Partial Digestion of a Yeast Lysine Transfer Ribonucleic Acid and Reconstruction of the Nucleotide Sequence†

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ABSTRACT: The production, isolation, and analysis of the large oligonucleotides that were used to reconstruct the nucleotide sequence of a yeast lysine transfer RNA are described. Since only one large fragment was obtained by partial digestion with ribonuclease T1, reconstruction of the nucleotide sequence was carried out with large fragments isolated after partial digestion with pancreatic ribonuclease. None of the fragments produced by partial digestion with pancreatic ribonuclease terminated in the anticodon loop.

The nucleotide sequence of baker's yeast $tRNA_2^{Lys}$ has been reported (Madison *et al.*, 1972). The accompanying paper describes the oligonucleotides produced by RNase T1 and RNase 1. The present report describes the large oligonucleotides produced by partial digestion with RNase I and RNase T1 and the reconstruction of the nucleotide sequence.

Materials and Methods

Materials. RNase T1 was obtained from Calbiochem,[1] Enzite-Ribonuclease from Miles-Seravac, and pancreatic RNase (Code R) from Worthington.

Partial Nuclease Digestion. About 20 mg of $tRNA_2^{Lys}$ was incubated with 750 units (Takahashi, 1961) of RNase T1 for 30 min at 4° in 0.09 M Tris (pH 7.5) and 5 mM $MgCl_2$ in a total volume of 2 ml. The RNase T1 was removed by extracting two times with an equal volume of phenol followed by five extractions with ether.

Partial RNase I[2] digestions of about 30 mg of $tRNA_2^{Lys}$ were carried out with 30 mg of Enzite-RNase in 2 ml of 0.15 M NaCl–5 mM $MgCl_2$ for 30 min at 23° with constant stirring. The Enzite-RNase was removed by filtering through Whatman No. 40 filter paper.

Isolation of Large Oligonucleotides. The partial digests were made 7 M in urea and chromatographed on 0.35 × 120 cm columns of DEAE-cellulose. Elution was carried out with a NaCl gradient in 7 M urea–0.02 M morpholinopropanesulfonic acid (pH 7.0), at room temperature with a flow rate of about 15 ml/hr. The purity of the large oligonucleotides was checked by rechromatography on a 0.35 × 30 cm column of DEAE-cellulose in 0.1 M formic acid–7 M urea (Rushizky and Sober, 1964) or on a 0.35 × 120 cm column of DEAE-cellulose at 55° in 7 M urea–0.02 M morpholinopropanesulfonic acid (pH 7.0), elution being carried out with a linear gradient of NaCl. Urea and salts were removed by desalting on DEAE-cellulose (Rushizky and Sober, 1962).

Oligonucleotides were analyzed as in the preceding paper (Madison *et al.*, 1974).

Results

The pattern obtained when a partial RNase I digest was chromatographed on DEAE-cellulose is shown in Figure 1. Peaks 1–9 were rechromatographed in formic acid–urea; peaks 10 and 11 were rechromatographed at 55°. The basis for the sequences assigned to the various oligonucleotides is shown in Table I. Fragments TA and TB were produced by RNase T1; all the others were produced by RNase I.

Figure 2 shows how the partial nuclease fragments were assembled to reconstruct the complete nucleotide sequence. Although overlaps between nucleotides 8–9, 13–14, 20–21, 48–49, and 74–75 were not observed, the arrangement of the partial digestion products shown is correct. Nucleotides 7 and 8, U-U-, must go with A-m^2G since U-U-A-m^2Gp is the only RNase T1 fragment to begin U-U-. The nucleotide sequence, 49–74, is the only large fragment that has the 3′ terminus G-Cp that could account for the presence of CpC_{OH} in the RNase T1 digest and the incorporation of [^{14}C]ATP into C-C-A_{OH}.

Fragments P4 and P10 could possibly be arranged so that P10 was to the left of P4. The arrangement shown, however, puts the dihydrouridine residues, m_2^2G, t^6A, and the m^7G-D-m^5C sequence in positions where they have been found in many other tRNAs. In addition, a fragment containing all the components of fragments P1A, P4, and P5, except for a 3′-terminal pyrimidine, was isolated. This fragment was not found in later partial digestions so it is possible that it was a result of an anomalous cleavage. Even without this overlap it seems certain that P4 must be to the left of P10 as shown. The nucleotide sequence is shown in the cloverleaf arrangement in Figure 3.

