Identification of Sites of 4′-(Hydroxymethyl)-4,5′,8-trimethylpsoralen Cross-Linking in *Escherichia coli* 23S Ribosomal Ribonucleic Acid†

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ABSTRACT: The reagent 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen (HMT) was used to cross-link 23S rRNA from *Escherichia coli* under 50S ribosomal subunit reconstitution conditions. Following partial digestion of the RNA with ribonuclease T_1, two-dimensional diagonal electrophoresis in denaturing polyacrylamide gels was used to isolate fragments derived from the cross-linked sites. These fragments were analyzed by digestion with ribonucleases T_1 and A and their positions in the 23S RNA sequence identified. Fragment a1 (positions 1325–1426) is cross-linked to a2 (positions 1574–1623); fragment b1 (positions 1700–1731) is cross-linked to b2 (positions 1732–1753); and a cross-link is formed within fragment c (or c′) (positions 863–916). In the latter case, the cross-link was located precisely, linking residues C_{867} and U_{913}. All three HMT-mediated cross-links are consistent with a proposed secondary structure model for 23S RNA [Noller, H. F., Kop, J., Wheaton, V., Brosius, J., Gutell, R. R., Kopylov, A. M., Dohme, F., Herr, W., Stahl, D. A., Gupta, R., & Woese, C. R. (1981) *Nucleic Acids Res. 9*, 6167–6189].

The importance of bacterial 23S rRNA,[1] and of its analogues in mitochondria, chloroplasts, and eukaryotic cells, for both the structure and function of ribosomes has become increasingly apparent in recent years. Resistance to the antibiotic thiostrepton has been shown to involve methylation of 23S rRNA at position 1067[2] (Thompson et al., 1982a); chloramphenicol resistance in mitochondrial ribosomes can be conferred by single base changes in the region 2449–2054 (Dujon, 1980; Kearsey & Craig, 1981; Blanc et al., 1981a,b); and erythromycin resistance can arise in bacteria by methylation of 23S rRNA (Lai & Weisblum, 1971) and in mitochondria by mutations mapping within the large subunit RNA at positions 2058 and, approximately, 2610 (Sor & Fukuhara, 1982). Eukaryotic ribosomes are inactivated by cleavage of a single phosphodiester bond at position 2661 by α-sarcin (Veldman et al., 1981; Endo & Wool, 1982), and peptidyl transferase activity can be abolished by treatment with the guanine-specific agents RNase T_1 (Cerna et al., 1973) and kethoxal (S. Turner, R. A. Atchison, and H. F. Noller, unpublished results). Furthermore, certain kethoxal-reactive guanines in 50S ribosomes become protected in 70S ribosomes, suggesting that these residues may make contact with 30S subunits (Herr & Noller, 1979). Finally, numerous affinity labeling experiments have demonstrated that some region(s) in the 3′-half of 23S rRNA lie(s) within a few angstroms of the aminoacyl end of tRNA [reviewed by Ofengand (1981)].

Since publication of the nucleotide sequence of *Escherichia coli* 23S rRNA (Brosius et al., 1980), several independent models for its secondary structure have been proposed mainly on the basis of comparative sequence analysis (Glotz et al., 1981; Branlant et al., 1981; Noller et al., 1981). While generally similar, these models differ in many of the details of base pairing. In an effort to gain further insight into these interactions, we have employed the psoralen 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen (HMT) as a photoactivated cross-linking reagent.

The photochemistry of psoralens (furocoumarins) has been extensively reviewed (Pathak et al., 1974; Song & Tapley, 1979; Hearst, 1981). They are known to intercalate in double-stranded nucleic acids and form C_4-cycloaddition adducts with pyrimidines when irradiated with light of 300–400-nm wavelength. If two pyrimidines on opposite strands occupy adjacent base pairs, a covalent cross-link can be formed via the psoralen. Such a cross-link can be photoreversed by irradiation with UV light of 220–260-nm wavelength, greatly simplifying the isolation of cross-linked oligonucleotides.

