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The nucleotide sequence of phenylalanine tRNA from the cytoplasm of Neurospora crassa

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ABSTRACT

The phenylalanine tRNA from the cytoplasm of Neurospora crassa has been purified and sequenced. The sequence is:

pGCGGGUUUAm^{2}GCUCA(N)GDDGGGAGAGCm$^2_2$GψCAGACmUGmAAYAψm^{5}CUGAAGm^{7}GDm^{5}CGUGUGTψCGm^{1}AUCCACACAAACCGCACCA$_{OH}$. Both in the nature of modified nucleotides which are present in this tRNA and in the overall sequence, this tRNA resembles more closely phenylalanine tRNAs of eukaryotic cytoplasm than those of prokaryotes. The sequence of this tRNA differs from those of the corresponding tRNAs of wheat germ and yeast by only 6 and 7 nucleotides respectively out of 76 nucleotides.

INTRODUCTION

As a part of our studies on the structure, function and evolution of tRNAs from eukaryotic organelles, we have previously reported on the sequences of tRNA^{Phe} from the chloroplasts of Euglena gracilis (1) and of several tRNAs from N. crassa mitochondria (2-4). One of the objectives in these studies is to determine whether these organelle tRNAs are more closely related in sequence to the corresponding tRNAs from eukaryotic cytoplasm or from prokaryotic sources. An important control for this is the sequence of the tRNA from the cytoplasm of the same species. We have, therefore, recently been involved in sequence analysis of tRNAs also from the cytoplasm of N. crassa (5) and of E. gracilis (6). In this paper we describe the sequence of tRNA^{Phe}

from the cytoplasm of N. crassa.

In an accompanying paper, Selker and Yanofsky (7) report the DNA sequence of the N. crassa gene corresponding to this tRNA. They have found that the $tRNA^{Phe}$ gene in N. crassa contains an intervening sequence (8,9). A knowledge of the sequence of N. crassa $tRNA^{Phe}$ has enabled these investigators to identify the nucleotides in the intervening sequence which are most likely excised during the processing of the $tRNA^{Phe}$ precursor (10,11).

MATERIALS AND METHODS

N. crassa cytoplasmic $tRNA^{Phe}$ was purified by RPC-5 chromatography essentially as described for the corresponding initiator tRNA (5). The sequence of this tRNA was established by using methods which involve the in vitro ^{32}P-labeling of tRNA and of oligonucleotide fragments as described in detail elsewhere (12).

RESULTS

1. Analysis of Oligonucleotides Present in Complete T_1-RNAse and Pancreatic RNase Digests.

The tRNA was digested with T_1-RNase or pancreatic RNase and the oligonucleotides produced were labeled at their 5'-ends with [^{32}P] (13). The 5'-[^{32}P] oligonucleotides were separated by two-dimensional electrophoresis (14) (Fig. 1A and B) and their sequences were determined by partial digestion with snake venom phosphodiesterase and/or nuclease P1 (15) followed by analysis of partial digestion products by electrophoresis on DEAE-cellulose paper and/or two-dimensional homochromatography (16,17).

