Multiple crosslinks of proteins S7, S9, S13 to domains 3 and 4 of 16S RNA in the 30S particle

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ABSTRACT: Functionally active 70S ribosomes containing 4-thiouracil in place of uracil (substitution level 2%) were prepared by an in vivo substitution method. RNA-protein crosslinks were introduced by 366nm photoactivation of 4-thiouracil in the purified 30S subunits. Seven single stranded M13 probes containing rDNA inserts complementary to domains 3 and 4 of 16S RNA were constructed. These inserts approximately 100 nucleotides long starting at nucleotide 868 and ending at the 3' OH terminus were used to select contiguous RNA sections. The proteins covalently linked to each selected RNA section were identified by 2D gel electrophoresis. Proteins S7, S9, S13 were shown to be efficiently crosslinked to multiple sites belonging to both domains.

INTRODUCTION. In order to analyse at the molecular level the set of events occurring within a complex ribonucleoprotein particle such as the ribosome during its functioning in protein synthesis one should have a detailed knowledge of the RNA-protein interaction sites within the 30S and 50S subunits. A first goal is therefore the "reconstruction" of tridimensionnal models of these particles using in peculiar both RNA-proteins and RNA-RNA crosslinks. This challenge led us to the development of a new intrinsic photoaffinity labelling method based on the photochemical properties of the base analogue 4-thiouracil (s4U) (1-2). 4 thiouracil is introduced in vivo into E. coli RNAs and can (in principle) statistically replaces U at every sites. It can be selectively photoactivated in the native ribosomal particles to introduce RNA - protein crosslinks (and RNA-RNA crosslinks). The advantage of using this intrinsic probe has been discussed in the preceeding papers (1-2) and we report here its use in a preliminary localization of RNA protein contacts in the domains 3 and 4 (nucleotide 868 to 1542) of E. coli 30S ribosomes subunit.

The localization of RNA crosslinking points in RNA-protein complexes
has met many technical difficulties and up to now very few sites have been determined (for a review see 3-4). The method used by all groups was based upon limited RNAse digestion of the covalent complexes in order to limit the RNA length to a small section protected by the protein or to sections which due to their secondary structure are slowly processed by the RNase. This approach is unfortunately limited by the efficiency of the protein protection which in most cases leaves an oligonucleotide much too short to unambiguously localize the crosslink site upon the RNA by sequencing technic. To solve these problems we have developed a method which is based upon the protection of a given RNA region by a complementary single-stranded DNA probe. The DNA probe fulfills two functions; it first selects a small section in a large RNA molecule and second protects it from RNAses used to eliminate the non selected sections. The use of cloned DNA fragments has recently been described by several groups for selection of fragments of *E. coli* large ribosomal RNAs, for secondary and tertiary structure analysis of the 58/S15 binding domain (5) as well as for localization of peptidyl transferase center by modified aminoacyl tRNA (6-7) or chemical probing of RNA conformation (8). All these probes were double stranded DNA derived from the plasmid pKK 3535 (9).

The probes we use in this study are derived from the same plasmid. It was cleaved at selected restriction sites to obtain sections about 100 nucleotides long and the fragments cloned into the *E. coli* DNA phage M13 mp 10 (10). The orientation of the cloned fragments inserted into the M13 polylinker were selected so that the infectious M13 single strand form carried the strand complementary to the 16S RNA. We report here the use of 7 such single strand DNA probes complementary to sections of domains 3 and 4 for the detection of protein contacts.

**MATERIALS AND METHODS**

**Ribosomes preparation and crosslinking reaction.**

The preparation of thiolated 70S ribosomes and subunits; photocrosslinking reaction and purification of RNA protein covalent complexes were already described. It was purified from non crosslinked proteins and the crosslinked proteins labelled with $^{125}$I to give the (RNA-$^{125}$I protein) complex (1-2).

**Obtention of M13 single strand DNA probes.**

(a) Enzymes. Restriction enzymes; the Klenow fragment of DNA polymerase I; T4 DNA ligase were purchased from different suppliers: Boehringer

4010
Mannheim; Amersham and Appligene. The reactions were performed under conditions recommended by the suppliers.

(b) Strains and plasmids. *E. coli* strain 7118 was the pUC8 host strain; JM103 was the M13 host strain (11). Plasmid pKK3535 (9), pUC8 and M13 mp10 vectors (10) have been described before. The general methods used for cloning were described in (12-13). Obtention of competent cells and transformation were performed according to (14).

