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박테리오파지 T4 tRNA의 프로세싱에 관여하는 몇가지 RNase들

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Some RNases Involved in the Processing of Bacteriophage T4 RNA

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요 약. RNase III, RNase E, 및 RNase P가 각각 홀로 또는 복합적으로 결핍되는 E. coli 돌연변이 균주들 내에서의 박테리오파지 T4 tRNA의 전구 RNA로 부터의 합성을 연구하였다. RNase E⁻균주에서는 9S RNA로 볼 수 있는 한 RNA 띠가 축적되었으며 RNase P⁻균주에서는 6S 이중띠의 하부띠가 축적되었다. RNase III⁻균주에서는 T4 tRNA 유전인자 떼 (cluster)에 의하여 코드되는 (coded) $tRNA^{Gln}$의 생성이 심하게 억제되며 T4 DNA에 의하여는 코드되지만 T4 tRNA 유전인자떼에 의하여는 코드되지 않는 species 1 RNA의 양도 다소 억제된다. 그와 동시에 T4 tRNA 유전인자 떼에 의하여 코드 되는 6S 이중 띠의 상부 띠는 RNase III⁺ 균주의 경우에 비하여 더 크게 축적된다. 그러나 6S 이중 띠의 상부 띠 RNA와 $tRNA^{Gln}$ 사이에는 precursor-product 관계가 없다고 판단되며 RNase III이 precursor RNA을 가수분해 절단 한다고 생각하는 개념을 지지할만한 근거가 없음을 지적할 수 있다.

ABSTRACT. Bacteriophage T4 tRNA processing in *E. coli* mutant strains defective in RNase III, RNase E, and RNase P, respectively, singly or in combinations, was investigated. In RNase E⁻ strains, a RNA band, which would be referred as 9S RNA, accumulates, while in RNase P⁻ strains, lower band of 6S double band is accumulated. In RNase III⁻ strains, the production of $tRAN^{Gln}$ coded by T4 tRNA gene cluster, is severely depressed and also production of species 1 RNA, which is coded by T4 DNA but not by the tRNA gene cluster, is in somewhat depressed amounts; on the other hand, at the same time, an upper band of 6S double bands, coded by T4 tRNA gene cluster, is accumulated in rather greater amounts as compared to the RNase III⁺ strain. The upper band RNA of the 6S double band, however, does not appear to be a precursor to the $tRNA^{Gln}$. The present work points to the lack of evidence for an essential cleavage role of RNase III, although there must be a role for the RNase III in the T4 tRNA processing.

INTRODUCTION

In the biosynthetic pathway of functional transfer RNA from the products of DNA transcription, a series of events including enzymological cleavage and trimming of the precursors of the respective mature tRAN species occurs. The large, unmodified polynucleotide precursors containing extra nucleotide on the 5'and 3'ends and in the inter-tRNA spacer regions,

in addition to the mature tRNA sequence, must be processed by various cellular enzymes to yield the mature tRNA[1,2]. However, the identities and functional activities of RNases involved are not well understood, and only one enzyme, RNase P[3,4] has been shown to perform an obligatory function in the endonucleolytic processing of tRNA precursor molecules. The mechanism and rationale of the post-transcriptional processing of tRNA remain one of the least understood area of molecular biology.

In the bacterium *E. coli*, the processing of *E. coli* tRNA precursors occurs so fast that it is difficult to get detailed information. In the present work, since the processing of T4 tRNA precursors in cells is known to be dependent upon host cellular RNases, T4 tRNA synthesis in *E. coli* mutant strains carrying the mutated genes for RNases was investigated. The *E. coli* mutants employed in the present work were those defective in the enzymes, RNase III(rnc), RNase E(rne)[7], and RNase P(rnp), in singly or in combinations. Comparative studies of the cases using a common mutant lesion of an enzyme, *e.g.*, rnc, rnp, and rncrnp, will not only give some useful insight into the elucidation of the effect of an enzyme on the functional activity of the other, but also can be an effective and convenient approach to the elucidation of the functional activity of the RNases. The bacteriophage T4 have been reported to code for eight unique species of tRNA, and this system has been exploited as a useful system for the study of tRNA synthesis[7].

While a functional role and mechanisms of RNase III in the T4 tRNA processing remains obscure, the current prevailing concept[6] prescribing an essential cleavage role by this enzyme of a precursor RNA containing $tRAA^{Gln}$ lacks supporting evidence. In this paper, a notion that RNase III may participate indirectly in the tRNA processing by affecting the conformation of the RNA molecule to be recognized and cleaved by a secondary RNase species, *e.g.*, RNase P, will be presented.

