MODIFICATION OF 18 S rRNA IN THE 40 S RIBOSOMAL SUBUNIT OF YEAST WITH DIMETHYL SULFATE

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1. Introduction

A current study of a ribosome is rather restrained by a scarcity of data about rRNA topography in a ribosome [1]. The majority of up-to-date data concerns prokaryotic ribosomes [2–4] while almost nothing is known about eukaryotic rRNA topography. At the same time a comparison of these two types of ribosomes and their rRNAs should reveal common features which may be attributed to a ribosome as such, no matter of its origin.

This paper describes the chemical modification of 18 S rRNA in the 40 S subunit of yeast ribosomes with dimethyl sulfate. An identification of methylated sites has been done using a combination of method of RNA sequencing developed in [5] with procedure of T_1 RNase hydrolysis of 40 S subunits of yeast ribosomes published in [6].

The data obtained have been used to test the model of the secondary structure of eukaryotic 18 S rRNA proposed in [6,7].

2. Materials and methods

40 S Cytoplasmic ribosomal subunits of yeast *Saccharomyces cerevisiae* 197-4D were isolated as in [6,8]. RNase, extracted from the cobra *Naja oxiana* [9] was a generous gift of Dr S. Vasilenko. RNase T_1 was purchased from Sankyo (USA), T4-RNA-ligase was SKTB BAV (Novosibirsk) product [5′-^{32}P]pCp was from Amersham (2000–3000 Ci/mmol).

2.1. *RNA modification in 40 S subunits*

Freshly redistilled dimethyl sulfate (3 μl) was added to 100 μl 40 S ribosomal subunits (3–4 mg/ml) in a buffer containing 50 mM Na-cacodilate (pH 7.6), 10 mM $MgCl_2$, 60 mM NH_4Cl and 0.6 mM spermidine. The mixture was incubated for 10 min at 20°C, mercaptoethanol was added up to 50 mM and subunits were precipitated with 1.5-fold volume of cold ethanol for 15 min at −40°C. After reprecipitation from the buffer 'A' (50 mM Tris–HCl (pH 7.6), 10 mM $MgCl_2$, 60 mM NH_4Cl, 0.6 mM spermidine, 10 mM mercaptoethanol), the pellet of subunits was dried down in vacuum and dissolved in the buffer 'A'.

2.2. *Identification of methylated sites in RNA*

Methylated 40 S subunits were hydrolyzed either with RNase T_1 [6] or RNase from *Naja oxiana* [9]. rRNA fragments were 3′-end labeled by ligation with [5′-^{32}P]pCp (after dephosphorylation for RNase T_1 fragments) [10] and then separated by two-dimensional polyacrylamide gel electrophoresis as in [6]. RNA sequence determination as well as specific cleavage at modified RNA residues were done as in [5,11].

3. Results and discussion

A chemical modification has already been used for a structural study of prokaryotic rRNA (see [2] for references) but the localization of modified residues in the RNA polynucleotide chain was very puzzling.

In [11], a new approach was offered for a study of secondary and tertiary structures of small RNAs, based on a chemical modification of G and C residues with dimethyl sulfate and A residue with diethylpyrocarbonate and subsequent cleavage of RNA at modified residues. To apply this approach for large RNAs it is necessary to have a suitable method for a fragmentation of RNA to ~100 nucleotide long segments

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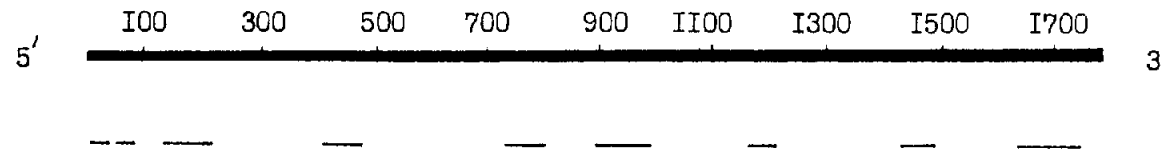

Fig.1. The scheme of distribution of identified modified segments in the 18 S rRNA.

and a separation of these fragments. The appropriate procedure for 18 S rRNA in 40 S subunits of yeast ribosomes have been developed [6].