**Hybridization of 16S RNA with M13 SS DNA probes.**

Typically 20 μg of purified (RNA-125I protein) complex was hybridized with 400 ng of M13 SS DNA probe in 50% formamide, 0.5 X S.C.E. buffer in 250 μl final volume. Hybridization was started at 75°C and temperature lowered to 35°C in about 50 min. Nucleic acids were recovered by ethanol precipitation and dried. (1 X S.C.E. buffer : Na citrate 10 mM pH 7.0; NaCl 150 mM; EDTA (Na2) 10 mM).

**RNAse T1 hydrolysis of the RNA - DNA hybrid.**

The ethanol pellet was resuspended in 1 X SCE buffer and digested with RNAse T1. Typically 1 μg hybridized RNA was digested with 1.25 unit of RNAse T1 in 12.5 μl for 1 h. at 37°C. This step gives the "RNAse T1 - digested hybrid" or "trimmed - hybrid". The "trimmed - hybrid" was precipitated by ethanol and dried.

**Gradient Purification of the RNAse T1 - digested hybrid.**

The pellet was dissolved in thiethanolamine (HCl) 10 mM pH 7.6; SDS 0.1%, LiCl 100 mM (buffer A) 0.1 to 0.2 ml and in order to purify the "trimmed hybrid" from unprotected (oligo) nucleotides and proteins the sample was centrifuged on a 4 ml 5-20% sucrose gradient in the same buffer in SW60 Ti rotor 50 000 rpm; 20°C for 145 min. The gradient was collected. The 125Iodine protein radioactivity and A260 unit of each fraction were measured. The samples containing the hybrid were pooled adjusted to 0.3 M NaCl and the hybrid recovered by ethanol precipitation.

**Separation and recovery of crosslinked proteins from the "trimmed hybrid."**

a) **Hydrolysis of RNA.** The "trimmed hybrid" RNA protein was hydrolysed by sodium hydroxide by the following method. The sample recovered from the gradient was resuspended in 100 μl 0.1% SDS; carrier 305 protein in 8 M Urea were added (16 μg) and NaOH adjusted to 0.4 M final. Hydrolysis was carried at 37°C for 1 h, and stopped by neutralization.

b) **Separation from DNA.** The sample was centrifuged on a 5-20% sucrose gradient in buffer A (in SW60 Ti rotor for 145 min at 20°C). Proteins (and mononucleotides) were recovered on the top of the gradient by acetone.
precipitation (5 volumes) and dried.
c) Separation of proteins from nucleotides. The pellet was dissolved in 50μl
8 M urea and separated from nucleotides in a Biogel P2 column in 60% acetic
acid. The proteins in the void volume fractions were lyophilised in a Speedvac
evaporator for two dimension gel analysis.

2D gel analysis.
Proteins were analysed on two dimensions gels. In the first dimension
the "soft gel" described in (15) was used. The second dimension was a
modified Laemmli gel 15% Acrylamide ; 0.4% Bisacrylamide ; 2 M urea ;
0.375 M Tris pH 8.8 (16). The control proteins were stained with Coomassie
brilliant blue and the 125I radioactive crosslinked proteins detected on dried
gels by autoradiography. The radioactive spots were cut out and counted
in a γ counter.

RESULTS
The 30S ribosomal subunits used in this study were obtained from the
E. coli strain AB1157 pyrD sflA grown in medium supplemented with uridine
and 4-thiouridine. The conditions for in vivo incorporation of s^4U,
characterization and preparation of ribosomal subunits were described in the
preceeding papers (1-2) We define these ribosomes by their thiolation level x
which is the percentage replacement of uracil by s^4U. These particles are
called thiolated particles or s^4U particles. Thiolated 70S ribosomes were
purified from non associated subunits on a first gradient and their subunits
obtained on a second gradient in dissociating conditions. The 30S subunits
used here were prepared with a thiolation level x = 2%, corresponding to 7
s^4U residues per 16S RNA.

