Incorporation of Six Additional Proteins to Complete the Assembly Map of the 50 S Subunit from *Escherichia coli* Ribosomes*

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The isolation of 70 S ribosomes, ribosomal subunits, and 23 and 5 S rRNA from *E. coli* K12 followed the procedure of Nierhaus and Dohme (1979). Highly purified proteins active in reconstitution were isolated according to Wystup et al. (1979), with the improvements described by Schulte and Nierhaus (1982).

**Materials and Methods**

The intermediates R10(1) and R10(2) contain an identical set of components but differ drastically in their S value. 18 proteins together with the 5 S rRNA were found in these particles in addition to 23 S rRNA. The protein moiety becomes completed during the transition R10(1) → R10(2), since the latter particle contains a full complement of 50 S components. The R10(2) particle is, however, functionally still totally inactive. Six 50 S proteins formerly could not be assigned to either set of protein additions, which occur during the steps 23 S rRNA → R10(1) or R10(1) → R10(2), respectively (Dohme and Nierhaus, 1976).

The assembly sequence and interdependences were analyzed by component and compiled into a 50 S assembly map, which previously comprised the 23 and 5 S rRNAs and 25 out of 32 proteins (Rohl and Nierhaus, 1982). Here we present the complete assembly map, which now contains all the 50 S components (except for L26, which is equivalent to S20) and takes into account a complete analysis of the proteins present in the first reconstitution intermediate R10(1).

Three defined reconstitution intermediates are formed consecutively in the course of total reconstitution in vitro of the 50 S subunit from *Escherichia coli* ribosomes, viz. R10(1) with a sedimentation coefficient of 33 S, R10(2) with a coefficient of 41 S, and R10(2) with a coefficient of 48 S (Dohme and Nierhaus, 1976). This sequence resembles the assembly process in vivo, where three precursor particles also occur, the P50S with an S value of 32 S, the P50S with a value of 43 S, and the P50S with an S value of about 50 S (Schlessinger, 1974).

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**Isolation of the R10(1) Particle-50**

The isolation of 50 A260 units of (23 S + 5 S) rRNA were incubated for 30 min at 0 °C in a volume of 2 ml with amounts of TP50* that were optimal for the total reconstitution of active 50 S subunits (molar ratio rRNA:TP50 = 1:1.3). The ionic milieu was the same as that of the first step of the two-step procedure (rec-4 buffer: 29 mm Tris/HCl, pH 7.5, 4 mm magnesium acetate, 400 mm NH4Cl, 4 mm β-mercaptoethanol; see Nierhaus and Dohme, 1979). The mixture was layered onto a 10–30% (w/v) sucrose gradient in rec-4 buffer and centrifuged in a SW 60 rotor for 3.5 h at 360,000 × g at 4 °C. The pellet was resuspended in rec-4 buffer, and the proteins were extracted with acetic acid. One-third of the protein recovered was used per two-dimensional gel electrophoresis, as described by Roth and Nierhaus (1975). In order to achieve optimal separations of the basic proteins, the sample was polymerized near to the anode end of the gel tube in the first dimension. For a correspondingly optimal separation of the acide proteins, the sample was polymerized more toward the cathode end (see Fig. 1).

**Assembly Mapping Experiments—**The procedure of Röhl et al. (1982) was followed, with slight modifications. For the identification of the bound proteins by SDS-gel electrophoresis, 5 A260 units of 23 S rRNA were incubated for 30 min at 44 °C in rec-4 buffer with the double stoichiometric amount of each of the isolated proteins under observation in a volume of 200 μl. The sample was layered onto a 10–30% (w/v) sucrose gradient in rec-20 buffer (the same as rec-4, except that the Mg2+ concentration was 20 mM). After centrifugation in a SW 60 rotor for 2 h and 45 min at 250,000 × g, the tubes were pumped out, the absorption was recorded at 290 nm, and the fast-running "heavy" half of the ribonucleoprotein peak was collected. 0.1 volume of 50% (v/v) trichloroacetic acid was added, and the ribonucleoproteins precipitated at 0 °C overnight. After a low speed centrifugation (10 min at 5,000 × g), the pellets were resuspended in 30 μl of SDS sample buffer. The mixture was heated for 5 min at 90 °C and applied to an SDS-polyacrylamide gel as described (Röhl et al., 1982).