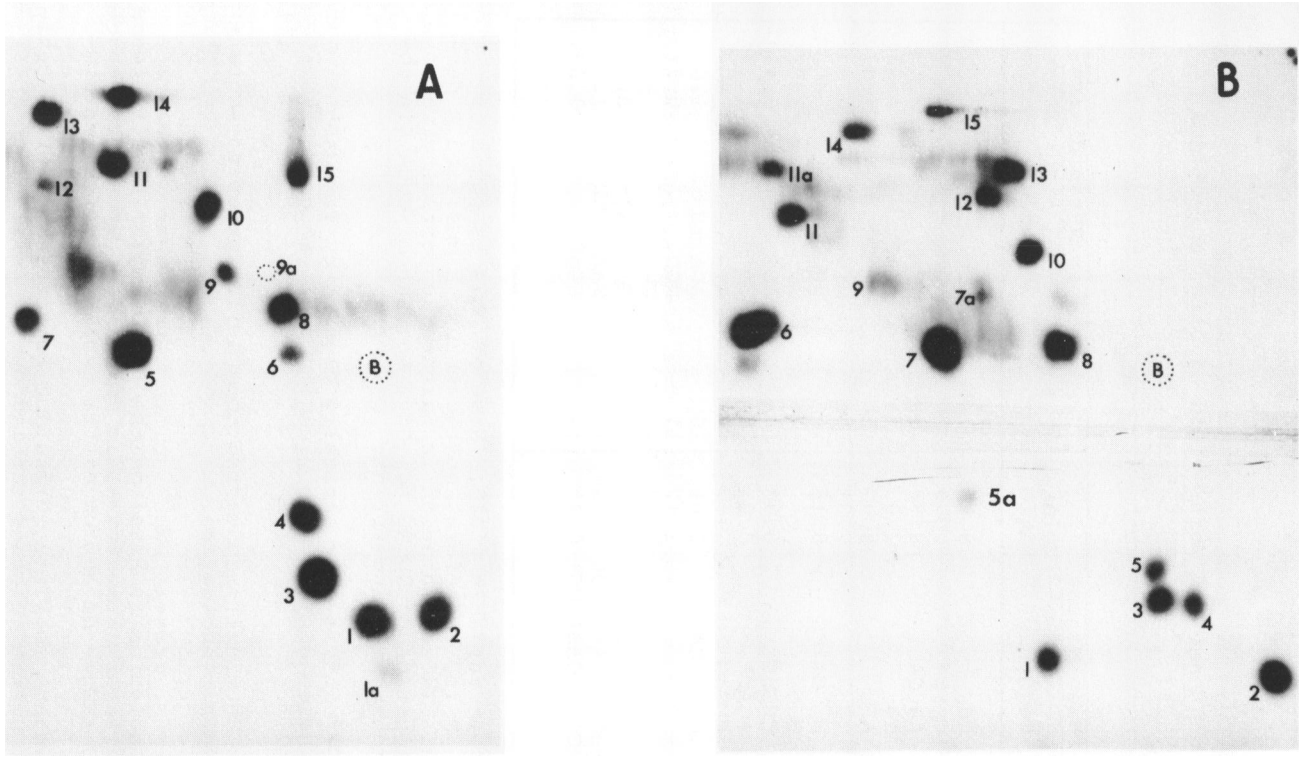

Figure 1. Autoradiographs of fingerprints of T_1-RNase (A) and pancreatic RNase (B) digests of N. crassa cytoplasmic $tRNA^{phe}_1$. Circled B, xylene cyanole blue tracking dye.

Table I lists the sequences and molar yields of these oligonucleotides.

TABLE I. Sequence and molar yield of oligonucleotides present in fingerprints of RNase digests of N. crassa cytoplasmic tRNA^{Phe}

T_1-RNase digest			Pancreatic RNase digest		
Spot Number	Sequence	Molar Ratio	Spot Number	Sequence	Molar Ratio
1	CG	0.98	1	pCp	-
1a	$Cm^2_\psi G$*	0.06	2	AC	3.17
2	CACCA	0.88	3	GC	2.05
3	AG	2.03	4	AAAC	1.02
4	AAG	0.93	5	Am^2GC	1.07
5	UG	2.31	5a	$m^2_\psi G\Psi$	0.35
6	m^7GDm^5CG	0.18	6	pUp	-
7	pGp	-	7	GU, GT	3.01
8	CUCNG	0.93	7a	AGD	0.44
9	ΨCAG*	0.21	8	Gm^1AU	1.15
9a	CUCAG	†	9	ATP	-
10	$Cm^2_\psi G\Psi CAG$*	0.66	10	AGACmU	1.0
11	TΨCG	1.12	11	NGD	0.71
12	DDG	0.10	11a	G-	-
13	$UUUAm^2G$	0.99	12	GmAAYAΨ++	0.51
14	$ACmUGmAAYA\Psi m^5CUG$	0.61	13	$GAAGm^7GD$	1.0
15	$m^1AUCCACACAAACCG$+	0.45	14	GGGU	0.33
			15	GGGAGAGC	0.24

† Present in variable yield. Most of this is found as CUCNG. N is, therefore, most probably a modified A derivative. This assumption is further supported by the close location of CUCNG relative to that of CUCAG in the two dimensional fingerprint.

* The $m^2_\psi G\Psi$ bond is only partially cleaved by T_1-RNase under the conditions used yielding small amounts of $Cm^2_\psi G$ and ΨCAG.

