Assembly of the 30S Subunit from *Escherichia coli* Ribosomes Occurs via Two Assembly Domains Which Are Initiated by S4 and S7†

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ABSTRACT: A protein which initiates assembly of ribosomes is defined as a protein which binds to the respective rRNA without cooperativity (i.e., without the help of other proteins) during the onset of assembly and is essential for the formation of active ribosomal subunits. The number of proteins binding without cooperativity was determined by monitoring the reconstitution output of active particles at various inputs of 16S rRNA, in the presence of constant amounts of 30S-derived proteins (TP30): This showed that only two of the proteins of the 30S subunit are assembly-initiator proteins. These two proteins are still present on a LiCl core particle comprising 16S rRNA and 12 proteins (including minor proteins). The 12 proteins were isolated, and a series of reconstitution experiments at various levels of rRNA excess demonstrated that S4 and S7 are the initiator proteins. Pulse–chase experiments performed during the early assembly with ^{14}C- and ^{3}H-labeled TP30 and the determination of the $^{14}C/^{3}H$ ratio of the individual proteins within the assembled particles revealed a bilobal structure of the 30S assembly: A group of six proteins headed by S4 (namely, S4, S20, S16, S15, S6, and S18) resisted the chasing most efficiently (S4 assembly domain). None of the proteins depending on S7 during assembly were found in this group but rather in a second group with intermediate chasing stability [S7 assembly domain; consisting of S7, S9, (S8), S19, and S3]. A number of proteins could be fully chased during the early assembly and therefore represent "late assembly proteins" (S10, S5, S13, S2, S21, S1). These findings fit well with the 30S assembly map. Our data, together with the assembly map, imply that S8 and S5 play an important role in the interconnection of the two assembly domains.

The small subunit of *Escherichia coli* ribosomes contains 21 different proteins (S proteins), 12 of which can bind independently to 16S rRNA in vitro under reconstitution conditions (Mizushima & Nomura, 1970; Hochkeppel et al., 1976). If these proteins were also to bind independently in vivo, they should be able to initiate 12 different assembly nuclei; i.e., they all would represent assembly-initiator proteins. This would have the consequence that, in the situation where rRNA is present in excess over ribosomal proteins, the 12 assembly-initiator proteins would be distributed independently of each other over all the rRNA molecules, thus dramatically reducing the output of active particles. This is not what has been observed. Under unfavorable growth conditions the molar ratio of rRNA to ribosomal proteins can reach values of 3:1 but is still accompanied by a significant synthesis of ribosomes (Gausing, 1977). Therefore, the actual number of assembly-initiator proteins must certainly be less than 12. In fact, an early analysis of the assembly cooperativity led to the conclusion that two or three proteins or protein complexes initiate the assembly, although the nature of these proteins could not be uncovered (Nomura et al., 1969).

In this paper we demonstrate that only two proteins initiate the 30S assembly. The proteins are identified as S4 and S7. Furthermore, evidence is presented that each assembly-initiator protein initiates its own assembly domain. The results are in good agreement both with the 30S assembly map data (Mizushima & Nomura, 1970) and the spatial arrangement of the S proteins as deduced by neutron scattering (Moore et al., 1986).

Materials and Methods

Ribosomes, their subunits, and 16S rRNA were isolated from *Escherichia coli* K12, strain D10, as described (Nierhaus & Dohme, 1979). Isolation of the total proteins from the 30S subunit (TP30)[1] followed the protocol given for 50S subunits (Schulze & Nierhaus, 1982). TP30 was labeled by means of reductive methylation (Jentoft & Dearborn, 1979) with the following modifications: For the labeling with [^{14}C]formaldehyde, 1 mL of TP30 (35 A_{230} units) in Hepes buffer [100 mM Hepes (pH 7.0), 10 mM magnesium acetate, 20 mM KCl, 4 M guanidinium hydrochloride] was added to 100 μL of $NaBCNH_3$ (12.6 mg/mL) in the same buffer containing 3 μL of aqueous [^{14}C]formaldehyde (50 μCi with a specific activity of 55 mCi/mmol). This mixture was incubated at 37 °C for 30 min. The proteins were separated from the reaction mixture by Sephadex G-25 gel filtration. For labeling with [^{3}H]formaldehyde, 4 mL of TP30 (90 A_{230} units) was supplemented with 400 μL of $NaBCNH_3$ solution and 8 μL of aqueous [^{3}H]formaldehyde (250 μCi with 78 mCi/mmol). The specific activity of [^{3}H]TP30 was 141 000 cpm per A_{230} unit, and that of [^{14}C]TP30 was 230 000 cpm per A_{230} unit. One A_{230} unit of TP30 is equivalent to about 250 μg of protein or to 8 equiv units (eu, 1 eu of TP30 is that amount of protein present on 1 A_{260} unit of 30S subunits).

