METHYLATION OF PROTEINS IN 40 S RIBOSOMAL SUBUNITS FROM *SACCHAROMYCES CEREVISIAE*

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1. Introduction

Ribosomal proteins are subject to post-translational modification. Although the biological significance of this phenomenon is not understood, it is conceivable that acetylation or methylation of amino groups in ribosomal proteins may play a vital physiological role.

Methylated amino acids have been detected in ribosomal proteins from *Escherichia coli, Blastocladiella emersonii, Euglena gracilis,* cultured muscle cells and HeLa cells [1–11]. Several of these studies have indicated that methylated amino acids occur in the larger of the two ribosomal subunits. Thus in *E. coli* at least six proteins in the 50 S particle are methylated [5–7] with methylation of proteins L11 and L18 being very apparent. Methylated proteins also occur in 60 S particles from *Saccharomyces cerevisiae* [12]. In particular protein L15 (their nomenclature) was found to be heavily methylated, the extent of methylation vastly exceeding that shown by any of the ribosomal proteins from *E. coli*.

The situation with respect to the smaller ribosomal subunit is more confused. Although it was claimed [5] that methylated amino acids in *E. coli* ribosomal proteins were confined predominantly to 50 S particles, the possibility of methylation in proteins S10 and S16 was conceded – although data for these proteins were unconvincing. In HeLa cells dimethylarginine and di- and trimethyllysine could be detected in both ribosomal subunits although the proteins involved were not identified [9]. Methylation was, however, higher in the 60 S particle and in fact exposure of 40 S particles to high salt (0.6 M KCl) led to a loss of some of the arginine-associated methyl groups. Better evidence that methylated amino acids occur in the small ribosomal subunit is in [13] where *N*-α-monomethylated amino acids appear to occur at the N-terminus of the *E. coli* ribosomal proteins S11, L16 and L33.

We have studied here the methylation of proteins from the 40 S ribosomal subunit of *S. cerevisiae*. Our results indicate that 2 and possibly 3 other proteins possess methylated amino acids. However, in all cases the level of methylation is very low and does not approach the levels observed for certain proteins of the 60 S ribosomal subunit [12].

2. Materials and methods

S. cerevisiae strain A224A (leucine requiring) was maintained on agar plates and grown in complete synthetic medium [12]. For labelling of ribosomes, cells were grown in 200 ml complete synthetic medium, containing both 20 μCi [1-^{14}C]methionine and 200 μCi [*methyl*-^{3}H]methionine until A_{550} 0.8–0.9. Sodium azide (final conc. 1 mM) was added

and the culture placed on ice to allow 'run off' of polyribosomes. Cells were then harvested by centrifugation, washed once with 20 mM Tris–HCl buffer, pH 7.4, containing 15 mM magnesium acetate and 60 mM KCl (buffer A) and frozen. The frozen pellet was mixed with twice its weight of washed sea sand and the components were ground for 5–10 min at 0–4°C. The paste was then extracted with 10 ml buffer A supplemented with 1 mM dithiothreitol and the mixture was centrifuged at 10 000 × *g* for 15 min in a MSE 18 centrifuge. The supernatant liquid was removed and centrifuged for 3 h at 40 000 rev./min in a Spinco model SW50.1 rotor to pellet ribosomes. These were resuspended in 0.5 ml buffer solution containing 50 mM Tris–HCl, pH 7.4, 5 mM magnesium acetate, 500 mM KCl and 1 mM dithiothreitol (buffer B), any aggregates being removed by low speed centrifugation (5000 × *g* for 10 min). The ribosome suspension was layered onto a 26 ml 15–40% sucrose gradient prepared in buffer B and centrifugation was carried out for 16 h at 22 000 rev./min at 5°C in a Spinco model SW25.1 rotor. Gradients were analyzed at 254 nm in an Isco model DUA-2 gradient analyzer and fractions (20 drops) were collected. Fractions corresponding to each ribosomal subunit were combined and diluted 2-fold with buffer B. Subunits were sedimented by centrifugation for 20 h at 42 000 rev./min in a Spinco model 50Ti rotor, resuspended in 0.2 ml 60 mM magnesium chloride solution and extracted for 50 min at 0–4°C by shaking with 2 vol. glacial acetic acid. The resultant precipitate was removed by centrifugation for 15 min at 10 000 × *g*. The supernatant liquid (containing the ribosomal proteins) was collected and freeze dried and the residue was dissolved in a solution containing 8 mM urea and 10 mM dithiothreitol. Ribosomal proteins (approx. 1 mg) were analyzed and assayed for radioactivity as in [12]. [1-^{14}C]Methionine (57 mCi/mM) and [*methyl*-^{3}H]-methionine (6 Ci/mM) were obtained from The Radiochemical Centre, Amersham.