In experiments where urea gels were used for the identification of the proteins, the assay was scaled up 2-fold. After addition of trichloroacetic acid, the sample was polymerized near to the anode end of the gel tube in the first dimension. For a correspondingly optimal separation of the acide proteins, the sample was polymerized more toward the cathode end (see Fig. 1).
RESULTS

Proteins Present on the First Reconstitution Intermediate \( \text{RI}_{10}(1) \)

The \( \text{RI}_{10}(1) \) particle already forms at 0 °C under the ionic conditions of the first step (rec-4 buffer) of the two-step reconstitution procedure (Dohme and Nierhaus, 1976). Accordingly, 50 \( \text{A}_{260} \) units of 23 S rRNA were incubated with the total proteins of the 50 S subunit (TP50) for 30 min at 0 °C in rec-4 buffer, and then pelleted through a sucrose gradient (10–30%, w/v) in the same ionic milieu. The particles were resuspended in rec-4 buffer, and the proteins were extracted by the acetic acid method and identified by two-dimensional gel electrophoresis. In the first dimension, the sample was polymerized near the anode end for an optimal development of the basic proteins (Fig. 1A), and in a second duplicate run near the cathode end for an optimal development of the acidic proteins (Fig. 1B). The following proteins were found (Fig. 1, A and B, left panels; proteins given in parentheses were found in substoichiometric amount): L1, L2, L3, L4, L5, L7/12, L9, L10, L11, L13, L15, L17, L18, L20, L21, L22, L23, L24, L26, L29, L33, and L34.

Assembly Mapping Experiments

Binding Dependence of L14—Formerly, we did not succeed in the isolation of pure L14, this protein being obtained only in a mixture with L24 (Wystup et al., 1979). In an equimolar mixture of L14 and L24, protein L14 bound to the 23 S rRNA, and since L14 was not known to be an rRNA binding protein, we concluded that L24 mediates the binding of L14 (Röhl and Nierhaus, 1982).

In the meantime, we have been able to isolate pure L14 from a protein fraction termed SP 3.5\( \text{S}_{10}(1.3) \) using a shallow gradient on a CM 52 column (see Wystup et al., 1979). Fig. 2A demonstrates that L14 binds directly to 23 S rRNA, i.e. it is an rRNA binding protein, and addition of L24 does not stimulate the L14 binding. L14 did not bind to poly(U) or \( \text{S rRNA}, \) which indicates that the binding of this protein is specific for 23 S rRNA. Accordingly, L14 is connected to 23 S rRNA with a thin arrow in the revised assembly map (Fig. 3A).

Binding Dependences of L28—Incubation of 50 S subunits with 4 M LiCl splits off most of the proteins including L28 and 5 S rRNA and leaves the “4.0c” core, which contains 23 S rRNA and 11 proteins (L2, L3, L4, L13, L17, L20, L21, L22, L23, L29, and L34; Homann and Nierhaus 1971; Spillmann and Nierhaus, 1978). The 4.0c core is able to bind L28 (Fig. 2B, lanes 1 and 2). To investigate this property in detail, the protein pattern of the 4.0c core was reconstructed from single proteins. L15 was added since it is sometimes seen on the 4.0c as a minor component, and single omission experiments were performed with this protein group and 23 S rRNA in the presence of L28. The binding of L28 was reduced when L3, L15, or L17 were omitted. Accordingly, the binding of L28 was tested with these three proteins (Fig. 2B, Lanes 3–8). L28 alone binds weakly to 23 S rRNA (lane 3), but both L15 and L17 strongly stimulate the L28 binding (lanes 5 and 6, respectively), and a weak stimulation is found with L3. Again, controls with poly(U) and 5 S rRNA revealed that L28 binds exclusively to 23 S rRNA (not shown). Consequently, L28 is incorporated into the assembly map with four arrows: two thin arrows from 23 S rRNA and L3, respectively, and two thick arrows from L15 and L17, respectively, to L28.

