Nature **314**, 311 (1981); G. Likens *et al.*, *Spektrum Wiss.* **1**, 18 (1981); B. Nilgard, *Ambio* **14**, 2 (1985); M. Ashmore *et al.*, *ibid.* **14**, 81 (1985); L. W. Blanck *et al.*, *Nature* **336**, 27 (1988).
4. O. Kandler, *Naturwiss Rundschau* **11**, 488 (1983); in *Waldschäden*, G. von Kortzfleisch, Ed. (Oldenburg Verlag, München, 1985), pp. 19–61; K. E. Rehfuess, *Allg. Forstz.* **38**, 601 (1983).
5. W. Zech and E. Popp, *Forstwiss. Centralbl.* **102**, 50 (1983); C. Bosch, E. Pfannkuch, U. Baum, K. E. Rehfuess, *ibid.*, p. 167; H. W. Zöttl and E. Mies, *Mitt. Dtsch. Bodenkundl Ges.* **38**, 429 (1983).
6. B. Ulrich and J. Pankrath, Eds., *Effects of Accumulation of Air Pollutants in Forest Ecosystems* (Reidel, Dordrecht, 1983); B. Ulrich, *Ecol. Stud. Anal. Synth.* **61**, 11 (1987); *Adv. Environ. Sci.*, in press.
7. H. Ziegler, *Proceedings of the 14th International Botanical Congress*, Berlin, 1987 (Koeltz, Königstein, 1988); P. Böger and H. Mohr, *Allg. Forst. Zeit.* **27**, 691 (1987).
8. R. Oren, K. S. Werk, J. Meyer, E.-D. Schulze, *Ecol. Stud. Anal. Synth.*, in press; T. S. Kuhn, *International Encyclopedia of Unified Science*, vol. 2 (Univ. of Chicago Press, Chicago, 1970); H. W. Ambrose III and K. P. Ambrose, *A Handbook of Biological Investigation* (Hunter, Winston-Salem, NC, ed. 3, 1977); M. R. Ashmore, in *Air Pollution and Ecosystems*, P. Mathy, Ed. (Reidel, Dordrecht, 1988), pp. 284–287; H. R. Wallace, *Annu. Rev. Phytopathol.* **16**, 379 (1978).
9. E.-D. Schulze, O. L. Lange, R. Oren, Eds., *Air Pollution and Forest Decline*, vol. 77 of *Ecol. Stud. Anal. Synth.* (1989).
10. This article is based mainly on research conducted by the Bavarian Research Group on Forest Toxicology, in which 23 departments of seven universities worked on the same plots of a healthy and declining forest site on phyllite in the Fichtelgebirge, northeast Bavaria, Federal Republic of Germany (50°N, 12°E). Five plots randomly distributed at the healthy site showed no signs of decline, whereas five plots randomly distributed at the declining site showed great variability in decline symptoms ranging from plots with severe damage and tree death to plots that showed no apparent decline symptoms. See (*11*) for a detailed site description and (*9*) for the comprehensive results.
11. R. Oren *et al.*, *Oecologia* **75**, 25 (1988).
12. United Nations Economic Commission for Europe (ECE), ECE Critical Levels Workshop, Bad Harzburg, 14 to 18 March 1988 (Umweltbundesamt, Berlin, 1988).
13. A. Laisk *et al.*, *Planta* **173**, 230 and 241 (1987); J. N. Boyer *et al.*, *Eur. J. For. Pathol.* **16**, 293 (1986); O. L. Lange *et al.*, *Forstwiss Centralbl.* **104**, 186 (1985); R. Zimmermann *et al.*, *Oecologia* **76**, 513 (1988); N. M. Darrall, *Plant Cell Environ.* **12**, 1 (1989).
14. O. L. Lange, U. Heber, E.-D. Schulze, E. Ziegler, *Ecol. Stud. Anal. Synth.*, in press.
15. K. Rost-Siebert, *Ber. Forschungszentrums Waldökosysteme-Waldsterben Göttingen* **12** (1985); E. G. Mulder, *Plant Soil* **7**, 341 (1956); A. Jacob, *Magnesia der Fünfte Pflanzenhauptnährstoff* (Ferdinant Enke Verlag, Stuttgart, 1955); H. Matsumoto and T. Yamaya, *Soil Sci. Plant Nutr.* **32**, 179 (1986); J. R. Cumming, R. T. Eckert, L. S. Evans, *Can. J. Bot.* **63**, 1099 (1985).
16. H. Sandermann, personal communication; E.-D. Schulze, I. McCracken, R. Zimmermann, U. Benecke, *Oecologia*, in press.
17. W. A. H. Asman and A. J. Janssen, *Atmos. Environ.* **21**, 2099 (1987); W. Verhoeven, R. Herrmann, R. Eiden, O. Klemm, *Theor. Appl. Climatol.*, in press.
18. B. Ulrich and H. Meyer, *Ber. Forschungszentrums Waldökosysteme-Waldsterben Göttingen B* 6 (1987).
19. L. Hallbäcken and C.-O. Tamm, *Scand. J. For. Res.* **1**, 219 (1986).
20. U. Falkengren-Grerup, *Oecologia* **70**, 339 (1986).
21. H. Hauhs and R. F. Wright, *Water Air Soil Pollut.* **31**, 463 (1986).
22. N. von Bremen *et al.*, *Plant Soil* **75**, 283 (1983); W. de Vries and A. Breeuwsma, *Water Air Soil Pollut.* **35**, 293 (1987); J. M. Kelly and R. C. Strickland, *ibid.* **34**, 167 (1987); M. Bredemeier, *Ber. Forschungszentrums Waldökosysteme-Waldsterben Göttingen A* **33** (1987).
23. E.-D. Schulze *et al.*, *Water Air Soil Pollut.*, in press; J. Nilsson and P. Grennfelt, "Critical loads for sulfur and nitrogen," ISBN 91-7996-096-0.
24. A. W. Boxman *et al.*, in *Critical Loads for Sulfur and Nitrogen*, I. Nilsson, Ed. (Nordic Council of Ministers and United Nations Economic Commission for Europe, Uppsala, 1988), pp. 295–322; J. G. M. Roelofs *et al.*, *Plant Soil* **84**, 45 (1985); A. W. Boxman *et al.*, *Water Air Soil Pollut.* **31**, 517 (1986); B. Adams, *Soil Biol. Biochem.* **18**, 45 (1986); M. Senser and K. A. Höpker, *Proceedings of the "Forest Damage" Workshop of the GSF*, Munich, 27 February to 1 March 1989 [Gesellschaft für Strahlenforshung (GSF), Munich, in press].
25. E.-D. Schulze and G. Gebauer, *Proceedings of the "Forest Damage" Workshop of the GSF*, Munich, 27 February–1 March 1989 [Gesellschaft für Strahlenforschung (GSF), Munich, in press]; E. Mitterhuber *et al.*, *Plant Cell Environ.* **12**, 93 (1989); C. Katz *et al.*, *Trees*, in press.
26. V. R. Timmer and G. Armstrong, *Soil Sci. Soc. Am. J.* **51**, 1082 (1987).
27. F. S. Chapin III, K. Van Cleve, P. R. Tryon, *Oecologia* **69**, 238 (1986).
28. J. Meyer *et al.*, *ibid.* **76**, 7 (1988).
29. T. Ingestad, *Physiol. Plant* **12**, 568 (1959); *ibid.* **54**, 373 (1979); *Plant Cell Environ.* **5**, 443 (1982); *Geoderma* **40**, 237 (1987); *Scand. J. For. Res.*, in press; S. Linder, *Ecol. Stud. Anal. Synth.* **61**, 180 (1987).
30. R. Oren, E.-D. Schulze, K. S. Werk, J. Meyer, *Oecologia* **75**, 163 (1988); E. K. S. Nambiar and D. N. Fife, *Ann. Bot. (London)* **60**, 147 (1987).
31. S. E. Lindberg *et al.*, *Science* **231**, 93 (1986); G. M. Lovett *et al.*, *ibid.* **218**, 1303 (1982); G. M. Lovell and S. E. Lindeberg, *Biogeochemistry* **2**, 137 (1986).
32. M. Hauhs and R. F. Wright, *Air Pollut. Res. Rep. 11* (Commission of the European Communities, Geneva, 1988).
33. F. Nienhaus, *Jül-Spez-364 ISSN 0343-7639*, 33 (1986); K. E. Rehfuess and H. Rodenkirchen, *Forstwiss Centralbl.* **103**, 248 (1984); J. Suske and G. Acker, *Can. J. Bot.* **65**, 2098 (1987); E. M. Davison, *GeoJournal* **17**, 239 (1989).
34. H. Doehler and M. Wiechmann, *Proceedings of the 4th International CIEC Symposium on Agricultural Waste Management and Environmental Protection*, E. Welte and I. Szabolcs, Eds. (Belgrad, 1987); U. Holzer, H. Doehler, R. Aldag, *VDLUFA-Schriftenreihe* **23**, 265 (1987).
35. Scientific support by H. Mohr, P. J. Crutzen, B. Ulrich, and O. L. Lange in preparing this manuscript is greatly appreciated. Furthermore I thank J. Pate, N. C. Turner, B. Richards, and I. McCracken for editing my English.

