INVOLVEMENT OF 50S RIBOSOMAL PROTEINS L6 AND L10 IN THE RIBOSOME DEPENDENT GTPase ACTIVITY OF ELONGATION FACTOR G

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SUMMARY: CsCl-prepared 50S cores in the presence of groups of individual split proteins were tested for their capacity to support EF-G dependent GTP hydrolysis. The activity of cores prepared at 40 mM Mg^{2+} could be restored by adding L7/L12 and L10 together, each in an amount of two copies per 50S particle, which abolishes the difference in activity between L7 and L12. In the range of 20 - 2 mM Mg^{2+}, 50S cores lose the protein L6, which is also required for GTP hydrolysis. L10 cannot replace L6, or vice versa.

INTRODUCTION

Recently, much emphasis has been placed on the possible function of two acidic 50S ribosomal proteins, L7 and L12, in elongation factor G (EF-G) dependent hydrolysis of GTP (1 - 5). The GTPase activity of 50S CsCl cores supplemented with L7 or L12 was reconstituted more efficiently with L12 than with L7 (1, 5), the latter being the N-acetylated form of L12 (6). However, at least a tenfold excess of L7/L12 was needed for maximal reconstitution of the GTPase activity (5), whereas much smaller amounts of split protein were sufficient to give particles active in polypeptide synthesis (7). This aspect of artificial GTPase reconstitution with L7/L12 suggests that besides these proteins other 50S split components may play a role in the GTPase activity of the intact particle.

With respect to the possible binding site of GDP on the ribosome, Bodley and Lin (8) reported the need of a 50S basic protein in the binding reaction induced by EF-G and fusidic acid, and other workers (3, 4) found that thiostrepton still binds to 50S cores deprived of L7/L12. On this basis there seems to be a complex interplay between a cluster of ribosomal proteins which together induce the splitting of GTP.

To find other 50S proteins possibly participating in GTP hydrolysis, we used an approach originally developed by Meselson and coworkers (9): 50S ribosomes were stripped gradually with 5 M CsCl solutions of decreasing Mg^{2+} concentrations. The resulting core preparations were tested for GTPase activity in the presence of 30S ribosomes, EF-G, and different combinations of L7, L12, and single proteins from these split fractions. In this way two additional proteins, L6 and L10, were identified as contributing to the GTPase reaction.

MATERIALS AND METHODS

Ribosomal particles

Ribosomes from E.coli MRE 600 and ribosomal subunits were isolated as described elsewhere (10). 50S CsCl core particles were prepared at 40, 20, 10, 8, 5 and 2 mM Mg^{2+}, according to Meselson et al. (9). The densities of these core preparations were 1.657, 1.666, 1.677, 1.679, 1.686, and 1.696 g/cm^3, respectively. One A_{260} unit was taken to represent 25 pmoles of 70S, 39 pmoles of 50S, or 67 pmoles of 30S particles (5).

Proteins

Selective removal of the two proteins L7 and L12 from the 50S CsCl split fractions was achieved by chromatography on DEAE-cellulose at pH 5.7 as described earlier (11).

L7 and L12 were isolated separately and purified as described previously (11). The proteins L6 and L10 were isolated by LiCl-urea extraction of 50S ribosomes according to Hindennach et al. (12), followed by chromatographic purification essentially according to Mora et al. (13). A single band or spot was observed after polyacrylamide gel electrophoresis at pH 3.5 (14), gel electrophoresis in the presence of SDS (15), and two-dimensional polyacrylamide gel electrophoresis (16). In addition isoelectric focussing (17, 18) of a sample of L10 gave one band in the pH 3-10 range (Ampholine, L.K.B. Instruments). The amino acid compositions were in agreement with published values (13, 19).