Discussion

Partial digests with RNase T1, which have been so useful with other tRNAs, gave only one large fragment (TA). Digests were tried with and without Mg^{2+}, with varying amounts of enzyme, and at 0 and 23°. Except in the case of TA, the products contained all the oligonucleotides in the intact tRNA (except for the 3′-terminal CpC), or they were the

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[1] Trade names and company names are included for the benefit of the reader and do not infer any endorsement or preferential treatment by the U. S. Department of Agriculture of the product listed.

[2] Abbreviations used are: RNase I, beef pancreas ribonuclease; mcm^5S, 2-thio-5-carboxymethyluridine methyl ester (can also be named 2-thiouridine-5-acetic acid methyl ester); mcm^5S*, modified form of mcm^5S; t^6A, *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]-L-threonine (also can be named threonylcarbamoyladenosine); in Table I and Figures 2 and 3, the symbols for internucleotide phosphates have been omitted.

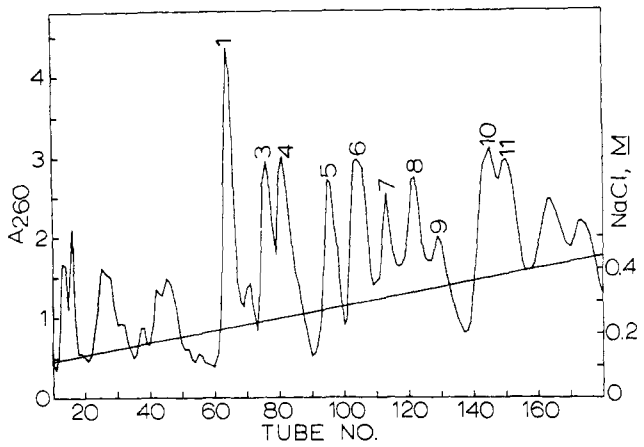

FIGURE 1: Chromatography on DEAE-cellulose in 7 M urea of a partial Enzite-RNase digest of tRNA$_2^{Lys}$. The numbers correspond to the fragments described in Table I and Figure 2.

oligonucleotides found after complete digest with RNase T1.

The only useful overlap that could be reconstructed from combining the RNase I and RNase T1 digests was A-G-G-G-G-T-ψ-C-Gp. The large fragments necessary to reconstruct the sequence were isolated after partial digestion with RNase I. The use of the insoluble derivative, Enzite-RNase, made it much easier to isolate large fragments. With RNase I most of the tRNA was completely digested.

None of the large fragments isolated after partial digestion terminated in the anticodon region. It is not surprising that RNase T1 does not give large fragments as a result of cleavage in the anticodon loop, since there are no Gp residues located there. But since there are four pyrimidine internucleotide bonds susceptible to cleavage with RNase I (the mcm^{5}S*-U bond is not cleaved even under conditions of complete digestion) in the anticodon loop, it is surprising that no fragments that resulted from hydrolysis of one of these bonds were found.

It seems unlikely that Enzite-RNase, although capable of cleaving other parts of the tRNA, was incapable of attacking the anticodon region because the enzyme was attached to carboxymethylcellulose. It seems more likely that hydrolysis of one of the bonds in the anticodon loop made the ψ-ψ-C sequence to the left and the C-C sequence to the right of the anticodon loop so labile that large fragments did not survive. However, it is also possible that the tRNA was isolated in a configuration in which the anticodon was not accessible.

Since the codons for lysine are A-A-A and A-A-G, the anticodon is expected to be U-U-U or C-U-U. There is no U-U-U sequence, and the only C-U-U sequence is near the 5′ terminus, so mcm^{5}S*-U-U is undoubtedly the anticodon. There has been some confusion about the coding properties of yeast lysine tRNA. Mitra *et al.* (1971) found that both major species of brewer's yeast tRNA^{Lys} responded to A-A-G, but not to A-A-A. Woodward and Herbert (1971) found that tRNA$_2^{Lys}$ incorporated lysine into all the lysine positions in a hemoglobin synthesizing system. Sen and Ghosh (1973), however, have found that tRNA$_2^{Lys}$ responded to poly(A) much better than to poly(AAG). It is possible that different modifications of mcm^{5}S (which we believe to be present in the native tRNA) are the cause of the different coding responses cited.

The results of Sen and Ghosh (1973) correspond to what has been found with tRNA^{Glu} from yeast (Yoshida *et al.*, 1970, 1971) and *Escherichia coli* (Ohashi *et al.*, 1970, 1972), where the tRNA^{Glu} with a 2-thiouridine derivative in the first position of the anticodon codes with G-A-A, but not G-A-G.