S proteins were purified with a combination of salt washing procedures (Homann & Nierhaus, 1971) and (carboxymethyl)cellulose column techniques (Wystup et al., 1979; Moore, 1979). The proteins were characterized and their purity was checked by 2D gel electrophoresis (Roth & Nierhaus, 1975).

Reconstitution of the 30S subunit was performed in Rec-20 buffer (20 mM Tris-HCl, pH 7.5, 20 mM magnesium acetate, 400 mM NH_4Cl, 2 mM β-mercaptoethanol) at 40 °C for 40 min if not otherwise indicated; 1.5 eu of TP30 was incubated with 1.25 A_{260} units of 16S rRNA (or, if indicated, a multiple

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[1] Abbreviations: TP30, total proteins of the 30S subunit; eu, equivalent unit (1 eu is that amount of protein present in 1 A_{260} unit of 30S subunits).

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of this amount) per 100 μL. Aliquots of 40 μL were withdrawn and supplemented with 1 A_{260} unit of 50S subunit, 16S rRNA was added to match the highest 16S rRNA amount present in the corresponding experiment, and the mixtures were tested for poly(U)-dependent poly(Phe) synthesis in the presence of 11 mM Mg^{2+} at 30 °C for 45 min [for further details, see Nierhaus and Dohme (1979)]. Controls indicated that, during the incubation for poly(Phe) synthesis, only insignificant amounts of 30S subunits could assemble in the presence of a 16S rRNA excess. The corresponding values were taken as background and were subtracted.

The TP30 fractions were tested by total reconstitution followed by a poly(U)-dependent poly(Phe) synthesis assay. For the test of the ^{3}H-labeled TP30, the assay system was set up with [^{14}C]phenylalanine and vice versa. For the pulse–chase experiment, 15 A_{260} units of 16S rRNA was incubated with 10.5 eu of [^{14}C]TP30 in 1 mL in "Rec-20 conditions" at 29 °C (this relatively low temperature was chosen to slow down the assembly process). After 1 min, 42.0 eu of [^{3}H]TP30 was added and the incubation continued for 100 min at 29 °C. For the 0-min control, the [^{14}C]- and [^{3}H]TP30 fractions were mixed before addition to rRNA. The assay was subjected to a sucrose gradient run (10–30% sucrose in Rec-20 buffer; SW40 at 20 000 rpm for 18 h). The 30S particles were isolated, the proteins separated by 2D gel electrophoresis, and the ^{3}H and ^{14}C isotopes separately counted as described (Nowotny & Nierhaus, 1982).

Results

The Total Proteins of the 30S Subunit Contain Two Assembly-Initiator Proteins Which Are Still Present on the 3.0 M LiCl Core. Assembly-initiator proteins initiate "assembly nuclei" which merge in the course of the assembly and finally form the active particle. These proteins, therefore, have to bind to rRNA without cooperativity (i.e., without the help of other proteins) at the onset of ribosomal assembly and are essential for the formation of active particles. This definition leads to a simple relationship:

$$A = E^{1-n}$$

where the output of active particles (A, active fraction) depends on the stoichiometric ratio E = 16S rRNA:TP30 to the power of $1 - n$ [n = number of initiator proteins; for details, see Nowotny and Nierhaus (1982)]. A double logarithmic plot of the active fraction A versus the excess E of rRNA should yield a straight line, the slope of which is $1 - n$, and thus gives the number of initiator proteins.

The straight lines in Figure 1A indicate the theoretical decrease of the output of active particles with increasing excess of 16S rRNA over TP30, with values of n from 1 up to 10. The points in this figure represent the results of a corresponding experiment, where the amount of TP30 has been kept constant (1.5 eu/100 μL) while increasing amounts of 16S rRNA were added. The results clearly demonstrate that the TP30 contains two initiator proteins.

