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Modification of Adenine Residues of Mouse 5S Ribosomal Ribonucleic Acid with Monoperphthalic Acid: the Secondary Structure of 5S Ribosomal Ribonucleic Acid (Nucleosides and Nucleotides. LVI)[1]

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The chemical modification of adenine moieties of mouse 5S ribosomal ribonucleic acid (RNA) was carried out with monoperphthalic acid in order to define the secondary structure of mouse 5S ribosomal RNA. The analysis of the modified 5S ribosomal RNA showed that the adenine residues at positions 11, 13 (or/and 16), 22, 23, 49, 50, 54, 55, 77, 83, 88, 90, 100, 101 and 103 were modified to adenine N-oxide residues. This result is consistent with a secondary structure of mouse 5S ribosomal RNA consisting of 5 loops and 5 helices with three bulge structures involving cytosine and adenine residues.

Keywords——chemical modification; monoperphthalic acid; adenine residues; mouse 5S ribosomal RNA secondary structure; bulge structure

Numerous studies on the higher-order structures of eukaryotic 5S ribosomal RNA (rRNA) or 5S rRNA in ribonucleoprotein complex have been performed in order to elucidate the functional roles of 5S rRNA in protein synthesis and the phylogenic diversity of organisms. At present, the secondary structure is believed to contain 5 loops and 5 helices, as first proposed by Nishikawa and Takemura.[2]

Recently, we have presented a secondary structure of mouse 5S rRNA based on the results of chemical modifications of the cytosine and guanine residues with hydrogen sulfide and kethoxal, respectively.[3,4] This secondary structure consists of 5 loops and 5 helices containing bulge structures, being slightly different from that reported by Nishikawa and Takemura.[2] The cytosine-specific chemical modification provided evidence that the cytosine residue located at position 63 was bulged out, as also found in the Nishikawa and Takemura model. A major difference was the local structure involving the consecutive adenine residues at positions 49 and 50. The bulge structure has been suggested to be universal in 5S rRNA and to have important roles in the function and structure of 5S rRNA.[5,6]

In this report, therefore, we describe the chemical modification of adenine residues in mouse 5S rRNA with monoperphthalic acid in order to define the involvement of the adenine residues in the secondary structure. The analysis of the modified 5S rRNA demonstrated the presence of bulge structures involving the adenine residues located at positions 49, 50 and 83.

Materials and Methods

Materials——Ribonucleases (RNases) A, T_1, T_2 and nuclease P_1 were obtained from Sankyo Co., Ltd., Sigma Chemical Co. and Yamasa Shoyu Co., Ltd. The materials for the two-dimensional electrophoresis were obtained from the sources described in a previous paper.[3] Monoperphthalic acid was prepared according to the reported method[7] and its oxidizing activity was analyzed before use. 5S rRNA was prepared from mouse kidney cells

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(C3H2K) as described in a previous paper.[3)]

Modification of 5S rRNA with Monoperphthalic Acid——^{32}P-Labeled 5S rRNA (about 1000000 cpm) containing 100 μg of carrier yeast tRNA was dissolved in 120 μl of 100 mM phosphate–10 mM $Mg(OAc)_2$ (pH 7.0) containing 1.6 μmol of monoperphthalic acid and incubated at 35 °C for 2 h. Then, the reaction mixture was applied to a Sephadex G-25 column equilibrated with 20 mM *N*-hydroxyethylpiperazine-*N*′-2-ethanesulfonate (HEPES)–KOH (pH 7.3), and 5S rRNA was eluted with 20 mM HEPES buffer. The fractions containing 5S rRNA were combined and 5S rRNA was precipitated by addition of 2.5 volumes of ethanol and 0.8 M NaCl at −20 °C overnight or at −70 °C for 30 min. The precipitate was recovered by centrifugation at 10000 rpm for 30 min. The recovered 5S rRNA was purified by repeated ethanol precipitation (4—5 times). Recovery of the modified 5S rRNA was about 90%.

Determination of the Modification Sites——The analysis of the modified 5S rRNA was carried out according to Sanger's fingerprinting method as described in previous papers.[3,4)] After two-dimensional electrophoresis of the RNase T_1 or RNase A digest of the modified 5S rRNA, newly appeared oligonucleotides in the fingerprint were extracted and analyzed by successive enzymatic digestion followed by two-dimensional cellulose thin layer chromatography (TLC).