Psoralens have been used to probe the secondary structure of various ribosomal RNAs, including *Drosophila melanogaster* 5S RNA (Thompson et al., 1981) and *E. coli* 5S (Rabin & Crothers, 1979) and 16S RNAs, the latter having been examined both in solution (Wollenzein et al., 1979; Turner et al., 1982) and in the ribosome (Wollenzein & Cantor, 1982). Here we describe the use of HMT to probe the solution structure of *E. coli* 23S rRNA.

Materials and Methods

Isolation of ^{32}P-labeled RNA, photoreaction with psoralen, and isolation and analysis of cross-linked oligonucleotides have been described in detail elsewhere (Turner et al., 1982). Briefly, ^{32}P-labeled 23S rRNA from *E. coli* MRE-600 cells was derived from purified 50S subunits by $NaDodSO_4$/phenol extraction followed by sucrose gradient centrifugation (Fellner, 1969). The RNA was taken up to a concentration of 80 μg/mL in 50S subunit initial reconstitution buffer (Dohme & Nierhaus, 1976), containing 20 mM Tris-HCl (pH 7.4), 2 mM magnesium acetate, and 400 mM NH_4Cl, and incubated at 40° C for 30 min. HMT, a generous gift from Dr. J. E. Hearst, was added from a concentrated stock solution in dimethyl sulfoxide to a final concentration of 30 μg/mL and the solution irradiated with light of 340–380-nm wavelength at 15 °C. A second aliquot of HMT (final concentration 20 μg/mL) was added and irradiation repeated.

RNA was precipitated with ethanol, extracted with phenol, and partially digested with RNase T_1 (1:200, enzyme to RNA)

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[1] Abbreviations: rRNA, ribosomal ribonucleic acid; RNase, ribonuclease; tRNA, transfer ribonucleic acid; HMT, 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen; UV, ultraviolet; Tris, tris(hydroxymethyl)-aminomethane; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; DEAE-cellulose, diethylaminoethylcellulose; cpm, counts per minute.

[2] All nucleotide positions given correspond to those of the analogous residues of *E. coli* 23S rRNA (Brosius et al., 1980). References cited should be consulted for actual numbering used by the authors for individual sequences.

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at 0 °C for 1 h. Digestion was stopped by the addition of $NaDodSO_4$ and diethyl pyrocarbonate followed by extraction with phenol. Digestion products were ethanol precipitated, taken up in gel-loading buffer containing 8 M urea, 90 mM Tris–borate (pH 8.3), 2.5 mM Na_2EDTA, and 20% sucrose, and incubated at 60 °C for 10 min and then quick cooled on ice. Two-dimensional electrophoresis on denaturing polyacrylamide gels was as described (Turner et al., 1982) except the first dimension was run at room temperature and the second dimension at 6 °C. To reverse HMT cross-links between electrophoretic dimensions, the gel was irradiated with 254-nm UV light (Turner et al., 1982) for 3–30 min, depending on the experiment.

Appropriate off-diagonal spots were excised, and the RNA was eluted from the gel with a solution of 0.5 M NH_4Cl, 0.5% $NaDodSO_4$, 0.1 mM Na_2EDTA, and 10 μg/mL carrier tRNA. Each eluted RNA fragment was then digested with RNase T_1 and electrophoresed on DEAE-cellulose in 7% formic acid (Barrell, 1971). The resulting products were eluted from the paper and further digested with RNase A and submitted to a second electrophoresis at pH 3.5 (Barrell, 1971). For each off-diagonal fragment eluted from the gel, certain RNase T_1 generated oligonucleotides gave rise to RNase A digestion products that could correspond only to unique sequences within the 23S rRNA when compared to a computer-generated catalog of RNase T_1 oligomers derived from the complete nucleotide sequence of the 23S rRNA gene (Brosius et al., 1980). RNase A analysis of the remaining oligonucleotides was consistent with the expected results for the surrounding sequences. In this manner, each off-diagonal element could be fitted to a specific segment of the 23S rRNA molecule.

