Incubation of 50 S subunits with 4.2 M LiCl leads to 4.2c cores and the complementary split protein fraction SP4.2, the latter containing quantitatively L24. L24 was removed from the split fraction by means of CM-cellulose chromatography. Partial and total reconstitution experiments performed with this protein preparation in the absence and presence of L24 demonstrate the crucial role of L24 in the early stage of assembly. However, this protein is dispensable for the subsequent steps of the in vitro assembly.

50 S subunits lacking L24 are fully active in the translation of artificial (poly(U)) and natural (R17 RNA) mRNA, indicating that L24 is not involved in any function of protein synthesis of the mature ribosome. It is therefore an assembly protein.

Three reconstituted intermediates (RI501 particles) were found during the course of in vitro assembly of the 50 S subunit (1), and this process is analogous to the assembly in vivo where three precursors were detected (for review, see Ref. 2). The conversion from the first to the second reconstituted intermediate (RI50 (I) → RI50* (I)) is a drastic conformational change (33 S → 41 S). This conformational change occurs exclusively in the first incubation of the two-step procedure (1) which is necessary for the reconstitution of active 50 S subunits (3).

Five proteins are essential for this conformational change, L24 being one of these key proteins (4). L24 binds specifically to the 23 S RNA (5) near the 5' end (6) and forms a remarkably tight complex with the RNA; an extensive digestion of the 50 S subunit with trypsin and ribonucleases liberates nondegraded L24 bound to an RNA fragment more than 100 nucleotides in length (7).

In this paper, we analyze the role of L24 by reconstitution experiments. The results demonstrate that L24 is essential for the early assembly but is not important for either the late assembly steps or the protein synthesizing functions of the mature ribosome.
The Ribosomal Protein L24 Is an Assembly Protein

The proteins extracted from the 4.2 cm core and reconstituted in the second step only (3). The incubation at 50°C is essential, no active particle being formed at 0°C (Table I, Experiment 1). This finding indicates that the 4.2c core has a configuration corresponding either to the RI*50 (1) particle, the RI50 (2), or some intermediate; in all these cases, the second incubation step is sufficient for the formation of active particles (1). Thus, L24 is absolutely required for achieving the RI*50 (1) conformation in that reconstitution, but L24 (together with other proteins) can be removed from the 50 S subunit without destroying a conformation which is at least equivalent to that of the RI*50 (1) particle.

In order to analyze the conformation of the 4.2c core in more detail, the S values of some reconstituted particles were compared to those of the reconstituted intermediates (RI50 particles). The 4.2c core has an S value of 42 S (Fig. 1A) similar to the RI*50 (1) intermediate (41 S, Ref. 1). However, incubation of the 4.2 cm core at 0°C with TP50 (4 mM Mg2+) shifts the S value to 46 S, and further incubation at 44°C for more than 20 min does not change this S value (Fig. 1B and C, respectively) similar to the RI50 (2) particle (Ref. 1). In contrast, in the total reconstitution procedure, the conversion of the RI*50 (1) particle to the RI50 (2) intermediate needs a 10-min incubation at 44°C or 5-min incubation at 50°C (4 and 20 mM Mg2+, respectively, Ref. 1). Since the 4.2c core particle can assemble the missing proteins to form an RI50 (2)-like particle in the cold, the 4.2c core conformation is more related to that of the RI50 (2) particle than to the RI*50 (1) particle.

The proteins extracted from the 4.2c core and reconstituted with the 23 S RNA under the conditions of the first incubation step cannot achieve the RI*50 (1) conformation unless L24 is present (compare Fig. 1, D (33 S) and E (40 S), respectively), a result which is in agreement with the functional analysis (see below). The dependence of the RI*50 (1) formation on L24 is not influenced by a prolongation of the incubation time of the first step. A prolongation of more than 4-fold has no effect on the activity and, therefore, on the RI*50 (1) formation (Table I, Experiment 2).

When the 4.2c core is incubated at 44°C and 4 mM Mg2+, the 42 S particle unfolds to more slowly sedimenting particles, with about 30% of the 42 S particle being unfolded (Fig. 1F) after 20 min incubation. This unfolding is prevented by the addition of L24 (Fig. 1G).

We conclude that L24 is essential for the formation of the RI*50 (1) particle but not for the maintenance of the RI50 (2) conformation. However, L24 stabilizes this conformation.

L24 and the Functions of the Mature Ribosome—Taking advantage of the 4.2c core which lacks L24 but has the RI50 (2) conformation, we can analyze the role of L24 during the subsequent assembly steps and in the functions of the mature ribosome.