To induce the formation of RNA protein crosslinks (and RNA-RNA
crosslinks which will not be discussed here) the thiolated 30S subunits were
illuminated at 366nm to selectively photoactivate 4 thiouracil. The
crosslinked subunits were then stripped from non crosslinked proteins by a
two steps SDS gradient, the RNA protein complexes recovered and the
covalently linked proteins labelled with 125I iodine (for details see (2)). We
have previously shown that in 4 to 5% thiolated ribosomes proteins are found
covalently linked to RNA before photoactivation (dark reaction) and that upon
photoactivation in the crosslinking efficiency increased by a factor of 2 to 3.
In the 30S particles the same proteins are involved with variable efficiency
in both reactions. The overall crosslinking efficiency measured on 4-5%
thiolated ribosomes was evaluated to be 7.5 to 10%. This overall value was
FIGURE 1: Schematized derivation of the seven M13 DNA probes.

The pKK3535 plasmid is schematized with the two Mlu sites used to obtain the half terminal section of 16S rDNA. This section is inserted into pUC8. pKK3535 number-ring is indicated above and 16S RNA number-ring below with the different restriction sites used to obtain the seven fragments. The RNA sections protected by the seven M13 probes are presented with 16S RNA number-ring and the number of RNA bases protected. The M13 probe 7 has an insert, 168bp long which protects the 100 bases at 3'OH terminal.

Preparation of M13 DNA probes.

As a first step to precise localization of RNA proteins crosslinking sites we have analyzed the proteins crosslinked to short selected RNA sections (generally less than 100 nucleotides long). This selection was obtained by hybridization of crosslinked 16S RNA with different M13 single strand DNA probes. Seven probes P1 to P7 were used in this study. For
convenience the complementary RNA sections protected by the seven probes P1 to P7, will be referred here as section 1 to 7.

The obtention of the M13 single strand DNA probes derived from pKK3535 is schematically described in Figure 1. A preparation of the pKK3535 (1186bp) plasmid (9) was digested with Mlu I to give 3 fragments 11037bp, 745bp and 82bp. The 745bp fragment coding for the 3'OH half of 16S rRNA was purified on agarose gel; it was rendered blunt by treatment with the Kleenow fragment of DNA pol I and cloned into pUC8 at Hinc II site. The selected plasmid clone called pUC8-C7 was characterized and prepared in large scale; it was digested with Bam HI and Hind III to recover the inserted rDNA fragment.

Five different enzyme digestions were performed to cut the DNA into fragments about 100 nucleotides long. These fragments are numbered 1 to 7 according to their position on the 16S rDNA gene. Fnud II digest gave three fragments from which the 104bp is fragment 1. Alu I + Xmn I digest gave four fragments from which the 68bp is fragment 3 and 169bp is fragment 7 with 100bp coding for the 3'OH end of 16S RNA. Alu I + Fnud II + Hae III digest gave 10 fragments from which the 97bp is fragment 2. Xmn + Ava II digest gave four fragments from which the 95bp is fragment 5. AluI + Ava II digest gave five fragments from which the 145bp is fragment 4 and 65bp is fragment 6. Fragments 1, 2, 6, 7 have blunt ends; fragments 3, 4, 5 were rendered blunt. All fragments were cloned into M13 mplO at Hinc II site. The M13 clones carrying the inserted DNA were characterized for the presence of the DNA complementary to RNA in the single stranded form.

Preparation of proteins bound to the different RNA sections.

16S RNA crosslinked 125I proteins were hybridized to single strand DNA probe in excess of DNA. The DNA section which is complementary to RNA is sufficiently long to prevent the linked protein from interfering significantly with the hybrid stability. Indeed we expect that for each protein-RNA interaction a maximum of ~ 10 nucleotides length is involved. When treated by RNase T1 the hydrolysed sections of the hybrids are expected to be fully eliminated and the hybridized sections fully protected. This was first checked with 32P labelled 16S RNA derived from non thiolated MRE 600 ribosomes (not shown). Probes 1 to 6 behave accordingly to these predictions and the protected fragments correspond to predicted size. However several G situated in terminal sections of hybridized RNA are more or less accessible to RNase T1 probably due to local breathing. This leads in some hybrids to a few bands migrating on gels around the predicted length.
FIGURE 2  Gradient separation of "T1 treated DNA-RNA hybrid" from proteins and (oligo) nucleotides non protected by the probe.
!