Results

Isolation of Cross-Linked RNA Fragments. Two-dimensional electrophoresis has previously been used to isolate psoralen-cross-linked oligonucleotides in 16S rRNA (Turner et al., 1982). Figure 1A shows the pattern obtained with 23S rRNA from *E. coli*. Oligonucleotides mutually cross-linked in the first dimension are expected to appear as collinear elements below the diagonal and present in equimolar yields. Thus, we conclude that spot a1 is cross-linked to a2 and b1 to b2. Spots appearing as single entities above or below the diagonal such as c and c′ are interpreted as arising from hairpin loops individually cross-linked by HMT, as reported previously for 16S rRNA (Turner et al., 1982). In addition to those we discuss here, a number of off-diagonal elements are present in yields too low to allow sequencing by the methods employed in this paper.

It should be noted that a number of spots appear that are not attributable to photoreversal of HMT adducts, as they are also evident in gels of control samples not exposed to HMT. Those above the diagonal arise from the presence of a UV-photolabile nucleotide(s) that has (have) been observed within the sequence 2430–2529 of *E. coli* 23S rRNA (S. Turner, unpublished results). Other spots migrating immediately below the diagonal and also present in control gels are of unknown origin. They are seen reproducibly with different preparations of RNA and thus are not caused by possible nuclease contamination introduced during handling of the gel strips between dimensions. These will not be discussed further. Only those off-diagonal spots not present in control gels do we interpret as arising from reversal of HMT cross-links.

Sequence Analysis. Analysis of off-diagonal spots a1, a2, b1, and b2 by the sequencing methods described under Materials and Methods was straightforward, and these oligonucleotides were readily fitted to the known sequence of 23S rRNA (Brosius et al., 1980).

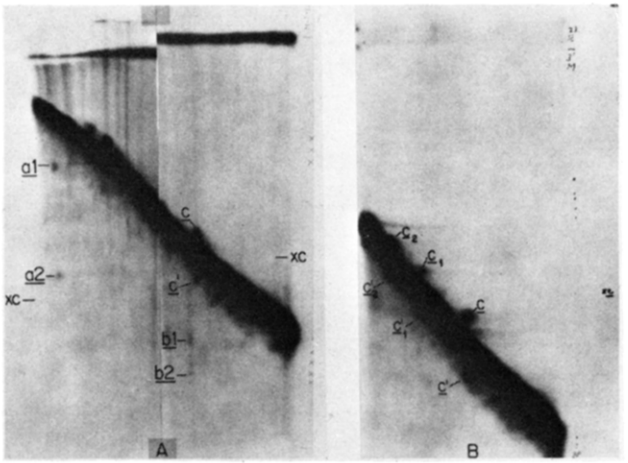


FIGURE 1: Autoradiographs of two-dimensional denaturing polyacrylamide gels of a partial RNase T_1 digest of HMT-cross-linked 23S rRNA. Photoreversal with short-wavelength UV light was carried out for 10 min between dimensions. First dimension is left to right; second dimension is top to bottom. (XC) Distance migrated by blue dye (xylene cyanol FF) in second dimension. (A) Upper two-thirds of 12% acrylamide gel [30:1, monomer to bis(acrylamide)] showing the HMT-cross-linked fragments discussed in the text. (B) Central one-third of 10.5% acrylamide gel [30:1, monomer to bis(acrylamide)] showing increased resolution of fragments c and c′. c_1 and c_2 and c'_1 and c'_2 are fragments arising from the same region as c and c′, respectively, but with extended 5′- and 3′-termini. The scale of (B) is 2 times that of (A).

Spot a1 is comprised of the segment of 23S rRNA extending from U_{1325} to $G_{1422-1426}$ and a2 the segment from C_{1574} to $G_{1622-1623}$, the exact 3′-termini not being established. Spots b1 and b2 represent contiguous segments of 23S rRNA, the former extending from A_{1700} to G_{1731} and the latter from C_{1732} to G_{1753}.

As shown in Figure 1B, spots c and c′ are better resolved in gels of slightly different composition than those used in Figure 1A. Their alignment in the second dimension attests to their comigration during first-dimension electrophoresis. Digestion of c and c′ with RNase T_1 followed by one-dimensional DEAE-cellulose electrophoresis resulted in the pattern seen in Figure 2A. RNase A analysis of the RNase T_1 digestion products of fragments c and c′ is presented in Table I. The RNase A products of c-7, -9, and -10 can be fit only to unique T_1 oligonucleotides in 23S rRNA, and the identical composition of those T_1 oligonucleotides common to c and c′ demonstrates that these fragments are from the same region of the molecule, namely, the sequence from A_{863} to G_{916}. The absence of oligonucleotides c-6, c-7, and c-8 in the T_1 pattern of c′ and the concomitant appearance of c′-12 suggest that the former oligonucleotides are possibly cross-linked via HMT in fragment c′ but not in c.

