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Correlation of 30S Ribosomal Proteins of *Escherichia coli* Isolated in Different Laboratories

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Summary. Ribosomal proteins isolated from 30S subunits of *E. coli* in four laboratories have been correlated by using two-dimensional gel electrophoresis, immunological techniques, amino acid compositions and molecular weights. The results are given in the Table. A common nomenclature for naming 30S ribosomal proteins and their genetic loci is proposed.

Introduction

The isolation and characterization of the ribosomal proteins of *E. coli* have been pursued in a number of laboratories (Kaltschmidt *et al.*, 1967; Traut *et al.*, 1967; Fogel and Sypherd, 1968; Moore *et al.*, 1968; Hardy *et al.*, 1969; Craven *et al.*, 1969; Nomura *et al.*, 1969; Dzionara *et al.*, 1970; Kaltschmidt *et al.*, 1970a). At present there is general agreement that the 30S ribosomal subunits contain twenty-one different proteins and that the 50S subunits contain another nonverlapping set of 30–35 proteins (Traut *et al.*, 1969; Kaltschmidt and Wittmann, 1970b; Mora, Thamana, Donner, Lutter, Craven and Kurland, manuscript in preparation).

The simultaneous development of *in vitro* reconstitution techniques for the ribosomal subunits (Traub and Nomura, 1968) as well as the identification of genetic loci which specify the structures of some of these proteins (see recent review by Nomura, 1970) makes it extremely awkward for each laboratory to retain its own nomenclature for the identification of this large number of proteins. Therefore, we wish to adopt a uniform nomenclature that can be used by all laboratories studying the ribosomal proteins of *E. coli*.

The implementation of any arbitrarily chosen uniform nomenclature would be an improvement over the present use of individual numbering systems. It would be particularly helpful if the uniform nomenclature were based on a fractionation system that is both simple and yet sufficiently powerful to resolve all of the ribosomal proteins in a single fractionation step. Then, any laboratory wishing to identify a given protein could do so with a minimum of effort. From this point of

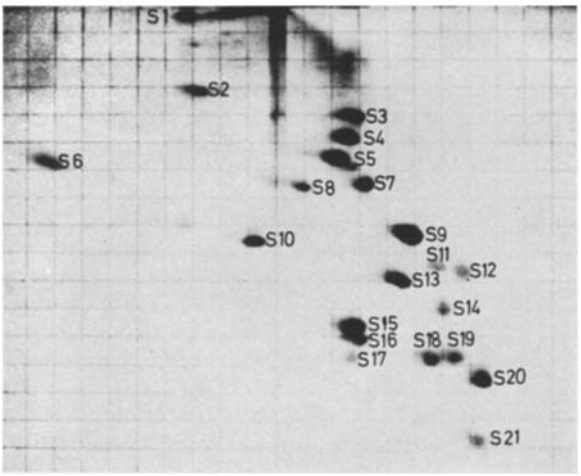

Fig. 1. Proteins S1–S21 from *E. coli* C after two-dimensional polyacrylamide electrophoresis. Conditions as described by Kaltschmidt and Wittmann, (1970a)

view, the two-dimensional gel electrophoresis procedure of Kaltschmidt and Wittmann (1970a) is nearly ideal. Therefore, we wish to adopt the numbering system that has grown out of this technique. The present communication describes the correlations between the numbering systems for 30S ribosomal proteins used by several laboratories. In addition, a rational system for the designation of genetic loci that specify the structures of ribosomal proteins is presented.

Comparison of Ribosomal Proteins

Once ribosomal proteins have been purified, there are a number of straightforward ways to compare the samples obtained in different laboratories. If rather large quantities of each protein are available (3–5 mg) the amino acid compositions, the molecular weights and the finger prints can be compared. With small amounts (0.3–5 μg) of the proteins, sensitive immunochemical methods (Stöffler, 1969; Wittmann *et al.*, 1969; Traut *et al*, 1969; Stöffler and Wittmann, 1971; Ahmad-Zadeh and Traut, to be published) can be employed if specific antibodies are available. Small quantities of many ribosomal proteins can also be identified by analysis of radioactive protein with a combination of chromatographic and electrophoretic techniques (Otaka, Itoh and Osawa, 1968) or by radioimmuno-assays (Stöffler, to be published). In the intermediate range (20–80 μg) samples can be compared by co-electrophoresis according to the two-dimensional electrophoresis of Kaltschmidt and Wittmann (1970a).

