Identification of the proteins interacting with tRNA in rat liver small ribosomal subunits

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Irradiation at 254 nm of the rat liver 40 S ribosomal subunit–poly(U)–Phe[^{32}P]tRNA complex induced a covalent linkage of tRNA with a limited number of ribosomal proteins. After RNA hydrolysis, S10 was found to be the protein most highly labeled by radioactive nucleotides. Some radioactivity was also associated with protein S6–6a, S3a, S2 and S13–15.

Ribosome *tRNA* *Rat* *Liver* *Ultraviolet* *Irradiation*

1. INTRODUCTION

Up to now, only one method has been employed for locating the site of non-enzymatic tRNA-binding on the isolated mammalian 40 S ribosomal subunit. That method is affinity chromatography of proteins from the rat liver ribosomal 40 S subunit on immobilized rRNAs [1,2]. This approach however, has the limitation that these proteins are not integrated within the ribosomal structure. We here report results obtained by a direct method which was applied to intact subunits. It consists of the identification of proteins covalently linked to tRNA after irradiation of the purified ribosomal subunit–poly(U)–Phe [^{32}P]tRNA complex at 254 nm.

2. MATERIALS AND METHODS

Total [^{32}P]tRNA (9×10^5 cpm/A_{260} unit) was isolated from *Escherichia coli* cells labeled in vivo and aminoacylated with phenylalanine.

2.1. *Subunit labeling with [^{32}P]tRNA*

Small ribosomal subunits were prepared as in [3] as described in [4]. The small ribosomal subunit–poly(U)–Phe[^{32}P]tRNA complex was formed in 10 mM HEPES (*N*-2-hydroxy-ethyl-piperazine-*N'*-2 ethane sulfonic acid), pH 7.4, 80 mM KCl, 14 mM $MgCl_2$ (buffer A), as described in [5] and washed of the excess of poly(U) and tRNA by centrifugation through a 14% sucrose layer in the same buffer (4°C, 5 h at 150000 × *g* in a Beckman SW 50 rotor). The pellets suspended in buffer A were irradiated at 254 nm (4°C) using 1.08×10^{18} quanta which we have found to be a dose sufficiently low to leave the tertiary structure of small subunits unchanged [4]. Aliquots of control and irradiated samples were taken out, incubated 20 min at 20°C in the presence of 20 mM EDTA, which was found to dissociate most of the non-crosslinked complex, and then filtered (Millipore HAWP, 0.45 μM pore size); the remainder was used for the identification of tRNA-binding proteins. The low-pressure mercury lamp used has a maximum output at 253.7 nm and produces 2.9×10^2 erg.mm^{-2}.s^{-1} at the distance of the sample (10 cm). Incident radiation doses were determined by ferrioxalate actinometry [6].

2.2 *Identification of labeled proteins*

Proteins from irradiated 40 S subunit–poly(U)–Phe[^{32}P]tRNA complexes were obtained essentially as in [7]. RNAs were digested by ribonuclease T_1 and ribonuclease A, and the proteins containing ^{32}P-labeled oligonucleotides were dialyzed against