Results

The modification of 5S rRNA with monoperphthalic acid was carried out at 35 °C. The analysis of the modification sites was performed by Sanger's fingerprinting method.[3,4)] Because of the different mobility of adenine N-oxide nucleotide from adenine nucleotide on electrophoresis and chromatography, RNase-derived oligonucleotides containing modified adenine residue(s) migrated separately from the corresponding original oligonucleotides. Therefore, the analysis of newly appeared spots on the fingerprints could identify the sites of modified adenine residues.

The RNase T_1 fingerprint of modified 5S rRNA revealed 8 new spots (Tn-1—Tn-8, Fig. 1). The RNase T_2 digestion of oligonucleotides Tn-1—Tn-8 and subsequent two-dimensional cellulose TLC showed that these oligonucleotides contained 1 mol eq of 3′-adenylate N-oxide (A*p) (data not shown). In addition, analysis of the base composition of the originally present oligonucleotides revealed that the 9th oligonucleotide (Tn-9) containing an adenine N-oxide residue comigrated with spot t-9, 10.

The sequence analysis of oligonucleotides containing an adenine N-oxide residue was performed by successive enzymatic digestion followed by two-dimensional TLC.

Tn-1 gave A*p and Gp in an equimolar ratio on RNase T_2 digestion, showing Tn-1 to be A*pGp. The digestion of Tn-2 with RNase T_2 gave A*p, Cp and Gp in a molar ratio of 0.8 : 2.0 : 1.3. RNase A digestion gave A*pCp, Cp and Gp (molar ratio, 1.2 : 0.9 : 1.0). Therefore, the sequence of Tn-2 was determined as A*pCpCpGp or CpA*pCpGp. Since ApCpCpGp is the only oligonucleotide which can give ApCp, Cp and Gp on digestion with RNase A, the sequence A*pCpCpGp can be assigned for Tn-2. The digestion of Tn-3 with RNase T_2 and nuclease P_1 produced Ap, A*p, Cp and Gp, and pA, pA*, pC, pG and inorganic phosphate, respectively. Therefore, the sequence of Tn-3 was determined as A(A*)pA*(A)pCpGp. Tn-4 was assigned as A*pUpGp on the basis of the digestion with RNase A giving A*pUp and Gp (molar ratio, 1.1 : 0.9). The digestion of Tn-5 with RNase A gave Cp, Up and A(A*)pA*(A)pGp (molar ratio, 1.2 : 0.8 : 1.0). Since the only oligonucleotide giving Cp, Up and ApApGp is CpUpApApGp (t-51), Tn-5 can be assigned as CpUpA(A*)pA*(A)pGp. Tn-6 was digested with RNase A to give A*(A)pA(A*)pUp, A*pCp, ApApUp, ApCp, Cp and Gp in a molar ratio of 0.7 : 0.08 : 0.08 : 0.9 : 0.8 : 1.1 and was determined to be a mixture of three nucleotides, A*pApUpApCpCpGp, ApA*pUpApCpCpGp and ApApUpA*pCpCpGp. Tn-7 was also found to be a mixture and the sequences were determined to be CpCpA*pUpApCpCpApCpCpCpUpGp and CpCpApUpA*(A)-pCpCpA(A*)pCpCpCpUpGp from the following evidence. Tn-7 was digested with RNase A to give Cp, Up, Gp, ApCp, ApUp, A*pUp and A*pCp in a molar ratio of 5.7 : 1.0 : 1.2 : 1.7 : 0.4 : 0.5 : 0.05, which showed that CpCpApUpApCpCpApCpCpCpUpGp (t-55)

