably three, copies per 50S subunit (18, 26, 28, 29)§§, while the majority of ribosomal proteins appear to be present in one or fewer copies per particle (3, 28)§§.

GTP is hydrolyzed in at least three distinct steps of protein synthesis. During initiation, it is required for the binding of fMet-tRNA to 30S subunits, and its hydrolysis is mediated by initiation factor IF-2 and requires the addition of 50S subunits. In elongation, GTP hydrolysis occurs during EF-Tu-mediated aminoacyl-tRNA binding, and during EF-G-mediated translocation. In the first case hydrolysis requires the 50S subunit, and in the second it can occur entirely upon this subunit. Three independent lines of evidence suggest that at least these latter two hydrolytic events involve a common region of 50S subunits. (*i*) Thiostrepton, an antibiotic that binds to 50S subunits, prevents both the EF-G- and EF-Tu-associated hydrolyses (30). (*ii*) Protein L7 and/or L12 are required for both reactions (15, 16). (*iii*) The two

§§ Thammana, P., Weber, H. J., Deusser, E., Maschler, R., Kurland, C. G., Stöffler, G. & Wittmann, H. G., *Nature*, in press.
¶¶ Terhorst, C., Möller, W., Laürsen, R. & Wittmann-Liebold, B., *Eur. J. Biochem.*, submitted for publication.

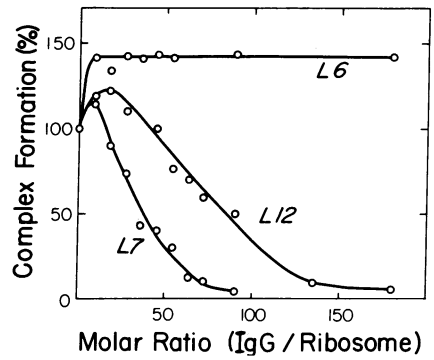

FIG. 1. Concentration dependence of the effect of IgG on the formation of the ribosome·EF-G·[^{3}H]GDP complex. Conditions are described in the text.

hydrolytic reactions cannot occur simultaneously on a single ribosome (2, 11–13). Additionally, it has recently been suggested that GTP hydrolysis during initiation might also involve this ribosomal site (31).

In the light of these relationships and the observations reported here, an attractive hypothesis is that proteins L7 and L12, perhaps present as a triad, constitute a "universal" GTPase region on the ribosome. In combination with either EF-G or EF-Tu, proteins L7 and L12 would participate in GTP hydrolysis and, in some way, transduce the energy derived from these hydrolyses to the ribosome so as to promote the conformational changes required for translocation and for aminoacyl-tRNA binding, respectively.

Whatever the case, it is clear that ribosomal protein-specific antibodies will be of considerable importance in defining the functional and structural topology of the ribosome.

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