MATERIALS AND METHODS

Organisms: All bacteriophage and *E. coli* strains were obtained from Dr. D. Apirion (University of Washington, U.S.A.). The characteristics of deletion of tRNA genes in bacteriophage mutant strains used are as follows: *Δ*27, deletion of all tRNA genes except for those encoding $tRNA^{Gln}$ and $tRNA^{Leu}$: *Δ*33, deletion of all tRNA genes: *Δ*8, deletion of all tRAN genes except those encoding $tRNA^{Leu}$, $tRAN^{Gln}$, $tRNA^{Arg}$, $tRNA^{Ileu}$, and $tRNA^{Thr}$. *E. coil* strains used were: N2099, wild type: N 2097, rnc (RNase III$^-$): N2021, rnp (RNase P$^-$): N2018, rncrnp (RNase III$^-$ and RNaseP$^-$): N3421, rne (RNase E$^-$): N3589, rnc-rne (RNase III$^-$ and RNase E$^-$): N3522, rnernp (RNase E$^-$ and RNase P$^-$).

Media and Cell Growth: The basal medium in Tris buffer (pH 7.4) was adapted from Landy *et al.*[8] using the guidelines of Neidhardt *et al.*[9] The medium contains, per liter, 6.05 g of Tris base, 1.50 g of KCl, 1.0 g of ammonium sulfate, 5 m*l* of a 200X concentrate of trace salts[9], pH adjusted to 7.4. For labelling with ^{32}Pi, the medium was supplemented with 0.2 % glucose and 0.6 % peptone (15 μg PO_4/m*l*).

Overnight cultures of *E. coli* strains were made by inoculating a single colony of each strain to a test tube of rich medium and incubating at 30°C. Experimental cultures were made by transferring the overnight culture to the Tris-glucose medium containing 0.6 % peptone and incnbating in a water bath at 30 °C with shaking for at least 3 doublings.

Bacteriophage Infection: At an A_{560} of about 0.5 (*ca.* 3×10^8cells/m*l*), cells were infected,

at zero time, by bacteriophage at the MOI (multiplicity of infection) of 10 and at the time of 4 min again infected (superinfected) at the MOI of 10. Temperature sensitive *E. coli* mutants were transferred to the nonpermissive temperature of 43 °C, 90 minutes before the infection with the bacteriophage.

Labeling of RNA and Preparation of Samples for Electrophoresis: Then, eight minutes later, the bacteriophage-infected cells were labeled with ^{32}Pi by the addition of neutralized $^{32}PO_4$ (Amersham) to a final concentration of 1.0~2.0 mCi/m*l*. At 20 minutes after the labeling, was added 4 volumes of ice-cold 80 % ethanol containing 1 % diethylpyrocarbonate (DEP) and was left at -20 °C for several hours. To the precipitate was added 1.0 m*l* of lysis buffer (0.02 *M* Tris-HCl, pH 7.4: 0.01 *M* EDTA: 1 % sodium dodecylsulfate (SDS)), and the precipitate in the lysis buffer was lysed for 2 minutes in boiling water bath at 95 °C. After cooling to room temperature, equal volume (1.0 m*l*) of phenol saturated with the lysis buffer was added, and the mixture was shaken vigorously on a Vortex test tube mixer for 30 minutes at room temperature. The aqueous phase, separated by centrifugation and collected was further phenol-extracted. After the phenol extraction, the nucleic acid was precipitated by the addition of 2 volumes of ethanol containing 0.1 *M* sodium acetate, pH 5.0. The RNA was allowed to precipitate out of the extract for 15 minutes at −20 °C, and then recovered by centrifugation. For the purpose of gel electrophoresis, the RNA was redissolved in boiling sample buffer (0.02 *M* Tris-HCl, pH 7.6: 0.002 *M* EDTA: 20 % glycerol: 0.2 % SDS and ca. 0.005 % bromphenol blue dye).

Polyacrylamide Gel Electrophoresis of RNA: ^{32}P-labeled RNA samples in the sample buffer were fractionated on SDS-containing polyacrylamide gel slabs, using the system described by Studier[10]. For analytical purposes, 5~15 %, 5~12 % or 5~10 % tandem polyacrylamide (acrylamide: bisacrylamide=30:0.8) gels were used. Tris-glycine buffer (pH 8.3) with 0.1 % SDS was used as the running buffer. The gels were run at 4 °C for 30 minutes at 100 volts, then 4 hours at 150 volts. For autoradiography, the wet gel was covered with plastic wrap and exposed to Kodak XR-5 film.

