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Locations and sequences of tobacco chloroplast genes for $tRNA^{Pro}$(UGG), $tRNA^{Trp}$, $tRNA^{fMet}$ and $tRNA^{Gly}$(GCC): the $tRNA^{Gly}$ contains only two base-pairs in the D stem

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ABSTRACT

The nucleotide sequences of tobacco chloroplast genes for $tRNA^{Pro}$(UGG), $tRNA^{Trp}$, $tRNA^{fMet}$ and $tRNA^{Gly}$(GCC) have been determined. None of these genes contains an intron. One unusual feature is that the $tRNA^{Gly}$ contains only two base-pairs (A-U, G-U) in the D stem. These four tRNA genes were located in the known physical map of tobacco chloroplast DNA. Hybridization analysis to chloroplast tRNA revealed that all four tRNA genes are transcribed in vivo.

INTRODUCTION

Chloroplasts contain their own tRNAs which are believed to be coded in their chloroplast genomes (1). Eleven, twelve, six, and sixteen tRNA genes have been sequenced so far in tobacco (2,3,4,5), maize (2,6), spinach (2) and Euglena (2,7,8) chloroplast DNA, respectively, and these show features of both prokaryotic and eukaryotic tRNA genes.

To analyze the organization and fine structures of tRNA genes of tobacco chloroplasts, we have determined nucleotide sequences of the chloroplast DNA fragments which hybridized to its chloroplast tRNAs (2,3,4). However, their locations on a physical map of tobacco chloroplast DNA have not been determined. In this report, we show the map locations and nucleotide sequences of $tRNA^{Pro}$(UGG), $tRNA^{Trp}$, $tRNA^{fMet}$ and $tRNA^{Gly}$(GCC) genes of tobacco chloroplasts. The $tRNA^{Gly}$(GCC) deduced from the DNA sequence has only two base-pairs in the D stem.

MATERIALS AND METHODS

The recombinant plasmid pTS9, which contains a 5.7 kbp SalI fragment, and pTcS-2, which contains a 6.6 kbp EcoRI fragment, of Nicotiana tabacum (var. Bright Yellow 4) chloroplast DNA have been constructed as described (9). pTS9 DNA was digested with SalI and the 5.7 kbp fragment was separated by electrophoresis in a 5% polyacrylamide gel. pTcS-2 DNA was digested with

EcoRI and the 6.6 kbp fragment was separated as above. DNA sequence was determined by the method of Maxam and Gilbert (10). For Southern blot hybridization, DNA fragments were transferred to nylon membrane (Biodyne A Pall) and the membrane was immersed for 1hr at 65°C in the hybridization buffer (0.9 M NaCl, 50 mM sodium phosphate buffer (pH 7.7), 5 mM EDTA, 0.2% SDS, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin and 10 μg/ml denatured calf thymus DNA). Hybridization was carried out at 65°C for 16hr in the hybridization buffer containing [5' ^{32}P]-labeled tobacco chloroplast tRNAs.

RESULTS AND DISCUSSION

tRNA^{Pro} and tRNA^{Trp} genes

The 5.7 kbp SalI fragment (S9) of tobacco chloroplast DNA is located on a SalI cleavage map of tobacco chloroplast DNA (11) as shown in Fig. 1. On digestion with BglII and MluI, the 5.7 kbp fragment yields 3.4, 1.1, 0.8 and 0.4 kbp sub-fragments (Fig. 2a). Only the 1.1 kbp BglII-MluI sub-fragment