Binding Dependences of L30—The 4.0c core is also able to bind L30 and L32 (Fig. 2C, lanes 1 and 2). Single omission tests as described in the preceding section revealed that L30 did not bind when L20 or L21 were omitted. Assembly mapping experiments with these proteins showed that whenever L21 was present a weak L30 binding was found (lanes 4 and 6), whereas L20 alone had no effect (lane 5). The binding of
L21 is mediated by L20, which itself is stained only poorly in this gel system (Röhl et al., 1982). We now demonstrate that L21 binds directly but weakly to 23 S rRNA (compare lanes 6 and 7). Thus a thin arrow can be drawn from L21 to L30 and another one from 23 S rRNA to L21.

**Binding Dependences of L32**—The 4.0~ core binds L32 (Fig. 2C, lane 1). A series of experiments revealed that the proteins mediating L32 binding must be present within a group of

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**Fig. 3. Assembly map of the 50 S subunit.** A, revised 50 S assembly map. The main fragments of 23 S rRNA (13, 8, and 12 S) are indicated. The proteins are roughly arranged according to their binding regions on 23 S rRNA (Zimmermann, 1980). RNA → Lx, Lx is an RNA binding protein; → (→), binding of Lx is strongly (weakly) dependent on Ly. The broken arrow to L2 indicates that some L2 preparations bind strongly to 23 S rRNA while others bind weakly. **•••**, the encircled proteins are essential for mediating the binding of 5 S rRNA to 23 S rRNA (Röhl and Nierhaus, 1982). Proteins enclosed by **••••** are important or essential for the conformational change R1(l) → R1(0) (Spillmann et al., 1977). Components below **••••** are not present on the R1(0) particle. B, darkly shaded area, proteins of the 4.0~ core; lightly shaded area, additional proteins of the 2.0~ core (Homann and Nierhaus, 1971). C, the proteins combined in one box are coded in one transcriptional unit.

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**Fig. 2. Assembly mapping experiments.** A, the binding of L14 (SDS gel stained with Coomassie Brilliant Blue). B, the binding of L28. Lanes 1 and 2, assembly experiments with the 4.0~ core in the presence and absence of L28, respectively; lanes 3-8, binding experiments with 23 S rRNA and the proteins indicated (SDS gels with silver staining). C, the binding of L30. Lanes 1 and 2, urea gels stained with silver; assembly experiments with 4.0~ core in the presence and absence of L30 and L32; lanes 3-8, binding experiments with 23 S rRNA and the indicated proteins; L20 can be stained only poorly in SDS gels (Röhl et al., 1982). D, the binding of L32 (SDS gels stained with silver); assembly experiments with 23 S rRNA and the proteins indicated. E, the binding of L31 and L33 (SDS gels stained with silver). F, the binding of L34 (lanes 1-4, area gels; lanes 5-11, SDS gels stained with silver). G, the binding of L17 (SDS gels stained with Coomassie Brilliant Blue). H, 5 S rRNA binding proteins (SDS gels stained with Coomassie Brilliant Blue).
seven proteins (L3, L4, L13, L17, L20, L22, and L24). Furthermore, L15 seemed to stimulate the L32 binding. Fig. 2D documents that the group of seven proteins enables strong binding of L32 (lane 1) to occur. Three proteins of this group induced binding of L32, even when added as individual proteins, namely L4 (lane 2), L24 (lane 4), and L17 (lane 6). The same was found with L15 (lane 7). Since the L4 preparation used was slightly contaminated with L23 (lane 2), we also tested L23, which was, however, ineffective (lane 3). The effects of L4 and L24 were additive (lane 5), as were those of the other mediating proteins (not shown). L32 alone did not bind to 23 S rRNA.

Accordingly, four thin arrows were drawn to L32 from L4, L15, L17, and L24, respectively.

**Binding Dependences of L31 and L33**—We began the analysis with a protein mixture containing L31 and L33 in a molar ratio of 30:70. Neither of the proteins was able to bind to 23 S rRNA (Fig. 2E, lane 1), but binding of both proteins was found in the presence of TP50 lacking all the proteins smaller than L26 (TP50 = (L26-L34), see lane 2). Addition of L28 seems to stimulate the binding (lane 3). Addition of 5 S rRNA did not influence the binding of either protein (not shown). Single omission tests revealed that omission of L3 strongly reduced the binding of both proteins, whereas omission of L15 weakly reduced the binding of L33, and the omission of other proteins had no effect. Accordingly, L3, L15, and L28 were analyzed individually with respect to the L31/L33 binding. L15 enabled a weak (lane 4) and L28 a strong binding of L33 (lane 5) to occur, while the presence of L3 induced a strong binding of L33 and a weak binding of L31 (lane 6).