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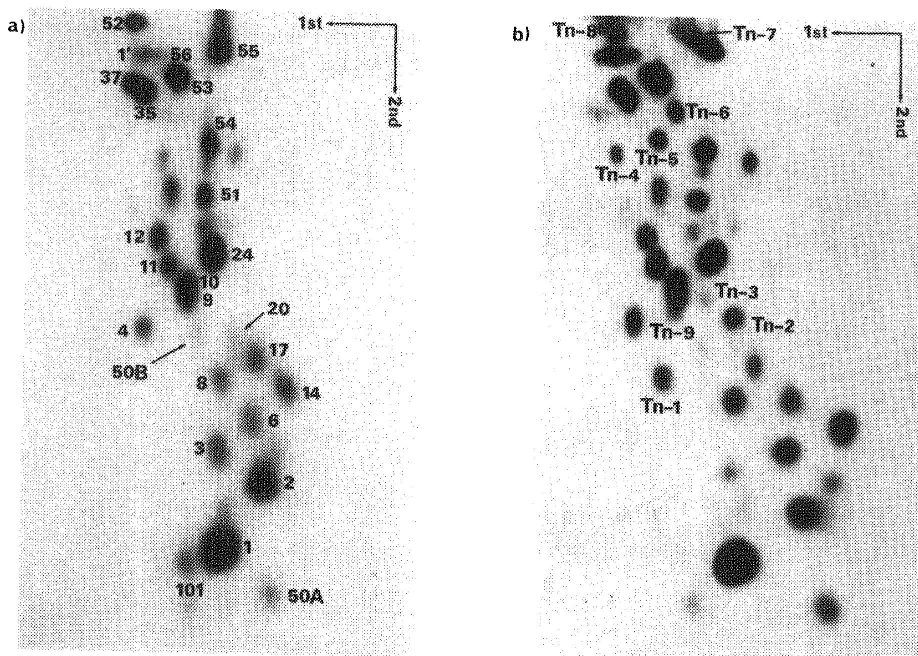

Fig. 1. RNase T_1 Fingerprints of 5S rRNAs

(a) Untreated 5S rRNA; (b) 5S rRNA treated with monoperphthalic acid.

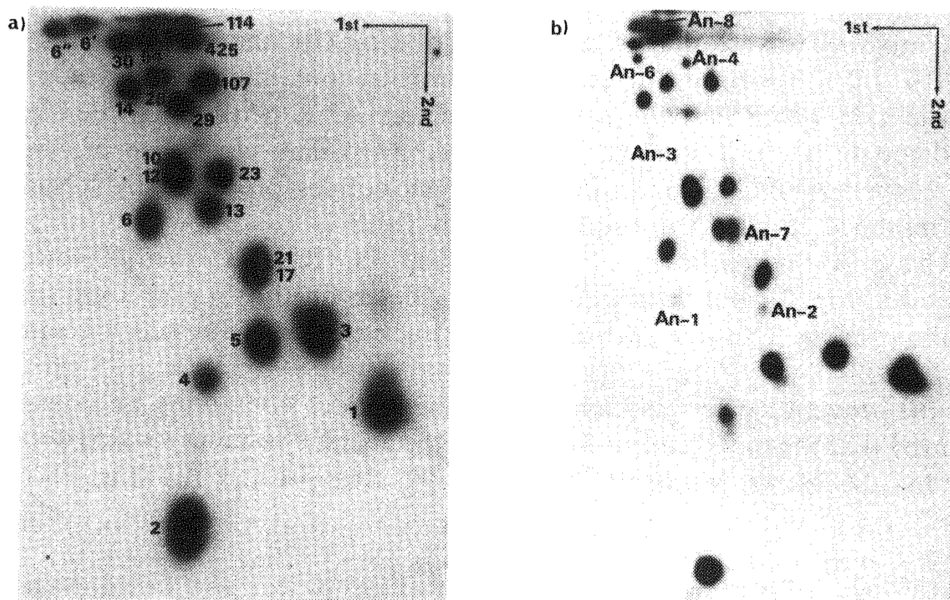

Fig. 2. RNase A Fingerprints of 5S rRNAs

(a) Untreated 5S rRNA; (b) 5S rRNA treated with monoperphthalic acid.