In order to diminish the number of possible candidates for the assembly-initiator proteins, ribosomal proteins were successively washed off the 30S subunits by 1, 2, or 3 M LiCl. The resulting 1, 2, or 3 M LiCl cores were pelleted and the proteins isolated by the acetic acid method. With each of these three protein groups a series of reconstitution experiments equivalent to that of Figure 1A was performed. Following the reconstitution TP30 was added in the cold, and after supplementation with native 50S subunits, the poly(Phe) synthesis was measured at 30 °C for 45 min (for further details, see Materials and Methods). The results obtained with the 1.0,

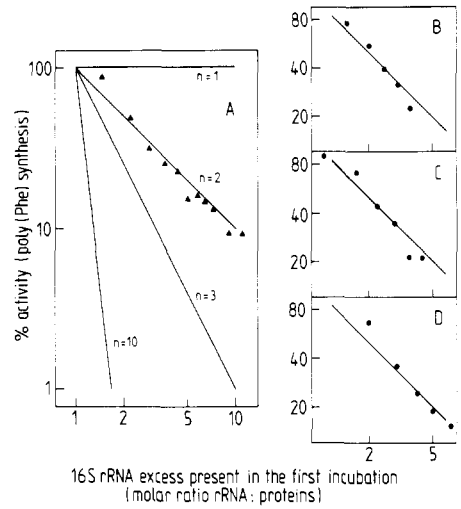

FIGURE 1: Determination of the number of initiator proteins for the assembly of the 30S subunit. (A) TP30. The straight lines are calculated for 1, 2, 3, or 10 initiator proteins. (B–D) Analysis of the proteins derived from 1.0, 2.0, or 3.0 M LiCl cores, respectively. The straight line gives the theoretical activity for the presence of two initiator proteins. 100% is equivalent to 16 143 cpm, which corresponds to an incorporation of statistically 40 Phe per 70S ribosome. Native 30S subunits yielded 15 760 cpm after complementation with native 50S subunits. For details see text.

2.0, and 3.0 core proteins are shown in panels B–D of Figure 1, respectively. The theoretical line for two initiator proteins is given in addition. Clearly, the proteins derived from the 3.0 core still contain the two assembly-initiator proteins. According to protein analyses on overloaded 2D gels, the 3.0 core contains proteins S3, S4, S5, S7, S8, S9, S12, S15, S16, S17, S18, and S20, including those proteins present only in minor amounts.

The Two Assembly-Initiator Proteins Are S4 and S7. The proteins found on the 3.0 core were purified. In addition to the 12 proteins, we also had to include S19 and S21, since these proteins were found as impurities in some protein preparations. Therefore, only seven proteins (S1, S2, S6, S10, S11, S13, and S14) are not further considered as candidates for assembly-initiator proteins in the next experiments.

During the reconstitution incubation the reconstitution intermediate RI^*_{30} must be formed, in order to allow the formation of significant amounts of active 30S subunits at 0 °C after addition of TP30 (Traub & Nomura, 1969). Five proteins are essential for RI^*_{30} formation, namely, S4, S7, S8, S16, and S19 (Held & Nomura, 1973), which therefore must be present during the reconstitution incubation. In order to avoid interference between the search for the assembly-initiator proteins and the need of the five essential proteins, we pursued the following reconstitution strategy:

16S rRNA (1× to 6×) +

$$\sum\text{P minus selected proteins} \xrightarrow[\text{first incubation}]{1\text{ min}/37\text{ °C}}$$

+ 16S rRNA (5× to 0×) +

$$\text{selected proteins} \xrightarrow[\text{second incubation}]{20\text{ min}/37\text{ °C}} + \text{TP30 at 0 °C} \rightarrow$$

poly(Phe) synthesis

$\sum$P means the mixture of all isolated proteins. In the first incubation a subgroup of the isolated proteins ($\sum$P minus selected proteins) was incubated with various amounts of 16S rRNA (molar ratio rRNA:proteins = 1:1 to 6:1); then, the 16S rRNA amount was adjusted in each tube to a molar ratio rRNA:proteins = 6:1 before the missing proteins were added. After a second incubation TP30 was added, and since this step always takes place in the presence of the 6-fold excess of