Since in three different experiments the L3-dependent L33 binding varied considerably, we draw a thin arrow from L3 to L33 in the assembly map. L33 receives two additional arrows, a weak one from L15 and a thick one from L28. L31 has not yet been tested individually, since we have not been able to isolate it in sufficient amounts. We conclude from the foregoing experiments that the L3 mediates the binding of L31 (thin arrow from L3 to L31).

The Binding Dependences of L34—L34 was identified as a component of the 4.0% core (compare the L34 content on the 4.0% core with that in TP50; Fig. 2F, lanes 1 and 2, respectively) and was isolated from that particle. A series of assembly experiments demonstrated that L34 was present. It is evident that one or more protein(s) in addition is (are) involved in the binding of L17.

A first experimental series demonstrated that the putative additional protein(s) responsible for the binding of L17 are among the four proteins L10, L15, L16, and L18. In a single omission experiment (Fig. 2G) only the omission of L15 drastically reduced the L17 binding (lane 3), and L15 alone was able to mediate the binding of L17 (lane 6, thick arrow from L15 to L17).

**Binding of Proteins to 5 S rRNA**—The binding of L5, L18, and L25 to 5 S rRNA has been reported under various ionic conditions (Spierer and Zimmermann, 1978; for review see Garrett et al., 1981). Since we did not observe any binding of L25 to 5 S rRNA in a previous attempt (Rohli and Niehaus, 1982), we decided to recheck the binding of L-proteins to 5 S rRNA under reconstitution conditions (4 mM Mg²⁺, conditions of the first incubation of the two-step procedure).

In a control experiment 5 S rRNA was incubated with TP50, and the 5 S RNA-protein complex was isolated in a sucrose gradient run. Only three proteins were found, namely L18, L25, and, in lower amounts, L5 (Fig. 2H, lane 1), in agreement with earlier observations (Horne and Erdmann, 1972). Accordingly, we focused our attention on the binding dependences of these three proteins. All three bind to 5 S rRNA (lane 2) in amounts equivalent to the TP50 experiment. L18 and L25 bind equally well when added as individual proteins (lanes 6 and 7, respectively), whereas the weak binding of L5 alone (lane 8) is significantly increased in the presence of either L18 (lane 9) or L25 (lane 4). The effects of L18 and L25 are not additive (compare lanes 5 and 4, respectively, with lane 2). We conclude that all three proteins bind to 5 S rRNA under reconstitution conditions and that L18 and L25 bind noncooperatively, whereas the binding of L5 is stimulated by both L18 and L25. Accordingly, we draw two thick dashed arrows from 5 S rRNA to L18 and L25, respectively, and a thin one to L5. The latter is amplified by a connection with both L18 and L25.

**DISCUSSION**

The assembly of ribosomes from E. coli in vivo takes only a few minutes at 37°C (Schlessinger, 1974), whereas in vitro the two-step procedure for the total reconstitution of the large ribosomal subunit, for example, requires very long incubation times at high temperatures (20 min/44°C → 90 min/50°C). The enormous difference between these assembly conditions poses a number of questions. Which criteria have to be obeyed in order to keep the possibility of observing artifacts as small as possible? Can any physiological conclusions be drawn from the results of in vitro assembly studies? Are there plausible explanations for the drastically different incubation requirements for the in vitro assembly?

An absolute requirement for in vitro studies of ribosomal assembly is the application of conditions of ionic strength and temperature which lead to the formation of active particles. The efficiency of forming active 50 S subunits ranges from 50 to 100% of the input components, if the standard two-step procedure is applied (Niehaus and Dohme, 1979; Schulze and Niehaus, 1982). Working under these conditions, a number of results have been obtained with obvious physiological relevance.

1) As already mentioned in the Introduction, three reconstitution intermediates are formed in the course of the in vitro 50 S assembly, which are very similar in their S-values and compositions to the three 50 S precursors of the in vivo assembly. This finding strikingly indicates that the overall path of assembly and the rate-limiting steps are very similar or even identical in vitro and in vivo (for review see Niehaus, 1980). A complete analysis of the components present on the
first reconstitution intermediate particle is presented in this paper (Table I) and incorporated into the assembly map (Fig. 3A).