The nucleotide sequence of tRNA$_2^{Lys}$ has been compared (Madison *et al.*, 1972) to the sequence of a tRNA^{Lys} isolated from a haploid yeast (Smith *et al.*, 1971). The tRNA^{Lys} studied by Smith *et al.* probably corresponds to tRNA$_1^{Lys}$ in commercial baker's yeast, since they both code with A-A-G, but not A-A-A.

Yeast tRNA$_2^{Lys}$ is extremely rich in pseudouridine, containing five residues, including a 5′-terminal pψp. pψp has also been found at the 5′ terminus of brewer's yeast tRNA$_{II}^{Arg}$ (Weissenbach *et al.*, 1972). These two species of tRNA could very well account for the 2–4% of pψp Gray and Lane (1967)

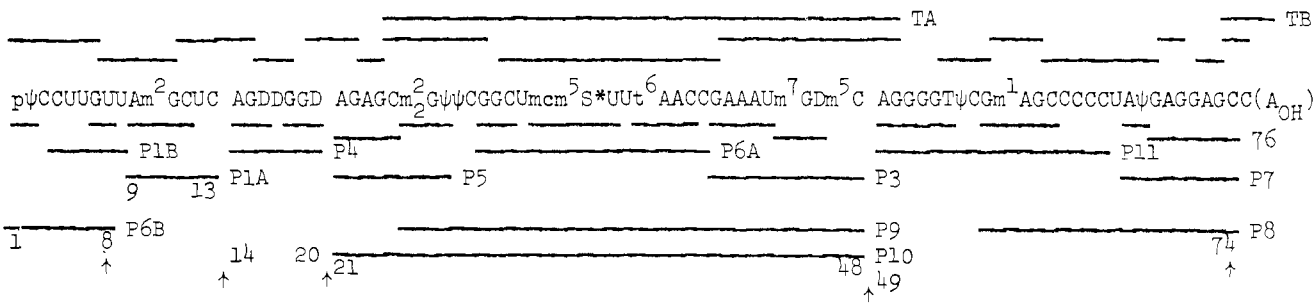

FIGURE 2: Oligonucleotides of yeast tRNA$_2^{Lys}$. RNase T1 fragments are shown above the nucleotide symbols, RNase I fragments below. Partial RNase I digestion products are labeled P1–P11. The crucial overlaps discussed in the text are shown with arrows.

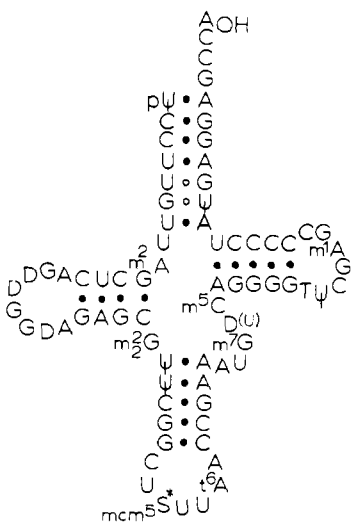

FIGURE 3: Cloverleaf arrangement of yeast tRNA$_2^{Lys}$.

strengths[2] is the fact that there are ten different intercalation sites in the double helical DNA molecule (Gabbay *et al.*, 1972) which could interact quite differently with the NA. From Figure 3 it can be seen that on increasing ionic strength, Nap decreases to a value near 0.14 and shows only a slight decrease after this point. This could indicate that some NA intercalation sites are more strongly affected by ionic strength (or Na^+) than others and are essentially eliminated at an ionic strength of 0.10. This results in a lower Nap value and a Scatchard plot of much less curvature since the intercalation sites are now more homogeneous. It should be mentioned that this is consistent with Gabbay's work since Rosenberg *et al.* (1973) found specific binding of Na^+ ion to double helical AU and not to GC. From the X-ray work it seems that any specific Na^+ binding in the minor groove of a double helix composed of mixed AT and GC base pairs would be very weak and would not interfere with NA binding.