Table I: Identification of the Assembly-Initiator Proteins for the 30S Assembly[a]

	first incubation (10 min/40 °C)					second incubation (20 min/40 °C)		
		proteins						
expt	RNA: proteins (molar ratio)	S4	S7	S8	S20	RNA: proteins (molar ratio)	proteins	poly(Phe) synthesis (cpm)
1	1×	−	+	+	+	5×	S4	1575
2	1×	+	−	+	+	5×	S7	1413
3	1×	+	+	−	+	5×	S8	2143
4	1×	+	+	+	−	5×	S20	2313
5	1×	−	−	+	+	5×	S4, S7	845
6	1×	+	+	−	−	5×	S8, S20	1933

[a] The values are the average of double determinations. After the second incubation TP30 was added in the cold, the samples were supplemented with 50S subunits, and poly(Phe) synthesis was measured. The experiment was repeated four times and yielded identical results qualitatively. However, the individual values varied significantly. Therefore, the experiment should be interpreted only qualitatively.

rRNA, it causes completion of the assembly of the preformed $RI^{\bullet}_{30}$ intermediates, rather than triggering ab initio assembly of active particles.

Only if the subgroup in the first incubation was able to generate complete initiation complexes (i.e., if it contains *both* assembly-initiator proteins) will the rRNA added for the second incubation not affect the number of assembly initiation complexes. These complexes will now at the latest form $RI^{\bullet}_{30}$ intermediates with the help of the selected proteins. However, if the subgroup contains only one or none of the assembly-initiator proteins, these proteins will be confronted with the 6 molar excess of 16S rRNA in the second incubation, thus giving rise to only very small amounts of complete assembly-initiation complexes. These samples will therefore show only low activity.

Figure 2A–D shows the results of the first experiment. In panel A all 14 isolated proteins were present, containing, as expected, both initiator proteins. Qualitatively, the same results were obtained when all the proteins except S3 and S5 were present during the first incubation (Figure 2B). Therefore, neither S3 nor S5 can be an assembly-initiator protein, and both were excluded from the following experiments. However, if the five proteins indicated in Figure 2C or the four indicated in Figure 2D were omitted from the first incubation, complete initiation complexes could not be formed, as indicated by the low activity under all conditions. It follows that the three proteins, S12, S15, and S21, common to both experiments can also be excluded, whereas each of the two selected groups indicated in panels C and D, respectively, should contain one initiator protein.

In the next experiment (Figure 2E–H) we followed the same strategy but with different subgroups of proteins. Figure 2E presents the control with all proteins present in the first incubation. In the experiment shown in Figure 2F three proteins (S9, S18, and S19) were omitted during the first incubation but the remaining subgroup still contains the two assembly-initiator proteins. Therefore, these three proteins (S9, S18, and S19) can be discarded. Similarly, the omission of S15, S16, and S17 did not affect the formation of complete initiation complexes during the first incubation (Figure 2G); i.e., none of these three proteins is an initiator protein. Consequently, the remaining four proteins, S4, S7, S8 and S20, were omitted in the next experiment during the first incubation, resulting in consistently low activities (Figure 2H). It follows that the two assembly-initiator proteins must be among S4, S7, S8 and S20.

For the analysis of the last four proteins the reconstitution procedure was slightly modified. Up to three proteins of this group were incubated with 16S rRNA and the other proteins (S2, S9, S11, S13, S15, S16, S17, S18, and S19) in equimolar amounts (Table I); then, the 16S rRNA amount was raised

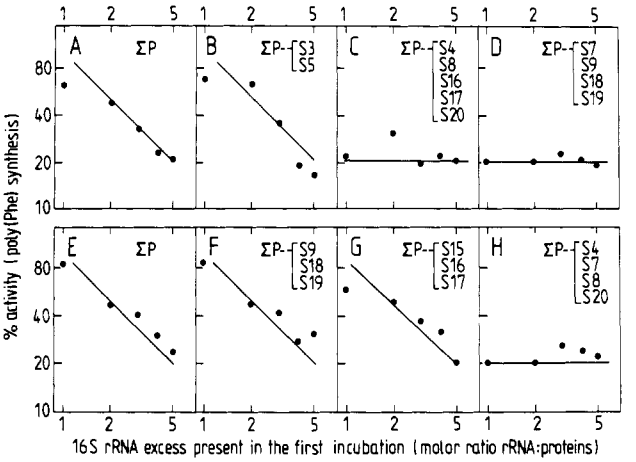

FIGURE 2: Determination of the number of assembly-initiator proteins in various groups of S proteins. ΣP, the proteins S3, S4, S5, S7, S8, S9, S12, S15, S16, S17, S18, S19, S20, and S21. The proteins omitted from this group are given in the respective panel; e.g., in experiment F all proteins of group ΣP except S9, S18, and S19 were present during the first incubation. The straight line gives the calculated expectation for two (A, B, E–G) or one initiator protein (C, D, and H). The 100% activity corresponded to 520 (A–D) and 560 cpm (E–H); the background (minus reconstituted particle) has been subtracted.