- $^{125}$I iodine protein radioactivity; $\Box$ $A_{260}$ absorption of the DNA probe; arrow indicate the direction of migration. A- Centrifugation control M13 single strand DNA after hybridization with 16S crosslinked $^{125}$I proteins and subsequent RNase T1 treatment. B- Same as in Fig. A with M13 single strand DNA probe 4.

Nota : the radioactivity migrating at the bottom of tubes results from partial autohybridisation of DNA.

<table>
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<tr>
<th>Section</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<td>6.3</td>
<td>5.2</td>
<td>6.4</td>
<td>4.0</td>
<td>5.3</td>
<td>5.5</td>
<td>37</td>
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</tbody>
</table>
FIGURE 3: 2D gel analysis of protein-crosslinks obtained in RNA section 1 to 7.

The position of reference proteins are indicated in the left panels and the corresponding autoradiographies on the right panels. In top left corners arrows indicate the direction of the first and second dimensions. The RNA section selected by the probe is indicated by the number in the left bottom corner. Graduations on the frames of both panels are added for a precise comparison of the localization of radioactive spots with reference proteins. The first dimensions were left attached to the 2nd dimension gels. It can be seen that an important part of the radioactivity remains trapped in it. In gel 7 the radioactive spot in left bottom corner is a reference marker. The slight differences in dimensions of left and right panels are due to enlargements of the photographs.
value (not shown).

The T1 treated hybrids were separated from non protected (oligo)nucleotides and proteins on SDS gradient and recovered by ethanol precipitation. Position of DNA was determined by \(A_{260}\) measurement and proteins by \(^{125}\text{I}\) counting. When single strand DNA M13 carrying no Insert was used in control hybridization, no \(^{125}\text{I}\) label migrated in the gradient at the position of the DNA indicating that 16S RNA hybridization does not occur and furthermore that no protein could be trapped by the M13 DNA (Fig. 2). The fraction of \(^{125}\text{I}\) radioactivity migrating with the DNA peak was measured for each hybrid and expressed in percent of total radioactivity on the gradient. Table I expresses the \(s^4\text{U}\) crosslinking efficiency for protein in each RNA section. The values obtained with \(s^4\text{U}\) are not very different from hybrid to hybrid, varying between 4% (hybrid 5) and 6.5% (hybrid 4). This means that \(s^4\text{U}\) does not operate strong site selection in contrast to the soluble carbodiimide EDC see (17). Cumulating the values obtained with the seven probes, a 37% efficiency is obtained for 44% of the 16S RNA length showing no special efficiency for domains 3 and 4. This is somehow surprising when we consider that proteins S7 and S9 are representing obviously the major crosslinked proteins. [See the 2D gel analysis in (2)]. We can conclude that the 63% \(^{125}\text{I}\) radioactivity crosslinked to the non screened domains 1 and 2 will very probably be more evenly distributed between the different proteins species.

Proteins isolation.

Usually for protein analysis the trimmed hybrids were directly incubated in sodium hydroxyde and SDS. Then proteins and nucleotides were separated from DNA on a 5-20% sucrose-SDS-LiCl gradient, and recovered by acetone precipitation. The protein samples were freed from (oligo)nucleotides on a Biogel column and the proteins analysed on 2D gels.

Analysis of the proteins crosslinked to sections 1 to 7.

The 2D gel analysis of crosslinked proteins is shown in Fig. 3a with probes 1 to 4, and in Fig. 3b with probes 5 to 7. The main characteristic of these gels is the systematic occurrence of the same major radioactive spots which correspond to proteins S7, S9 (possibly S11), S13 and protein S15 (or S18); the pattern of crosslinked proteins is more complicated in section 7. A visual inspection of the different autoradiographies is not sufficiently precise, and a better evaluation of each protein contribution was obtained by measurement of the radioactivity content in each spot. The distribution of radioactivity was then expressed in % of the total radioactivity counted in...
The radioactivity for each protein is expressed in percent of total radioactivity in each gel shown in figure 1 (radioactivity remaining in the first dimension is not taken into account). Columns 1 to 7 correspond to analysis of crosslinks in RNA section 1 to 7. Maximum counts was 1600 cpm for spot 59/11 in section 4 and minimum was 16 cpm for 53 and S19/20 in Hybrid 7 (background subtracted) this is twice the background value (500 counts minimum were counted for statistical significance).