Of note is the analysis of c-8, which appears to be a HMT adduct of c-7 modified at U_{913}. This assignment is based on (a) the low yield of c-7, compensated for by the appearance of c-8, (b) the fact that RNase A is unable to cleave on the 3′-side of pyrimidines containing a HMT moiety (Bachellerie & Hearst, 1982), and (c) the presence of a RNase A digestion product of c-8 consistent with the presence of such an adduct (cf. Table I).

The absence of c-6 in the T_1 pattern of c′ also implicates its participation in the HMT cross-link of this region. The RNase A analysis of c-6 and the absence of both U and AC and the presence of G as digestion products of c′-12 indicate C_{867} and not U_{868} as being linked to HMT in c′-12. The

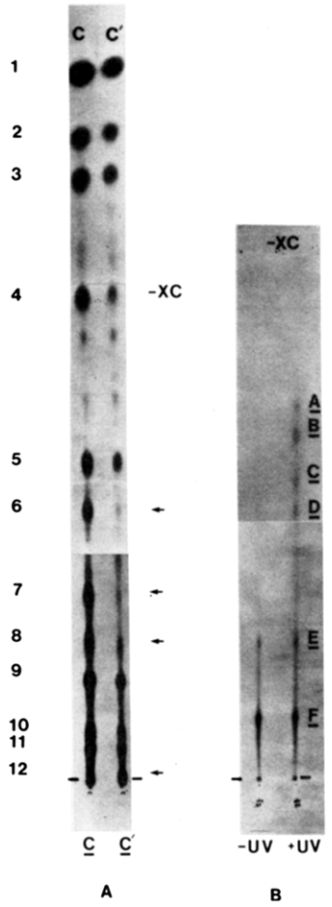


FIGURE 2: Autoradiographs of one-dimensional DEAE-cellulose electrophoresis of RNase T_1 digests of cross-linked fragments. Origins are indicated by straight lines. (XC) Position of blue dye (xylene cyanol FF). (A) Pattern of products of c and c′ run in 7% formic acid (Barrell, 1971). Arrows indicate differences between the patterns as discussed in the text. (B) Patterns of UV photolysis products of T_1 product c′-12 electrophoresed in the buffer system of Uchida et al. (1973). Identity of products A–F is discussed in the text. (+UV) c′-12 irradiated with short-wavelength UV light. (–UV) Unirradiated c′-12 run as a control.

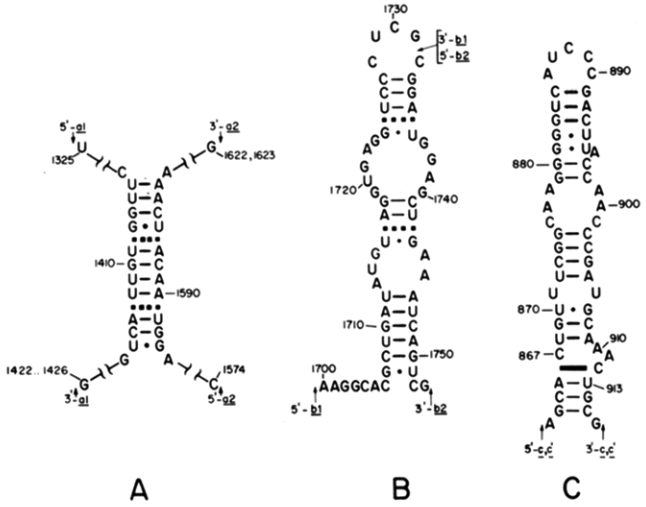


FIGURE 3: Secondary structures adapted from Noller et al. (1981) for HMT-cross-linked 23S rRNA fragments. Stippling represents helices considered proven by phylogenetic comparison. RNase T_1 cut sites producing 5′- and 3′-termini are indicated. (A) Fragments a1 and a2. Potential sites of HMT cross-linking are indicated by broken bars. Other possible sites are discussed in the text. (B) Fragments b1 and b2. Symbols are similar to those for A. (C) Fragments c and c′. The solid bar represents the site of HMT cross-linking between C_{867} and U_{913} at the top of the three base pair helix discussed in the text.

sequences for the HMT-cross-linked fragments we report are summarized in Table II.