RNA-Protein Interactions in 30*S* Ribosomal Subunits: Folding and Function of 16*S* rRNA

SETH STERN,* TED POWERS, LI-MING CHANGCHIEN,† HARRY F. NOLLER

Chemical probing methods have been used to "footprint" 16*S* ribosomal RNA (rRNA) at each step during the in vitro assembly of twenty 30*S* subunit ribosomal proteins. These experiments yield information about the location of each protein relative to the structure of 16*S* rRNA and provide the basis for derivation of a detailed model for the three-dimensional folding of 16*S* rRNA. Several lines of evidence suggest that protein-dependent conformational changes in 16*S* rRNA play an important part in the cooperativity of ribosome assembly and in fine-tuning of the conformation and dynamics of 16*S* rRNA in the 30*S* subunit.

RIBOSOMES ARE LARGE RIBONUCLEOPROTEIN (RNP) structures that are responsible for translation of the genetic code (*1, 2*). As emphasized by Woese (*3*), their biological role links genotype with phenotype, and therefore the evolutionary origins of ribosomes are closely tied to the origin of life as we know

S. Stern, T. Powers, and H. F. Noller are at the Thimann Laboratories, University of California, Santa Cruz, CA 95064. L.-M. Changchien is at the Laboratory of Molecular Biology, University of Wisconsin, Madison, WI 53706.

*Present address: Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.
†Present address: Wadsworth Center for Research, Rockefeller Plaza, Albany, NY 12201.

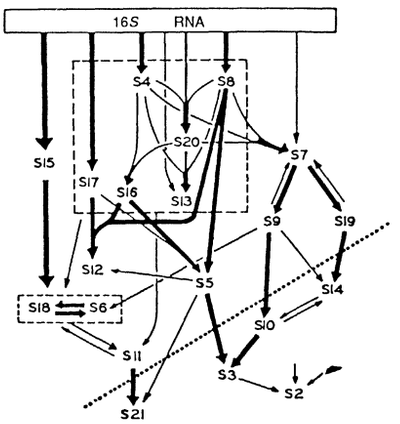

Fig. 1. Assembly map of *E. coli* 30*S* ribosomal proteins. Arrows between proteins indicate a facilitating effect of one protein on another in in vitro assembly of 30*S* subunits; a thick arrow indicates a major effect. Proteins above the dotted line are required for the formation of activated reconstitution intermediate (RI*) particles. [Reprinted from (*43*) with permission, copyright 1973 American Society of Biologic Chemists]

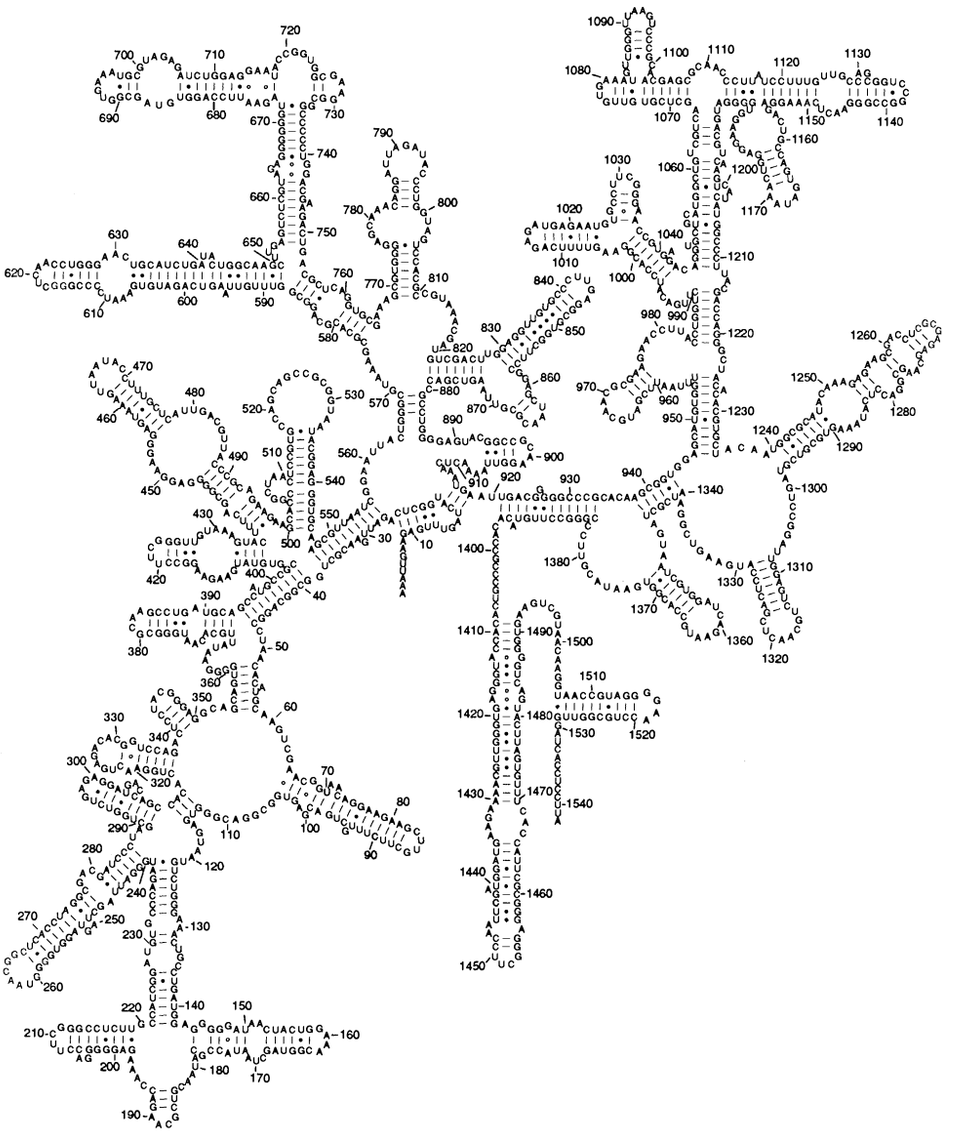

Fig. 2. Secondary structure of *E. coli* 16*S* rRNA, as deduced by comparative sequence analysis (*28*).

it. A deeper understanding of the structure and function of ribosomes is therefore likely to lead not only to a description of the complex interplay of events in protein synthesis, but also to have an important influence on theories of molecular evolution. As a complex RNP, the ribosome serves as a prototype for the study of an increasing number of newly discovered functional RNPs, such as spliceosomes (*4*), signal recognition particle (*5*), ribonuclease P (*6*), and telomerase (*7*).