to a ratio of 6:1, the remaining omitted proteins were added, and a second incubation followed. Next, TP30 was added in the cold, and the active fraction was assessed as described for the preceding experiments. In the first set of reconstitutions only one out of four proteins (S4, S7, S8, S20) was omitted, and the lowest activities were observed when S4 and S7 were absent (Table I, experiments 1 and 2, respectively). Only marginal activity was found when both S4 and S7 were omitted during the first incubation (experiment 5), whereas in the presence of these two proteins appreciable activity was found (experiment 6). The experiments in Table I identify S4 and S7 as the assembly-initiator proteins for the 30S assembly.

S4 and S7 Each Initiate an Assembly Domain. To extend the results of the preceding section we performed pulse–chase experiments during the early assembly. 16S rRNA is incubated with substoichiometric amounts of [^{14}C]TP30 for 1 min at 29 °C, then excess of [^{3}H]TP30 is added, and the incubation is continued for 100 min at 29 °C. The low incubation temperature of 29 °C slows down the assembly process by about an order of magnitude and thus ensures that the chasing interferes with the very early assembly reactions. The reconstituted particles were isolated, the proteins separated on 2D gels, and the ^{14}C and ^{3}H contents of each protein determined.

The expectations are the following: If the rRNA-binding affinities of both initiator proteins are sufficiently large, they should resist the chasing effect better than the other proteins.

Table II: ^{14}C and ^{3}H Label of the Assembled Proteins[a]

	0 min		1 min	
	^{14}C (cpm)	^{3}H (cpm)	^{14}C (cpm)	^{3}H (cpm)
S1	523.5	2242.6	1523.7	7781.1
S2	3147.5	10646.4	3118.7	11436.9
S3	914.5	5324.1	1095.9	4898.4
S4	873.5	4985.6	3458.4	2396.1
S5	1071.3	5855.1	1110.4	6324.9
S6	3833.5	10781.9	9042.2	8488.9
S7	1068.0	6530.4	1353.2	4007.9
S8	4322.8	8333.6	6107.9	6474.9
S9	849.8	3324.4	1126.7	2423.6
S10	1449.3	4659.9	1524.9	5008.4
S13	445.0	1744.1	323.4	1344.4
S15	175.8	560.6	1039.9	699.9
S16	304.3	897.4	1869.7	1151.4
S17	52.0	84.4	299.9	259.9
S18	124.0	378.1	280.9	366.1
S19	2207.8	10077.1	2423.9	6944.9
S20	591.3	3152.1	4439.4	3009.9
S21	342.3	1523.1	364.9	1794.9
blank	22.0	58.9	20.6	35.4

[a] For the 0-min control [^{14}C]- and [^{3}H]TP30 were mixed before being added to the 16S rRNA (molar ratio 16S rRNA:[^{14}C]TP30:[^{3}H]-TP30 = 1:0.7:2.8). For the 1-min value the 16S rRNA was incubated with [^{14}C]TP30 (molar ratio 1:0.7) for 1 min at 29 °C; then, [^{3}H]TP30 (2.8-fold) was added and the incubation continued at 29 °C for 100 min. After reconstitution was completed the nonbound proteins were separated from the reconstituted particles by sucrose gradient centrifugation. The proteins from the reconstituted 30S were isolated by 2D PAGE, and the ^{14}C and ^{3}H label content in the individual proteins was determined. Proteins S11, S12, and S14 were not found on the gels. The given blank values have been subtracted. The $^{14}C/^{3}H$ ratios of the 0-min values differ from 0.53 (S8) to 0.16 (S7). This is probably due to protein–protein complexes present (to a different extent) in the corresponding two TP30 preparations during the labeling procedure. Protein–protein complexes have been observed in various ribosomal protein preparations (Wystup et al., 1979).