Two-dimensional gel electrophoresis was carried out according to the technique developed by Ikemura and Dhalberg and modified by Gegenheimer and Apirion[11]:

RNA Oligonucleotide Analysis: The minifingerprinting technique of Volkaert *et al.*[12] was employed. The spots were eluted according to them, and the pancreatic RNase redigestion and DEAE electrophoresis as described by Barrell[13] were followed. Base composition of marker oligonucleotides from DEAE paper was determined after digestion with RNase T2 (Calbiochem) and chromatography on PEI thin-layer plate in 1.0 *M* LiCl.

RESULTS

The autoradiograms of ^{32}P-labeled RNA extracted from T4-infected cells of *E. coli* mutant strains defective in the RNases in singly or in combination and fractionated by electrophoresis on a 12 % polyacrylamide gel are shown in *Fig.* 1. In the autoradiogram, one can see that a RNA band, which would be referred as 9S RNA, appears in the *E. coil* strains defective in RNase E, *i.e.*, rne, rncrne, and rnernp, while in the strains defective in RNase P, *i.e.*, rnp, rncrnp, and rnernp, double bands corresponding to the size of 6S RNA accumulate. In the counterpart RNase P^+ Strain, *i.e.*, rnc and wild strain (N 2099), the lower band of the 6S double bands occurs in relatively reduced

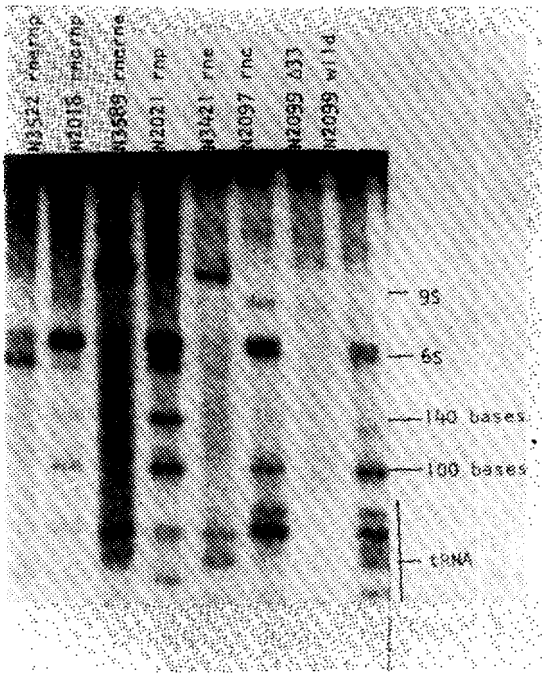

Fig. 1. Gel-electrophoretic patterns of RNA from T4-infected *E. coli* mutant cells defective in the enzymes, RNase III (rnc), RNase P (rnp), and RNase E (rne), in singly or in combinations. Each lane is labeled with both the strain number and the symbol of mutant, characteristic for the defective enzyme system. The picture shown here is of an autoradiogram of the 12 % portion of the gel. Experimental procedures are described in the text. The band, labeled as "140 bases", is referred as "species 1 RNA" in the text.

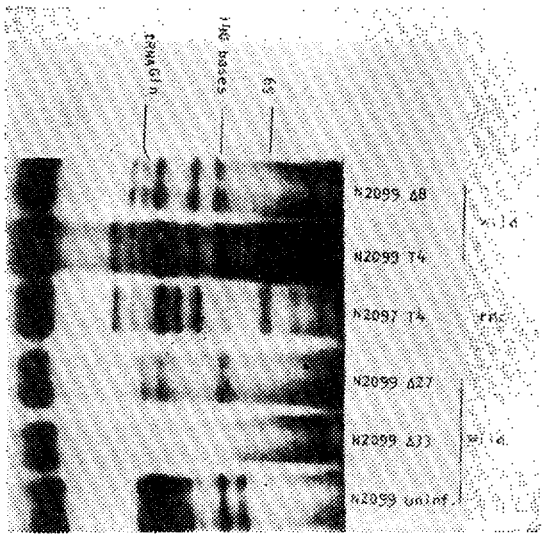

Fig. 2. Gel-electrophoretic patterns of RNA from wild type (N2099) and rnc (N2097) cells infected by bacteriophage T4 strains, *Δ*8, *Δ*27, *Δ*33, and wild type. This picture is an autoradiogram of the 15 % portion of the gel. See the text for the experimental procedures.