\+ m^1A was chemically converted to m^6A (12) prior to sequencing of this nucleotide.

++ The Y in N. crassa tRNA^{Phe} differs from that in yeast. This conclusion is based on the following: (a) difference in thin layer chromatographic mobility of Yp's obtained after T_2-RNase digestion of the tRNA^{Phe}s in the two-dimensional system described previously (5) and (b) differences in the mobility shifts due to removal of pY in analyses of partial digests carried out in parallel on [^{32}P] GmAAYAΨ isolated from N. crassa and from yeast tRNA^{Phe} (details in ref. 12).

Since preliminary analyses indicated a substantial similarity in the sequence of this tRNA to that of yeast $tRNA^{Phe}$, oligonucleotides isolated from corresponding fingerprints of yeast $tRNA^{Phe}$ were also analyzed in parallel and used as markers. Such analyses showed that while N. crassa $tRNA^{Phe}$, similar to yeast $tRNA^{Phe}$, also contains a fluorescent Y nucleoside (18) adjacent to the anticodon, in the sequence GmAAYAΨ, the Y in N. crassa $tRNA^{Phe}$ is different (see legend to Table I) from the corresponding one in yeast (19).

Sequence analysis of the remainder of the oligonucleotides present in complete nuclease digests of N. crassa $tRNA^{Phe}$ was relatively straight forward except for spot 8 of Fig. 1A which was characterized as CUCNG. N is most likely a modified derivative of A, T_1-RNase fingerprints contain variable amounts of a related sequence which was characterized as CUCAG (spot 9a of Fig. 1A, see legend to Table I).

2. Derivative of Total Sequence

The oligonucleotides listed in Table I were aligned into a unique sequence on the basis of the following lines of evidence: (i) analysis of sequences at the 5'- and 3'- termini of tRNA using 5'- and 3'- [^{32}P] labeled tRNA (15); (ii) sequence analysis of large oligonucleotides present in partial pancreatic RNase digests following their labeling with [^{32}P] at the 5'- ends and separation by two-dimensional polyacrylamide gel electrophoresis (12) and (iii) sequence analysis of the 3'-terminal 30 nucleotide long fragment obtained by specific cleavage of phosphodiester bond adjacent to m^7G in the tRNA (20). Figure 2 shows the linear sequence of the tRNA along with a list of oligonucleo-

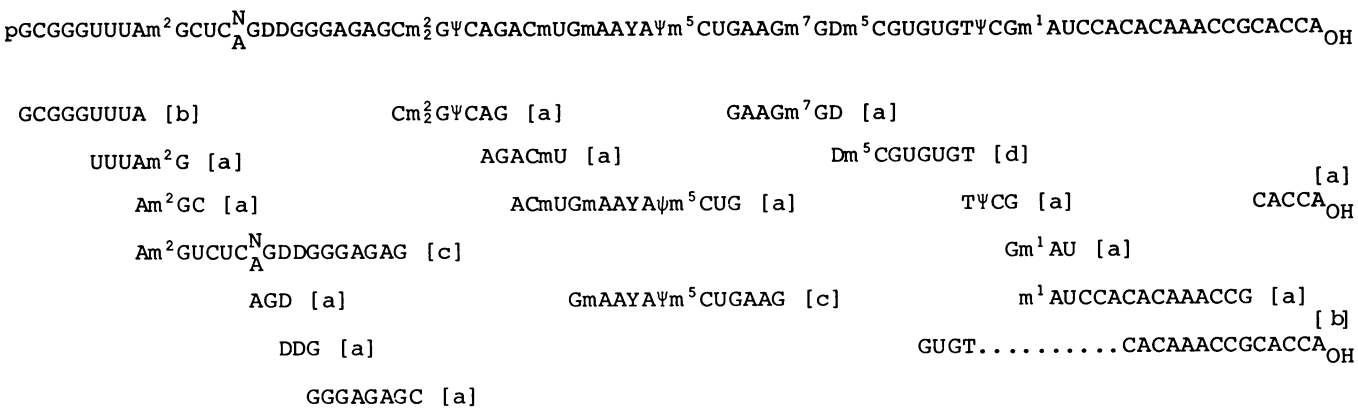

Figure 2. Sequence of N. crassa $tRNA^{Phe}$ in linear form (top) and oligonucleotides necessary for establishment of the sequence. [a], Fragments present in either complete T_1-RNase or pancreatic RNase digest of the tRNA; [b] sequence derived by analysis of 5'- or 3'-end labeled tRNA; [c], sequence derived by analysis of oligonucleotides present in partial pancreatic RNase digests and [d], sequence at the 5'-end of a 30 nucleotide long fragment obtained by specific cleavage of the tRNA.

tides necessary for establishment of the sequence. Figure 3 shows this sequence in the standard clover-leaf form.