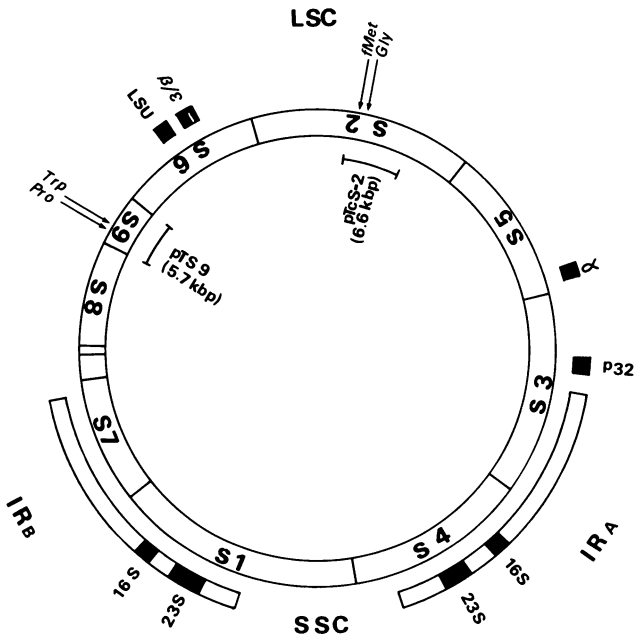


Fig. 1. Positions of the cloned fragments and the genes for tRNA^{Pro}, tRNA^{Trp}, tRNA^{fMet} and tRNA^{Gly} on the SalI cleavage map of tobacco chloroplast DNA (11). Arrows, tRNA genes; IRA and IRB, inverted repeat sequence (5); LSC and SSC, large and small single copy regions.

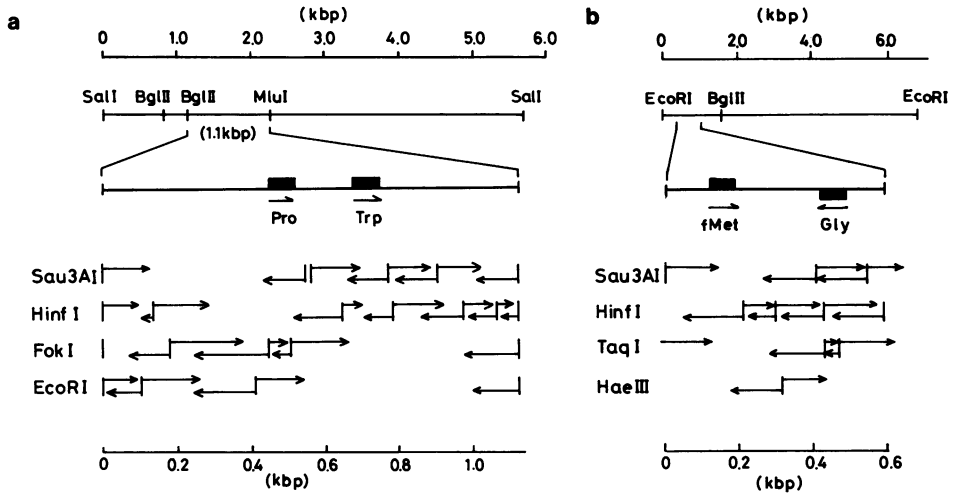
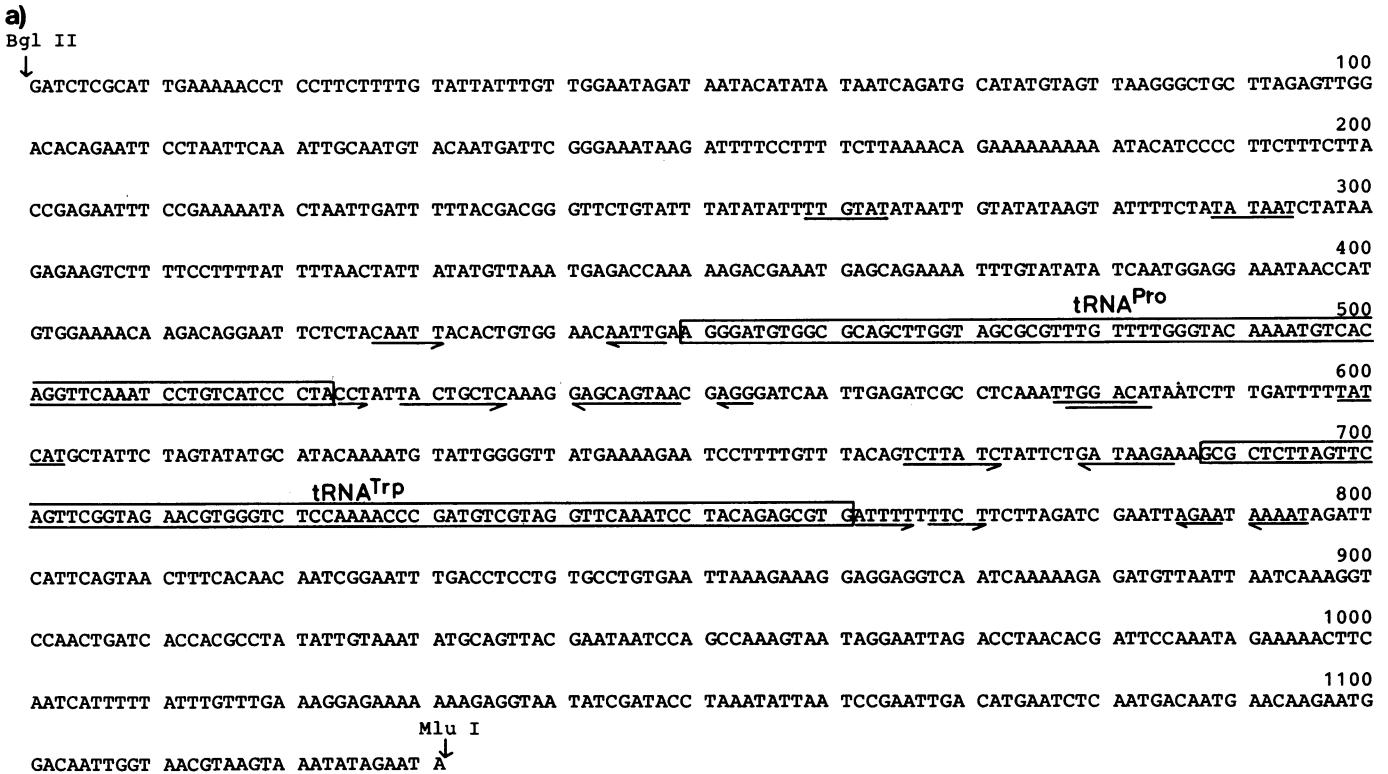