Dimethyl sulfate proved to be a useful reagent in investigation of the structure of ribosomes:

(i) The limited modification of *E. coli* ribosomes with dimethyl sulfate does not disturb their functional activity [12];
(ii) Modification of rRNA with dimethyl sulfate was very sensitive to alteration in rRNA conformation [13].

The modification of the 40 S ribosomal subunit was carried out in the buffer providing a compactness of ribosomal particles. An excess of the reagent was removed by ethanol precipitation. To obtain a suitable set of rRNA fragments modified 40 S subunits were hydrolyzed either with RNase T_1 [6] or RNase from *Naja oxiana* [9]. That allowed us to gain information about the distribution of the methylated sites in ~50% of the 18 S rRNA chain at once (fig.1). It is worth noting that the RNase cleavage pattern is indistinguishable for both the methylated 40 S subunit and the unmodified one.

After the isolation by two-dimensional gel electrophoresis each fragment was divided into 3 portions: one was used for a chemical sequence analysis and two others for the specific cleavage at G and C residues modified with dimethyl sulfate in the 40 S subunit.

All samples were loaded onto the same slab of polyacrylamide gel, so it was possible to read off directly the position of modified sites in the 18 S rRNA primary structure (fig.2).

Positions of identified methylated sites are shown on the model of the secondary structure of 18 S rRNA (fig.3). Dimethyl sulfate modifies cytosines in single-stranded regions of RNA and guanines N7 of which are not involved in a formation of hydrogen bonds. Thus the modification of G residue reveal its location near a 'surface' of the 40 S ribosomal subunit and the modification of C residues indicates, (besides that mentioned above for G) that this exposed nucleotide is in a single-stranded conformation. So this kind of data allows not only to map the exposed regions of 18 S rRNA in the 40 S subunit but also to test their secondary structure organization.

The RNase hydrolysis of the 40 S ribosomal subunit leads to the formation of several rather long fragments which have internal sequences inaccessible to the RNase in the subunit. The chemical modification approach enabled us to test the structure and topography of such regions in the ribosome. For example the distribution of modified cytosines in the long hairpin 1645–1751 is in a very good correlation with the secondary structure proposed for this region. Taking also into account the modification of G residues in this structure one may conclude, that it is located at or near the 'surface' of the 40 S subunit. Analogous topography of corresponding region was predicted for the *E. coli* 16 S rRNA [2].

The modification of the only two cytosines in the

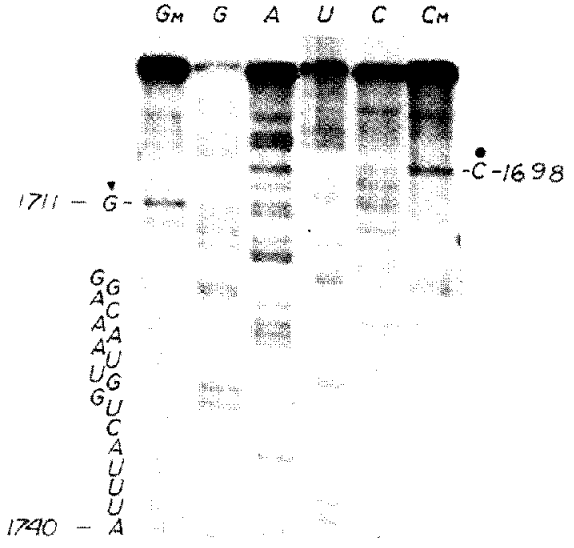

Fig.2. An example of the sequence determination of the modified fragment of 18 S rRNA.

