Journal of Supramolecular Structure 2:163–165 (1974)

ASSEMBLY OF BACTERIAL RIBOSOMES

Masayasu Nomura

Institute for Enzyme Research, Departments of Biochemistry and Genetics, University of Wisconsin, Madison, Wisconsin 53706

The work from the author's laboratory on the assembly of bacterial ribosomes is reviewed in this article.

RECONSTITUTION AND FUNCTIONAL ANALYSIS OF MOLECULAR COMPONENTS

One example of reconstitution and functional analysis is the identification of the ribosomal components involved in the initiation of natural mRNA translation (1). While Escherichia coli 30S subunits can initiate translation of the coat cistron of RNA phage R17 with high efficiency in vitro, 30S subunits from Bacillus stearothermophilus cannot. To identify the ribosomal components responsible for this difference, reconstitution was performed using E. coli 16S RNA and a mixture of purified E. coli proteins with B. Stearothermophilus components, singly or in combination, substituted for the corresponding E. coli components. It was found that among 17 B. stearothermophilus proteins examined individually, only substitution of B. stearothermophilus S12 resulted in a significant decrease (50%) in the translation of R17 RNA relative to that of poly U. None of the other proteins showed such an effect. In addition to S12, substitution of B. stearothermophilus 16S RNA also caused a 40 to 50% reduction in this ability, and substituion of both B. stearothermophilus S12 and 16S RNA caused a reduction (85%) to the level exhibited by native B. stearothermophilus 30S subunits. These and other experiments indicate that S12 is not only crucial in the general initiation function (2), but also plays an important role, in conjunction with 16S RNA, in determining the efficiency of initiation at the coat cistron of R17 RNA (1). A possible direct interaction between 16S RNA and mRNA initiation sites is suggested.

MECHANISM OF ASSEMBLY IN VITRO

An assembly map of the 30S subunit of E. coli has been constructed from studies on the sequence of addition of 30S ribosomal proteins to 16S RNA in vitro (3, 4). The map shows cooperative effects among the various 30S ribosomal proteins in the assembly reaction. We originally suggested that the assembly map reflects topological relationships between ribosomal proteins in the organized ribosome structure (3). This suggestion was further discussed in the light of recent experiments by other workers on the three-dimensional structure using other approaches, such as the use of bifunctional reagents. It is emphasized that the assembly map would not be expected to predict *all* "neighborhood relationships." Nevertheless, positive agreement between many of the cross-linked pairs and the pairs connected in the assembly map support the topological interpretation of the interrelationships between 30S ribosomal proteins shown in the assembly map.

The question of whether the assembly map really shows the temporal sequence of events during the in vitro assembly reaction was also discussed.

COMPARISON OF ASSEMBLY IN VITRO AND IN VIVO

Although it is highly probable that the information obtained from the in vitro assembly system is pertinent to the in vivo assembly mechanism, the assembly mechanism in vivo is certainly not identical to that in vitro. However, two types of experiments can support the hypothesis that the sequence of addition of ribosomal proteins to 16S RNA in vivo is probably very similar to that found in the in vitro experiments. The first is the analysis of protein compositions of "intermediate" ribonucleoprotein particles isolated in vivo (5, 6), and the second is the recent analysis of the 30S proteins required for the methylation of 16S RNA from a kasugamycin-resistant mutant in vitro (7, 11).
In the latter studies, the proteins required for methylation were determined by adding purified 30S proteins, singly or in combination, to the methyl-deficient 16S RNA under reconstitution conditions, and then testing whether the resulting particles could be methylated. Eight proteins (S4, S8, S16, S17, S15, S18, S6, and S11) were found to be essential (and together, sufficient) for methylation of the 16S RNA. Other proteins (S9, S14, S10, and S3, present together) were found to inhibit methylation of CsCl core particles. The proteins required for methylation are strongly interrelated and occupy early parts of the assembly map while the proteins required for inhibition of methylation, also strongly interrelated, occupy a distal portion of the map. Thus, it is highly likely that the methylating enzyme also acts in vivo on intermediate particles which contain the essential eight proteins and perhaps some other proteins, but lack some or all of the "inhibitory" proteins.

These and other experiments suggest that the assembly of 30S ribosomes in vitro is similar in many respects to that observed in vivo.

FROM GENES TO RIBOSOMES

Problems related to the possible coordinated synthesis of ribosomal components (three RNA molecules and about 50 protein molecules) were discussed (see Ref. 8). The genetic approaches to the problems taken in the author's laboratory were summarized (e.g., see Ref. 9). In addition, recent experiments on the in vitro synthesis of a number of E. coli 30S ribosomal proteins were described. A cell-free system for ribosomal protein synthesis has been developed consisting of ribosomes, initiation factors, RNA polymerase, a fraction containing soluble enzymes and factors, and E. coli DNA (10). A requirement for E. coli DNA for the synthesis of ribosomal proteins has been demonstrated. This system may be useful for the study of the mechanism of the possible coordinated expression of ribosomal protein genes and rRNA genes in growing bacteria.

ACKNOWLEDGMENTS

The author wishes to thank his former as well as present coworkers who have participated in the work described here. The work has been supported by grants from the National Science Foundation (#GB–31086X) and from the National Institutes of Health (#GM–20427–01).

REFERENCES

1. Held, W. A., Gette, W. R., and Nomura, M., Biochemistry 13:2115 (1974).
2. Ozaki, M., Mizushima, S., and Nomura, M., Nature 222:333 (1969).
3. Mizushima, S., and Nomura, M., Nature 226:1214 (1970).
4. Held, W. A., Ballou, B., Mizushima, S., and Nomura, M., J. Biol. Chem. 249:3103 (1974).
5. Nashimoto, H., Held, W., Kaltschmidt, E., and Nomura, M., J. Mol. Biol. 62:121 (1971).
6. Nierhaus, K. H., Bordasch, K., and Homann, H. E., J. Mol. Biol. 74:587 (1973).
7. Helser, T. L., Davies, J. E., and Dahlberg, J. E., Nature New Biol. 235:6 (1972).
8. Davies, J., and Nomura, M., Ann. Rev. Genet. 6:203 (1972).
9. Nomura, M., and Engbaek, F., Proc. Nat. Acad. Sci. U.S. 69:1526 (1972).
10. Kaltschmidt, E., Kahan, L., and Nomura, M., Proc. Nat. Acad. Sci. U.S. 71:446 (1974).
11. Thammana, M., and Held, W. A., Nature, (in press).