Fig. 2. Physical maps of the cloned 5.7 kbp SalI (a) and 6.6 kbp EcoRI (b) fragments from tobacco chloroplast DNA and the strategy for sequencing parts of them. Coding regions are shown by thick lines and arrows (⟶) below them indicate the direction of transcription.

was hybridized with total tobacco chloroplast tRNA (data not shown). We then sequenced the 1.1 kbp sub-fragment by the sequence strategy shown in Fig. 2a. Figure 3a shows the nucleotide sequence of the 1131 bp BglII-MluI sub-fragment.

A $tRNA^{Pro}$(UGG) gene was found between positions 450 and 523 (Figs. 3a & 4a). The $tRNA^{Pro}$(UGG) deduced from the DNA sequence shows 93% sequence homology with spinach chloroplast $tRNA^{Pro}$(UGG) (12). E. coli $tRNA^{Pro}$ sequence has not been reported (2,14). A $tRNA^{Trp}$ gene (688 to 761) was found 165 bp downstream from the $tRNA^{Pro}$ gene in the same orientation (Figs. 3a & 4b). The $tRNA^{Trp}$ deduced from the DNA sequence shows 96%, 87% and 67% sequence homologies with spinach chloroplast (13), Euglena chloroplast (7) and E. coli (14) $tRNA^{Trp}$, respectively.