These methods, especially immunological techniques and two-dimensional electrophoresis, were used to correlate the 30S ribosomal proteins described by the groups in Berlin (Kaltschmidt and Wittmann, 1970b), in Uppsala formerly Madison (Kurland *et al.*, 1969), in Madison (Nomura *et al.*, 1969) and in Geneva (Traut *et al.*, 1969). The results are given in the Table. We agree to use a common nomenclature (S1–S21 as shown in Fig. 1) for 30S ribosomal proteins of *E. coli* in

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

Berlin	Uppsala					Madison					Geneva				
Code	Code	e	i	a	m	Code	e	i	a	m	Code	e	i	a	m
S1	1	+	+	+	+	P1	+				13			+	+
S2	4a	+	+	+	+	P2	+				11	+	+	+	+
S3	9 (+5)	+	+	+	+	P3	+				10b	+	+	+	+
S4	10	+	+	+	+	P4a	+				9	+	+		+
S5	3	+	+	+	+	P4	+				8a	+	+	+	+
S6	2	+	+	+	+	P3b + P3c	+	+			10a	+	+	+	+
S7	8	+	+	+	+	P5	+				7	+	+	+	+
S8	2a	+	+	+	+	P4b	+				8b	+	+		+
S9	12	+	+	+	+	P8	+	+			5	+	+	+	+
S10	4	+	+	+	+	P6	+				6	+	+	+	+
S11	11	+	+	+	+	P7	+	+	+		4c	+	+	+	+
S12	15	+	+	+	+	P10	+								
S13	15b	+	+			P10a	+								
S14	12b	+	+	+	+	P11	+								
S15	14	+	+	+	+	P10b	+				4b	+	+	+	+
S16	6	+	+	+	+	} P9	+	+			4a			+	+
S17	7	+	+	+	+						3a	+	+		+
S18	12a	+	+	+	+	P12	+				2b	+	+		+
S19	13	+	+	+	+	P13	+				2a	+	+	+	+
S20	16	+	+	+	+	P14	+				1	+	+	+	+
S21	15a	+	+	+	+	P15	+				0				+

Correlation with S1–S21 were done by: e: two-dimensional polyacrylamide gel electrophoresis; i: immunological techniques, especially by Ouchterlony's double diffusion test (Fig. 2); a: amino acid compositions; m: molecular weights.

Furthermore, migration in polyacrylamide disc electrophoresis and elution from CM-cellulose or cellulose phosphate columns have been used for correlation.

Proteins S11, P7 and 11 (from Uppsala) give two spots in two-dimensional electrophoresis: one at position of S11 and the other at position of S9. Protein 4c (from Geneva) gives one spot at position of S11 (Fig. 3). Protein S11, P7, 11 (from Uppsala) and 4c (from Geneva) are immunologically identical and give no cross reaction with anti-S9.

Protein 0 from Geneva is similar to S21, P15 and 15a with respect to migration in disc electrophoresis and in molecular weight. New results (R.R. Traut, unpublished) give a lower molecular weight (15500 daltons) for protein 0 than previously reported (Traut *et al.*, 1969). This new value is in better agreement with data of other groups.

Protein 3b (Traut *et al.*, 1969) corresponds to one of three proteins (S12, S13 or S14) based on comparison of molecular weights and mobility in disc electrophoresis. Protein P3a (Nomura *et al.*, 1969) and protein 12 (Traut *et al.*, 1969) correspond to none of the proteins S1–S21. Although protein P3a gives a spot at a position almost identical to the S5 position, P3a can be clearly distinguished from S5 in the immunological tests and by two-dimensional gel electrophoresis.

future publications. Correlations with 30S ribosomal proteins studied by other laboratories are intended.

The methods used by the Madison, Uppsala and Geneva groups to define their nomenclatures include chromatography on phosphocellulose and carboxymethylcellulose, and gel electrophoresis in urea and sodiumdodecylsulfate. These methods have been fully described in the publications of each group. The correlations in the Table make it possible in most cases for laboratories using any of the identifi-