was a possible original oligonucleotide. In addition, it is clear that the modification rates of the 2nd and 3rd adenine residues from the 5′-end of this oligonucleotide should be very much lower because of the lower ratio of A*pCp. However, the differences of susceptibilities between the 2nd and 3rd adenine residues could not be estimated. The sequence of Tn-8 was determined to be UpA*pCpUpUpGp on the basis of RNase A digestion giving A*p-Cp, Up and Gp and RNase T_2 digestion giving A*p, Cp, Up and Gp in a molar ratio of 1.0 : 1.1 : 3.1 : 0.8. The separation of Tn-9 and t-9, 10 was not successful, and these fragments were analyzed by RNase T_2 digestion and subsequent two-dimensional TLC. The oligo-

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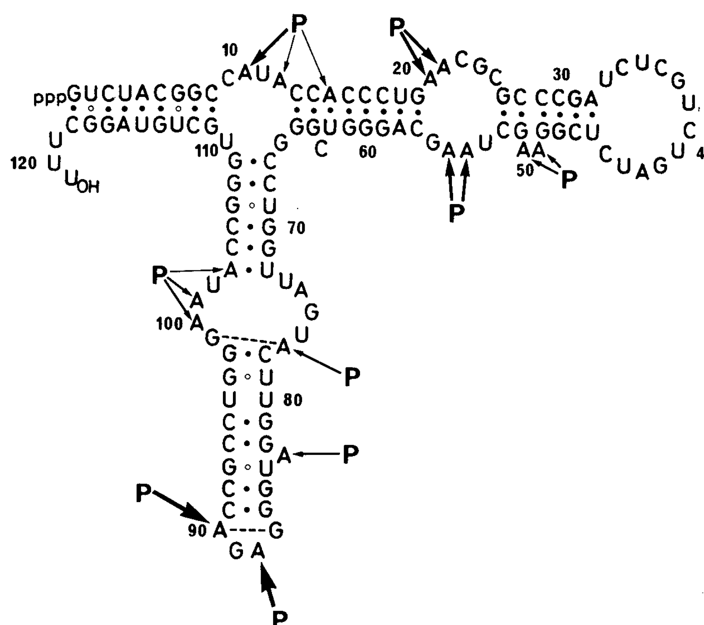

Fig. 3. The Secondary Structure of 5S rRNA from Mouse Kidney Cells

Capital P and an arrow indicates the modification sites. The thickness of the arrows corresponds to the relative susceptibility. A broken line between guanine and adenine residues indicates a novel guanine: adenine pair.

nucleotides mixture was composed of Ap, A*p, Cp, Up and Gp in a molar ratio of 0.18 : 0.14 : 2.0 : 1.9 : 1.8. RNase A digestion of the mixture containing Tn-9 gave Cp, Up, Gp and an oligonucleotide which had the same mobility as A*(A)pA(A*)pGp derived from the RNase A digest of Tn-5. Therefore, A*(A)pA(A*)pGp may have been present in spot t-9, 10. The sequences and molar ratios of oligonucleotides produced by RNase T_1 digestion of the modified 5S rRNA are summarized in Table I.

The RNase A fingerprinting of the modified 5S rRNA showed 9 newly appeared oligonucleotides (Fig. 2), and their sequences were determined by a procedure similar to that described above using RNase T_1, RNase T_2 and nuclease P_1. The results are summarized in Table II.

Thus, the sequence determination of oligonucleotides containing an adenine N-oxide residue showed that the modification occurred at the adenine residues located at positions 11, 13 (or/and 16), 22, 23, 49, 50, 54, 55, 77, 83, 88, 90, 100, 101 and 103 (Fig. 3).

Discussion

Nishikawa and Takemura have proposed a secondary structure model consisting of 5 loops and 5 helices with two bulge nucleotides at positions 63 and 83 for Tolula yeast 5S rRNA.[2)] In our previous papers,[3,4)] we have reported the results of chemical modifications specific for cytosine and guanine residues of mouse 5S rRNA. Our results have supported the Nishikawa and Takemura model with a slight modification. However, it remains unclear whether some adenine residues are involved in the bulge structure and whether other adenine residues located in the loops are susceptible to a chemical reaction specific to the adenine moiety.