A "Pribnow box"-like sequence TATAAT (289 to 294) and a "-35 region"-like sequence TTGTAT (269 to 264) were found in the region upstream from the $tRNA^{Pro}$ gene (Fig. 3a). Likewise in the region upstream from the $tRNA^{Trp}$ gene a "Pribnow box"-like sequence TATCAT (598 to 603) and a "-35 region"-like sequences TTGGACA (577 to 583) were found (Fig. 3a). These tRNA genes may be transcribed separately. Inverted repeat sequences (427 to 448, 524 to 554, 666 to 685, and 762 to 795) were found in both flanking


a)
Bgl II
100
GATCTCGCAT TGAAAAACCT CCTTCTTTTG TATTATTTGT TGGAATAGAT AATACATATA TAATCAGATG CATATGTAGT TAAGGGCTGC TTAGAGTTGG
200
ACACAGAATT CCTAATTCAA ATTGCAATGT ACAATGATTC GGGAAATAAG ATTTTCCTTT TCTTAAAACA GAAAAAAAAA ATACATCCCC TTCTTTCTTA
300
CCGAGAATTT CCGAAAAATA CTAATTGATT TTTACGACGG GTTCTGTATT TATATATTTT GTATATAATT GTATATAAGT ATTTTCTATA TAATCTATAA
400
GAGAAGTCTT TTCCTTTTAT TTTAACTATT ATATGTTAAA TGAGACCAAA AAGACGAAAT GAGCAGAAAA TTTGTATATA TCAATGGAGG AAATAACCAT
tRNAPro
500
GTGGAAAACA AGACAGGAAT TCTCTACAAT TACACTGTGG AACAATTGAA GGGATGTGGC GCAGCTTGGT AGCGCGTTTG TTTTGGGTAC AAAATGTCAC
600
AGGTTCAAAT CCTGTCATCC CTACCTATTA CTGCTCAAAG GAGCAGTAAC GAGGGATCAA TTGAGATCGC CTCAAATTGG ACATAATCTT TGATTTTTAT
700
CATGCTATTC TAGTATATGC ATACAAAATG TATTGGGGTT ATGAAAAGAA TCCTTTTGTT TACAGTCTTA TCTATTCTGA TAAGAAAGCG CTCTTAGTTC
tRNATrp
800
AGTTCGGTAG AACGTGGGTC TCCAAAACCC GATGTCGTAG GTTCAAATCC TACAGAGCGT GATTTTTTTC TTCTTAGATC GAATTAGAAT AAAATAGATT
900
CATTCAGTAA CTTTCACAAC AATCGGAATT TGACCTCCTG TGCCTGTGAA TTAAAGAAAG GAGGAGGTCA ATCAAAAAGA GATGTTAATT AATCAAAGGT
1000
CCAACTGATC ACCACGCCTA TATTGTAAAT ATGCAGTTAC GAATAATCCA GCCAAAGTAA TAGGAATTAG ACCTAACACG ATTCCAAATA GAAAAACTTC
1100
AATCATTTTT ATTTGTTTGA AAGGAGAAAA AAAGAGGTAA TATCGATACC TAAATATTAA TCCGAATTGA CATGAATCTC AATGACAATG AACAAGAATG
Mlu I
GACAATTGGT AACGTAAGTA AATATAGAAT A

