Functional Units in Protein Biosynthesis

edited by
R. A. Cox and
A. A. Hadjiolov

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FUNCTIONAL UNITS IN PROTEIN BIOSYNTHESIS

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R. A. COX

National Institute for Medical Research, Mill Hill, London England

and

A. A. HADJIOLOV

Biochemical Research Laboratory, Bulgarian Academy of Sciences, Sofia Bulgaria

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Nomenclature

The proceedings of this symposium reveal the complexity of the ribosome and other macromolecules involved in protein biosynthesis and also the need for standard nomenclature. Three codes were used to describe the same ribosomal proteins and these are collected by Dr. Wittman on p. 6. Although a standard has been agreed this is not yet generally used. Two codes were used to describe initiation and other factors. The description of subribosomal particles is confusing and there are no commonly accepted abbreviations as there are for nucleic acids. We suggest that when applied to ribosome the term subunit should have the same meaning as it has in protein chemistry; namely, a subunit is a single molecule that forms part of a larger structure such as an enzyme. On the basis of this definition an *E. coli* ribosome comprises about 60 subunits, i.e. about 56 protein subunits and at least three RNA subunits and can be compared with haemoglobin which has four subunits.

It seems appropriate that by analogy with RNA, the abbreviation for ribosomes should be RS leading to the abbreviations given in the table

Table 1. Suggested abbreviations for ribosomes, polyribosomes, subribosomal particles and ribosomal RNA.

Species of particle	Proposed abbreviation
Ribosomes	RS
Mitochondrial ribosomes	MRS
Chloroplast ribosomes	ChRS
polyribosomes	poly RS
small (biologically active) subribosomal particle	S-sRS
larger (biologically active) subribosomal particle	L-sRS
smaller (biologically inactive) subribosomal particle prepared by treatment with EDTA	S-sRS(EDTA)
The designation of s value might be informative e.g.	S-sRS(EDTA-16S)
smaller ("core") particle prepared by treatment with 4M CsCl	S-sRS(4M CsCl)
RNA subunit of the smaller subparticle	S-rRNA
largest RNA subunit of the large subparticle	L-rRNA or Ll-rRNA
5S-RNA subunit of the larger subparticle	L2-rRNA
RNA subunit analogous to the 7SRNA of Pene, Knight and Darnell	L3-rRNA

The use of the abbreviation S-sRS and L-sRS is restricted to biologically active subparticles and others are described by the additional information given in parentheses. Thus a larger subparticle lacking 5S-rRNA becomes L-sRS (-5SRNA) and a particle lacking specific proteins, e.g. L15 and L16 (agreed code) can be written as L-sRS(-L15, –L16). Other examples are given in the Table.

There remains the problem of designating the RNA subunits. The proteins are designated as either S or L according to their origin and are given numbers according to their position in two-dimensional electrophoretograms. It seems more appropriate to number RNA subunits in historical order. Moreover rRNA is frequently used to denote ribosomal RNA. Accordingly the RNA subunit of the smaller subparticle is designated S-sRNA. There are two or three possible RNA subunits of the larger subparticle. We propose that the largest subunit be designated (which was the first to be discovered) L-rRNA (formally L1-rRNA). Formally 5S-rRNA should be designated L2-rRNA but the former is so well established that it is unlikely to be superseded. Thus the other species known (e.g. analogues of the 7S RNA of Pene, Knight and Darnell) are designated L3-rRNA.

We believe that a systematic nomenclature offers convenience as well as precision.

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Introduction

FRITZ LIPMANN

The Rockefeller University, New York, New York, U.S.A.

With this Symposium there is a good opportunity to survey the various aspects of functional units in protein biosynthesis. Quite generally, at present there is a phase of consolidation; the framework of the overall process has been mapped out and it is the refinement of detailed mechanisms with which workers are becoming more and more involved. Clearly dominating is the study of the bacterial, and in particular the *E. coli*, ribosomal system, but a fair amount of work will be reported in this symposium on eukaryote systems.

There is now an understanding of the machinery that operates in the bacterial, i.e. prokaryote system, which has been elaborated in great detail, and now appears to serve us well as the foundation of the lay-out in all living systems. The eukaryote protein synthesis differs much less from the prokaryote one than it had seemed it would some time ago. Only relatively recently it has been shown that in eukaryote cells one is actually dealing with two protein synthesizing systems: the cytoplasmic dominates, but eukaryote cells contain another one in their mitochondria and chloroplasts that strongly resembles the bacterial system. Most extracts of eukaryote cells tend to contain both systems because no care is taken to separate the cytoplasm and organelles. Therefore, in view of the interchangeability of the mitochondrial and bacterial systems a certain confusion has arisen.