Ribosomes are divided, both structurally and functionally, into a large and a small subunit. The best understood ribosomal particle is the small (30*S*) subunit, which is involved in the early steps of translational initiation and is the site of codon-anticodon interaction (*1, 2*). During the course of protein synthesis, it interacts with messenger RNA (mRNA), transfer RNA (tRNA), initiation factors, and the large (50*S*) subunit and is involved in regulating the accuracy of translation. The *Escherichia coli* 30*S* subunit is composed of 16*S* ribosomal RNA (rRNA) and 21 different ribosomal proteins (r-proteins), which are assembled into a complex three-dimensional structure held together by noncovalent interactions. In recent years, the focus has shifted from protein to RNA, as a result of a growing body of evidence that RNA is itself the function-determining molecule in ribosomes. Apart from the established interaction between the 3′ terminus of 16*S* rRNA and the initiation region of mRNA (*8*), the presence of universally conserved elements of rRNA structure at or near the site of codon-anticodon interaction (*9, 10*), the peptidyl transferase region (*11, 12*), the sites of interaction of elongation factors EF-Tu and EF-G (*13*), and the interaction sites of various ribosome-directed antibiotics (*14, 15*) argues strongly for the direct involvement of rRNA in translation. In contrast to experience with smaller catalytic RNAs, it has proven difficult to demonstrate function capabilities in protein-free rRNA (*16*). This difficulty may be due to a requirement for structure-stabilizing proteins that are involved in crucial adjustments of the higher order structure of the RNA. Accordingly, an understanding of the molecular mechanism of action of the 30*S* subunit depends on detailed knowledge of the three-dimensional structure of 16*S* rRNA and its interactions with r-proteins.

At present, there is a consensus among electron microscopists about the large-scale morphology of ribosomes and their subunits (*17–20*), and immuno-electron microscopy (IEM) and related methods have allowed identification of the approximate positions of individual r-proteins (*17, 18*) and specific features of rRNA (*17, 18,*

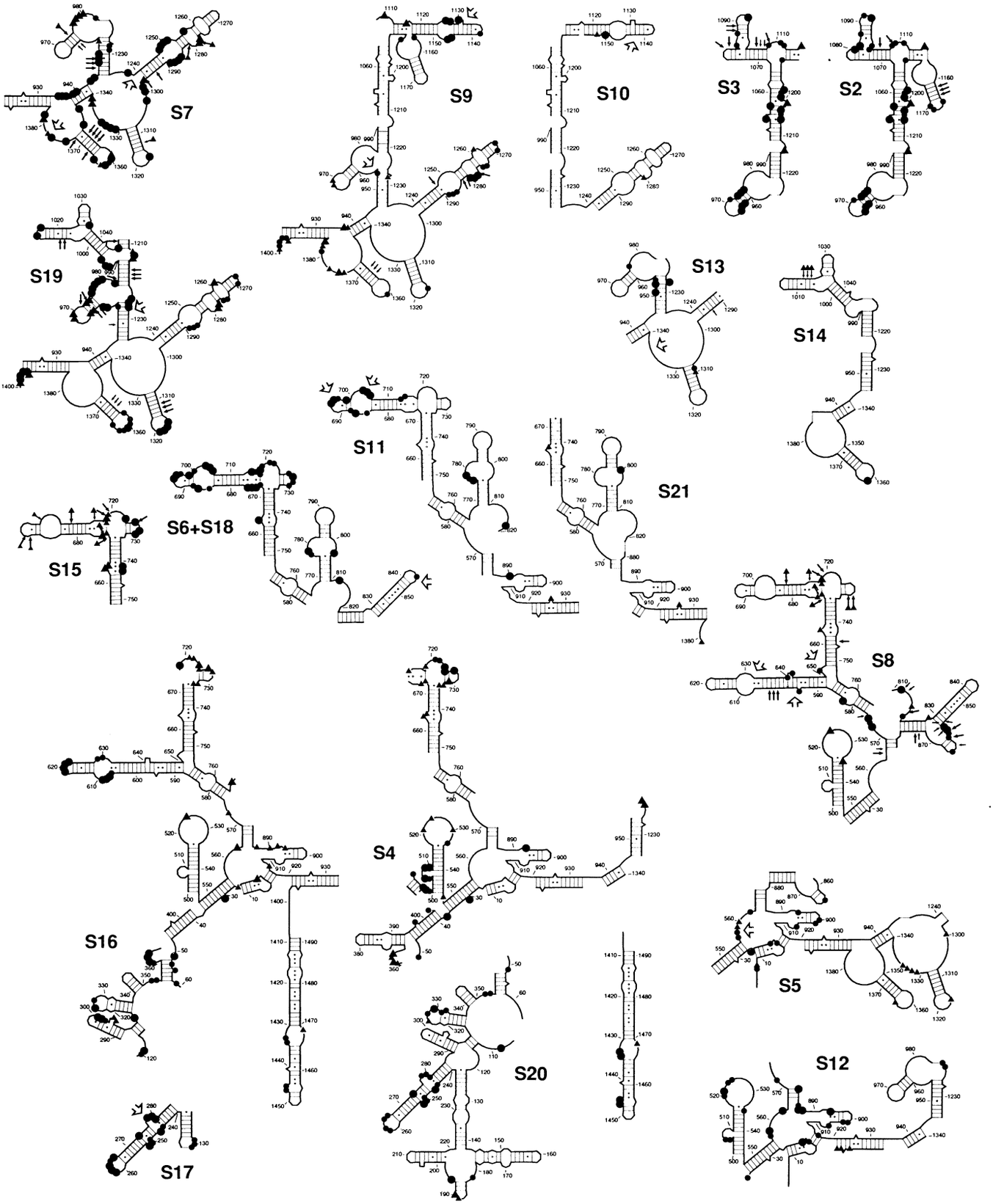

Fig. 3. Effects of assembly of individual 30*S* subunit ribosomal proteins on the reactivities of bases in 16*S* rRNA (*35–40*). Protein-dependent protection from (●) or enhancement of (▲) attack by chemical probes are indicated, as are protection or enhancement of nuclease attack (arrows or arrows with triangles, respectively). Chemical probes (and their base specificities) are kethoxal (G), dimethyl sulfate (A and C), and 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (U and G). Nuclease probes are RNase (ribonuclease) T_1 (G), RNase A (C and U), and RNase V_1 (double strand–specific). Positions where individual proteins have been cross-linked to 16*S* rRNA (*47*) are shown by large open arrows. The proteins are grouped appoximately according to whether their main effects are on the 3′ (top), central (middle), or 5′ (bottom) domain of 16*S* rRNA. Assembly map proximities between proteins (Fig. 1) are often reflected in structural proximities of their effects on the RNA.