Each gel. The radioactivity remaining in the first dimension was not taken into account. These results are summarized in Table 2.

In section 1 the main spot belongs to S9/S11; although we favour S9, the slight displacements of the radioactive spots compared to the reference proteins cannot exclude S11. Two other spots belong with no ambiguity to S7 and S13; a faint spot corresponds to S3 and a faint spot is found situated between the control proteins S18 and S15.

In section 2 the same proteins are detected with a closely related distribution.

In section 3 only S7, S9/11 and S15/18 are detected S7 being very weakly bound.

<table>
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<th>RNA section</th>
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<th>2</th>
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<th>4</th>
<th>5</th>
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<td>5</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>S7</td>
<td>15</td>
<td>9</td>
<td>8</td>
<td>15</td>
<td>42</td>
<td>84</td>
<td>24</td>
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<tr>
<td>S9/(11)</td>
<td>56</td>
<td>53</td>
<td>56</td>
<td>71</td>
<td>44</td>
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<td>16</td>
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<td></td>
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</tr>
<tr>
<td>S13</td>
<td>16</td>
<td>18</td>
<td>5</td>
<td>14</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>S15/18</td>
<td>9</td>
<td>13</td>
<td>36</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td></td>
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<tr>
<td>S15/16/17</td>
<td>8</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>S19/20</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<td>100</td>
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**Table 2** Distribution of 125 Iodine radioactivity in proteins analysed on 2D gels.
**TABLE 3** Distribution of $^{125}$I radioactivity in each protein as a % of total radioactivity crosslinked to 16S RNA. Hybrids correspond to sections 1 to 7.

<table>
<thead>
<tr>
<th>Hybrid proteins</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>TOTAL</th>
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<tr>
<td>S3</td>
<td>0.17</td>
<td>0.44</td>
<td>-</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>0.7</td>
<td>1.90</td>
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<tr>
<td>S4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.44</td>
<td>0.45</td>
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<td>S5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.3</td>
<td>0.30</td>
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<td>S7</td>
<td>0.64</td>
<td>0.57</td>
<td>0.4</td>
<td>0.96</td>
<td>1.7</td>
<td>4.4</td>
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<td>S9/11</td>
<td>2.4</td>
<td>3.3</td>
<td>2.9</td>
<td>4.6</td>
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<td>0.4</td>
<td>0.9</td>
<td>1.17</td>
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<tr>
<td>S13</td>
<td>0.7</td>
<td>1.1</td>
<td>-</td>
<td>0.3</td>
<td>0.56</td>
<td>0.26</td>
<td>0.4</td>
<td>3.5</td>
</tr>
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<td>S15/18</td>
<td>0.4</td>
<td>0.8</td>
<td>1.9</td>
<td>0.3</td>
<td>-</td>
<td>0.2</td>
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<td>S15/16/17</td>
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<td>-</td>
<td>-</td>
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<td>0.4</td>
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<td>-</td>
<td>-</td>
<td>0.1</td>
<td>0.1</td>
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</table>

In section 4 S9/11 is the major spot followed by S7; S13 and S15/18 are significantly bound. Two faint spots are detected also, one belongs to S3 which is at the very limit of detection and the other could correspond to S10. In section 5 the radioactivity is almost evenly distributed among 3 proteins only S7, S9/11 and S13.

In section 6 S7 is very dominant followed by S9/11; S13 and S15/18.

In section 7 the radioactivity is distributed among many proteins again S7 is dominant followed by S9/11 and S3 which is there very significantly above the background (contrary to what is obtained in section 1-2). There are again two spots corresponding to S13 and S15/18 and new spots which did not appear in the other sections: S4 and S5 another close to the spot named S15/18 could belong to S15/16 or 17 and a very faint spot in the region of S19/20.