The modification of the adenine residues was carried out with monoperphthalic acid, which has been used as a probe for adenine residues in functional and structural studies of tRNA and rRNA.[8,9)] The analysis of the modified 5S rRNA by the fingerprinting method with RNases allowed us to locate the modified adenine residues. When this result is related to

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TABLE I. Oligonucleotides Produced by Ribonuclease T_1 Digestion of Monoperphthalate-Treated and Untreated 5S rRNAs

Spot No.	Sequence	Molar ratio		
		Treated	Untreated	Theoretical
1	Gp	16.3	16.5	15
2	C–Gp	2.0	2.4	1
3	A–Gp	0.5)	1.3)	1
Tn-1	A*–Gp	0.7)	—)	
4	U–Gp	1.1	1.1	1
6	C–A–Gp	0.9	1.0	1
8	A–A–Gp	0.8)	1.0)	1
Tn-9	A*–A–Gp and/or A–A*–Gp	(0.3))	—)	
9, 10	U–C–Gp + C–U–Gp	2.0	2.3	1 + 1
11	U–A–Gp	1.2	1.0	1
12	A–U–Gp	0.8)	1.1)	1
Tn-4	A*–U–Gp	0.3)	—)	
14	C–C–C–Gp	1.0	1.0	1
17	A–C–C–Gp	0.5)	0.9)	1
Tn-2	A*–C–C–Gp	0.5)	—)	
20	A–A–C–Gp	0.3)	0.5)	1
Tn-3	A*–A–C–Gp and/or A–A*–C–Gp	0.2)	—)	
24	C–C–U–Gp	2.3	2.3	2
35	U–C–U–Gp	1.2	1.4	1
37	U–U–A–Gp	1.4	1.1	1
51	C–U–A–A–Gp	0.4)	0.9)	1
Tn-5	C–U–A*–A–Gp and/or C–U–A–A*–Gp	0.4)	—)	
52	U–A–C–U–U–Gp	0.7)	0.9)	1
Tn-8	U–A*–C–U–U–Gp	0.3)	—)	
53, 56	A–U–C–U–C–Gp + U–C–U–A–C–Gp	2.7	2.7	2 + 1
54	A–A–U–A–C–C–Gp	0.5)	0.8)	1
Tn-6	A*–A–U–A–C–C–Gp and/or A–A*–U–A–C–C–Gp and/or A–A–U–A*–C–C–Gp	0.3)	—)	
55	C–C–A–U–A–C–C–A–C–C–C–U–Gp	0.5)	0.9)	1
Tn-7	C–C–A*–U–A–C–C–A–C–C–C–U–Gp and/or C–C–A–U–A*–C–C–A–C–C–C–U–Gp and/or C–C–A–U–A–C–C–A*–C–C–C–U–Gp	0.3)	—)	
1′	pppGp	1.1	1.0	1
1″	ppGp			
50-A	C–U–U_{OH}	0.7	0.8	1
50-B	C–U–U–U_{OH}			

the secondary structure of mouse 5S rRNA proposed previously,[3,4] the modified adenine residues at positions 11, 13, 22, 23, 54, 55, 77, 88, 91, 100 and 101 are found to be located in the loop regions, suggesting that the 5 loops and 5 helices structure model is adequate (Fig. 3). The moderate susceptibility of the adenine residue at position 83 and the intactness of cytosine and guanine residues in the sequences G_{81}–G–A–U–G–G_{86} and C_{91}–C–G–C–C_{95} as reported previously[3,4] can be explained by assuming that both sequences form a stem structure with a bulged adenine residue. The other bulge structure involving consecutive adenine residues at positions 49 and 50 is now evident as both adenine residues are modified by adenine-specific modification. The guanine and cytosine residues in the sequences G_{27}–C–C–C–G–A_{32} and U_{45}–C–G–G–A–A–G–C_{52} are considered to be involved in the formation of G:C pairs because they are not susceptible to guanine-specific modification.[4]

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Table II. Oligonucleotides Produced by Ribonuclease A Digestion of Monoperphthalate-Treated and Untreated 5S rRNAs