Identity of c and c′. To further test the possibility that c and c′ are representative of the same HMT cross-link, two additional experiments were done. In the first, gel strips from the first electrophoretic dimension were irradiated with short-wavelength UV light for different periods, and the amount of radioactivity in c and c′ was determined following second-dimension electrophoresis. The results presented in Table III are consistent with conversion of c′ to c upon longer periods of photoreversal.

In a second experiment, a fraction of RNase T_1 product c′-12 (Figure 2A) eluted from the DEAE-cellulose was dissolved in water and irradiated for 15 min with short-wavelength UV light. Subsequent electrophoresis on DEAE-cellulose with the buffer system of Uchida et al. (1973) gave rise to the pattern of photolysis products illustrated in Figure 2B. Spot F is indicative of unphotolyzed c′-12. We interpret spots A–D as arising from photoreversal of a HMT cross-link in c′-12. Their respective mobilities in this electrophoretic system suggest that spots A and C may correspond to T_1 oligonucleotides c-6 and c-7, respectively, while spots B and D are likely to be the respective HMT monoadducts. Spot E is an unidentified artifact present at the electrophoretic origin of the RNase T_1 digestion products of c′ (Figure 3) and may arise from polyacrylamide- or polyacrylate-bound radioactivity derived from the gel.

Similar treatments of control T_1 oligonucleotides arising from other off-diagonal spots did not give rise to subfragments, showing that the appearance of spots A–D (Figure 2B) is not likely to be an artifact of irradiation of the nucleotide bases themselves.

From these data, then, and those of the sequence analysis, we conclude that both c and c′ are from the region of 23S rRNA extending from A_{863} to G_{917} but that c′ appears to retain a cross-link in the second dimension between nucleotides C_{867} and U_{913}.

Discussion

A number of cross-linking reagents and techniques have been used to study the structure of ribosomal RNAs, including UV irradiation (Zwieb & Brimacombe, 1980; Glotz et al., 1981; Steige et al., 1983), 1,4-phenylenediglyoxal (Wagner & Garrett, 1978; Hancock & Wagner, 1982), sulfur and nitrogen mustards (Malbon & Parish, 1971; Zwieb et al., 1978; Stiege et al., 1982), and psoralens (Rabin & Crothers, 1979; Wollenzein et al., 1979; Thompson et al., 1981; Turner et al., 1982; Wollenzein & Cantor, 1982).

For larger RNAs, two-dimensional gel electrophoresis has been particularly useful in the isolation of cross-linked oligonucleotides (Zwieb & Brimacombe, 1980; Glotz et al., 1981; Turner et al., 1982; Steige et al., 1982). Figure 1 displays the pattern of off-diagonal spots arising from HMT-cross-linked *E. coli* 23S rRNA, and the location of cross-linked RNA fragments is presented in Table II.

Fragments a1 and a2. Sequence analysis shows fragment a1 to be the segment of 23S rRNA extending from U_{1325} to $G_{1422-1426}$ and a2 that from C_{1574} to $G_{1622-1623}$. Published secondary models of 23S rRNA differ in base-pairing schemes for much of this area (Glotz et al., 1981; Branlant et al., 1981; Noller et al., 1981), but all have in common nucleotides 1405–1415 base paired with 1587–1597, as shown in Figure 3A. Within this helix are two potential sites of HMT cross-linking, on the basis of the greater reactivity of psoralens with