2) A compelling example of the thesis that in vitro reconstitution can reveal essential features of the in vivo assembly is the finding of the "assembly gradient" (Spillmann et al., 1977; Nierhaus, 1980). The term "assembly gradient" signifies that in vivo the early assembly reactions already start with a small number of r-proteins shortly after the onset of rRNA synthesis and that the progress of rRNA synthesis dictates the progress of assembly. This conclusion was derived from the observation that the five proteins essential for the early assembly reactions (RI(1) formation) bind exclusively near to the 5'-end of 23 S rRNA. These proteins are boxed in Table I (an exception is L3, which was only stimulating rather than essential) and are grouped in the upper left corner of the assembly map (Fig. 3).

The concept of an assembly gradient leads to two important conclusions. First, the assembly in vivo starts soon after onset of the rRNA synthesis, using the relatively short 5'-region of nascent rRNA and the five early assembly proteins, whereas in vitro the assembly process has to operate with the complete mature rRNA molecule and 32 different proteins. It follows that the entropic situation in the cell is much simpler than that in the test tube, thus giving one important reason why the assembly process requires 1.5 h at 50 °C in vitro, whereas the cell achieves the same within a few minutes at 37 °C. Second, the entropic advantage of the assembly gradient, i.e., the coupling of rRNA synthesis and ribosomal assembly, might be of such importance that the assembly gradient principle had to be maintained in the eukaryotic cell. This requirement would explain the complicated mode of assembly of these cells. rRNA genes are located in the nucleoli, whereas the r-proteins are encoded outside the nucleoli, and their rRNA molecules have to be exported to the cytoplasm, where the r-proteins are synthesized. Subsequently the r-proteins have to be reimported into the nucleoli, where the coupled processes of rRNA synthesis and ribosomal assembly take place. A small separated compartment for the assembly process also favors the occurrence of high concentrations of specific assembly proteins, which facilitate the assembly reactions and are absent from the mature particles (Kumer and Subramanian, 1975). Nucleoli are abundant in this type of assembly proteins, which are unknown in prokaryotes. In procaryotes the assembly proteins such as L20 (Nowotny and Nierhaus, 1980) and L24 (Spillmann and Nierhaus, 1978) are also found on the mature particles.

3) In spite of the large number of proteins (20) which can bind to the naked 23 S rRNA in vitro (arrows starting from 23 S rRNA beam in Fig. 3), only two proteins, viz. L24 and L3, can initiate the assembly process (Nowotny and Nierhaus, 1982). If 23 S rRNA is present in excess, then only those rRNA molecules which contain both initiation proteins can be used for the formation of active particles. Surprisingly, a temperature-sensitive mutant has been described which lacks the initiation protein L24 (Dabbs, 1982). The growth of the mutant is drastically reduced at permissive temperatures (below 36 °C), and the 50 S assembly is severely hampered (molar ratio 50 S:30 S = 0.5:1). A recent reconstitution analysis was able to explain satisfactorily the phenotypic features of the mutant. At optimal temperatures L24 is absolutely required for the assembly of active 50 S subunits. However, this requirement is relaxed at lower temperatures, where another protein could take over the functions of L24, although not as efficiently as L24 (Herold et al., 1986). Thus, the temperature sensitivity of the mutant is a direct consequence of the assembly constraints induced by the lack of the assembly initiation protein L24. Recently, we were able to identify the protein which replaces L24 in the mutant at permissive temperatures as L20.1

4) A final instructive example is the identification of the proteins essential for the reconstruction of the peptidyltransferase center by means of single omission tests. One analysis has been performed with 50 S subunits from E. coli (Hampel et al., 1981; Schulze and Nierhaus, 1982) and another with those from Bacillus stearothermophilus (Auron and Fahnstock, 1981). These two bacterial species separated about 1.2 × 10⁹ years ago (Osawa and Hori, 1980). An identical set of corresponding proteins was found in both cases (with the exception of L15, which was essential in the E. coli analysis only) strongly arguing for the significance of the reconstitution results. In E. coli, proteins L2, L3, L4, L15, and L16 were identified, and a glance at the assembly map (Fig. 3) shows that all these proteins are interconnected via strong assembly dependences. If a single protein exerts the peptidyltransferase activity, then it must necessarily be one of these five proteins. Among these, L2 is a primary candidate for the peptidyltransferase protein, since it has been reported that isolated L2 can hydrolyze AcPhe-tRNA (Remme et al., 1985). Furthermore, it is the most conserved protein in the ribosome (Schmid et al., 1984), which is not unexpected for a protein of outstanding functional importance.