The split proteins SP4.2 contain a full complement of protein L24 (see Fig. 2B), and this protein fraction was freed of L24 by means of CM-cellulose chromatography (SP4.2 – L24, for details, see "Materials and Methods"). The complete removal of L24 was checked by two-dimensional electrophoresis: when the SP4.2 – L24 proteins were added to those extracted from the 4.2c core and subjected to gel electrophoresis, no traces of L24 were detected (TP50 – L24, Fig. 2C). On the 5-fold overloaded gel (Fig. 2C), L24 would be detectable if it amounted to as little as 2% of the amount of one of the other proteins. Thus, the TP50 – L24 preparation contains at most 2% of L24 as compared to other proteins. When the 4.2c core was reconstituted with SP4.2 – L24 in the second step, full activity was obtained regardless of whether L24 was present or not (Table I, Experiment 3). This result strikingly demonstrates that the late assembly steps following the RI50 (2) formation do not require L24. Furthermore, L24 plays no important role during the elongation cycle of the protein biosynthesis, as shown by the poly(U) assay (Table I). In contrast, when a total reconstitution was performed, starting with the proteins derived from the 4.2c core in the first incubation, the activity was dependent on the presence of L24 during this incubation step. This confirms that the RI*50 (1) formation, which occurs exclusively during the first incubation step and which is essential for a successful reconstitution of an active 50 S subunit (1), does require L24 as already described (4).

Finally, we tested whether L24 is involved in the ribosomal functions of initiation or termination, or both. Twenty-five A260 units of the 4.2 cm core were reconstituted in the second step with the SP4.2 – L24 fraction in the presence and absence of L24. The reconstituted particles were isolated and tested in a protein synthesis system using the RNA from the phage R17 as natural mRNA (R17 system). In addition, the activity in the poly(U) system was tested again. Table II shows that in both protein synthesis systems the activities are independent of L24. Therefore, L24 is important neither for initiation nor termination.

**DISCUSSION**

The results presented in the preceding section demonstrate that L24 is essential for RI*50 (1) formation but plays no important role in either the last step of the assembly (RI50 (2) → 50 S) or in the protein synthesizing functions of the mature ribosome.

The analysis of a thermosensitive mutant with an impaired assembly *in vivo* of the 50 S subunit at 42°C revealed that the mutant contains an altered L24 (11). These findings indicate that this protein is involved in an early assembly step *in vitro* in nice agreement with our observation that L24 (among other components) is essential for the first assembly step *in vivo*. 

![Fig. 1](http://example.com/fig1.png)
The Ribosomal Protein L24 Is an Assembly Protein

Recently, a LiCl/methanol splitting technique and subsequent partial reconstitution was used to investigate the role of some ribosomal proteins (12). To the mixture of cores and split proteins, a 40-fold molar excess of a purified protein modified with fluorescein isothiocyanate was added, and after reconstitution, some ribosomal activities were tested. With modified L24, the particles showed a residual activity of 20 to 30%. The authors inferred that L24 is possibly involved in determining the overall structure of the particles. However, the results are difficult to interpret because no controls were made using an equivalent excess of the unmodified protein. A serious disadvantage in these experiments (12) was the heterogeneity of the cores with respect to L24; most of this protein is still present on the core (13). Furthermore, the possibility cannot be excluded that the label on protein Lx influences neither the assembly nor the function of this protein, but rather does affect the assembly or function, or both, of a neighboring ribosomal component Ly or RNA.

In the case of the small ribosomal subunit, two proteins have been described, namely S16 and S18, which are involved in the in vitro assembly of the 30 S subunit but are not functionally important (14). The main effect of S18 seems to be the stabilization of the binding of two proteins S11 and S21 in accordance with the assembly map (15), whereas S16 notably increases the rate of assembly. It is likely that S16 affects at least the R1*30 formation (16), but the step(s) in the assembly influenced by S16 has not yet been identified. In contrast to L24, both proteins S16 and S18 are not essential for assembly and, according to the assembly map (15), are not involved in the early assembly.
The Ribosomal Protein L24 Is an Assembly Protein

Table I
Reconstitution experiments with the 4.2 c core and its proteins

Partial reconstitutions were performed with the 4.2 c core and the indicated proteins under the conditions of the second incubation of the two-step procedure; for total reconstitutions (23 S + 5 S) RNA was used. "4.2 c HAc" means the acetic acid-extracted proteins from the 4.2 c core, "SP4.2 - L24" the split proteins SP4.2 freed of L24. Three to four equivalents of proteins were incubated with 2.5 A260 units of RNA or cores, respectively. For details see "Materials and Methods." Except where otherwise indicated, the incubation time in the first incubation was 20 min, and the temperature in the second one was 50°C.