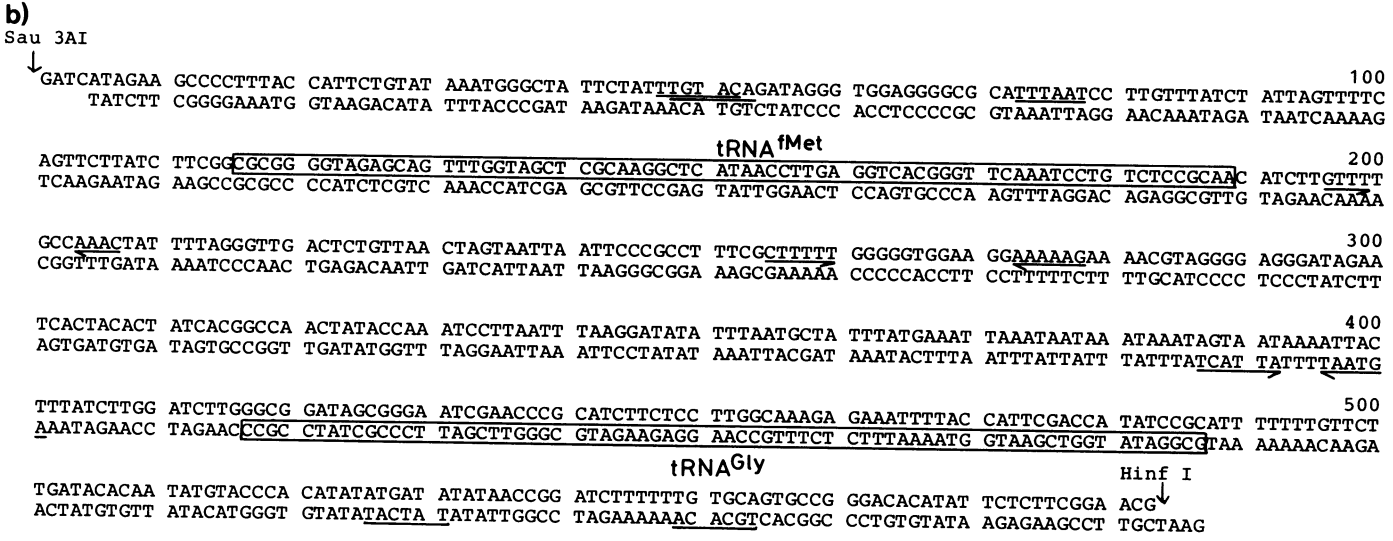


Fig. 3. Nucleotide sequences of the regions containing $tRNA^{Pro}$(UGG) and $tRNA^{Trp}$ genes (a), and $tRNA^{fMet}$ and $tRNA^{Gly}$(GCC) genes (b). Coding regions are boxed. "Pribnow box"-like sequences and "-35 region"-like sequences are underlined. Horizontal arrows indicate inverted repeat sequences.