L15 can be excluded from the group of peptidyltransferase candidates, since, as mentioned above, it has not been found in B. stearothermophilus and, furthermore, an E. coli mutant lacking L15 could be isolated (Lotti et al., 1983). The existence

Table I

| 23 S + 5 S rRNA + 22 proteins | RI(1) | 33 S |
|-------------------------------|------|-----|
| L1 (L2), L3, L4, L5, L7/L12, L9, L10 | 0 °C, 4 mM Mg²⁺ | RI(1) | 33 S |
| L11 (L13), L15, L17 (L18), L20, L21 | 44 °C, 4 mM Mg²⁺ | RI(1) | 41 S |
| L22, L23, L24 (L26), L29 (L33), L34 | 50 °C, 20 mM Mg²⁺ | RI(2) | 48 S |

TABLE I

Gross assembly in vitro of the 50 S subunit from E. coli ribosomes

L8 is a complex of L7/L12 and L10 and has not been included. Proteins in parentheses have been found in substoichiometric amounts, which means that they are either not fully assembled or the RI(1) particle or have been removed during the sucrose gradient run of the RI(1) particle. The boxed-in proteins are essential or important for the RI(1) formation (Spillmann et al., 1977).
of this mutant is a surprise in view of the dominant role L15 plays in both the reconstitution of active ribosomes (Teraoka and Nierhaus, 1978; Schulze and Nierhaus, 1982) and in the large assembly of the 50 S subunit (see Fig. 3A), although the observed side reactions in the course of the total reconstitution would explain a 50 S assembly in the absence of L15. However, it remains to be seen whether in fact the E. coli cells which we use as wild type in our reconstitution assays (K12, strain A19 or D10; Gesteland, 1966) can survive without L15, unless the lack of this protein is compensated by changes in other proteins. Evidence for such compensation comes from the observations that the L15-lacking mutant contains in addition a modified L4 and L30 (indicated by an altered electrophoretic mobility; Herold, 1986) and that we have not so far succeeded in transferring the lack of L15 into an A19 genetic background.3

The examples outlined above, where the physiological relevance of the reconstitution results is clearly evident, support the view that the assembly dependences, identified by the identical reconstitution procedure and compiled in the assembly map (Fig. 3A), also reflect relationships in the in vivo assembly. Furthermore, it is hard to imagine the existence of a completely artificial assembly path, which can be demonstrated reproducibly in vitro, but which is not related to the in vivo assembly of such a complicated particle.

In this paper the positions of the remaining six proteins within the map are assessed. Altogether 21 additional binding dependences have been incorporated into the assembly map of the 50 S subunit shown in Fig. 3A. Only one of the previous binding dependences, namely L24 — L14, has had to be removed, since, as shown in this paper, L14 is itself an rRNA binding protein, and its binding is not stimulated by L24. With L14, L21, and L28 the number of 23 S rRNA binding proteins has risen to 20, namely L1, L2, L3, L4, L9, L20, L23, and L24 as “strong binders,” and L7/L12, L10, L11, L14, L15, L16, L17, L18, L21, L22, L28, and L29 as “weak binders” (Marquardt et al., 1979; and this paper). Including those proteins which bind to the 5 S RNA, the 50 S subunit thus contains 23 proteins which bind to rRNA.

The significance of the assembly map becomes clear by a number of comparisons. First, the map should be related to the spatial distribution of the ribosomal proteins within the ribosome. The expectation is that two proteins are progressively more likely to depend on each other during assembly, the smaller the distance between them becomes. This has been demonstrated in the 30 S subunit (Nomura, 1973; Moore et al., 1986). For the 50 S subunit this can be tested by a comparison with the surface topology of ribosomal proteins, which has been studied by means of immune electron microscopy. In fact, an excellent agreement between the neighborhoods of epitopes from L-proteins on the surface of 50 S subunits and assembly dependences has been noted (Stöffer and Stöffer-Meilicke, 1986).