amounts or disappears completely, and this observation is reproduced again in the *Fig.* 2. A comparison of the RNA patterns of rnp vs. rncrnp, and rnc vs. the wild strain will show that the upper band of the 6S double bands occurs in large amounts in the RNase III$^-$ strains, as compared to RNase III$^+$ strains. The deletion of their characteristic RNA bands in their tRNA regions can also be confirmed in these pictures. Thus, while the final verification remains to be attained by additional studies of the relationship between the precursor RNA and the product tRNA bands in fingerprints and in vitro processing experiments, the results based on the figures of autoradiograms of ^{32}P-labeled RNAs, as summarized, may lead to the speculation that the 9S RNA band is affected by RNase E, the upper band of the 6S double band is affected by RNase III, and that the lower band of the 6S double band is affected by RNase P respectively.

In an effort to improve the knowledge of the possible functional role of RNase III in the processing of the tRNA precursors, *Δ*27 and A8 deletion phage mutants were used in the infection of the *E. coli* cells of the wild strain (N2099) and the rnc strain. *Fig.* 2 shows the ^{32}P-labled RNA gel electrophoresis (15 % acrylamide) patterns of wild type and rnc cells infected with T4 and deletion mutant phages respectively. The deletion mutant phages carry a deletion in the tRNA operon[1]. Infection of the

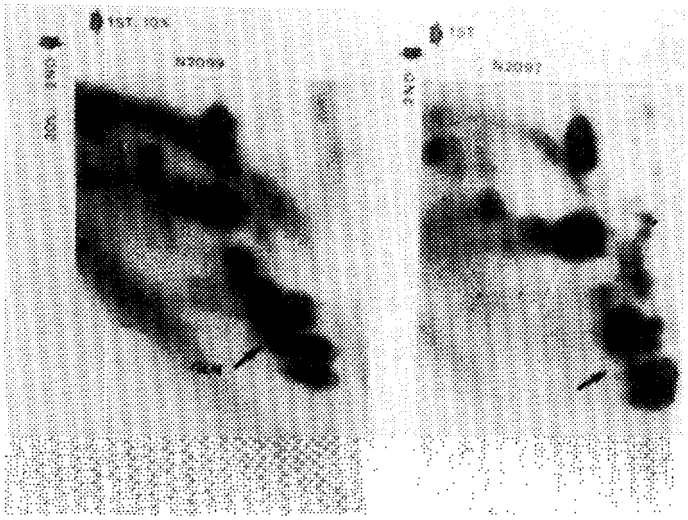

Fig. 3. Two-dimensional gel electrophoresis of tRNA region of RNA from T4-infected wild type and rnc *E. coil* strains respectively. The technique developed by Ikemura and Dahlberg, and modified by Ghora and Apirion[3] was employed in the experimental procedures. The arrow in the N2099 gel indicates the spot of $tRNA^{Gln}$, and the arrow in the N2097 gel indicates the corresponding position, where the $tRNA^{Gln}$ spot is missing.

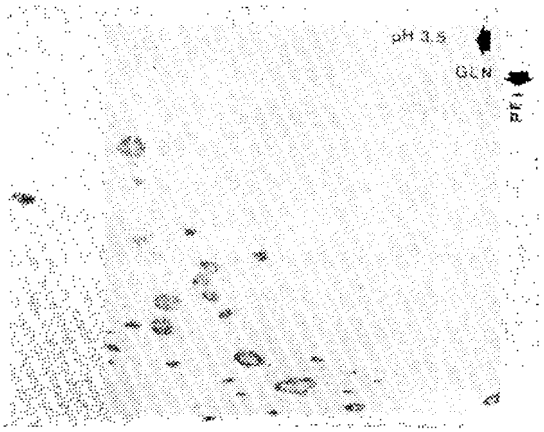

Fig. 4. RNase T1 two-dimensional fingerprint of $tRNA^{Gln}$ spot extracted from the gel of wild type strain (N2099) shown in *Fig.* 3. The first dimension was electrophoresis, followed by homochromatography in homomixture ClO (10 min hydrolyzed homomixture C)[8] in the secound dimension.