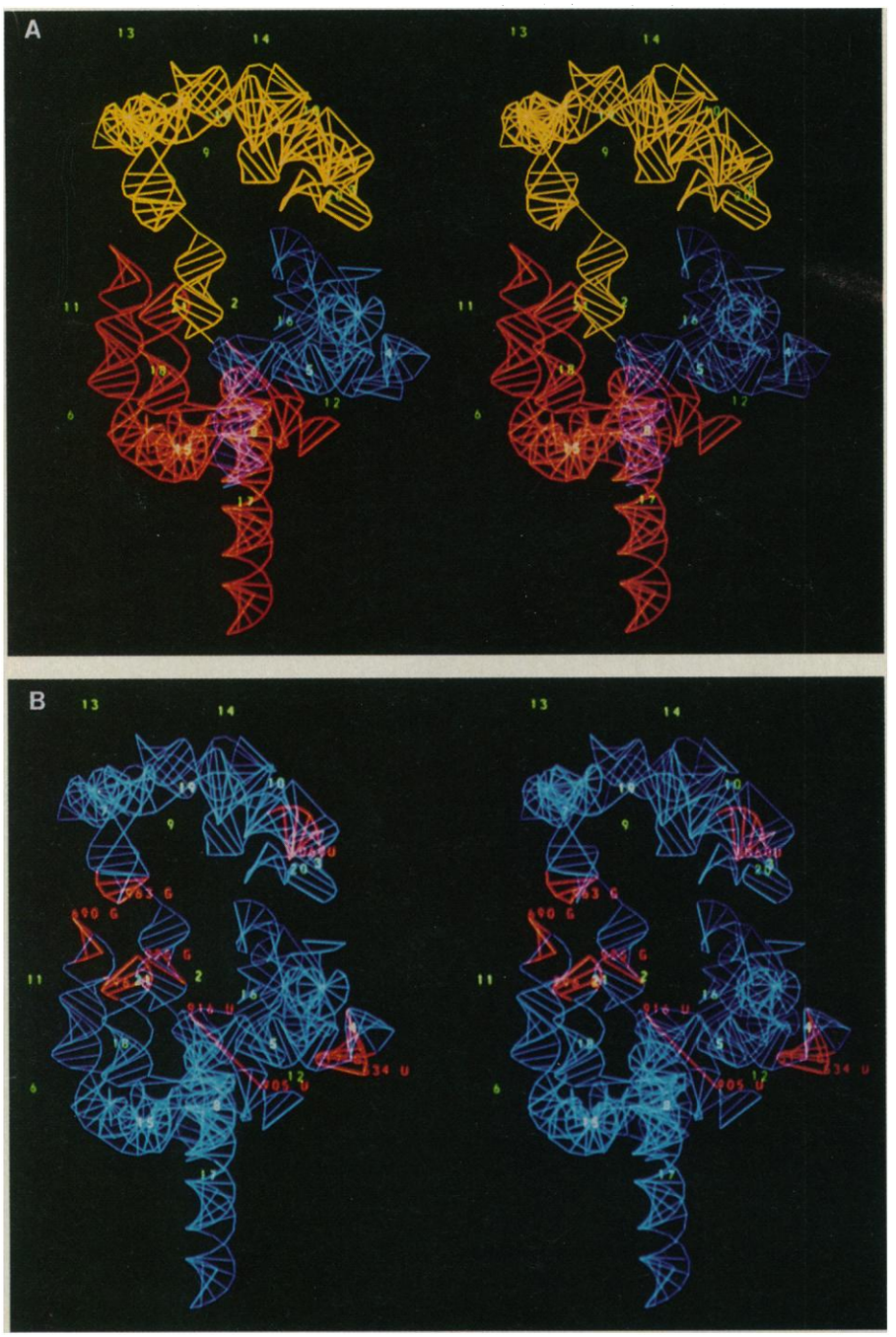

Fig. 4. (**A**) Stereo pair showing a model for the folding of 16*S* rRNA (*48*), viewed from the side away from the 30*S* to 50*S* subunit interface. The 5′, central, and 3′ major domains are shown in blue, red, and yellow, respectively. Numbers show the positions of the centers of mass of the individual ribosomal proteins (*27*). (**B**) As in (A), except that the tRNA-protected regions (*10*) around positions 693, 790, 926, and 966 (upper left); the 530 loop (far right); and the streptomycin- and spectinomycin-protected regions (*14*) around positions 910 and 1064, respectively, are shown in red.

21–24), mRNA (*25*), and tRNA (*26*). Moore and co-workers (*27*) have used neutron diffraction methods to deduce the positions of the centers of mass of all 21 of the small subunit r-proteins, providing the most complete and accurate set of three-dimensional structural data now available for the ribosome. However, relatively little has been learned so far with physical methods about how 16*S* rRNA is folded in ribosomes. In spite of this, a definitive secondary structure has been deduced for 16*S* rRNA on the basis of comparative sequence analysis (*28*) and supported by extensive biochemical evidence (*28–33*).

The structure of the assembled ribosome depends on numerous protein-RNA interactions. For example, if specific contact sites in 16*S* rRNA could be identified for individual 30*S* r-proteins, this information could be used in conjunction with the neutron map of the protein locations to deduce the three-dimensional folding of 16*S* rRNA in 30*S* ribosomal subunits. We therefore used a rapid "footprinting" method, with RNA-specific chemicals and enzymes to probe specific protein-RNA complexes, monitored by primer extension (*34*). This study has yielded extensive, detailed information on the interactions between r-proteins and 16*S* rRNA (*32, 35–40*). Apart from providing the basis for a model for the folding of 16*S* rRNA, these data provide insight into a number of long-standing problems concerning the mechanism of ribosome assembly and function, including the effects of r-proteins on rRNA conformation, the nature of the cooperativity of assembly, specific roles for the various proteins in the assembly process, and whether the reported functional effects of certain ribosomal proteins may be attributable to their influence on the conformation of the RNA.

In Vitro Reconstitution

Successful total in vitro reconstitution of active 30*S* subunits was achieved by Traub and Nomura (*41*). A detailed pathway for in vitro assembly, called the 30*S* assembly map (Fig. 1) was then elucidated by Nomura and his collaborators using step-wise reconstitution with purified r-proteins (*42, 43*). The assembly map describes a complex set of interdependencies between r-proteins during assembly of 30*S* subunits and gives a sense of the high degree of cooperativity of this process. Several proteins, including S4, S7, S8, S15, S17, and S20, bind directly and independently to 16*S* rRNA (Fig. 1). We refer to these as primary binding proteins. Other proteins, which otherwise show no specific affinity for 16*S* rRNA, are capable of assembly contingent on the presence of one or more primary binding proteins (we call these other proteins secondary binding proteins). For example, the presence of the primary binding protein S7 is both necessary and sufficient for assembly of the secondary binding proteins S9 and S19 (Fig. 1). Assembly of the remaining proteins (tertiary binding proteins) requires the presence of one or more secondary binding proteins and sometimes other tertiary proteins, as for protein S21 (Fig. 1). Incorporation of proteins into the assembling RNP particle is accompanied by changes in its hydrodynamic and scattering properties, indicative of a progressive folding into the compact structure of the active subunit. These earlier reconstitution experiments provide a picture

of the assembly process that is intricately cooperative, reflecting the structural complexity of the ribosome itself. More recent studies have been directed toward an understanding of the molecular mechanism of ribosome assembly, and in particular the role of rRNA.