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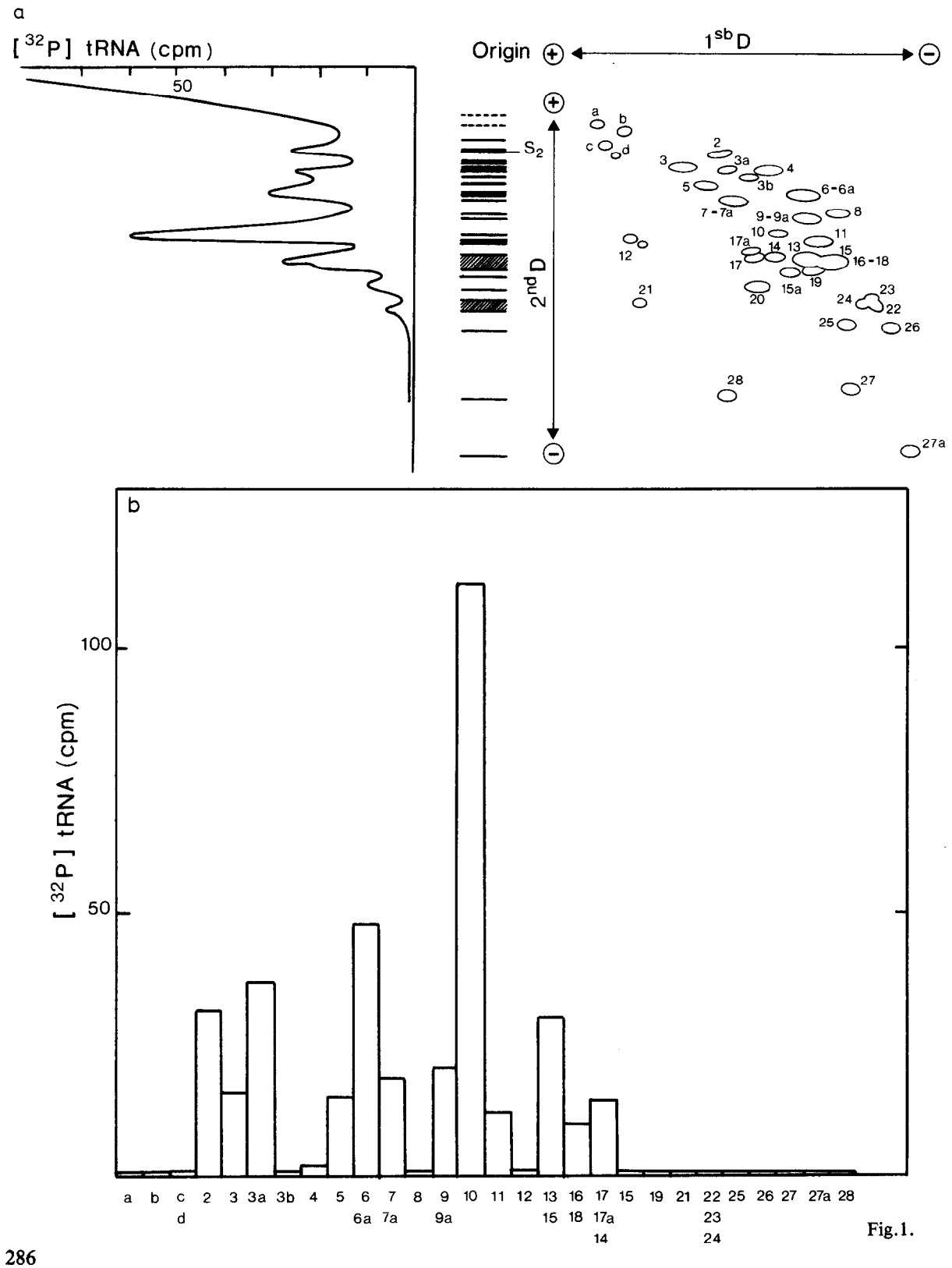
a
[32P] tRNA (cpm)
50
Origin
1sb D
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S2
a
b
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3
3a
4
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5
6-6a
7-7a
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27a
b
[32P] tRNA (cpm)
100
50
a b c 2 3 3a 3b 4 5 6 6a 7 7a 8 9 9a 10 11 12 13 15 16 18 17 17a 14 15 19 21 22 23 24 25 26 27 27a 28

Fig.1.

acetic acid and lyophilized. Subsequently they were treated with iodoacetamide and analyzed on polyacrylamide gel electrophoresis. Identification problems, raised by the possibility of displacement of proteins bearing a small piece of [^{32}P]tRNA, were solved by analyzing the same samples of ribosomal proteins simultaneously in one-dimensional (1-D) and two-dimensional (2-D) polyacrylamide gel electrophoreses. This method has already been shown to be appropriate for precise identification of labeled products is affinity labeling experiments [8]. 1-D and 2-D gel electrophoreses were performed in acidic and acidic–acidic systems, respectively [9]. Gel strips were sliced into about 2-mm slices which were incubated with tissue solubilizer and counted. The radioactivity of each band was then compared to that of the corresponding radioactive spots in the 2-D polyacrylamide gel plate. Because the [^{32}P]tRNA used had a low specific radioactivity, the position of [^{32}P]oligonucleotide-containing proteins could not be determined autoradiographically, but by counting the radioactivity recovered from either 4 1-D gel strips or 8 2-D polyacrylamide gel electrophoresis plates, which necessitated 0.25 mg and 1.4 mg protein, respectively. The code adopted for numbering the proteins corresponds to the 'uniform nomenclature' [10].