| Experiment | Proteins | Incubation time | Poly(U) system |
|------------|----------|-----------------|----------------|
|            |          | min             |                |
| 1          | Core     | +               | 50°C           | 100 (7,491)
|            | Core     | -               | 0°C            | 5
| 2          | RNA      | +               | +              | 10
|            | RNA      | -               | +              | 5
|            | RNA      | +               | +              | 100 (5,716)
| 3          | RNA      | +               | +              | 72
|            | RNA      | -               | +              | 100 (6,463)
|            | Controls | +               | +              | 84
|            | Core     | -               | +              | 60
|            | Core     | +               | +              | 9
|            | RNA      | +               | -              | 8
|            | Core     | +               | +              | <1

Controls

| Reconstituted particle | R17 system | Poly(U) system |
|------------------------|------------|----------------|
|                        | Complete   | Without R17 RNA |
|                        | cpm        | cpm             |
| 4.0 c + SP4.2 + L24    | 6,940      | 995             |
|                        | 18,405     | 18,405          |
| 4.0 c + SP4.2 - L24    | 6,160      | 619             |
|                        | 16,828     | 16,828          |
| Control                | 30 S alone | 352             |

Activity of the particle lacking L24 in the natural mRNA system (R17 system)

| Reconstituted particle | R17 system | Poly(U) system |
|------------------------|------------|----------------|
|                        | Complete   | Without R17 RNA |
|                        | cpm        | cpm             |
| 4.0 c + SP4.2 + L24    | 6,940      | 995             |
|                        | 18,405     | 18,405          |
| 4.0 c + SP4.2 - L24    | 6,160      | 619             |
|                        | 16,828     | 16,828          |
| Control                | 30 S alone | 352             |

Acknowledgments—We thank Drs. H. G. Wittmann and R. Brimacombe for discussions and reading the manuscript and Drs. H. Schulze and H. Roth for help and advice.

REFERENCES
1. Dohme, F., and Nierhaus, K. H. (1976) J. Mol. Biol. 107, 585-599
2. Schlessinger, D. (1974) in Ribosomes, Cold Spring Harbor Monograph Series (Nomura, M., Tissieres, A., and Lengyel, P., eds) pp. 295-416, Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
3. Nierhaus, K. H., and Dohme, F. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 4713-4717
4. Spillmann, S., Dohme, F., and Nierhaus, K. H. (1977) J. Mol. Biol. 115, 513-523
5. Stöffler, G., Daya, L., Kak, K. H., and Garrett, R. A. (1971) J. Mol. Biol. 62, 411-414
6. Branlant, C., Krol, A., Sriwidada, J., Fellner, P., and Crichton, R. (1973) FEBS Lett. 35, 265-272
7. Crichton, R. R., and Wittmann, H. G. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 655-668
8. Homann, H. E., and Nierhaus, K. H. (1971) Eur. J. Biochem. 20, 249-257
9. Kaltschmidt, E., and Wittmann, H. G. (1970) Anal. Biochem. 36, 401-412
10. Funatsu, G., Nierhaus, K. H., and Wittmann-Liebold, B. (1972) J. Mol. Biol. 64, 201-209
11. Cabezón, T., Herzog, A., Petre, J., Yaguchi, M., and Bollen, A. (1977) J. Mol. Biol. 116, 361-374
12. Hernández, F., Vázquez, D., and Ballesta, J. P. G. (1977) Eur. J. Biochem. 78, 267-272
13. Hernandez, F., Vázquez, D., and Ballesta, J. P. G. (1975) Biochemistry 14, 1503-1508
14. Held, W. A., and Nomura, M. (1975) J. Biol. Chem. 250, 3179-3184
15. Held, W. A., Ballesta, B., Miuchina, S., and Nomura, M. (1974) J. Biol. Chem. 249, 3103-3111
16. Held, W. A., and Nomura, M. (1973) Biochemistry 12, 3273-3781
The ribosomal protein L24 of Escherichia coli is an assembly protein.
S Spillmann and K H Nierhaus

J. Biol. Chem. 1978, 253:7047-7050.

Access the most updated version of this article at http://www.jbc.org/content/253/19/7047

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/253/19/7047.full.html#ref-list-1