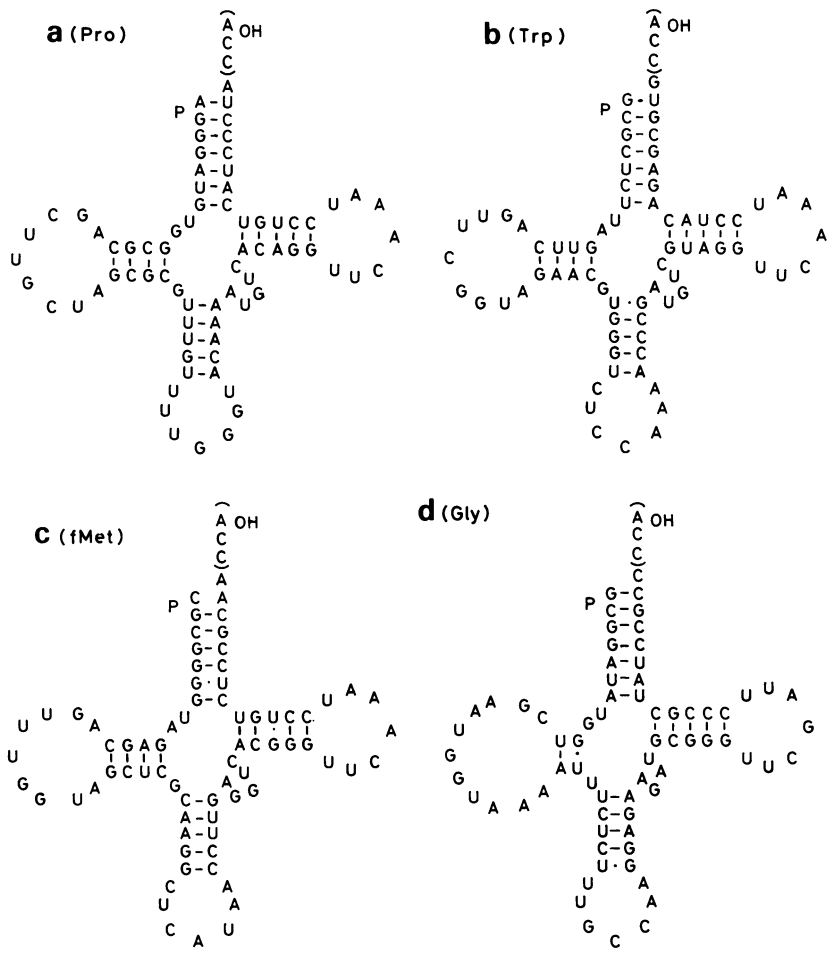


Fig. 4. Sequences of unmodified bases and cloverleaf structures of tobacco chloroplast $tRNA^{Pro}$(UUG) (a) $tRNA^{Trp}$ (b), $tRNA^{fMet}$ (c) and $tRNA^{Gly}$(GCC) (d) predicted from the DNA sequences.

regions of the $tRNA^{Pro}$ and $tRNA^{Trp}$ genes (Fig. 3a). These sequences may be related to tRNA processing and transcriptional termination.

$tRNA^{fMet}$ and $tRNA^{Gly}$ genes

The 6.6 kbp EcoRI fragment of tobacco chloroplast DNA is located in the SalI fragment 2 (S2) on the tobacco chloroplast DNA map (11) shown in Fig. 1. On digestion with BglII, the 6.6 kbp EcoRI fragment yields 5.0 and 1.6 kbp sub-fragments (Fig. 2b). The 1.6 kbp EcoRI-BglII sub-fragment was hybridized with total tobacco chloroplast tRNA (data not shown). We then

sequenced part of the 1.6 kbp sub-fragment by the sequence strategy shown in Fig. 2b. Figure 3b shows the nucleotide sequence of a 583 bp portion of it.

A $tRNA^{Met}$ gene was found between positions 116 and 189 (Figs. 3b & 4c). It is likely to be an initiator $tRNA^{Met}$ gene because its sequence is identical with that of spinach chloroplast initiator $tRNA^{Met}$ (15). The $tRNA^{fMet}$ deduced from the DNA sequence shows 84% and 83% sequence homologies with Euglena chloroplast (8) and E. coli (14) $tRNA^{fMet}$, respectively, but only 47% homology with the tobacco chloroplast elongator $tRNA^{Met}$ (3). A $tRNA^{Gly}$(GCC) gene (417 to 487) was found 228 bp downstream from the $tRNA^{fMet}$ gene in the opposite orientation (Figs. 3b & 4d). The $tRNA^{Gly}$(GCC) deduced from the DNA sequence shows 63%, 61% and 73% sequence homologies with Euglena chloroplast (8), E. coli (14) $tRNA^{Gly}$(GCC) and tobacco chloroplast $tRNA^{Gly}$(UCC) (4), respectively. The 3'-terminal CCA sequence is not found in any of the tRNA genes presented here.

A "Pribnow box"-like sequence TTTAAT (73 to 78) and a "-35 region"-like sequence TTGTACA (47 to 53) were found in the region upstream from the $tRNA^{fMet}$ gene (Fig.3b). Likewise the region upstream from the $tRNA^{Gly}$ gene contains a "Pribnow box"-like sequence TATCAT (531 to 526) and a "-35 region"-like sequence TGCACA (554 to 549) (Fig. 3b). Inverted repeat sequences (196 to 207, 255 to 278, and 387 to 401) were found in the regions downstream from the $tRNA^{fMet}$ and $tRNA^{Gly}$ genes. These sequences may be tRNA processing or transcriptional termination signals.