However, if the affinities are significantly different from one another, it might be possible to distinguish those proteins which are dependent on one or the other initiator protein.

The gross counts of the 1-min chasing experiment are listed in Table II together with a 0-min control, where the corresponding amounts of [^{14}C]- and [^{3}H]TP30 were mixed before they were added to 16S rRNA. The $^{14}C/^{3}H$ quotients were formed and the 1-min values normalized to the corresponding 0-min values (Table III). The theoretically lowest value is 1, indicating complete chasing (late assembly proteins). The larger the $^{14}C/^{3}H$ value for a protein, the better the protein has resisted the chasing effect.

The protein best resisting the chasing effect should be an initiator protein, and in fact, S4 is found (Table III). The next five proteins following S4 with regard to their chasing stability are S20, S16, S15, S6, and S18. The assembly of these proteins does not depend on S7 according to the assembly map (Figure 3). It follows that they must belong to an assembly nucleus which is initiated by S4, the S4-dependent assembly domain. The proteins following S7 with a $^{14}C/^{3}H$ value significantly larger than 1 are S9, S8, S19, and S3. The assembly of these proteins depends directly or indirectly on S7 with the exception of S8 (Figure 3). We conclude that a second assembly domain exists, the S7-dependent assembly domain, comprising the proteins S7, S9, S19, and S3.

Discussion

The results presented in the preceding sections demonstrate that the in vitro assembly of the 30S subunit is initiated by two assembly-initiator proteins which were identified as S4 and S7. The relative ease with which S7 could be chased at an early assembly stage, in contrast to S4, reflects its relatively

Table III: Normalized $^{14}C/^{3}H$ Quotients of the S Proteins at 1-min Pulse Time[a]

protein	1-min quotient	assembly group
S4	8.93	S4-dependent domain
S20	8.52	
S16	5.19	
S15	5.14	
S6	3.25	
S18	2.54	
S7	2.24	S7-dependent domain
S9	1.97	
(S8	1.97)	
S19	1.73	
S3	1.41	
S10	1.06	late assembly proteins
S5	1.04	
S13	1.02	
S2	1.00	
S21	0.98	
S1	0.91	

[a] The $^{14}C/^{3}H$ quotients of the 0- and 1-min data of Table II, respectively, were formed, and the resulting quotients of the 1-min data were divided by the corresponding values from the 0-min data. The radioactivity in S17 was too low (see Table II) and was not considered here.

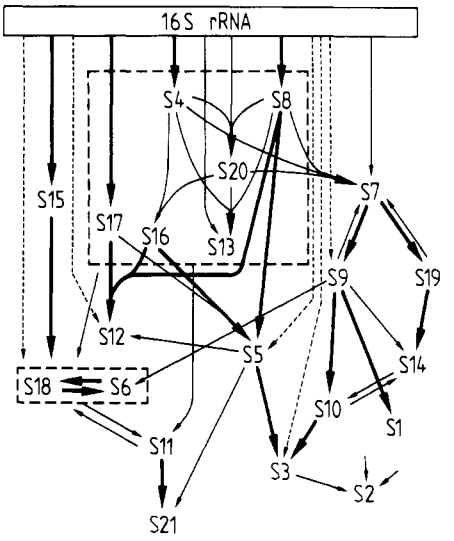

FIGURE 3: Assembly map of the 30S subunit. The map is adapted from Held et al. (1974) and supplemented with the results of Hochkeppel et al. (1976) (broken arrows).

low affinity for 16S rRNA, which had been already noted in early assembly-mapping experiments (see thick and thin arrow to S4 and S7, respectively, from the 16S rRNA bar in Figure 3). The difference in RNA-binding affinities of both initiator proteins obviously extends to the respective proteins which depend on each initiator protein. For example, S4, which has the highest chasing stability (Table III), is followed by S20 and S16, which depend on S4 during assembly (see Figure 3). Likewise, S9 and S19 follow S7 in this list (Table III) and during assembly (Figure 3). The excellent fit with the assembly map indicates two assembly domains: the S4-dependent domain comprises S4, S20, S16, S15, S6, and S18 and the S7-dependent domain S7, S9, S19, and S3.