Probing Strategy

We now summarize our efforts to monitor in detail changes in the higher order structure of 16*S* rRNA that occur during the course of in vitro assembly of the *E. coli* 30*S* ribosomal subunit. In our approach (*34*) we have used various chemical and enzymatic probes to measure the accessibility and reactivity of each nucleotide in 16*S* rRNA. Primer extension with reverse transcriptase and a set of complementary DNA oligomers allows us to scan rapidly the 16*S* rRNA chain to assess the precise positions and extent of attack of the probes. Since the chemical probes are relatively small, and their chemistry and structural specificity are well understood, the information obtained is quite detailed and of high resolution. For example, the reactivities of the N1 and N7 atoms of a given guanine base can be monitored independently.

For the primary binding proteins, the experiments are straightforward. A single protein is bound to 16*S* rRNA, and the results of probing the corresponding RNP and naked RNA are compared. For the secondary and tertiary binding proteins, we chose two strategies based on the in vitro assembly studies of Nomura and coworkers. (i) In the "sequential addition" experiments (*42, 43*) we build up RNP particles, one protein at a time, following the assembly map (Fig. 1). Each RNP is probed, and the reactivity pattern of 16*S* rRNA before and after assembly of each protein is compared. (ii) In "single component omission" experiments (*44*), reconstitution is carried out with a mixture of 16*S* rRNA and all of the proteins except one.

Effects of r-Proteins on Reactivities of Bases in 16*S* rRNA

At the outset, we compared the reactivity pattern of naked 16*S* rRNA with that of 30*S* subunits (*32*). The results of this study, which define the initial and final states of the assembly process, show that many changes in the reactivity of rRNA occur during assembly. These differences most likely reflect alterations in tertiary and quaternary structure, for the most part. This conclusion is based on a detailed understanding of the secondary structure of 16*S* rRNA, derived from comparative sequence analysis (*28*) (Fig. 2). Each helix (and, indeed, most of the individual base pairs) is supported by several sets of compensatory base changes found by comparison of 16*S* rRNA sequenced from different organisms. All of the more than 200 sequenced 16*S*-like rRNA molecules can be folded into this secondary structure. Although comparative analysis can provide convincing evidence for the existence of a helix in vivo, it does not address such questions as whether all such helices coexist in the ribosome at the same time or whether they depend on the presence of r-proteins for their stability. Chemical probing provides a strong test for the secondary structure; if two strands are base-paired, they must be unreactive at their Watson-Crick pairing positions. The results for both naked 16*S* rRNA and 30*S* subunits (*32*) are in almost perfect agreement with the predicted secondary structure and provide evidence that all of the predicted helices coexist simultaneously. The data show that in vitro assembly involves relatively little net alteration of RNA secondary structure. Many striking changes occur in unpaired regions and lead us to conclude that most assembly events involve tertiary and quaternary levels of RNA structure.

The effects of assembly are complex; in most cases, the reactivity of bases decreases, but in many instances reactivity is enhanced. We interpret the enhancement as evidence of conformational changes. Decreased reactivity, however, could also be due to rearrangement of the preexisting structure and could also be the result of contact between the protected base and a newly recruited protein. Many of the bases that show a net increase in reactivity in the finished 30*S* particle have been implicated in ribosome function.

All of the 30*S* subunit proteins cause changes in reactivity of specific nucleotides in 16*S* rRNA on assembly (Fig. 3) (*35–40*). These results show that each protein participates, either directly or indirectly, in specific interactions with 16*S* rRNA. Nearly every protein produces enhancements as well as decreases in reactivity, showing that most steps of 30*S* subunit assembly involve alterations of RNA conformation, as discussed below. It is evident that the effects of the primary and secondary binding proteins are generally more extensive than those of the tertiary binding proteins (Fig. 3). This suggests that late-assembling proteins may rely relatively less on protein-RNA interactions and more on protein-protein interactions.

There is good agreement between the observed protection patterns for the primary binding proteins S4, S7, S8, S15, S17, and S20 (*35–38*) and their "binding site fragments" obtained in earlier studies (*45*). The latter were isolated as nuclease-resistant fragments after partial ribonuclease digestion of RNA-protein complexes. With the exception of protein S8 (*37*), most of the protein-specific protections for a given protein are located within its corresponding binding site fragment. Moreover, the protections are usually contained within a localized subregion of the binding site fragment, suggesting that the proteins bind to relatively compact regions of the rRNA and that the large size of some of the binding site fragments may be due to the inherent nuclease resistance of the RNA itself.

Some proteins, such as S4, S8, S15, and S20 (*35–37*), produce effects outside of their binding site fragments. Since the binding constants for the interaction of S4 and S20 with their respective binding site fragments are similar to those measured for their interactions with 16*S* rRNA, it is unlikely that such distal effects represent direct protein-RNA contacts. More likely is the possibility that interaction of these proteins with their binding sites causes disruption of nonspecific RNA-RNA interactions. This interpretation is supported by the fact that many of these distal effects involve enhanced reactivities, such as the S4-dependent effects in the 660 to 735 and 1220 regions (*35–36*), which are outside the S4 binding site fragment. In the case of protein S8, whose binding site fragment has been localized to the 590 to 650 stem region (*44, 46*), enhancements are observed in the 530 and 720 regions (*37*), again suggestive of disruption of nonspecific interactions. In addition, however, there are many strong S8-dependent protections that are outside the binding site fragment, in the 560 to 590 and 810 to 880 regions, raising the possibility that previously unobserved S8 contacts occur in the lower part of the central domain (*37*). The widespread effects of protein S8 observed in our probing studies seem more in keeping with the prominence of its role in assembly (*42, 43*) (Fig. 1).

RNA Conformation and Cooperativity of Assembly

The results of in vitro reconstitution studies, most notably the assembly map, show that the 30*S* subunit assembly involves a complex set of cooperative interactions involving proteins and RNA. A central issue is the basis of the dependence of later

assembling proteins on earlier assembly events. One can imagine two extreme models to explain such dependencies. In one model, based purely on protein-protein interactions, protein A contains the binding site for protein B, explaining why prior assembly of protein A is obligatory for incorporation of protein B. At the other extreme is a model in which cooperativity is mediated by RNA. Binding of protein A produces a conformational change in 16*S* rRNA, unveiling a cryptic RNA binding site for protein B. (Analogous to this would be a third class of model, in which binding of protein A induces a conformational change in protein A, creating a new site for protein-protein contact.) Individual steps of assembly may well involve elements of both kinds of mechanism.

As shown previously by the neutron diffraction studies of Moore and co-workers (*27*), many of the proteins that are linked in the assembly map are near neighbors in the ribosome. Together with the earlier demonstration of specific protein-protein complexes (*47*), the involvement of protein-protein interactions in the assembly process seems likely. Correlations between the RNA probing results (Fig. 3) and the assembly relation between specific proteins (Fig. 1) suggests that protein-dependent conformational changes in 16*S* rRNA also contribute to the cooperativity of assembly.