Spot No.	Sequence	Molar ratio		
		Treated	Untreated	Theoretical
1	Cp	14.7	17.1	17
2	Up, U>p	15.3	14.5	13—14
3	A–Cp	4.9	5.6	5
An-2	A*–Cp	0.1	—	
4	A–Up	1.0	1.3	1
An-1	A*–Up	0.3	—	
5	G–Cp	4.4	4.1	4
6	G–Up	2.3	2.4	2
10, 12	A–G–Up + G–A–Up	3.5	3.7	1 + 1
An-3	G–A*–Up	0.2	—	
13	G–G–Cp	1.2	1.1	1
14	G–G–Up	1.2	1.2	1
17, 21	A–A–G–Cp + G–A–A–Cp	1.0	2.0	1 + 1
An-7	A*–A–G–Cp and/or A–A*–G–Cp + G–A*–A–Cp and/or G–A–A*–Cp	0.6	—	
23	A–G–G–Cp	1.0	1.0	1
28	G–G–A–Up	0.8	1.1	1
An-6	G–G–A*–Up	0.3	—	
29	G–G–G–Cp	0.6	0.9	1
30	G–G–G–Up	1.0	0.8	1
54	A–G–G–G–Up	0.9	1.2	1
107	G–G–A–A–G–Cp	0.7	0.8	1
An-4	G–G–A*–A–G–Cp and/or G–G–A–A*–G–Cp	0.3	—	
114	G–G–G–A–A–Up	0.8	0.6	1
An-8	G–G–G–A*–A–Up and/or G–G–G–A–A*–Up	0.5	—	
425	G–G–G–A–G–A–Cp	0.4	0.6	1
6′	pppG–Up	0.9	0.9	1
6″	ppG–Up			

The present study showed another interesting feature, *i.e.*, the adenine residues located at positions 77 and 90 were modified with monoperphthalic acid. In our previous study,[4] we indicated that the guanine residues located at positions 87 and 99 might be involved in guanine : adenine (G : A) pairs with the adenine residues at positions 90 and 77, respectively, based on the finding that these guanine residues were not modified by a guanine-specific chemical reaction, kethoxalation. Traub and Sussman have proposed[10] a G : A pair in which the adenine moiety is in a *syn* conformation to form the Hoogsteen-type base pair with the guanine residue. Therefore, it is reasonable that the adenine residues at positions 77 and 90 are susceptible to adenine-specific N^1-oxidation even though the guanine residues are resistant to the ketoxal modification.

Thus, the secondary structure model of mouse 5S rRNA consisting of 5 loops and 5 helices with three bulge structures of cytosine and adenine residues at positions 63, 49/50 and 83 is supported by the results of adenine-specific chemical modification in addition to previous findings.[3,4]

In contrast with several susceptible adenine residues involved in the loops and bulge structures of the secondary structure model reported here, two adenine residues, A_{42} and A_{74}, were not modified in spite of being present in the proposed loop regions. This may suggest that there is a tertiary interaction involving the sequences containing adenine residues A_{42} and A_{74}. We are currently undertaking the chemical modification of uracil residues of mouse 5S

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rRNA, and the results will be reported shortly.

References

1) Part LV: A. Ono, M. Sato, Y. Ohtani, and T. Ueda, *Nucleic Acids Res.*, **12**, 8939 (1984).
2) K. Nishikawa and S. Takemura, *FEBS Lett.*, **40**, 106 (1974).
3) K. Miura, S. Tsuda, T. Iwano, T. Ueda, F. Harada, and N. Kato, *Biochim. Biophys. Acta*, **739**, 181 (1983).
4) K. Miura, S. Tsuda, T. Ueda, F. Harada, and N. Kato, *Biochim. Biophys. Acta*, **739**, 281 (1983).
5) D. A. Peattie, S. Douthwaite, R. A. Garrett, and H. F. Noller, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 7331 (1981).
6) J. McDougall and R. N. Nazar, *J. Biol. Chem.*, **258**, 5256 (1983).
7) G. B. Payne, *J. Org. Chem.*, **24**, 1354 (1954).
8) F. Cramer, H. Doepner, F. v. d. Haar, E. Schlimme, and H. Seidel, *Proc. Natl. Acad. Sci. U.S.A.*, **61**, 1384 (1968).
9) F. Cramer and V. A. Erdmann, *Nature* (London), **218**, 92 (1968).
10) W. Traub and J. L. Sussman, *Nucleic Acids Res.*, **10**, 2701 (1982).

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