Table I: Sequence Analysis of c and c′ RNase T_1 Digestion Products

RNase T_1 digestion product[a]	RNase A analysis[b]	proposed sequence[b,c]	presence in 2-D gel spot[d] c	c′
1	G	G	~5	~5
2	C, G	CG	1	1
3	AG	AG	1	1
4	C, AAG	CAAG	1	1
5	G, AU	AUG	1	1
6	U, C, G, AC	CACUG	1	
7	U, C, G, AAAC	CAAACUG	~0.5	
8	C, "AAC", AAAC	CAAAC[*UG]	~0.5	
9	U, C, G, AU	UCAUCCCG	1	1
10	U, C, G, AC, AAC	ACUUACCAACCCG	1	1
11	U, C, G	UUUCG	1	1
12	C, G, AAAC, "AAAU"	C[ACU*UG]G		1

[a] Numbers refer to RNase T_1 products from Figure 2A. [b] Boxed products represent assignment of HMT-modified RNase A fragments that have electrophoretic mobilities similar to those oligonucleotides placed in quotes. Following the usual convention (Brownlee & Sanger, 1967), each underline in the RNase A analysis represents an additional residue of the underlined species. [c] An asterisk denotes a HMT-modified nucleotide(s). All sequences are written in the 5′ to 3′ direction. [d] Numbers represent estimated stoichiometric amounts present in each gel spot as determined by visual examination of autoradiographs of both RNase T_1 and RNase A digestion products. The relative amounts of 7 and 8 vary somewhat, depending on length of photoreversal.

uracil than with cytosine (Pathak et al., 1974; Thompson et al., 1981; Bachellerie & Hearst, 1982) and the affinity of psoralens for intercalation in weak helices having runs of uracils and/or U·G base pairs (Hearst, 1981). Thus U_{1409} could be cross-linked to U_{1594} and/or U_{1412} cross-linked to U_{1589}. Nevertheless, the possibility of U–C cross-links such as U_{1409} or U_{1411} to C_{1592}, C_{1414} to U_{1589}, or U_{1406} to C_{1595} cannot be ruled out, since such cross-links have been implicated in studies with 5S RNAs (Rabin & Crothers, 1979; Thompson et al., 1981), and one such cross-link has been established in this work (vide supra).

Fragments b1 and b2. Sequence analysis of b1 and b2 revealed them to be oligonucleotides from a continuous sequence of 23S rRNA proposed to form a hairpin loop stretching from A_{1700} to G_{1753}. The secondary structures of Noller et al. (1981) and Branlant et al. (1981) are identical for this region. That of Glotz et al. (1981) differs somewhat from these others. There are several sites of potential HMT cross-linking. Most attractive among them are U_{1716} to U_{1742} and U_{1725} to U_{1736}, as shown in Figure 3B. As is the case for a1 and a2, other sites of cross-linking involving cytosines are also possible such as C_{1706} or C_{1708} with U_{1751}.