The indication that mitochondria may be descendants of bacteria seems rather startling and most interesting. In yeast and in *Neurospora* the mitochondrial elongation factors have been separated from the cytoplasmic ones and assayed with bacterial ribosomes. Such separation was worked out for yeast by Richter in our laboratory, and by Küntzel for *Neurospora*. Furthermore, in several laboratories petite mutants of yeast were found to produce nearly as much mitochondrial factors as normal yeast. This suggests mitochondrial factors

to be encoded in nuclear rather than mitochondrial DNA, which would, in any case be much too small to code for all mitochondrial proteins. This reshuffling of coding between organelles and nucleus is quite remarkable.

In spite of distinct differences between eukaryote and prokaryote systems, one is inclined to emphasize today their rather essential similarity. Thus, initiation and termination in the eukaryote-cytoplasmic system, which had remained obscure for so long, have recently been found to differ relatively little from the bacterial mechanism.

Recent observations can now be given on a phase of protein synthesis that has long been of special interest to many, namely the part of the elongation cycle which is called translocation. This well known phase refers to the concerted movement of the messenger RNA and newly elongated polypeptidyl-tRNA from their temporary location, the aminoacyl or acceptor site, to the donor or peptidyl site on the ribosome. Part of the symposium will deal with these details but it is worth mentioning that recent experiments to be reported by Vazquez and his group indicate a common site for the attachment of the aminoacyl-tRNA-T_u-GTP complex and G factor or translocase. This seems to indicate a linkage between the function of GTP in aminoacyl-tRNA binding and in translocation, and to suggest that the same molecule of GTP might be involved in both binding and translocation. The energy released from GTP-breakdown may thus be used in the conjugated sequence of binding of aminoacyl-tRNA, transpeptidation, and translocation, a possibility we have often discussed in our laboratory.

Some generalities may now be given on the increased understanding of parallel features in the machinery that transacts different phases of genetic information transfer. Biochemically, the synthesis of a protein is, in a way, more complex than that of a nucleic acid. Therefore, it is surprising that a more profound understanding of protein synthesis preceded the working out of details in nucleic acid synthesis. It was discovered that in protein biosynthesis one divides the reaction flow into initiation, elongation and termination. It has recently been realized that the same three phases are also a feature of the preceding transcription of DNA to mRNA. With the discovery of sigma and rho factors as initiation and termination complements in the transcription from DNA, the same division into three phases again appears. This parallel design of consecutive sections in the overall scheme unveils an intrinsic feature of replication of a limited sequence from long templates. The special topic of the first section of this volume deals with the constituents of the ribosome, the small-sized organelle common to all living organisms, prokaryote as well as eukaryote.

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Ribosomal Proteins from Prokaryotes

H. G. WITTMANN

Max-Planck-Institut für Molekulare Genetik
Berlin-Dahlem, Germany

ABSTRACT

It was shown by two-dimensional polyacrylamide gel electrophoresis that *E. coli* 30S subunits contain 21 proteins and that 50S subunits contain 34 proteins. The proteins were isolated by a combination of various methods, namely zonal centrifugation, stepwise extraction by salt treatment, CM-cellulose chromatography and gel filtration and they were tested for purity. Pure proteins were characterized with respect to their chemical, physical and immunological properties.

Studies on protein-RNA interactions, on subunit assembly, on stepwise removal of proteins by salts and enzymes, on precursors and on the availability of antigenic determinants in the ribosome gave some information on the topography of *E. coli* ribosomes. The function of some ribosomal components was studied by immunological techniques or by chemical modification.

Proteins from mutants with altered ribosomes were isolated and the altered proteins were studied by methods of sequence analysis. It was found that amino acid replacements were clustered in small regions of the protein chains. Comparison of ribosomal proteins from four *E. coli* strains showed only two (S5 and S7) out of the 55 proteins to differ. There is a rather strong similarity among ribosomes from several genera of the Enterobacteriaceae, e.g. *Escherichia, Salmonella, Shigella, Aerobacter, Proteus, Erwinia* and *Serratia*, whereas only weak relationships exist between Enterobacteriaceae on one hand and other bacterial families, e.g. Bacillaceae, on the other. No immunological cross-reaction could be detected between bacterial ribosomes and those of *Neurospora* mitochondria or of chloroplasts from higher plants. The relationship among 80S ribosomes from different families of higher plants is much closer than that among ribosomes from different bacterial families.

INTRODUCTION

Although the general scheme of protein biosynthesis has been elucidated during the last 10-12 years little is known about the function of the ribosome at a molecular level during this process. This has mainly been due to a lack of information on the detail structure of the ribosomal particle. Only recently such information became available by intensive studies in a few laboratories.