3. Results and discussion

Proteins from both 60 S and 40 S yeast ribosomal subunits were labelled and separated by two-dimensional gel electrophoresis using conditions in [12]. The separation pattern for 40 S ribosomal proteins is illustrated in fig.1a and the diagrammatic representation of this separation (fig.1b) illustrates the numbering system employed to identify the individual proteins. As in our previous work on 60 S ribosomal proteins [12] the numbering system used is based upon that in [14] and again we have analyzed only the basic proteins. 25 protein spots are clearly visible on the photograph of the gel and with the possible exception of the spot labelled 15a the spots correspond to those detected in [14]. 30 individual proteins from salt-washed (500 mM KCl) yeast 40 S ribosomal subunits were detected [14] but of these proteins 6 were acidic. An additional protein (not numbered in [14]) could be detected by doubling the duration of the electrophoresis in the first dimension [14]. This protein, which probably hardly moves in the first dimension, may well correspond to S15a identified here. Separation of proteins from 60 S particles (results not shown) gave patterns consistent with [12].

Individual proteins were excised from the gels and the $^{3}H/^{14}C$ ratio was determined for each protein as in [5,12] with the results illustrated in table 1. The $^{3}H/^{14}C$ ratio (5:1) for the total unfractionated ribosomal protein mixture was taken as the control and an increase over this ratio for any given protein is taken as a measure of the methylation level. The 5:1 ratio compares favourably with the $^{3}H/^{14}C$ ratio (5.37:1) calculated from summation of the radioactivity in the separated proteins. However, for determinations of $\Delta^{3}H/^{14}C$ the former value is used since in the present experiments we have not measured the contribution made by the separated acidic proteins to the overall labelling pattern.

From the data of table 1 it can be seen that in no case is there evidence of heavy methylation in proteins from the 40 S ribosomal subunit. Indeed the highest level recorded is for proteins S10 and S25 with a $\Delta^{3}H/^{14}C$ of 1.9, with lower values of 1.2, 1.1 and 0.8 being calculated for proteins S15a, S4 and S21, respectively. Our results were essentially reproducible in 5 different experiments but clearly these methylation levels in no way approach the $^{3}H/^{14}C$ ratios found for several proteins from yeast 60 S ribosomal subunits [12]. These latter ratios were in fact checked in the present series of experiments (data not shown) with results essentially confirming

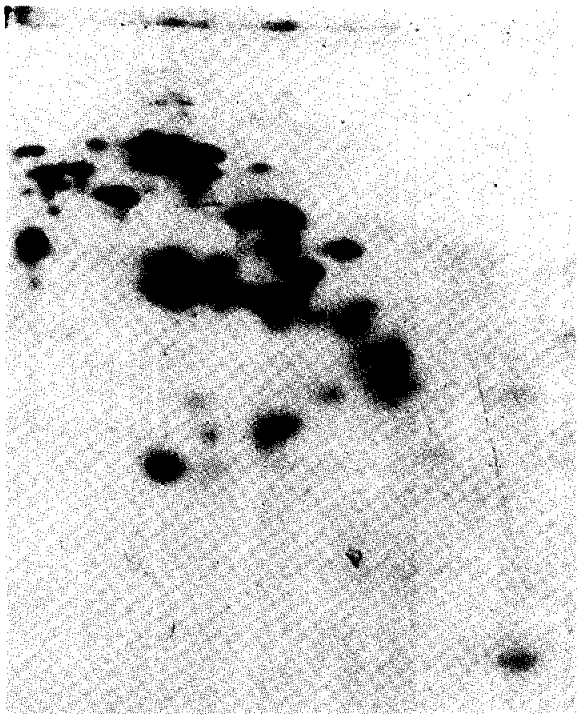

Fig.1a

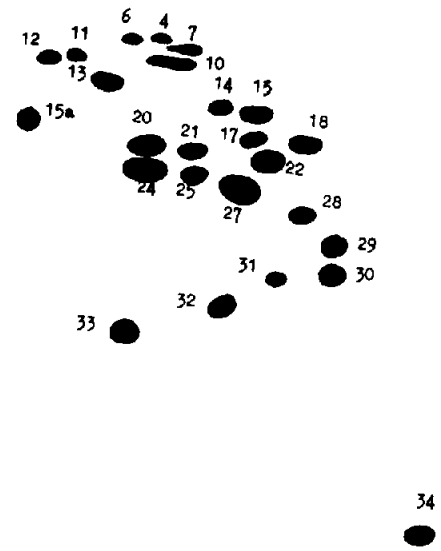

Fig.1b

Fig.1. Separation of ribosomal proteins from the small ribosomal subunit of *S. cerevisiae* by two-dimensional gel electrophoresis. The experimental conditions are described in section 2. (a) Electrophoretic separation of proteins. (b) Diagrammatic representation of the numbering system used to identify the individual ribosomal proteins. Migration in the first dimension was from left to right and in the second from top to bottom.