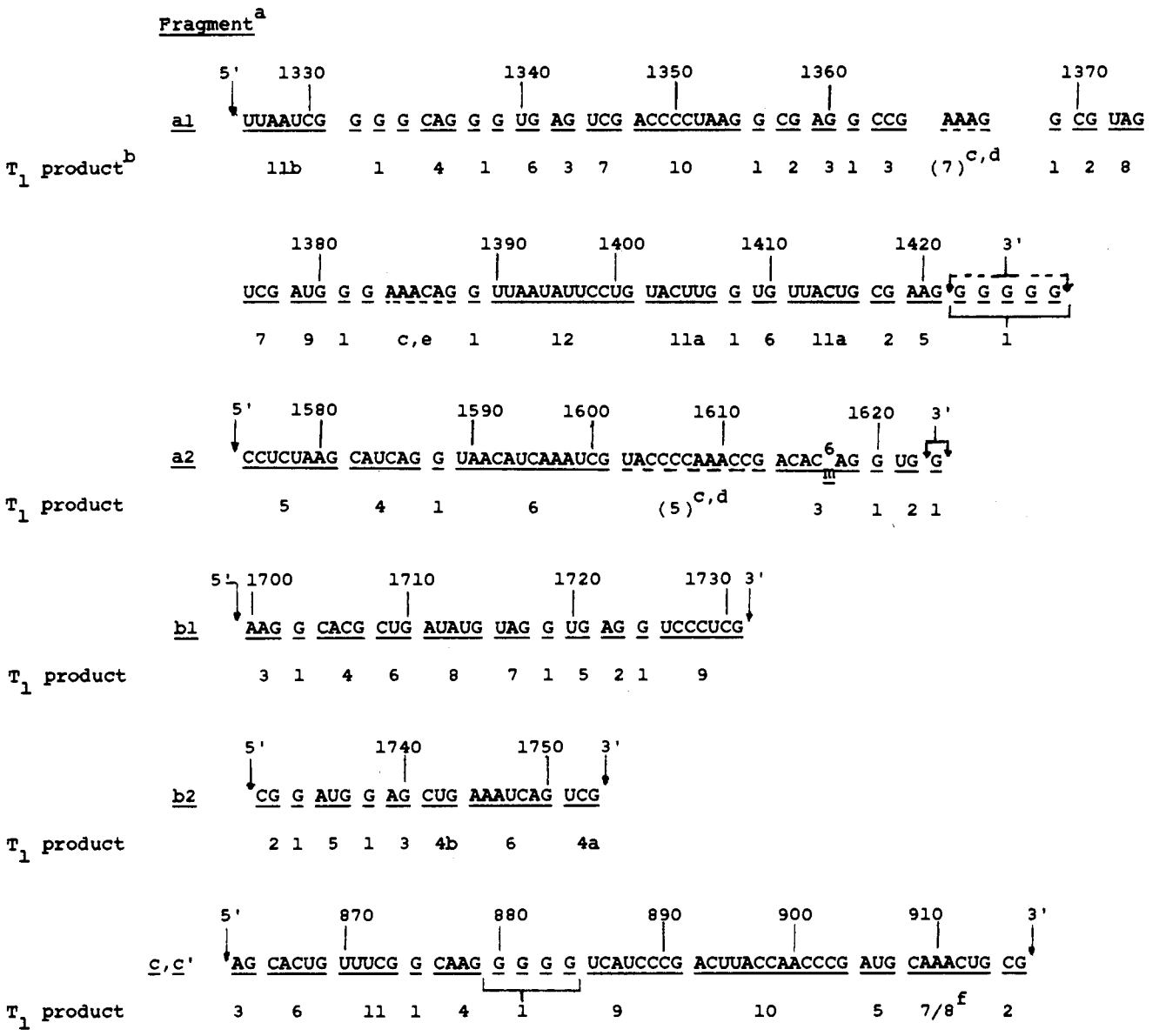
Fragments c and c′. Sequence analysis and photolysis experiments described above (Figure 2; Tables I and III) show that c and c′ are from the same sequence in 23S rRNA but that c′ retains a cross-link between C_{867} and U_{913}. To our knowledge, this is the first psoralen cross-link in a large rRNA in which the two cross-linked residues have been precisely identified. It occurs in a region depicted as being unpaired in published models of 23S rRNA secondary structure (Glotz et al., 1981; Branlant et al., 1981; Noller et al., 1981).

In the model of Noller et al. (1981), a weak three base pair helix may be formed in which C_{867} is juxtaposed to U_{913} in a suitable orientation for HMT cross-linking, as shown in Figure 3C. Cross-linking would trap the unstable base-paired portion, shifting the equilibrium to this form. However, such a structure cannot be drawn for homologous regions in other eubacterial 23S rRNAs, including those of *Bacillus stearothermophilus* (J. Kop, V. Wheaton, R. Gupta, C. R. Woese, and H. F. Noller, unpublished results), *Zea mays* chloroplast (Edwards & Kössel, 1981), and *Nicotiana tabacum* chloroplast (Takaiwa & Sugiura, 1982). In addition, G_{914}, putatively based paired in this minihelix, is reactive with the single strand specific reagent kethoxal in both the 50S subunits and 70S ribosomes of *E. coli* (Herr & Noller, 1979). In light of this, it appears unlikely that such a helix is of biological significance and may be a reflection of the relatively low temperature used in the photoreaction with HMT (15 °C) and/or the fact that the RNA is naked in solution and not involved in interactions with ribosomal proteins. The existence at low temperature of a similarly unstable base-paired region in a fragment of 16S rRNA has been established by other methods (Yuan et al., 1979).