The following is a progress report on studies in our laboratory on ribosomal proteins which were done since our report at the last FEBS Meeting in Madrid (Wittmann *et al.*, 1969). Therefore, mainly work done in our laboratory or in collaboration with other laboratories will be cited. A comprehensive review on structure and function of ribosomal proteins will appear elsewhere (Wittmann and Stöffler, 1972).

Number and Isolation of Ribosomal Proteins

It has been shown by a two-dimensional electrophoresis technique (Kaltschmidt and Wittmann, 1970 a, b) that *E. coli* 30S subunits contain 21 and 50S subunits

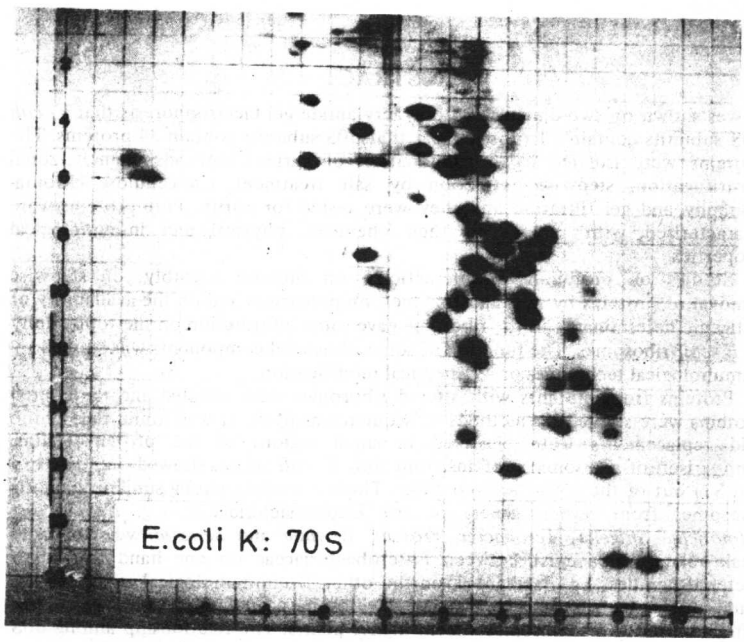

Figure 1. Separation of 70S ribosomal proteins of *E. coli* K by two-dimensional polyacrylamide gel electrophoresis. (For details see: Kaltschmidt and Wittmann, 1970 a, b.)

34 proteins (Fig. 1). These proteins have been isolated from 30S (Hindennach *et al.*, 1971a), from 50S (Hindennach *et al.*, 1971b) and from 70S particles (Kaltschmidt *et al.*, 1971). This was done by a combination of the following methods: Ribosomal subunits were separated by zonal centrifugation in B XV rotors. Proteins from 30S subunits were extracted and separated in CM-cellulose columns with pyridine formate gradients in the presence of urea (Fig. 2). Peaks with only one protein (as shown by disc electrophoresis) were desalted on Bio-Gel and lyophilized. When two or more proteins were present in one peak,

they were separated on Sephadex G 100. Starting from about 25 g of ribosomes relatively large quantities (up to 150 mg) of single ribosomal proteins per CM-cellulose run could be isolated.

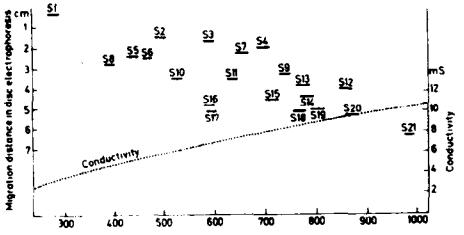

Figure 2. Separation of 30S proteins by CM-cellulose chromatography. Aliquots of fractions were tested for proteins in disc electrophoresis. (For details see: Hindennach *et al.*, 1971.)