DISCUSSION

The nucleotide sequence of N. crassa cytoplasmic $tRNA^{Phe}$ fits into the general pattern observed with other eukaryotic cytoplasmic $tRNA^{Phe}$ s in several respects. This tRNA also contains a large number of modified nucleosides including m^2G, m_2^2G, Y, m^5C, m^1A etc; which are absent in prokaryotic $tRNA^{Phe}_s$ but which have so far been found in all eukaryotic phenylalanine

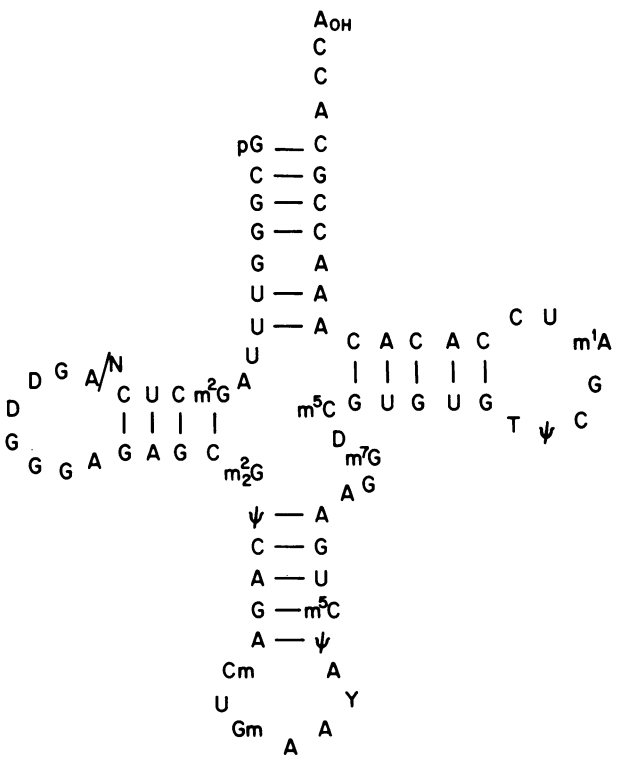

Figure 3. Sequence of N. crassa cytoplasmic $tRHA^{Phe}$ written in cloverleaf form.

tRNAs. The only exception is the $tRNA^{Phe}$ of D. melanogaster which lacks the hypermodified fluorescent Y nucleoside located next to the anticodon and which contains m^1G in place of Y (21). In terms of the overall nucleotide sequence also the N. crassa cytoplasmic $tRNA^{Phe}$ resembles much more $tRNA^{Phe}$s of eukaryotic cytoplasm than it resembles the corresponding tRNAs from prokaryotic sources (22), chloroplasts (1, 23) or mitochondria (24). Thus it differs from wheat germ and S. cerevisiae $tRNA^{Phe}$ by only 6 and 7 nucleotides out of a total of 76. The extent of homology of N. crassa cytoplasmic $tRNA^{Phe}$ varies between 76-92% for $tRNA^{Phe}$s of eukaryotic cytoplasm and between 61-68% for $tRNA^{Phe}$s of prokaryotes and chloroplasts. An exception is the $tRNA^{Phe}$ of the fission yeast S. pombe (25) with which the N. crassa $tRNA^{Phe}$ shares 64% homology, although it should be noted that this is about the extent of homology that exists between $tRNA^{Phe}$s of S. pombe and S. cerevisiae.

The gene for N. crassa cytoplasmic $tRNA^{Phe}$ has been cloned and sequenced by Selker and Yanofsky (7). They have found that the gene for this tRNA contains an intervening sequence. Based on the sequence of the tRNA reported in here, Selker and Yanofsky (7) have been able to define precisely the sites of excision of the intervening sequence from the putative precursor for this tRNA.

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