wild type *E. coli* with the △27 results in the production of only two tRNAs ($tRNA^{Gln}$ and $tRNA^{Leu}$) in equal amounts and species 1 RNA (140 bases)[15], whereas after infection of the RNase III⁻ strain (rnc) with he △27, the production of the $tRNA^{Gln}$ can not occur and, if any, only in minute amounts. By the comparison of the two-dimensional gel electrophoresis patterns of the ^{32}P-labeled RNA from T4-infected wild type and rnc cells in *Fig.* 3, the depressed production of $tRNA^{Gln}$ in rnc can be further confirmed. The ^{32}P-labeled RNA spot of N2099 (wild type) corresponding to $tRNA^{Gln}$ was extracted from the *Fig.* 3 and then its structure was analyzed by two-dimensional fingerprint after digestion with T1, pancreatic and T2 RNase. The result of the fingerprint analysis shown in *Fig.* 4 identifies the tRNA deleted to be the $tRNA^{Gln}$ (see also References 6 and 17). Further studies will be required for the elucidation of a specific role of the RNase III in the process of the $tRNA^{Gln}$ production.

DISCUSSION

The experimental results here, while confirming a previous observation[6,17] that the level of $tRNA^{Gln}$ and species 1 RNA is reduced in RNase III⁻ strains, are extended to show that also the level of the upper band of the 6S double band is incresed in RNase III⁻ strains. They also demonstrate the accumulation of a 9S RNA band in RNase E⁻ strain and the lower band of 6S double band in RNase P⁻ strain respectively. Whereas the 9S band, the 6S double band, and the species 1 RNA band are all coded by T4 DNA, the 9S and the 6S

bands only are coded by the tRNA gene cluster and not the species 1 RNA band, since the former two band species do not occur, while the latter band does, when the phage Δ33, which contains a deletion of all the tRNA gene cluster[18,19], is used (see *Fig.* 1 and *Fig.* 2.) Thus, the results obtained in the present work show that the role of RNase III is not limited only to the processing of $tRNA^{Gln}$ but also is implicated in the level of upper 6S band and species 1 RNA. These results prompted the author to look for any precursor-product relationship between the upper 6S band and the $tRNA^{Gln}$, employing the techniques of in vitro processing of the 6S RNA with an extract from an RNase III^+ strain and also with an RNase III enzyme preparation, but the experimental data obtained did not indicate any of such a relationship between the two RNA species. A separate experiment in the structural analysis of the 6S RNA, using digestions with T1 RNase followed by digestion with pancreatic or T2 RNase, again showed a negative result as to the precursor-product relationship between the 6S RNA and the $tRNA^{Gln}$. However, the structral analysis indicated that the 6S RNA contains $tRNA^{Pro}$ and $tRNA^{Ser}$. The effect of the RNase III on the 6S RNA and the species 1 RNA is not so striking, though as obvious, as on the $tRNA^{Gln}$ synthesis, and the previous reports[6,17] might have missed to recognize the RNase III effect on these RNA species.

We can notice in *Fig.* 1 rather little difference in their RNA patterns in rnc vs. rncrnp and vs. rncrne respectively. Thus one can speculate some delicate functional roles of RNase III other than drastic hydrolytic cleavage functions, and that RNase III may affect the conformational feature of the nucleic acid, instead of a hydrolytic cleavage role, thereby rendering the nucleic acid molecule to be recognized

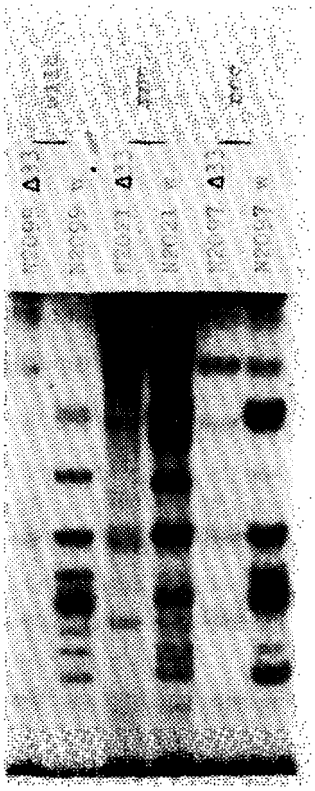

Fig. 5. Electrophoretic analysis (15 % acrylamide) of RNAS of wild type, rnc, and rnp, infected with bacteriophage T4 wild and Δ33 respectively. Experimental procedures are the same as those described in the legend of *Fig.* 2.