Assembly of proteins S5 and S12 shows a strong dependency on S16, and assembly of S16 depends in turn on the primary binding protein S4 (Fig. 1). Here, the probing data show a striking parallel to the assembly pathway. Binding of S4 causes enhancement of nucleotides 361 to 364 (*35*), which are then protected specifically on assembly of S16 (Fig. 3) (*36*). Protein S16 produces enhancements around the central loop of 16*S* rRNA at positions 21, 26, 563, 887, and 894 (*36*); these bases are in turn protected by assembly of S5 and S12 (*39*).

Another example is found in the central domain, where protein S15 is required for assembly of S6 + S18 (Fig. 1). Binding of S15 produces enhancements at positions 664, 673, 674, 717, 718, and 719 (*37*); these nucleotides are then protected on assembly of proteins S6 + S18 (Fig. 3).

Protein S7 is crucial for assembly of the proteins associated with the 3′ major domain and we have found that this protein is necessary and sufficient for assembly of the secondary binding proteins S9 and S19 (*38*). The S7 protein causes enhancement of the reactivity of bases in the 980 region, which are subsequently protected by S19, and also causes enhancements in the 1280 loop, where S9-dependent protections are observed (*38*).

These examples, representing proteins that interact with each of the three major domains, all involve enhancements of nucleotides by earlier binding proteins that are subsequently protected by the proteins that follow in the assembly sequence. We cannot say whether these reciprocal effects are manifestations of assembly events that are dependent on RNA conformational changes. It is clear, however, that changes in conformation occur during assembly in regions of 16*S* rRNA that interact specifically with pairs of proteins whose assembly is interdependent.

There are many examples of independent protection of the same nucleotides by different proteins. These effects, which we term polyspecific, provide further evidence for the participation of protein-dependent RNA conformational changes in assembly. Examples of polyspecific protections are S2 and S3 in the 960 and 1050 to 1200 regions (*40*), S5 and S12 in the 900 and adjacent regions (*39*), and S11 and S6 + S18 in the 700 region (*37, 39*) (Fig. 3). Since the different proteins cooperatively stabilize each others' binding, such protection effects cannot be the result of direct contact with these common nucleotides. We suggest that polyspecific effects reflect conformational changes that are stabilized by the binding of proteins to regions of the RNA adjacent to the observed effects, and that the proteins bind preferentially to the altered structure. The result would be to drive assembly forward in a cooperative manner; this is supported by the fact that, in all but one case [S17 and S20 protections in the 270 region (*36*)], proteins that share polyspecific effects are directly linked in the assembly map. This is, in effect, a bidirectional version of the mechanism described above, in which one protein produces a conformational change that is required for assembly of a subsequent protein.

Certain nucleotides are protected only by specific combinations of proteins, which we term cooperative effects. These effects are distinguishable from cooperativity of binding, as for example with proteins S6 and S18, which show very little capability for binding in the absence of each other (*42, 43, 46*). Cooperative protection is observed with proteins S2 and S3 (*40*). Each protein produces changes in the probing pattern independent of the other, but when both proteins are present, an additional set of effects is observed involving positions 1094, 1104, 1108, and 1111. Cooperative effects provide further evidence that protein-dependent conformational changes in 16*S* rRNA are important for ribosome assembly.

A Model for the Three-Dimensional Folding of 16*S* rRNA

We have used the neutron diffraction map for the three-dimensional positions of the r-proteins (*27*) and the phylogenetically established secondary structure for 16*S* rRNA (*28*) as a starting point for modeling studies directed toward deducing the pathway of 16*S* rRNA in the ribosome. The chemical footprinting data, corroborated by cross-linking results, provide a link between the RNA secondary structure and the neutron coordinates. Twelve of the 20 proteins have been localized by protein-RNA cross-linking on intact 30*S* subunits under physiological conditions (*48*), and in every case there is close agreement with the corresponding chemical protection data (Fig. 3). Thus, our protection results are useful in localizing the sites of interaction of proteins with 16*S* rRNA. Placing protected nucleotides within their corresponding protecting proteins strongly constrains the path of the RNA chain in three dimensions. Modeling was done with interactive computer graphics methods (*49*). We now briefly describe the resulting model and some implications for ribosome assembly, structure, and function.

There is a clear resemblance between the model and electron microscopic (EM) images of the 30*S* subunit (*17–20*). Well-known structural features, including the head, platform, cleft, and body are immediately recognizable (Fig. 4). The partitioning of 16*S* rRNA into three major domains, as defined by its secondary structure (*28*), appears to extend, to a large degree, to its three-dimensional structure. This is clearly seen in Fig. 4A, where the three domains are shown in different colors. The 3′ major domain (yellow) forms an autonomous structure corresponding to the head of the EM models. The platform is composed of elements from the central domain (red) and 3′ terminal region, and the body is made up of the 5′ domain (blue) and the remaining parts of the central domain (red).

Clues to the roles of some of the r-proteins in assembly can be inferred from their positions in the model relative to the RNA structure. Certain primary binding proteins such as S4 and S7 are located at the convergence of several helical elements. A potential role for such proteins may be to influence the relative orientation of these helices, a role in keeping with their importance for the assembly process. Other proteins, such as S15 and S19, are positioned in proximity to major bends in the RNA, and may participate in their formation or stabilization. Protein S9 interacts with the two extremities of the 3′ major domain (Fig. 3), possibly stabilizing a long-range interaction. Proteins that act late in assembly produce

subtle changes in regions of the RNA that have been implicated in specific functional roles, and therefore appear to be involved in maturation of the active conformation of these sites, as discussed below.

Implications for Ribosomal Function

Use of this same footprinting approach has led to the identification of sites of interaction of tRNA and antibiotics with 16*S* rRNA (*10, 14*) (Fig. 4B). Positions 693, 794 to 795, 926, and 966, which are protected by P site–bound tRNA or by the P site–directed antibiotic edeine, are clustered on the left side of the model. They line the cleft of the 30*S* subunit, which is the site of codon-anticodon interaction (*26, 50*), consistent with our finding that only the anticodon stem-loop of tRNA is required for protection of these bases (*10*). The A site–bound tRNA protects residues 1408 and 1492 to 1494, which are also constrained within the cleft region (although not constrained sufficiently for placement in our model at present), and residues 529 to 531, which are clearly remote (70 to 100 Å) from the cleft region (located at the right side of the model in Fig. 4B). Since these sites are also protected by the tRNA anticodon stem-loop fragment, which has a maximum dimension of around 25 Å, we conclude that protection of the bases in the 530 region is the result of an allosteric conformational change triggered by binding of the anticodon stem-loop to the A site. The 530 loop is one of the most highly phylogenetically conserved features of RNA; 15 of the 18 nucleotides in this loop are invariant (*28*). At present, there is no clear indication of what its role might be. The apparent proximity of the 530 loop region to the L7/L12 arm of the 50*S* subunit, from EM studies, suggests that it may be involved in some way with the functioning of the elongation factors EF-Tu and EF-G. A third group of bases (class III sites) is protected by tRNA, by certain antibiotics, or by 50*S* subunits at high magnesium ion concentrations (*10, 14*). Since these three ligands do not compete with one another for binding to ribosomes (indeed, they show positive cooperativity in their binding), we infer that protection of these sites is another example of a tRNA-induced conformational change. These class III sites are also generally clustered around the cleft region, but one is found at position 909 (shown at the bottom right in Fig. 4B), near the location of the 530 loop. The protection of class III sites in the cleft region could be explained by closing of the platform against the body when tRNA occupies the cleft region. The effect on position 909 may be related to the tRNA-dependent effects in the 530 loop. This possibility is supported by the fact that protein S12 causes perturbations in both regions (*39*).