One unusual feature is that the $tRNA^{Gly}$ deduced from the DNA sequence has only one Watson-Crick base-pair (A-U) and an additional G-U pair in the D stem (Fig. 4d). Transfer RNAs with such base-pairs in the D stem have not been reported, except for Xenopus mitochondrial $tRNA^{Asn}$ and mammal mitochondrial $tRNA^{Ser}$ which have no D stems (2,14). Wheat germ, rat and mouse $tRNAs^{Ser}$ contain one A-U pair and two additional G-U pairs in their D stems and a calf $tRNA^{Tyr}$ has two C-G pairs only in its D stem (2,14). The tobacco chloroplast $tRNA^{Gly}$(UCC) has been reported to contain a 691 bp intron in the D stem (4). Therefore the tobacco chloroplast $tRNA^{Gly}$ species are quite unique in their D stems.

Based on fine physical mapping of the 5.7 kbp SalI and 6.6 kbp EcoRI fragments and their neighboring regions (data not shown), the $tRNA^{Pro}$, $tRNA^{Trp}$ and $tRNA^{fMet}$ are coded for on the same strand as the α and β genes for H^+-ATPase (16, strand B) and the $tRNA^{Gly}$ gene on the same strand as the LS gene for ribulose-1,5-bisphosphate carboxylase (17, strand A) of the

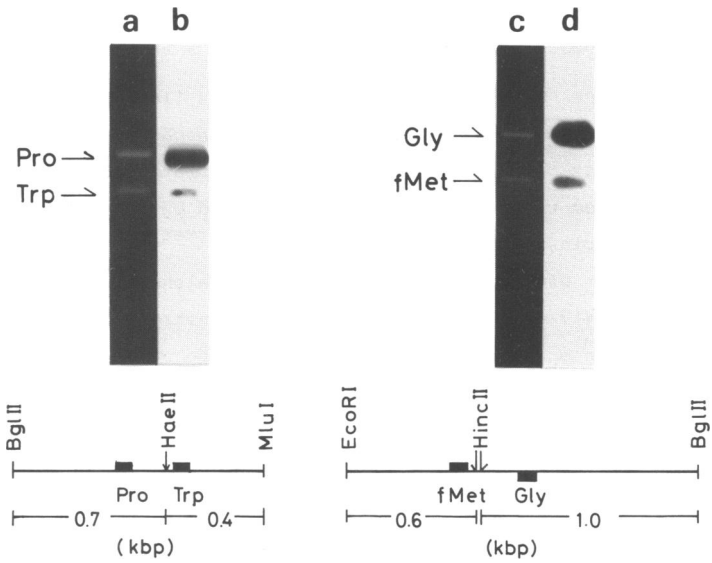


Fig. 5. 2% agarose gel electrophoresis of restriction fragments and autoradiographs of Southern blots hybridized with total tobacco chloroplast tRNAs. HaeII digest of the 1.1 bp BglII-MluI sub-fragment from the 5.7 kbp SalI fragment (a). HincII digest the 1.6 kbp EcoRI-BglII sub-fragment from the 6.6 kbp EcoRI fragment (c). Autoradiographs (b,d).

tobacco chloroplast DNA (Fig. 1). The locations of these four tRNA genes are consistent with the tRNA gene map obtained by the tRNA/DNA hybridization studies (18).

Expression of tRNA genes

To examine whether these four tRNA genes are expressed, Southern hybridization was carried out. The 1.1 kbp BglII-MluI sub-fragment of the 5.7 kbp SalI fragment was cut with HaeII into two fragments, one containing the $tRNA^{Pro}$ gene and the other containing the $tRNA^{Trp}$ gene. The 1.6 kbp EcoRI-BglII sub-fragment of the 6.6 kbp EcoRI fragment was cut with HincII into two main fragments, one containing the $tRNA^{fMet}$ gene and the other containing the $tRNA^{Gly}$ gene. Total tobacco chloroplast tRNAs hybridized to these four fragments (Fig. 5), indicating that these tRNA genes are expressed *in vivo*.

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