Second, the assembly map substantiates an old observation (Dohme and Nierhaus, 1976) that the “splitting-off” sequence of the L-proteins by increasing LiCl concentrations (Hommann and Nierhaus, 1981) roughly reflects the assembly process in reverse order. The stepwise removal of the proteins can be demonstrated reproducibly in vitro, but which is not related to the in vivo assembly of such a complicated particle.

Third, the L-proteins present in one operon or in a regulatory unit of an operon (for review see Nomura et al., 1984) depend on each other in nearly all cases during assembly (Fig. 3C). A possible explanation of this surprising observation is that the proteins in one regulatory unit represent assembly domains, since the common regulation of the proteins of one domain appears to be advantageous for the assembly process. This hypothetical relationship between assembly domain and operon structure would also explain why the operon structure of the ribosomal proteins appears to be a rather conserved feature during evolution (Dabbs, 1986).

The dependence of L33 and L28 reported here neatly fits with this concept, since both proteins are found in one operon (Lee et al., 1981). Further evidence for a common incorporation comes from a study of an E. coli mutant which synthesizes only half the amounts of L28 and L33, as compared to all other ribosomal proteins. This mutant accumulates a subpopulation of incomplete 50 S particles sedimenting with 47 S and totally lacking both proteins, i.e. either both proteins become incorporated or neither. In addition, the four proteins L16, L25, L26, and L27 were found in reduced amounts on the 47 S particle (Butler and Wild, 1984). The only exceptions to the operon-assembly relationship are L30, which occurs in the spc-operon and which does not belong to the assembly group around L15, and the small L1-L11 operon, from which L1 binds strongly to 23 S rRNA, thus masking possible assembly connections to other proteins.

Comparing the assembly process with the immune electron microscopy data so far available from the 50 S subunit (Stöffer and Stöffer-Meilicke, 1986), it is evident that proteins of the assembly nucleus (proteins boxed in the upper left corner of the assembly map), as well as strongly related proteins such as L4, L17, L20, L23, and L29, are concentrated at the rear lower region of the 50 S subunit. This observation suggests that the 50 S assembly starts in this region and proceeds along the arrows indicated in Fig. 4. The seemingly independent assembly of L1 and L9 corresponds to the isolated position of both proteins in the right protuberance.

The network of assembly dependences compiled in Fig. 3A seems to be too complicated to account on its own for the enormous efficiency of the assembly process. Rather, the map has to be combined with some qualitative features. For ex-

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3 E. R. Dabbs, personal communication.
example, as already mentioned, the map implies that 20 rRNA binding proteins exist, only two of which can initiate the assembly process. We know already that, based on the initiator proteins, the assembly proceeds in three or four steps, each of which is directed by one or two "assembly leader proteins" not yet identified. The assembly map together with each of which is directed by one or two "assembly leader proteins, the assembly proceeds in three or four steps, the sequence of the assembly leader proteins should provide