3. RESULTS AND DISCUSSION

Phe-[^{32}P]tRNA could be photoincorporated into the small ribosomal subunits by UV irradiation of the ternary complex formed in the presence of poly(U) and isolated after centrifugation through a sucrose layer. This was deduced from the fact that 5-times more radioactivity was retained on Millipore filters after treatment of the irradiated complex with EDTA as compared to the radioactivity recovered when using a non-irradiated complex (table 1). Since this radioactivity was much higher in the assays with poly(U) than in those without poly(U), it could be assumed that the

Table 1

Phe-[^{32}P]tRNA photoincorporation into small ribosomal subunits

Subunits	Phe[^{32}P]tRNA incorporated (cpm)	
	− poly(U)	+ poly(U)
Non-irradiated	182	1368
Irradiated	638	7031[a]

[a]This value represents 12% of the total radioactivity of the complex, measured before irradiation (58 600 cpm, see section 2)

40 S subunit–Phe[^{32}P]tRNA complexes prepared with or without poly(U) were irradiated with 1.018×10^{18} quanta. Following irradiation, aliquots of control and irradiated samples were incubated with 20 mM EDTA and filtered (see section 2). The results given are those obtained in 1 of 3 expt. The values in the two other experiments were in the same proportion, although not identical (due to the differences in ^{32}P-specific activity)

subunits were mostly labeled by the specific incorporation of Phe[^{32}P]tRNA^{Phe}.

In order to locate the site(s) of this incorporation, the irradiated 40 S subunit-poly(U)–Phe-[^{32}P]tRNA complex was dissociated into its protein and RNA moieties using the 2 M LiCl–4 M urea procedure. Almost no [^{32}P]tRNA was photoincorporated into the insoluble fraction containing 18 S RNA and poly(U). Therefore, most of the photoincorporated label must have been crosslinked to the protein moiety of the subunit. After digestion of the RNAs (see section 2), the proteins containing ^{32}P-labeled oligonucleotides were simultaneously identified by 1-D and 2-D gel electrophoreses (fig. 1a). The 1-D polyacrylamide gel pattern of proteins from tRNA-labeled 40 S subunits showed one main peak of radioactivity, which migrated 2.2-times further than protein S2 (a well-defined protein in this system), along with a few smaller peaks. The remainder of the radioac-

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Fig. 1. Electrophoresis analysis of proteins extracted from 40 S subunits crosslinked with [^{32}P]tRNA. The complex of ribosomal subunit–poly(U)–Phe[^{32}P]tRNA was irradiated at 254 nm, digested with ribonucleases and the resulting hydrolysate subjected to polyacrylamide gel electrophoresis (see section 2). (a) 1-D and 2-D electrophoretograms, with the recording of the radioactivity of the 1-D gel; (b) The radioactivities correspond to the stained protein spots from the 2-D gel. The average value (20 cpm) found outside the spots has been subtracted in each case.

tive material, most likely proteins still bearing large fragments of tRNA, was mainly recovered near the origin, in a lightly-stained region (fig. 1a). Two-dimensional electrophoretograms showed that radioactive spots coincided with the regions of the gel corresponding to the peaks mentioned above. The radioactivity of the main peak could be ascribed to protein S10 which was the major ^{32}P-labeled protein. Some radioactivity was also recovered with proteins S6-6a, S3a, S2 and S13–15 (fig. 1b). Other proteins than these might also have interacted with tRNA but through amino acids which were unable to photoreact with it. It it worth noting that the minor labeled proteins, S6, S3a and S13–15, which are in close vicinity (for reviews see [11,12]) might have been linked to tRNA either directly, which would agree with the fact that they are retained on a tRNA column [1,2], or indirectly through a very small oligo(U)-linker, since they are also assumed to interact with poly(U) [13,14]. The finding that S10 was the most labeled protein is of particular interest as this protein has been reported to be involved in the A-site organization and in EF_1- and EF_2-binding [15].

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