The most probable mode of binding of NA is by intercalation of the aromatic ring system between DNA bases with a high specificity for AT base pairs. The side chain probably lies in the minor groove of the double helix and exchanging a dibutylamino for a piperidyl side chain causes little difference in the binding. The interaction of the positive charge on the side chain with the DNA phosphate groups provides a significant portion of the binding energy at low ionic strength. It seems probable the side chain accounts for part of the AT specificity of the NA, but the reason for this base selection is not clear at this time. We have found using Pauling-Cory-Koltun molecular models and the above constraints on the NA-DNA interaction that a hydrogen bond could form between the hydroxyl group on the NA side chain and the thymine carbonyl oxygen in the DNA minor groove. This would help account for the AT specificity and the location of the NA side chain in the minor groove. This is the same interaction that Henry (1972) has proposed for aryl amino alcohols as a result of model building studies. Further studies on NAs with varied structure should allow determination of a detailed binding model.

Acknowledgments

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[2] At low ionic strength (curve a in Figure 3) curvature of the Scatchard plot leads to two Kap and two Nap values. The highest Kap value (with Nap of 0.27) is presumably due to intercalation, while the lower Kap and higher Nap values are probably associated with outside electrostatic binding of the NA to the DNA phosphate groups (Panter *et al.*, 1973).

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found at the 5′ terminus of bulk yeast tRNA. Even though ψ is very rare in the acceptor stem region of tRNA, $tRNA_2^{Lys}$ has two residues in this region.

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Isolation and Characterization of the Non-Histone Chromosomal Proteins of Developing Avian Erythroid Cells†

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ABSTRACT: A method originally devised for the isolation of acidic chromatin proteins from chick embryo brain [Graziano, S. L., and Huang, R. C. (1971), *Biochemistry 10*, 4770] was adapted for the isolation of non-histone chromosomal proteins from duck erythroid cells. The method involves isolation and repeated washing of cell nuclei and chromatin, dissociation of the chromatin components in 3 M NaCl, separation of soluble proteins from DNA by gel filtration on Bio-Gel A-50, and finally, separation of the histones from non-histone chromosomal proteins by chromatography on SP-Sephadex. Preliminary characterization of the non-histone proteins by both amino acid analysis and sodium dodecyl sulfate polyacrylamide gel electrophoresis indicates that they are a group of acidic proteins consisting of at least 16–22 components and ranging in size from 12,500 to 150,000 daltons. Both immature and mature erythroid cell populations manifested a major non-histone protein component of molecular weight 72,000. Non-histone proteins were also isolated from duck erythroid cells by an alternative method [Levy, S., Simpson, R. T., and Sober, H. A. (1972), *Biochemistry 11*, 1547] involving dissociation of the chromatin in 6 M urea–0.4 M Gdn·HCl, removal of DNA by ultracentrifugation, and separation of histones from non-histones by chromatography on Bio-Rex 70 resin. The latter procedure yielded at least 18–26 non-histone components with major components of apparent molecular weights 72,000 and 150,000 for both erythroid cell populations. Several significant qualitative and quantitative differences were noted in the gel electrophoretic patterns of the non-histone chromosomal proteins from immature *vs.* mature erythroid cells, suggesting that changes in the non-histone proteins may accompany the process of cellular differentiation.

In view of a number of studies, it appears that the non-histone chromosomal proteins probably play important roles in determining both the structure and function of the eukaryotic chromosome. Although many problems have been encountered in the isolation of non-histone chromosomal proteins (Graziano and Huang, 1971; Goodwin and Johns, 1972; Sanders, 1973), certain of these proteins have been shown (a) to exhibit tissue and species specificity (Teng *et al.*, 1970, 1971; Elgin and Bonner, 1970; Chytil and Spelsberg, 1971; Richter and Sekeris, 1972; MacGillivray *et al.*, 1972; Wu *et al.*, 1973), (b) to bind "selectively" to DNA (Kleinsmith *et al.*, 1970; Salas and Green, 1971; Teng *et al.*, 1971; van den Broek *et al.*, 1973), (c) to stimulate transcription *in vitro* and possibly to influence its specificity (Paul and Gilmour, 1968, 1969; Wang, 1971; Kamiyama and Wang, 1971; Spelsberg *et al.*, 1971; Stein *et al.*, 1972; Kamiyama *et al.*, 1972; Kostraba and Wang, 1972; Shea and Kleinsmith, 1973), and (d) to undergo specific changes in response to various hormones or phytomitogens (Teng and Hamilton, 1969; Shelton and Allfrey, 1970; Chung and Coffey, 1971; Levy *et al.*, 1973). In addition, several of the non-histone chromosomal proteins appear to function as enzymes, *e.g.*, histone acetyl transferases, deacetylases, kinases, and methylases; RNA polymerase, DNA polymerase, etc. (Wang, 1967; Howk and Wang, 1969; Kamiyama *et al.*, 1972; Vidali *et al.*, 1972).

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