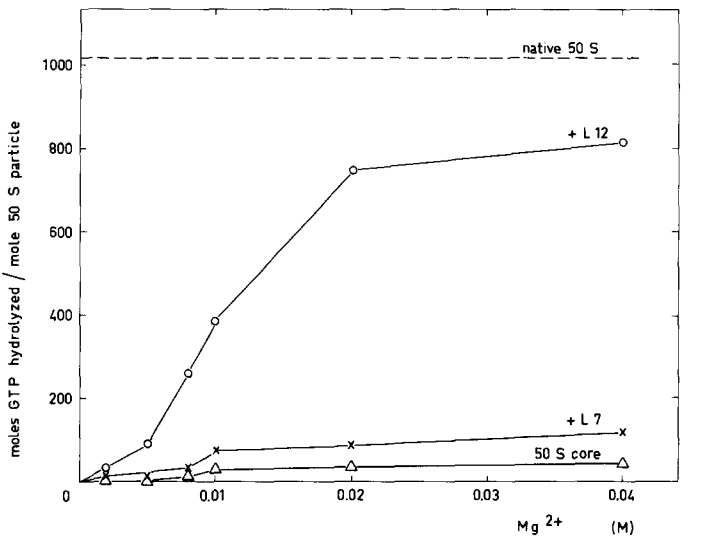

Fig. 1. Effect of the Mg^{2+} concentration in isopycnic CsCl gradient centrifugation of 50S ribosomes on the EF-G-dependent GTPase reaction of the resulting 50S cores (Δ - Δ), 50S cores in the presence of 0.45 nmoles (5.5 µg) L7 (× - ×), and 50S cores in the presence of the same amount of L12 (O - O). The dashed line gives the GTPase activity of native 50S ribosomes.

EF-G was prepared according to Parmeggiani et al. (20). The preparative polyacrylamide gel electrophoresis step was replaced by chromatography on DEAE-sephadex (21).

Protein was determined according to Lowry et al. (22).

Conditions of reconstitution and GTPase assay

Each 30 µl reconstitution mixture contained 0.03 M Tris-HCl (pH 7.6), 0.03 M $MgAc_2$, 0.26 M NH_4Cl, 0.003 M DTT, 0.75 A_{260} units of 50S particles (30 pmoles), and various amounts of split proteins. After a 10-min incubation at 37°, 1.3 A_{260} units of 30S ribosomes (90 pmoles), 3 µg EF-G and 50 nmoles $[\gamma\text{-}^{32}P]$ GTP (Amersham) with a specific activity of 0.5 Ci/mole were added. The final volume was adjusted to 100 µl, the end concentration being 0.015 M Tris-HCl (pH 7.6), 0.01 M $MgAc_2$, 0.08 M NH_4Cl and 0.001 M DTT. After a second 10 min incubation at 37°, the reaction was stopped by adding 100 µl 1 M $HClO_4$. Inorganic phosphate was determined essentially according to Kolakowsky et al. (23).