Results from our protein assembly experiments have an interesting bearing on these sites of protection by tRNA. One cluster of proteins, which includes S6, S11, S18, and S21, is located in the platform region. Assembly of these proteins affects the reactivities of bases in the 690 loop and adjoining helices and in the base of the 790 region, and enhances the reactivity of G926 (Fig. 3) (*37, 39*), corresponding to three of the four P site protections shown in Fig. 4B. Interestingly, these proteins were shown to be important for mRNA and tRNA binding in earlier experiments (*51*).

Proteins S2, S3, S10, and S14 form another cluster at the upper right of the model. This group of proteins affects the 960 loop region (which also contains a P site protection) in a polyspecific fashion, inducing its mature reactivity pattern (Fig. 3) (*40*). These four proteins have also been implicated in tRNA binding (*52*).

Finally, the cluster of proteins composed of S4, S5, and S12, located at the lower right of the structure shown in Fig. 4, has a well-known history. Mutations in protein S12 confer streptomycin resistance or dependence (*53*). Streptomycin dependence can be suppressed by certain S4 or S5 (*ram*) alleles, providing evidence for functional interaction between these three proteins (*54*). The assembly data (Fig. 3) show striking correlations, the most interesting of which involve the 530 and 900 loop regions which, as described above, are involved in tRNA-dependent conformational changes. Proteins S4 and S12 have antagonistic effects in the 530 loop; bases in this region are mainly enhanced by S4 and protected by S12 (*35, 39*). The S5 and S12 proteins produce several common polyspecific effects in the 900-loop region and in the flanking 20 and 560 regions, at the junction of the three major domains (*39*). Interestingly, a C to U change at position 912 has been reported to confer streptomycin resistance (*55*), and streptomycin itself protects bases in the 911 to 915 region (*14*). Thus, both S4 and S5 produce assembly effects that overlap those of S12, but in distinctly different regions of the structure.

Streptomycin induces misreading of the genetic code (*56*). The S12 alleles conferring streptomycin dependence give rise to ribosomes that are hyper-accurate in the absence of streptomycin (*57*). Ribosomes from S4 and S5 *ram* strains show a high error frequency (*54*). These observations imply that translational accuracy is somehow held in balance at a low level of misreading, by a mechanism that is perturbed by certain mutations in S4, S5, or S12. That these three proteins show overlapping assembly effects on regions of 16S rRNA, which undergo conformational changes on binding of tRNA, lends support to the interesting possibility that translational error frequency may be modulated by rRNA dynamics. It has recently been shown that a point mutation at position 523, in the 530-loop region, confers resistance to streptomycin (*58*).

Conclusions

Structure-specific chemical probing shows that assembly of 30*S* subunits involves changes mainly at the tertiary and quaternary levels of RNA structure. Most of the effects can be assigned to the primary and secondary binding proteins, suggesting that early assembling proteins make extensive interactions with 16*S* rRNA, whereas later assembling proteins tend to be more involved in interactions with other proteins. Ribosomal proteins appear to recognize irregular features of RNA structure, rather than sequences in helical regions. A three-dimensional model for the folding of 16*S* rRNA, based on these results, provides insights into the mechanisms of assembly and action of the 30*S* subunit. Finally, we provide evidence that some of the features of the assembly map and certain previously reported functional properties of ribosomal proteins may in fact be mediated by RNA conformational changes. We conclude that a major role of r-proteins lies in their ability to modulate the higher-order structure of rRNA.

REFERENCES AND NOTES

1. B. Hardesty and G. Kramer, Eds., *Structure, Function and Genetics of Ribosomes* (Springer-Verlag, New York, 1985).
2. G. Chambliss *et al.*, Eds., *Ribosomes* (University Park Press, Baltimore, MD, 1980).
3. C. R. Woese, *ibid.*, pp. 357–373.
4. E. Brody and J. Abelson, *Science* **228**, 963 (1985).
5. P. Walter and G. Blobel, *Nature* **299**, 691 (1982).
6. C. Guerrier-Takada, K. Gardiner, T. Marsh, N. Pace, S. Altman, *Cell* **35**, 849 (1983).
7. C. W. Greider and E. H. Blackburn, *ibid.* **51**, 887 (1987).
8. J. Shine and L. Dalgarno, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1342 (1974); J. Steitz, in (*2*), pp. 479–495; L. Gold *et al.*, *Annu. Rev. Microbiol.* **33**, 365 (1981).
9. J. B. Prince, B. H. Taylor, D. L. Thurlow, J. Ofengand, R. A. Zimmermann, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5450 (1982); J. Ofengand, J. Ciesiolka, R. Denman, K. Nurse, in (*1*), pp. 473–494.
10. D. Moazed and H. F. Noller, *Cell* **47**, 985 (1986).
11. A. Barta, G. Steiner, J. Brosius, H. F. Noller, E. Kuechler, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3607 (1984).