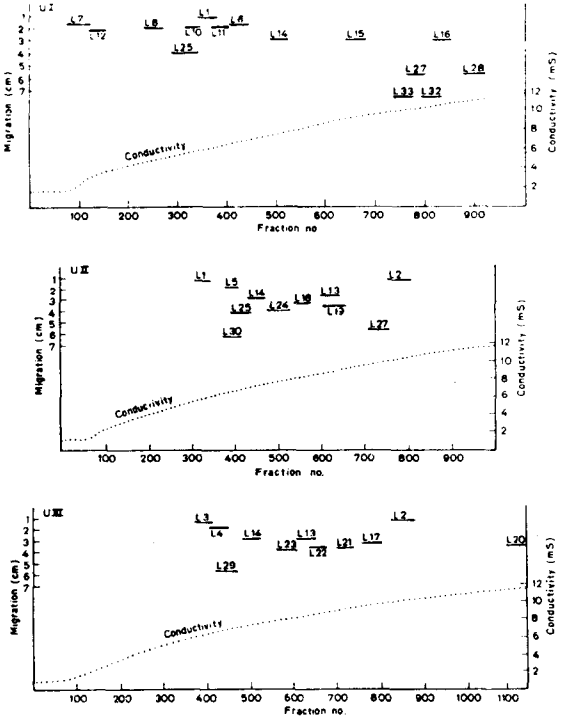

Figure 3. Separation of 50S proteins by CM-cellulose chromatography. 50S subunits were treated with various concentrations of LiCl in absence or presence of urea resulting in fractions UI-UIII. These were applied on CM-cellulose columns. Aliquots of fractions were tested for proteins in disc electrophoresis. (For details see: Hindennach *et al.*, 1971.)

In addition to the methods used for separation of 30S proteins, stepwise extraction of 50S proteins with LiCl in the absence and presence of urea was used as an additional fractionation step. In this way 50S proteins were fractionated into three groups (UI-UIII) which were then applied to CM-cellulose columns (Fig. 3). Further isolation of 50S proteins was done as described for 30S proteins.

The purity and identity of the isolated proteins were tested by two-dimensional polyacrylamide gel electrophoresis. This method and immunological techniques were used for the correlation of 30S proteins isolated in different laboratories which agreed on a common nomenclature, namely S1-S21 (Wittmann *et al.*, 1971). The correlation is given in Table 1.

Table 1. Correlation of 30S ribosomal proteins studied in four laboratories

Berlin	Uppsala	Madison	Geneva
S 1	1	P 1	13
S 2	4a	P 2	11
S 3	9 (+5)	P 3	10b
S 4	10	P 4a	9
S 5	3	P 4	8a
S 6	2	P3b + P3c	10a
S 7	8	P 5	7
S 8	2a	P 4b	8b
S 9	12	P 8	5
S10	4	P 6	6
S11	11	P 7	4c
S12	15	P10	
S13	15b	P10a	
S14	12b	P11	
S15	14	P10b	4b
S16	6	P 9	4a
S17	7		3a
S18	12a	P12	2b
S19	13	P13	2a
S20	16	P14	1
S21	15a	P15	0

Chemical, Physical and Immunological Properties of Proteins

The isolation of pure ribosomal proteins in relatively large quantities enabled us to do the following studies on the properties of these proteins:

(1) Molecular weights were done by two methods: Equilibrium sedimentation in an analytical ultracentrifuge and SDS gel electrophoresis. The values from both methods are in good agreement (Table 2) and show that the range for the

Table 2. Molecular weights of ribosomal proteins of *E. coli*

Protein	SDS-gel	Equil. sed.	Protein	SDS-gel	Sedimentation	Protein	SDS-gel	Sedimentation
S 1	65,000	n.d.	L 1	26,700	22,000	L21	13,900	14,000
S 2	28,300	24,000	L 2	31,500	28,000	L22	14,800	17,000
S 3	28,200	23,000	L 3	27,000	23,000	L23	12,700	12,500
S 4	26,700	23,000	L 4	25,800	28,500	L24	14,300	17,500
S 5	19,600	18,500	L 5	22,000	17,500	L25	12,000	12,500
S 6	15,600	15,500	L 6	22,200	21,000	L26	12,000	12,500
S 7	22,700	26,000	L 7	13,400	15,500	L27	12,700	12,000
S 8	15,500	15,500	L 8	17,300	19,000	L28	12,300	15,000
S 9	16,200	14,500	L 9	17,300	n.d.	L29	12,000	12,000
S10	12,400	18,000	L10	19,000	21,000	L30	11,200	10,000
S12	17,200	15,000	L11	19,600	19,000	L31	10,000	n.d.
S13	14,900	14,000	L12	13,200	15,500	L32	10,500	n.d.
S14	14,000	14,000	L13	17,800	20,000	L33	10,500	9000
S15	12,500	13,000	L14	16,200	18,500	L34	9600	n.d.
S16	11,700	13,000	L15	17,500	17,000			
S17	10,900	n.d.	L16	17,900	22,000			
S18	12,200	10,500	L17	16,700	15,000			
S19	13,100	14,000	L18	14,300	17,000			
S20	12,000	12,500	L19	14,900	17,500			
S21	12,200	13,500	L20	17,200	16,000			

molecular weights of all proteins (except protein S1) is about 10,000-30,000. The mean value is about 17,500 (Dzionara *et al.*, 1970).