by proper enzymes, *e.g.*, RNase P, or other factors. Indeed, Guthrie *et al.*[20] have shown that RNase P recognizes a conformational feature shared by all precursor molecules, and that loci far removed in space from the cleavage site can alter the enzyme-substrate recognition process. Pragai *et al.*[17] reported that a T4 tRNA precursor species (10S band), accumulated in RNase III^- strain, could be processed in vitro to $tRNA^{Gln}$ and $tRNA^{Leu}$ by an extract from RNase III^+ strain. Hower, as they did not carry out additional studies using purified RNase III and RNase P preparations separately and also together in combinations, the result they obtained can not be a supporting evidence

that TNase III has an essential cleavage role in the T4 tRNA processing.

As to the question how RNase III may function in the synthesis of only one T4 tRNA, $tRNA^{Gln}$, without significant effect on $tRNA^{Leu}$, it should be mentioned that all of the T4 tRNAs are synthesized in a single transcript having $tRNA^{Gln}$ nearest the 5′ end of the molecule and yet the relative amounts of different mature tRNA species can vary extensively, depending on the precise arrangement of sequences surrounding the T4 tRNA genes[16].

Verification of the possibilities presented here (1) that a role of RNase III is not limited only to the $tRAN^{Gln}$ metabolism but is extended over to the metabolism of more such RNA species as 6S upper band RNA and species 1 RNA, (2) that RNase E and RNase P are involved in the accumulation of 9S and 6S lower band RNA respectively, and (3) the evaluation of the postulation outlined above regarding to the plausible functional role of RNase III in the T4 tRNA processing, will require further extensive work, including isolation of precursor RNA species that accumlate in an RNase-defective strain, in vitro processing studies of the isolated precursors with appropriate RNase systems, structural analysis to establish precursor-product relationships, and physicochemical studies to test the plausible effect of RNase III on the conformational transition of nucleic acids.

REFERENCES

1. S. Altman, "International Review of Biochemistry, Biochemistry of Nucleic Acids II", Vol. 17, P. 19, B. F. C. Clark, Ed., University Park Press, Baltimore, U. S. A., 1978.
2. E. Bikoff, B. LaRue and M. Gefter, *J. Biol. Chem.*, **250**, 6248 (1975).
3. S. Altman and J. D. Smith, *Nature New Biol.*, **233**, 35 (1971).
4. H. D. Robertson, S. Altman and J. D. Smith, *J. Biol. Chem.*, **247**, 5243 (1972).
5. B. K. Ghora and D. Apirion, *Cell*, **15**, 1055 (1978).
6. W. H. McClain, *Biochem. Biophys. Res. Comm.*, **86**, 3 (1979).
7. W. H. McClain, C. Guthrie and B. G. Barrell, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 3703 (1972).
8. A. Landy, J. Abelson, H. M. Goodman and J. D. Smith, *J. Mol. Biol.*, **29**, 457 (1967).
9. F. C. Neidhardt, P. L. Bloch and D. F. Smith, *J. Bacteriol.*, **119**, 736 (1974).
10. F. W. Studier, *J. Mol. Biol.*, **79**, 237 (1973).
11. P. Gegenheimer and D. Apirion, *Cell*, **15**, 527 (1978).
12. G. Volkaert, W. Min Jou, and W. Fiers, *Anal. Biochem.*, **72**, 433 (1976).
13. B. G. Barrell, *Proc. Nucl. Acids Res.*, 2, P. 751, G. L. Cantoni and D. R. Davies, Eds., Harper and Row, New York, U. S. A., 1972.
14. J. H. Wilson and J. N. Abelson, *J. Mol. Biol.*, **69**, 57 (1972).
15. W. H. McClain, *Accts. of Chem. Res.*, **10**, 418 (1977).
16. J. Abelson, *Ann. Rev. Biochem.*, **48**, 1035 (1979).
17. B. Pragai, T.-S. Ko and D. Apirion, *Biochem. Biophys. Res. Comm.*, **95**, 1431 (1980).
18. J. H. Wilson, J. S. Kim and J. N. Abelson, *J. Mol. Biol.*, **71**, 547 (1972).
19. C. Guthrie, J. G. Seidman, M. M. Comer, R. M. Bock, F. J. Schmidt, B. G. Barrell. and W. H. McClain, *Brookhaven Symp. Biol.*, **26**, 106 (1974).