our earlier observations [12] where protein L15, for example, had a $\Delta^3H/^{14}C$ of 14.1. It must be remembered, however, that such high ratios have not been observed in *E. coli*. Thus maximum methylation in protein L11 was found [5], which had a $\Delta^3H/^{14}C$ of only 2.75 calculated above a control $^3H/^{14}C$ ratio of 4.12:1 – such a value being perhaps more in line with those calculated here for 40 S ribosomal proteins. It should be noted that similar conclusions to ours concerning the extent of methylation of 40 S ribosomal subunits of *S. cerevisiae* have been obtained from an analysis of methylation of 80 S ribosomes (D. Ursic, personal communication, Ph.D. thesis, University of Wisconsin, 1977).

In [12] we attempted to draw analogies between pro- and eukaryotic ribosomes by comparing methylation levels for different proteins. Thus L11 from *E. coli* and L15 from *S. cerevisiae* may correspond functionally since they are the most heavily methylated proteins in the two organisms. Such comparisons may provide a useful probe for studying the relatively poorly-characterized eukaryotic ribosome since, for example, protein L11 in *E. coli* has been implicated in both peptidyl transferase activity and EFG-dependent GTPase activity [15] and protein L15 in yeast may have a similar function. Our present results do not, however, allow an obvious comparison to be made in the case of the smaller ribosomal subunits. Methylated proteins could not be shown present in *E. coli* 30 S particles [5] although protein S11 from *E. coli* has a methylated N-terminus [13]. This protein has been implicated in a variety of functions involved in the translational process. Thus S11 may form part of the mRNA binding site on ribosomes, being concerned with codon presentation, and it is associated with the response of ribosomes to streptomycin and

Table 1
Localization of methylated ribosomal proteins in 40 S ribosomal subunits as detected by an increase in the $^{3}H/^{14}C$ ratio

Protein	^{14}C Incorporated (cpm)	^{3}H Incorporated (cpm)	$^{3}H/^{14}C$ ratio	$\Delta^{3}H/^{14}C$
S4	372	2269	6.1	+1.1*
S6	328	1443	4.4	−0.6
S7	1076	5487	5.1	+0.1
S10	1210	8349	6.9	+1.9*
S11	400	1760	4.4	−0.6
S12	132	634	4.8	−0.2
S13	501	2254	4.5	−0.5
S14	265	1325	5.0	0.0
S15	104	478	4.6	−0.4
S15a	1500	9300	6.2	+1.2*
S17	300	1560	5.2	+0.2
S18	172	860	5.0	0.0
S20	960	4800	5.0	0.0
S21	1560	9048	5.8	+0.8
S22	96	432	4.5	−0.5
S24	2600	12 220	4.7	−0.3
S25	1702	11 743	6.9	+1.9*
S27	680	2992	4.4	−0.6
S28	20	100	5.0	0.0
S29	50	250	5.0	0.0
S30	202	989	4.9	−0.1
S31	30	144	4.8	−0.2
S32	242	1137	4.7	−0.3
S33	1762	7929	4.5	−0.5
S34	200	900	4.5	−0.5

S. cerevisiae was grown in the presence of both [1-^{14}C]methionine and [*methyl*-^{3}H]methionine. Proteins from 40 S ribosomal subunits were prepared and separated and counted for both ^{3}H and ^{14}C as in section 2. The $^{3}H/^{14}C$ ratio for the total unfractionated ribosomal protein was 5:1. $\Delta^{3}H/^{14}C$ for any individual protein corresponds to the $^{3}H/^{14}C$ ratio minus 5. An asterisk marks the proteins which are considered to have methyl groups present in excess over methionine

hence the fidelity of translation. Protein S11 has also been identified near the ribosomal binding site for the initiation factors IF1 and IF2 [15].

Although it will be apparent from the above considerations that the biological significance of protein methylation is not currently understood, the data we have now accumulated from our studies on both of the subunits of *S. cerevisiae* ribosomes (present work and [12]) provide a firm basis for further investigations. Perhaps yeast ribosomal proteins will be shown methylated at specific sites within the amino acid sequences as is the case in *E. coli* [13] and these modifications may have important implications for structure–function relationships within the ribosome and/or regulation of ribosome biosynthesis and assembly. We are currently analyzing yeast mutant strains resistant to certain antibiotics, including trichodermin, cycloheximide or cryptopleurine in the hope that antibiotic resistance may sometimes be characterized by easily detectable changes in the methylation patterns of eukaryotic ribosomal proteins. Careful and detailed studies will be necessary since comparison of the methylation patterns of 80 S ribosomes from a wild type and a cryptopleurine-resistant mutant of *S. cerevisiae* yielded no significant differences (D. Ursic, personal communication, Ph.D. thesis, University of Wisconsin, 1977).

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