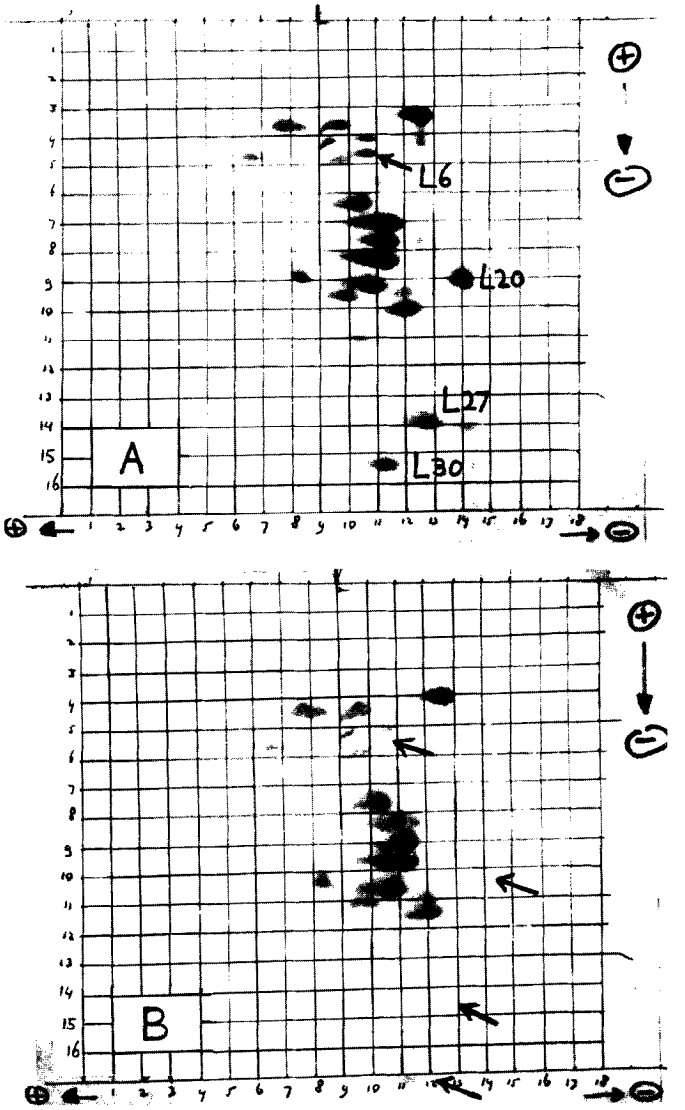

Fig. 2. Two-dimensional electrophoresis of 50S CsCl core proteins from cores prepared at 20 mM Mg^{2+} (A) and at 2 mM Mg^{2+} (B). The core proteins were extracted from 75 A_{260} units of cores according to Hardy et al. (33). 2-D electrophoresis was performed as described previously (16).

RESULTS

As shown in fig. 1, EF-G dependent hydrolysis of 50S cores, supplemented with L12, is highly dependent on the Mg^{2+} concentration used in the isopycnic CsCl gradient. In addition, the reconstituted GTPase activity is much higher with

Table 1. The effect of various split proteins on the EF-G-dependent GTPase activity of three different 50S CsCl cores, prepared at 2, 5 and 40 mM Mg^{2+}.

Experimental conditions	moles GTP hydrolyzed/mole 50S core		
	2 mM Mg^{2+}	5 mM Mg^{2+}	40 mM Mg^{2+}
50S core	19	22	34
" , + split	537	634	717
" , + (split - L7, - L12)	35	38	42
" , + L12	101	225	653
" , + L12, + (split - L7, - L12)	511	763	736
" , + L7	67	119	137
" , + L7, + (split - L7, - L12)	484	709	716

A 1.5 molar excess (13 µg) of the respective split protein fractions (split) was present where indicated. The amount of L7 or L12 added corresponded to 15 moles/mole 50S particle (5.5 µg). The GTPase activity of native 50S ribosomes was 845 moles GTP hydrolyzed/mole 50S particle.

L12 than with L7. To exclude the possibility of damage to cores prepared at "low" Mg^{2+} concentrations (2 or 5 mM Mg^{2+}), GTPase assays of such cores were performed in the presence of their total split protein fractions. The results of table 1 indicate that the cores remain intact and that "low" Mg^{2+} split fractions, contain other components, besides L7 and L12, which are needed for the hydrolysis of GTP. Since these components might remain attached to the "high" (20 and 40 mM) Mg^{2+} core but become detached at "low" Mg^{2+} concentration, the protein compositions of the "high" and "low" Mg^{2+} cores were compared by two-dimensional polyacrylamide gel electrophoresis. Fig. 2 shows that in the range between 20 mM Mg^{2+} and 2 mM Mg^{2+} four additional proteins, i.c. L6, L20, L27, and L30 are split off.

The L6 requirement in the GTPase reaction is shown in table 2; no further increase in GTPase activity of "low" Mg^{2+} cores was observed when amounts of L6 higher than 1 mole/mole 50S core were used. The possible contribution of L20, L27, and L30 to the GTPase activity has not yet been explored. A stimulatory action of L6 on 50S cores in the presence of L7 was marked but not comparable to that obtained in the presence of L12 (see table 2).

Table 2. The effect of L6 on the EF-G-dependent GTPase reaction of three different CsCl core preparations.