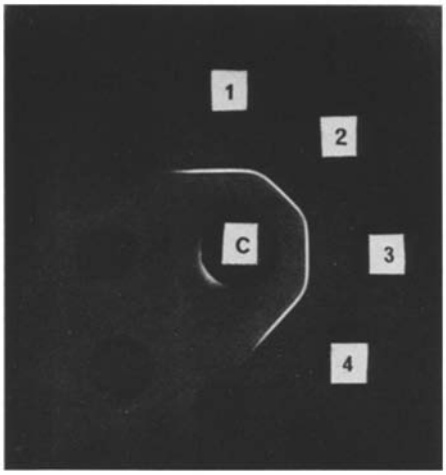

Fig. 2. Double diffusion test (Ouchterlony): 1.5% Agarose; 0.1 M Tris-HCl; pH 8.2; 1.0 M LiCl. Center well: Anti-S9 (rabbit 132). Peripheral wells: *1* protein S9 (Berlin), 2 μg; *2* protein 12 (Uppsala), 2 μg; *3* protein P8 (Madison), 2 μg; *4* protein 5 (Geneva), 1 μg

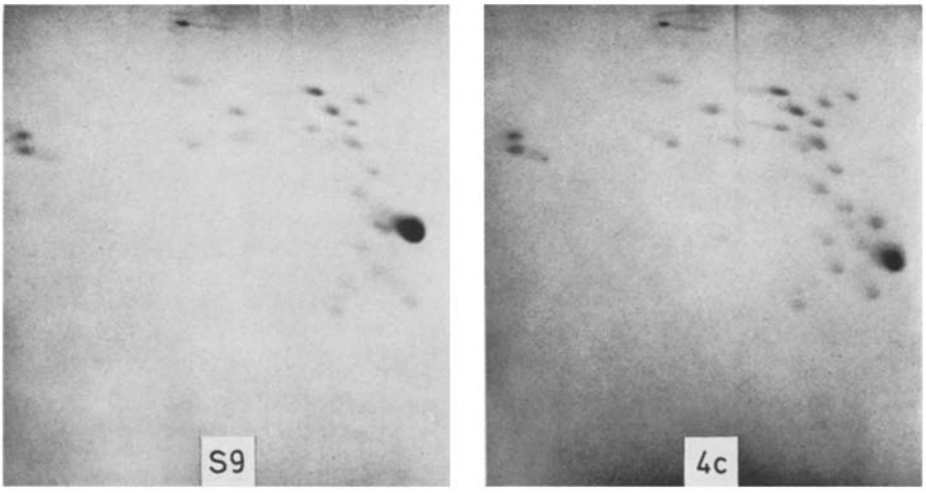

Fig. 3. Coelectrophoresis of proteins S9 (left) and 4c from Geneva (right) with an added background of 70S ribosomal proteins in two-dimensional electrophoresis

cation methods, employed prior to the availability of the two-dimensional technique, to relate their designations to the new nomenclature.

Designation of Genetic Loci

The genetic loci for some of the 30S ribosomal proteins have been positioned on the genetic map using mutants with altered ribosomal proteins. It has been established that altered forms of protein S4 give rise to a suppression of streptomycin dependence (Birge and Kurland, 1970; Deusser *et al.*, 1970), S5 to resistance

to spectinomycin (Bollen *et al.*, 1969; Dekio and Takata, 1969; Flaks *et al.*, 1966), S7 to the K-character (Leboy *et al.*, 1964; Mayuga *et al.*, 1968; Birge *et al.*, 1969; Sypherd, 1969, Osawa *et al.*, 1970; Kaltschmidt *et al.*, 1970b) and S12 to resistance to or dependence on streptomycin (Birge and Kurland, 1969; Ozaki *et al.*, 1969).

Some of these genetic loci have previously been labelled by three letter designations which bear some relation to the ways that the mutants have been selected. However, if this random procedure for naming genetic loci that specify the ribosomal proteins continues, we will end up with a collection of fifty or so completely unrelated designations. This would create an unnecessary degree of confusion for people working on the *E. coli* ribosome and it would restrict the accessability of this work to people outside of the field. Therefore, we wish to recommend a rational scheme to name the genetic loci for ribosomal proteins before the number of such genetic loci which have been identified becomes much larger.

It has been suggested that the uniform nomenclature for naming the ribosomal proteins also be employed in naming the corresponding genetic loci (Birge and Kurland, 1970). Thus, genetic loci for proteins S1 through S21 would be designated *rpx* A through *rpx* U. According to this nomenclature the genetic loci for proteins S4, S5, S7 and S12 would be *rpx* D, *rpx* E, *rpx* G and *rpx* L, respectively.

It would be impossible to use this procedure while the analysis of new ribosomal mutations is at an early stage. This small degree of confusion can be remedied as soon as the altered protein in the mutant has been identified. As a consequence, the earlier genetic designations such as *str* A for *rpx* L can be viewed in much the same manner that the initial protein designations were viewed when they were introduced by each separate laboratory, namely as a necessary tentative classification.

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