A direct comparison of the crosslinking efficiency of one protein S1
to the different RNA sections cannot be obtained directly from a comparison of the radioactivity contained in the different 2D gels. This is due to the fact that a variable fraction of the analysed proteins are retained in the first dimension gels. The crosslinking index can to a first approximation be obtained by ponderating the percentage of \(^{125}\text{I}\) radioactivity corresponding to protein Si bound to RNA section j (Table 2) by the percentage of total radioactivity crosslinked to this section (Table 1). In this way the distribution of \(^{125}\text{I}\) radioactivity corresponding to each bound protein can be obtained (Table 3). Notice that the crosslinking efficiency of proteins S7 and S9/11 to different RNA sections can vary by a factor of 10 and a factor of 3 only for S13 and S15/18.

**DISCUSSION**

Using a series of seven M13 DNA probes, we have been able to identify the 30S proteins crosslinked to RNA sections one hundred nucleotides long in the 3'OH half side of 16S RNA. Our selection procedure is based upon the T1 digestion of the non hybridized part of 16S rRNA. When a protein is crosslinked outside the duplex part of the RNA-DNA hybrid it should be lost excepted if the crosslink is close to one of the extremities. On the other hand, when the protein is internal to the duplex hybrid, it will in most cases be protected from RNase attack. It is nevertheless possible that in a few cases cuts occur on both sides of the protein. This would result in protein release and loss of information. The presence of internal cuts will be checked up when RNA is sequenced to precisely localize the RNA protein crosslink.

**Multiple RNA contacts.**

Unexpectedly we observed here that several proteins S7, S9, S13... have multiples RNA contact sites. For example S7 binds efficiently with sections 1, 4, 5, 6, 7 and possibly with sections 2 and 3 indicating at least five RNA contacts (Table 2 & 3). With EDC the contacts corresponding to sections 5, 6, 7 were also detected (17). S7 was already found crosslinked at three very different sites : the first being at position 1378 (18) situated at the junction of sections 5 and 6; the second site is at position 1240 (19) in section 4, the third at position 1265 (4) in section 4. These sites probably correspond to those detected here with sections 4, 5 and 6 which represent 9.6% (section 4), 17% (section 5) and 44% (section 6) of the total S7 linked in domains 3 and 4.
All sections excepted 6 are found efficiently linked with S9. This is confirmed by the EDC data where all sections are linked. Hence S9 have at least 6 possible RNA contacts. Protein S13 is unefficiently iodinated compared to S7 and S9, therefore the values presented in the tables underestimate its crosslinking efficiency by a factor close to 5. It is found crosslinked to all sections excepted section 3. On the other hand we were surprised not to detect S19 but possibly with section 7 although from numerous and consistent data S19 belongs to domain 3 (20).

RNA-proteins contact and the 3OS subunit conformational state.

Formation of a RNA-protein crosslink by 4-thiouridine photo-activation requires that the reactive groups i.e. either the 5-6 double bond or the carbon-sulfur bond of the excited s4U and a protein nucleophilic group are within bonding distance (less than 4 Å) and adequately oriented (1-2). A naive interpretation of our findings is that multiple crosslinking sites occur with proteins S7, S9 and S13 is that these proteins form a core around which domains 3 of 16S RNA is wrapped. Indeed these three proteins (with S19) are known, from a number of consistent data, to be close neighbours and situated at the top of the subunit. On the other hand the contacts between the putative reactive groups need not be permanent and transient interactions may readily led to the formation of a covalent bond. Hence the crosslinking sites reported here may reflect all the favourable contacts obtained in the different conformational states of the 3OS subunit allowed in solution.

Clear evidence for different 70S ribosome conformations has been detected by low angle X-rays scattering analysis of ribosomes in the pretranslocation or posttranslocation states (22). In addition evidence for local internal movements has been reported for both the 30S and 50S subunits (21). NMR analysis has shown that the L7/L12 stalk is mobile in the 50S particle and that internal motions attributable in part to protein S1 could be detected in the 30S subunit. Applying the approach described here to preselected states of the 30S particle may help define the extent of these conformational changes.

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