The only slight disharmony with the assembly map concerns S3. This protein shows a chasing stability above that of S5 and S10, which should bind first before S3 (Figure 3). Two not necessarily alternative explanations can be envisaged. A hitherto undetected assembly dependence exists (presumably to a member of the S7 family), and/or the direct binding of S3 to 16S rRNA contributes to the chasing stability.

The role of protein S8 is not yet clear. According to the assembly map S8 stabilizes the S7 binding together with S4 and S20. These stabilization effects obviously play no important role during initiation of the S7 assembly domain, since S7 has been identified as an assembly-initiator protein on the basis of its noncooperative binding during the onset of assembly. However, such a stabilization might become significant during a later assembly stage, where the two assembly domains must be connected and tightened to form the defined 30S particle. S8 seems to be predestined for such an integrating role, since it positively affects the assembly of components from both domains, namely, that of S20 and S13 from the S4 domain and S7 from the S7 domain. Furthermore, S8 strongly influences the assembly of S5, which likewise connects components from both domains (Figure 3). Therefore, it seems likely that one important role for S8 and S5 is the joining and tightening of both assembly domains during different stages of assembly.

The proteins required for the formation of the RI^{*}_{30} particle fit well with this view. RI^{*}_{30} is the essential, heat-dependent intermediate for the formation of active 30S subunits, and the minimal set of components required for RI^{*}_{30} formation consists of 16S rRNA and the proteins S4, S7, S8, S16, and S19 (Held & Nomura, 1973). Two of these proteins (S4 and S16) belong to the S4 domain and two (S7 and S19) to the S7 domain, and S8 connects both groups. It follows that a minimal organization of, and a defined connection between, both assembly domains is required for the formation of the RI^{*}_{30} particle.

The protein list in Table III represents a sequence of chasing stabilities observed during an early assembly stage in vitro. It does not necessarily reflect the time sequence of the assembly process per se. For example, one can imagine that in vivo the S4 and S7 domains assemble not in parallel but rather sequentially, since the assembly is most probably already initiated *during* the synthesis of 16S rRNA and S4 is known to bind to the 5′-region and S7 to the 3′-region of 16S rRNA (Zimmermann, 1980). Therefore, the assembly of the S4 domain should precede that of the S7 domain. In fact, a 30S precursor particle (p30S) isolated from wild-type *E. coli* cells has been described, which contains the proteins S4, S20, S16, and S15 from the S4 domain but completely lacks the S7 family (S7, S19, S14, S9, S10, S14, and S2; the assignment of S1 was questionable; Nierhaus et al., 1973). It seems likely therefore that the 30S assembly in vivo is governed by an assembly gradient, where the progress of the 16S rRNA synthesis and possibly the rRNA processing dictate the progress of 30S assembly. The existence of an assembly gradient for the 50S assembly has already been made evident [for review, see Herold and Nierhaus (1987)].

Proteins which could be completely chased (chasing value 1 in Table III) either easily exchange with external proteins or are not yet assembled. Exchangeability has indeed been reported for S1, S2, and S21 (Sillers & Moore, 1981; Subramanian & van Duin, 1977; Robertson et al., 1977). Moreover, since all these proteins are located without exception at the periphery of the assembly map (this statement also includes S13; see Figure 3), it seems to be appropriate to term this group "late assembly proteins" (Table III). The agreement with a recent report, where the 30S assembly was resolved into four successive protein-addition steps, is moderately satisfying. Of five late assembly proteins (S1 has not been considered), three are present in the last group, one (S13) is in the second group, and one (S5) is even in the earliest group (Datta et al., 1986). The authors interrupted the in vitro assembly at various times by the addition of a sulfonated polyaromatic dye (Cibacron Blue), making the assumption that the dye stops the assembly but does not interfere directly with the various assembled particles.

The bilobal structure of the 30S assembly, consisting of an S4 assembly domain at the 5′-end of the 16S rRNA and an S7 assembly domain at the 3′-end, corresponds well with the spatial distribution of ribosomal proteins as determined by neutron scattering. In fact, projecting the assembly map onto the 3D arrangement of these proteins has led already to the supposition that the 30S assembly starts with two assembly nuclei (Moore et al., 1986). The S4 domain dominates the assembly of the body and the S7 domain that of the head of the 30S subunit; the body comprises essentially the 5′-region and the head the 3′-region of the 16S rRNA.

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