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REFERENCES

Auron, P. E., and Fahnestock, S. R. (1981) J. Biol. Chem. 256, 10105–10110 Butler, F. D., and Wild, D. G. (1984) Eur. J. Biochem. 144, 649–654 Dabbs, E. R. (1982) Mol. Gen. Genet. 187, 453–458 Dabbs, E. R. (1986) in Structure, Function, and Genetics of Ribosomes (Hardesty, B., and Kramer, G., eds) pp. 733–748, Springer-Verlag New York Inc., New York Dohme, F., and Nierhaus, K. H. (1976) J. Mol. Biol. 107, 585–599 Garrett, R. A., Douthwaite, S., and Noller, H. F. (1981) Trends Biochem. Sci. 6, 137–139 Gesteland, R. F. (1966) J. Mol. Biol. 16, 67–84 GöröG, A., Postel, W., Weser, J., Schiwa, H. W., and Boesken, W. H. (1985) Sci. Tools 32, 5–9 Hampl, H., Schulze, H., and Nierhaus, K. H. (1981) J. Biol. Chem. 256, 2284–2288 Herold, M. (1986) Ph.D. thesis, Technische Universität Berlin Herold, M., Nowotny, V., Dabbs, E. R., and Nierhaus, K. H. (1986) Mol. Gen. Genet. 203, 281–287 Homann, H. E., and Nierhaus, K. H. (1971) Eur. J. Biochem. 20, 249–257 Horne, J. R., and Erdmann, V. A. (1972) Mol. Gen. Genet. 119, 337–344 Kaltschmidt, E., and Wittmann, H. G. (1970) Anal. Biochem. 36, 401–412 Kumar, A., and Subramaniam, A. R. (1975) J. Mol. Biol. 94, 409–423 Lee, J. S., An, G., Friesen, J. D., and Isono, K. (1981) Mol. Gen. Genet. 184, 218–223 Lotti, M., Dabbs, E. R., Hasenbank, R., Stöffler-Mellicke, M., and Stöffler, G. (1983) Mol. Gen. Genet. 192, 295–300 Marquardt, O., Roth, H. E., Wystup, G., and Nierhaus, K. H. (1979) Nucleic Acids Res. 6, 3641–3650 Moore, P. B., Capel, M., Kjeldgård, M., and Engelman, D. M. (1986) in Structure, Function, and Genetics of Ribosomes (Hardesty, B., and Kramer, G., eds) pp. 87–100, Springer-Verlag New York Inc., New York Nierhaus, K. H. (1980) in Ribosomes: Structure, Function, and Genetics (Chambless, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., and Nomura, M., eds) pp. 267–294, University Park Press, Baltimore Nierhaus, K. H., and Dohme, F. (1979) Methods Enzymol. 59, 443–449 Nomura, M. (1973) Science 179, 864–873 Nomura, M., Gourse, R., and Baughman, G. (1984) Annu. Rev. Biochem. 53, 75–117 Nowotny, V., and Nierhaus, K. H. (1980) J. Mol. Biol. 137, 391–399 Nowotny, V., and Nierhaus, K. H. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 7238–7242 Osawa, K., and Ebata, N. (1983) Anal. Biochem. 135, 409–415 Osawa, S., and Hori, H. (1980) in Ribosomes: Structure, Function, and Genetics (Chambless, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., and Nomura, M., eds) pp. 333–355, University Park Press, Baltimore Remme, J., Mezai, E., Mainets, T., and Vilems, R. (1986) FEBS Lett. 190, 275–278 Röhl, R., and Nierhaus, K. H. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 729–733 Röhl, R., Roth, H. E., and Nierhaus, K. H. (1982) Hoppe-Seyler’s Z. Physiol. Chem. 363, 143–157 Roth, H. E., and Nierhaus, K. H. (1975) J. Mol. Biol. 94, 111–121 Schlessinger, D. (1974) in Ribosomes (Nomura, M., et al., eds) pp. 393–416, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY Schmid, G., Strobel, O., Stöffler-Mellicke, M., Stöffler, G., and Böck, A. (1984) FEBS Lett. 177, 189–194 Schulze, H., and Nierhaus, K. H. (1982) EMBO J. 1, 609–613 Spierer, P., and Zimmermann, R. A. (1978) Biochemistry 17, 2474–2479 Spillmann, S., and Nierhaus, K. H. (1978) J. Biol. Chem. 253, 7047–7050 Spillmann, S., Dohme, F., and Nierhaus, K. H. (1977) J. Mol. Biol. 115, 513–523 Stöffler, G., and Stöffler-Mellicke, M. (1986) in Structure, Function, and Genetics of Ribosomes (Hardesty, B., and Kramer, G., eds) pp. 28–46, Springer-Verlag New York Inc., New York Teraoka, H., and Nierhaus, K. H. (1978) J. Mol. Biol. 126, 185–193 Wray, W., Boulikas, T., Wray, V. P., and Hancock, R. (1981) Anal. Biochem. 118, 197–203 Wystup, G., Teraoka, H., Schulze, H., Hampl, H., and Nierhaus, K. H. (1979) Eur. J. Biochem. 100, 101–113 Zimmermann, R. A. (1980) in Ribosomes: Structure, Function, and Genetics (Chambless, G., Craven, G. R., and Davies, J., Davis, K., Kahan, L., and Nomura, M., eds) pp. 135–169, University Park Press, Baltimore