(2) Isoelectric points. As expected from their electrophoretic behaviour most ribosomal proteins are very basic with isoelectric points of more than pH 8 (Kaltschmidt, 1971). Only a few proteins (S6, L7 and L12) have isoelectric points of about pH 5 (Table 3).

Table 3. Isoelectric points of ribosomal proteins of *E. coli*

30S		50S			
S 1	< 7.6	L 1	9.2	L24	10.7
S 2	6.7	L 2	> 12.0	L25	9.4
S 3	12.0	L 3	9.7	L26	n.d.
S 4	10.4	L 4	7.6	L27	> 12.0
S 5K	9.9	L 5	9.4	L28	n.d.
S 5B	10.4	L 6	10.0	L29	10.0
S 6	4.9	L 7	4.8	L30	> 12.0
S 7K	12.2	L 8	6.3	L31	n.d.
S 7B	12.3	L 9	6.4	L32	11.3
S 8	9.1	L10	7.5	L33	> 12.0
S 9	> 12.0	L11	9.7	L34	n.d.
S10	7.9	L12	4.9		
S11	> 12.0	L13	10.1		
S12	> 12.0	L14	12.3		
S13	> 12.0	L15	> 12.0		
S14	> 11.0	L16	> 12.0		
S15	> 12.0	L17	> 11.0		
S16	11.6	L18	12.0		
S17	9.7	L19	> 12.0		
S18	> 12.0	L20	> 12.0		
S19	> 12.0	L21	8.2		
S20	> 12.0	L22	11.5		
S21	> 12.0	L23	9.6		

(3) Amino acid compositions. As was expected from their electrophoretic mobilities most ribosomal proteins are very rich in lysine and/or arginine. Some of the proteins contain 25 to 35% of these two amino acids (Kaltschmidt *et al.*, 1970). Other proteins are rich in some other amino acids, e.g. proteins L7 and L12 in alanine (24%) and glutamic acid or glutamine (15%). In spite of these differences between some proteins the amino acid compositions of most of them is surprisingly uniform. Nevertheless all of them (with exception of L7 and L12) differ in their amino acid compositions. Recent studies on the primary structure of L7 and L12 (Terhorst *et al.*, 1972) indicate that the only difference between

them is a *N*-acetylserine in L7 and an unblocked serine in L12 at the *N*-terminal end of the protein chain.

(4) N-terminal groups. From 32 proteins studied by Edman degradation, 12 gave methionine, 10 alanine and 5 various other amino acids whereas no free N-terminus was found in 5 proteins (Wittmann *et al.*, 1969). The latter finding could have been caused by glutamine as the first amino acid or by blocked N-terminal amino acids. Formyl groups have been found in ribosomal proteins (Hauschild-Rogat, 1968) and it was estimated that two 30S proteins and nine 50S proteins are blocked by formyl groups. As mentioned above the 50S protein L7 has a *N*-acetylserine at the N-terminus (Terhorst *et al.*, 1972).

(5) Secondary structure. The α-helix content of ribosomal proteins was studied by circular dichroism and found to be in the range of 20-35% (Dzionara, 1970). Only two proteins of the 50S subunits, namely L7 and L12, have a considerably higher value of about 55%. This finding is in good agreement with the extraordinarily high content in these proteins of alanine and glutamic acid residues which are known to preferentially form α-helices.

(6) Peptide maps. Isolated proteins from 30S and 50S subunits were split by trypsin, the peptides chromatographed on a cation exchanger and the elution profiles after staining with ninhydrin monitored in a peptide analyser. It was found (Peeters *et al.*, 1971) that only two out of almost 50 investigated proteins gave identical peptide maps. These two proteins, L7 and L12, are shown to differ probably only in he N-terminal groups as mentioned above.

(7) Analyses of peptides. The tryptic peptides of 18 proteins were isolated on a preparative scale by column and paper chromatography and their amino acid compositions determined (Wittmann-Liebold, 1971 and unpublished). Furthermore, sequence analyses were done on a part of these peptides. The results show that there are no common peptides longer than three amino acids among the investigated proteins.

(8) Immunological studies. Antibodies were prepared against all of the 30S and most of the 50S proteins and used to study the immunological relationship among the ribosomal proteins. No cross reaction was found between any of the 30S proteins (Stöffler and Wittmann, 1971a, b) (Figs 4 and 5) or the studied 50S proteins with the exception of L7 and L12 (Stöffler, unpublished, Stöffler and Wittmann, 1971b). The two latter proteins gave complete cross reaction demonstrating at least a very high sequence homology. This finding is in very good agreement with the protein-chemical studies on these proteins.