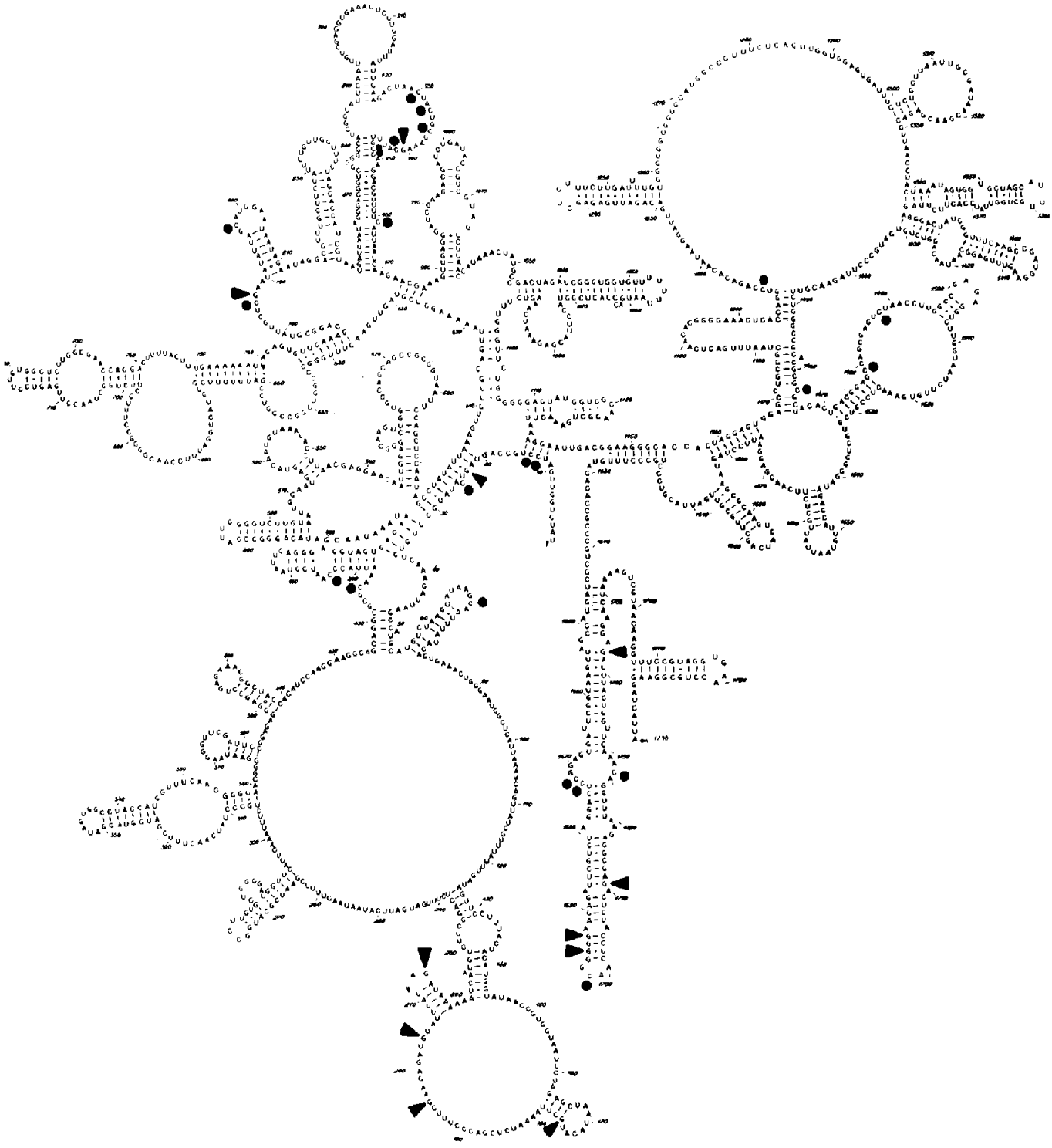

Fig.3. The revised model of the secondary structure of the 18 S rRNA of yeast. The methylated sites are shown with (●) C and (▲) G.

sequence 743–816 which has not analogous one in the prokaryotic 16 S rRNA indicates the highly-organized structure of this region. This conclusion is supported by the high accessibility of adjacent sequences to RNase *Naja oxiana* hydrolysis (unpublished).

In general the majority of data obtained in this study support the model of the secondary structure of the 18 S rRNA. Furthermore they portray 'surface' regions of the 18 S rRNA in the 40 S ribosomal subunit of eukaryotes which show close similarity to those of prokaryotes [2]. It is interesting that at least one of the so-called 'eukaryotic hairpins' [6] are also located on the 'surface' of the ribosome.

The described approach can be successfully used for studying a complex nucleoprotein structure, such as a ribosome.

When this work was completed we have learned that the similar approach had been used to study the structural organization of the 3′-end segment of 23 S rRNA in large subunits of prokaryotic ribosomes [14].

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