Experimental conditions	moles GTP hydrolyzed/mole 50S core		
	2 mM Mg^{2+}	5 mM Mg^{2+}	40 mM Mg^{2+}
50S core	10	22	23
" , + L6	10	28	23
" , + L12	73	225	677
" , + L12 + L6	273	494	633
" , + L7	50	119	143
" , + L7 + L6	87	131	170

1 µg L6 was added where indicated, corresponding to 1.5 moles/mole 50S core. The amount of L7 or L12 added corresponded to 15 moles/mole 50S particle (5.5 µg). In this experiment the GTPase activity of native 50S ribosomes was 810 moles GTP hydrolyzed/mole 50S particle.

Table 3. The effect of L10 on the EF-G-dependent GTPase activity of the 50S core prepared at 40 mM Mg^{2+}, in the presence of different amounts of L7 or L12.

Experimental conditions	moles GTP hydrolyzed/mole 50S core	
	- L10	+ L10
50S core (40 mM Mg^{2+})	36	38
" , + L12 (15 eq.)	677	873
" , + L12 (2 eq.)	200	710
" , + L7 (15 eq.)	178	773
" , + L7 (2 eq.)	72	759

Where indicated, 0.9 µg L10 (1.5 moles/mole 50S core) was added. 1 equivalent (eq.) L7 or L12 is defined as 1 mole added/mole 50S core. The amount of the GTPase activity of native 50S ribosomes was 907 moles GTP hydrolyzed/mole 50S particle.

Addition of individual split proteins in the presence of L7 or L12 reveals that L10 almost completely abolishes the difference between the reconstituted GTPase activity observed with L7 or L12 alone (see table 3). An impressive finding is that in the presence of L10, 2 moles of L7 or L12 per mole 50S core are sufficient to reconstitute a GTPase activity comparable to that obtained with

the intact 50S ribosome; neither L8 nor L9 showed any stimulatory effect. Although the L10 requirement is less stringent with a large excess of L12 (15 moles/mole 50S core), this does not hold for the same excess of L7 (table 3). However, in the presence of L10, 2 moles of L7/mole 50S core are sufficient to reach a GTPase activity comparable to fully reconstituted particles (cf. table 1). Similar effects were observed for 2 mM Mg^{2+} cores supplemented with L6 and L10 in the presence of either L7 or L12. A strict specificity was noted, L10 being unable to replace L6.

DISCUSSION

The results reported here point to the importance of the basic core protein L6 in the EF-G-dependent ribosomal GTP hydrolysis. Although the precise function of L6 in this process remains unclear, it is very interesting that L6 is also implicated in the binding of 5S RNA to the 50S particle (24) and that L6 is referred to as a protein essential for peptidyl transferase activity (25).

At present, at least six ribosomal proteins, S5, S9, L6, L7, L10, and L12 besides EF-G are known to be involved in the mechanism of GTP hydrolysis, two of which, namely S5 and S9, are thought to function at the 30S-50S interface, i.e. the region which possibly mediates GTPase activity (26). The marked increase of GTPase activity under the influence of L10 suggests that L10 facilitates the efficiency of the physical reconstitution of L7 and L12. Consistent with this proposal we noticed that in isolation the combination of L10, L7 and L12 shows a strong physical association. However, since the in vivo temporal order of assembly of L7, L10, and L12 is still controversial (27, 28), little more can be said about the possible role of L10 in the assembly process of L7 and L12. Nevertheless, the effects of L10 on the GTPase reaction complicate any simple explanation of the N-terminal acetyl group on L12 in terms of a regulatory mechanism of translocation (6, 29), particularly because L10 is reported to be a unit protein (30) in conditions of bacterial growth in both poor and rich media (31).

Whatever the actual function of L6, L7, L10, and L12 may prove to be, it remains remarkable that in studies with antibodies neither L6 and L10 nor S5 and S9 were found to have any measurable effect on the extent of binding of GDP to 50S ribosomes in the presence of EF-G and fusidic acid (32).

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