Several aspects of the analysis of c and c′ merit further comment. It is not clear why c′ should manifest itself as a spot below the diagonal, as it is cross-linked in both dimensions. This effect is also seen when both dimensions are run at 6 °C and, so, is not a result of running them under different electrophoretic conditions. The presence of psoralen monoadducts is known to retard the migration of oligonucleotides in high percentage polyacrylamide gels (Thompson et al., 1981; Bachellerie & Hearst, 1982). In addition to this effect, monoadducts within the sequence 865–917 may inhibit "snapback" and base pairing in the first dimension promoted by the cross-link between C_{867} and U_{913}. Removal of such monoadducts by irradiation between dimensions may enhance base pairing, thus tightening the structure and accelerating its migration in the second dimension.

The assignment of U_{913} as being involved in the cross-link was aided by the existence of its HMT adduct in T_1 oligonucleotide c-8 of Figure 2A and Table I. The persistence of this adduct after irradiation with short-wavelength UV light for as much as 30 min was uncharacteristic of other cross-links similarly analyzed (Turner et al., 1982; this work and unpublished results). There is no obvious explanation for this phenomenon, but Thompson et al. (1982b) have reported

Table II: Location of HMT-Cross-Linked Fragments in *E. coli* 23S rRNA



[a] Fragments correspond to off-diagonal elements shown in Figure 1. [b] Numbers correspond to RNase T_1 digestion products isolated by one-dimensional DEAE-cellulose electrophoresis for each fragment. Products that comigrate have the same number. [c] Some T_1 products appear as extended streaks due to depurination during electrophoresis (Barrell, 1971) and are not fully recoverable for RNase A analysis. [d] Present in less than stoichiometric amounts in this T_1 product. [e] Not analyzed with RNase A. Assigment is based upon position of T_1 product following DEAE-cellulose electrophoresis and the known sequence of *E. coli* 23S rRNA (Brosius et al., 1980). [f] Runs as two distinct T_1 products, one of which is HMT modified.

Table III: Time Course of Conversion of c' to c

period of photoreversal (min)	Cerenkov cpm in excised gel spot[a]		ratio (c/c')
	c	c'	
3	19 951	22 269	0.9
10	43 914	12 099	3.6

[a] Gel spots correspond to those in Figure 1.

evidence for two types of reaction of HMT with polyuracil, perhaps indicating a HMT adduct of a non-cyclobutane nature. In addition, the work of Johnston & Hearst (1981) showing psoralen cross-linking of DNA to occur via two separate pathways may have some bearing on this. However, it should be noted that the psoralen–pyrimidine adducts that have been stereochemically well analyzed to date have been of the cyclobutane variety (Straub et al., 1981; Kanne et al., 1982).

Several groups have reported the instability of psoralen–cytosine adducts (Musajo et al., 1967; Bachellerie et al., 1981; Straub et al., 1981; Kanne et al., 1982), the cytosine being readily deaminated to uracil. This was not encountered in the analysis of c, as the presence of C_{867} appeared undiminished and no additional uracil residue was found. However, the possible deamination of C_{867} to U in RNase T_1 product c'-12 cannot be ruled out.

Finally, the high reactivity of this region to HMT addition, as judged by relative yields of off-diagonal spots, is noteworthy. Such "hotspots" of psoralen reactivity have been observed in *E. coli* 16S rRNA (Turner et al., 1982; Youvan & Hearst, 1982). In view of this hotspot nature, the fact that the cross-link involves a cytosine residue is somewhat surprising,

since cytosine is known to be appreciably less reactive with psoralens than is uracil. Indeed, if the cross-link was not resistant to photoreversal, thus allowing the participating nucleotides to be identified, one might be inclined to assign the site of HMT intercalation and reaction to a region containing features more suitable for conventional psoralen cross-linking. We would caution, then, that in the absence of knowledge of the specific nucleotides involved or other independent data, base-pairing schemes inferred from less specific localizations of regions reactive with psoralens or other reagents should be considered tentative.

Acknowledgments

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Registry No. HMT, 62442-59-5.

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