12. D. Moazed and H. F. Noller, *Cell*, in press.
13. D. Moazed, J. M. Robertson, H. F. Noller, *Nature* **334**, 362 (1988).
14. D. Moazed and H. F. Noller, *ibid.* **327**, 389 (1987).
15. ———, *Biochimie* **69**, 879 (1987).
16. D. P. Burma *et al.*, *Arch. Biochem. Biophys.* **239**, 427 (1985).
17. M. Oakes, E. Henderson, A. Scheinman, M. Clark, J. A. Lake, in (*1*), pp. 47–67.
18. G. Stöffler and M. Stöffler-Meilecke, in (*1*), pp. 28–46.
19. M. Boublik *et al.*, in (*1*), pp. 68–86.
20. V. Knauer, R. Hegerl, W. Hoppe, *J. Mol. Biol.* **163**, 409 (1983).
21. S. M. Politz and D. G. Glitz, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1468 (1977).
22. M. R. Trempe, K. Ohgi, D. G. Glitz, *J. Biol. Chem.* **257**, 9822 (1982).
23. H. M. Olson and D. G. Glitz, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3769 (1979).
24. I. N. Shatsky, L. V. Mochalova, M. S. Kojoutiarova, A. A. Bogdanov, V. D. Vasiliev, *J. Mol. Biol.* **133**, 501 (1979).
25. A. G. Evstafieva *et al.*, *EMBO J.* **2**, 799 (1987).
26. P. Gornicki *et al.*, *J. Biol. Chem.* **259**, 10493 (1984).
27. M. S. Capel *et al.*, *Science* **238**, 1403 (1987).
28. C. R. Woese *et al.*, *Nucleic Acids Res.* **8**, 2275 (1980); H. F. Noller and C. R. Woese, *Science* **212**, 403 (1981); C. R. Woese, R. R. Gutell, R. Gupta, H. F. Noller, *Microbiol. Rev.* **47**, 621 (1983); R. R. Gutell, B. Weiser, C. R. Woese, H. F. Noller, *Prog. Nucleic Acid Res. Mol. Biol.* **32**, 155 (1985).
29. P. Stiegler, P. Carbon, J. P. Ebel, C. Ehresmann, *Eur. J. Biochem.* **120**, 487 (1981).
30. R. Brimacombe, J. Atmadja, A. Kyriatsoulis, W. Stiege, in (*1*), pp. 184–202; R. Brimacombe, P. Maly, C. Zwieb, *Prog. Nucleic Acid Res. Mol. Biol.* **28**, 1 (1983).
31. B. J. Van Stolk and H. F. Noller, *J. Mol. Biol.* **180**, 151 (1984).
32. D. Moazed, S. Stern, H. F. Noller, *ibid.* **187**, 399 (1986).
33. H. F. Noller, *Annu. Rev. Biochem.* **53**, 119 (1984).
34. S. Stern, D. Moazed, H. F. Noller, *Methods Enzymol.* **164**, 481 (1988).
35. S. Stern, R. C. Wilson, H. F. Noller, *J. Mol. Biol.* **192**, 101 (1986).
36. S. Stern, L.-M. Changchien, G. R. Craven, H. F. Noller, *ibid.* **200**, 291 (1988).
37. P. Svensson, L.-M. Changchien, G. R. Craven, H. F. Noller, *ibid.*, p. 301.
38. T. Powers, L.-M. Changchien, G. R. Craven, H. F. Noller, *ibid.*, p. 309.
39. S. Stern, T. Powers, L.-M. Changchien, H. F. Noller, *ibid.* **201**, 683 (1988).
40. T. Powers, S. Stern, L.-M. Changchien, H. F. Noller, *ibid.* p. 697.
41. P. Traub and M. Nomura, *Proc. Natl. Acad. Sci. U.S.A.* **59**, 777 (1968).
42. S. Mizushima and M. Nomura, *Nature* **226**, 1214 (1970).
43. W. A. Held, S. Mizushima, M. Nomura, *J. Biol. Chem.* **248**, 5720 (1973).
44. M. Nomura *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **34**, 49 (1969).
45. H. W. Schaup, M. Sogin, C. Woese, C. G. Kurland, *Mol. Gen. Genet.* **114**, 1 (1971); R. A. Zimmermann, A. Muto, P. Fellner, C. Ehresmann, C. Branlant, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1282 (1972); E. Ungewickell, C. Ehresmann, P. Stiegler, R. Garrett, *Nucleic Acids Res.* **2**, 1867 (1975); R. A. Zimmermann *et al.*, *ibid.*, p. 279.
46. R. G. Gregory *et al.*, *J. Mol. Biol.* **178**, 287 (1984).
47. J. Dijk, J. Littlechild, R. A. Garrett, *FEBS Lett.* 77, 295 (1977); M. F. Rhode, S. O'Brien, S. Cooper, K. C. Aune, *Biochemistry* **14**, 1079 (1975).
48. C. Zwieb and R. Brimacombe, *Nucleic Acids Res.* **6**, 1775 (1979); I. Wower and R. Brimacombe, *ibid.* **11**, 1419 (1983); J. Atmadja and R. Brimacombe, *ibid.* **13**, 6919 (1985); J. Atmadja *et al.*, *ibid.* **14**, 659 (1986); W. Stiege, J. Atmadja, M. Zobawa, R. Brimacombe, *J. Mol. Biol.* **191**, 135 (1986); A. Kyriatsoulis *et al.*, *Nucleic Acids Res.* **14**, 1171 (1986); M. Osswald *et al.*, *ibid.* **15**, 3221 (1987); B. Greuer, M. Osswald, R. Brimacombe, G. Stöffler, *ibid.*, p. 3241.
49. S. Stern, B. Weiser, H. F. Noller, *J. Mol. Biol.* **204**, 447 (1988).
50. M. Oakes *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 275 (1986).
51. M. Cantrell and G. R. Craven, *J. Mol. Biol.* **115**, 389 (1977); T. G. Fanning, M. Cantrell, C.-Y. T. Shih, G. R. Craven, *Nucleic Acids Res.* **5**, 933 (1978); C. Chang and G. R. Craven, *J. Mol. Biol.* **117**, 401 (1977); I. Fiser, K. H. Scheit, G. Stöffler, E. Kuechler, *FEBS Lett.* **56**, 226 (1975).
52. D. P. Rummel and H. F. Noller, *Nature New Biol.* **245**, 72 (1973); G. Thomas, R. Sweeney, C. Chang, H. F. Noller, *J. Mol. Biol.* **95**, 91 (1975); M. Shimizu and G. R. Craven, *Eur. J. Biochem.* **61**, 307 (1976); L. L. Randall-Hazelbauer and C. G. Kurland, *Mol. Gen. Genet.* **115**, 234 (1972).
53. M. Ozaki, S. Mizushima, M. Nomura, *Nature* **222**, 333 (1969); E. A. Birge and C. G. Kurland, *Science* **166**, 128 (1969).
54. L. Gorini, *Nature New Biol.* **234**, 261 (1971).
55. P. E. Montandon, R. Wagner, E. Stutz, *EMBO J.* **5**, 3705 (1986).
56. J. Davies, W. Gilbert, L. Gorini, *Proc. Natl. Acad. Sci. U.S.A.* **51**, 659 (1964).
57. L. Gorini, in *Ribosomes*, M. Nomura, A. Tissières, P. Lengyel, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1974), pp. 791–804.
58. P. Melancon, C. Lemieux, L. Brakier-Gingras, *Nucleic Acids Res.* **16**, 9631 (1988).
59. This work was supported by grant no. GM-17129 from the NIH (to H.F.N.) and by grant no. DMB-8521802 from the NSF Biological Instrumentation Program to the University of California at Santa Cruz Molecular Graphics Laboratory. We thank B. Weiser for computer programs and for generating figures, J. Silverthorne and K. Triman for their comments on the manuscript, and P. Svensson for his contribution to this work. We dedicate this paper to the memory of G. R. Craven.

Research Articles

G_{olf}: An Olfactory Neuron Specific–G Protein Involved in Odorant Signal Transduction

DAVID T. JONES AND RANDALL R. REED

Biochemical and electrophysiological studies suggest that odorants induce responses in olfactory sensory neurons via an adenylate cyclase cascade mediated by a G protein. An olfactory-specific guanosine triphosphate (GTP)–binding protein α subunit has now been characterized and evidence is presented suggesting that this G protein, termed G_{olf}, mediates olfaction. Messenger RNA that encodes $G_{olf\alpha}$ is expressed in olfactory neuroephithelium but not in six other tissues tested. Moreover, within the olfactory epithelium, $G_{olf\alpha}$ appears to be expressed only by the sensory neurons. Specific antisera were used to localize $G_{olf\alpha}$ protein to the sensory apparatus of the receptor neurons. $G_{olf\alpha}$ shares extensive amino acid identity (88 percent) with the stimulatory G protein, $G_{s\alpha}$. The expression of $G_{olf\alpha}$ in S49 cyc^- kin^- cells, a line deficient in endogenous stimulatory G proteins, demonstrates its capacity to stimulate adenylate cyclase in a heterologous system.

THE VERTEBRATE OLFACTORY SYSTEM IS EXQUISITELY adapted for the detection and recognition of small molecule odorants. For example, olfactory receptor cells can distinguish the subtle differences between chemical stereoisomers and are sensitive to some odorants at airborne concentrations of parts per trillion (*1*, *2*). Olfaction is probably the oldest means of sensory interaction with the external environment. Although our understanding of other sensory transduction systems (that is, vision and audition) is becoming substantial, little is known about the molecular basis of olfaction.

The vertebrate olfactory mucosa contains several million sensory neurons that reside in a psuedostratified columnar epithelium (*3*).

D. T. Jones and R. R. Reed are at the Howard Hughes Medical Institute, Department of Molecular Biology and Genetics, The Johns Hopkins School of Medicine, Baltimore, MD 21205