Hybridization of Maize Chloroplast DNA with Transfer Ribonucleic Acids†

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ABSTRACT: Hybridization of [^{125}I]tRNA to chloroplast DNA indicates that 0.60–0.75% of maize chloroplast DNA contains sequences complementary to maize tRNA, corresponding to 20–26 tRNA cistrons. Green maize seedlings contain about twice the amount of chloroplast DNA-hybridizable tRNA as etiolated maize seedlings. tRNA from green or etiolated maize seedlings was also aminoacylated in vitro with 21 labeled amino acids and then incubated with filters containing chloroplast DNA. tRNAs charging a total of at least 16 different amino acids hybridized with chloroplast DNA. Most of these plastid aminoacyl-tRNAs were present in higher concentrations in tRNA isolated from green maize seedlings, although there were several exceptions. The results are consistent with the hypothesis that a complete or nearly complete set of tRNAs can be transcribed from chloroplast DNA.

Chloroplasts from higher plants contain most of the metabolic machinery necessary for independent protein synthesis, including tRNA (Bogorad, 1967). In some cases, individual plastid tRNAs have been distinguished from isoaccepting cytoplasmic tRNAs (Burkard et al., 1970). Plastid tRNAs are also found in etiolated (nongreen) leaves of plants grown entirely in the dark (Burkard et al., 1973).

Following transfer of etiolated seedlings into light (greening), the chlorophyll and protein content of the plastids increases and synthesis of the photosynthetic apparatus is completed. The activity of the plastid RNA polymerase is enhanced during greening (Bogorad, 1967) as is the rate of synthesis of plastid ribosomal RNA.

We speculated that enhanced plastid RNA polymerase activity might also increase the synthesis of plastid tRNAs. These plastid tRNAs in turn might also influence the rate of plastid protein synthesis. In this paper, we attempted to measure the number of types of tRNAs transcribed from plastid DNA and the changes that occur during greening. tRNA was prepared from whole leaf tissue instead of isolated plastids to assure isolation of all tRNA species. Plastid tRNAs were identified by the sequence homology between tRNA and chloroplast DNA as demonstrated in DNA–RNA hybridization experiments.

The amount of tRNA in etiolated or green maize leaves that is complementary to plastid DNA was determined by hybridization of ^{125}I-labeled tRNA to excess amounts of chloroplast DNA. The amount of chloroplast DNA complementary to tRNA was also obtained, by incubating excess amounts of [^{125}I]tRNA with chloroplast DNA.

The total tRNA from green or etiolated maize leaves was labeled with a mixture of 21 labeled amino acids using a mixture of amino acid–tRNA ligases (aminoacyl-tRNA synthetases). The amounts of the different sets of isoaccepting plastid tRNAs for which genes occur in maize plants were determined by amino acid analysis of those labeled aminoacyl-tRNAs that hybridized specifically to chloroplast DNA.

Experimental Procedures

Materials. The following materials were obtained from the indicated sources: maize seeds (*Zea mays* FR9^{c}MS × FR37), Illinois Foundation Seeds, Inc. (Champaign, Ill.); all nucleases, Pronase, *M. lysodeikticus* DNA, Sigma Chemical Co. (St. Louis, Mo.); Sarkosyl (sodium *N*-lauroylsarcosinate), K & K Laboratories, Inc. (Plainview, N.Y.); carrier-free [^{125}I]-Aquasol, and most labeled amino acids, New England Nuclear (Boston, Mass); cesium chloride, Calbiochem (LaJolla, Calif.).

Growth of Maize Seedlings and Isolation of Maize DNAs. Maize seedlings were grown as previously described (Bottomley et al., 1971). Etiolated seedlings were grown in total darkness for 8 days. Green plants were obtained by illuminating 7 day old etiolated seedlings with fluorescent lamps for 17 h. Maize chloroplast DNA and "whole" maize DNA were isolated as described in the following paper in this issue (Haff and Bogorad, 1976).

Isolation of Whole Maize tRNA. Total nucleic acid was isolated from maize leaves by the method of Loening (1969). The total nucleic acid from 50 g of leaves was incubated at 37 °C for 30 min in 10 ml of a solution containing 25 mM Tricine–KOH (pH 7)–10 mM $MgCl_2$–100 μg/ml of DNase. After this incubation, Pronase was added to a final concentration of 100 μg/ml and the sample was incubated for an additional 15 min. Following these treatments, the solution of RNA was extracted twice with phenol and precipitated with ethanol. The pellet of RNA was dissolved in 2 ml of 25 mM Tricine–KOH and fractionated further by CsCl gradient centrifugation. About 25 mg of RNA was isolated per 100 g of etiolated green leaf tissue.

RNA preparations were purified by centrifugation of up to 15 mg of RNA in a block gradient of CsCl in a Spinco SW40 rotor. Each tube contained, from bottom to top: 2 ml of CsCl, density 1.75 g/cm^3, 2 ml of CsCl, density 1.60 g/cm^3, the sample, and mineral oil. All aqueous solutions contained 0.04% Sarkosyl. The rotor was centrifuged at 34 000 rpm for 19 h at 0 °C. Under these conditions, tRNA remained in the gradient (while higher molecular weight RNA pelleted), and was further purified by Sephadex and DEAE[1]-cellulose chromatography as described by Bock and Cherayil (1967).

Radioiodination of Maize tRNA. Reactions were carried

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[1] Abbreviations used are: SSC, 0.15 M NaCl–0.015 M trisodium citrate; DEAE, diethylaminoethyl; T_m, the temperature at which one-half of a hybrid nucleic acid is melted; C_0t and R_0t are defined as mol of DNA or RNA nucleotides per liter times seconds, respectively.

TABLE I: Labeled Amino Acids Used to Prepare Aminoacyl-tRNA.[a]

Amino Acid[b]	Label	cpm/Reaction Mixture ($\times 10^{-7}$)	cpm/mol ($\times 10^{-15}$)
Ala	3-^{3}H(N)	1.34	7.70
Arg	3-^{3}H(N)	1.18	12.1
Asn	2,3-^{3}H	4.21	0.652
Asp	2,3-^{3}H(N)	1.34	6.65
Cy-SH (D-L)	3-^{14}C	0.90	0.0054
Glu	3-^{3}H	2.10	9.85
Gln	^{14}C(G)	1.00	0.0030
Gly	2-^{3}H	0.670	1.15
His	3-^{3}H	0.250	1.23
Ile	4,5-^{3}H(N)	0.840	26.7
Leu	4,5-^{3}H	2.35	25.3
Lys	4,5-^{3}H(N)	1.01	2.11
Met	^{3}H(G)	3.24	0.160
Phe	3-^{3}H(N)	1.34	3.69
Pro	^{3}H(G)	0.840	1.84
Ser	^{3}H(G)	0.670	1.31
Thr	^{3}H(G)	0.840	0.76
Trp	^{3}H(G)	0.84	0.76
Tyr	3,5-^{3}H	0.67	0.25
Val	^{3}H(G)	1.35	0.630

[a] Abbreviations: (G), generally labeled; (N), nominally labeled. [b] All amino acids except Cy-SH were L-amino acids.

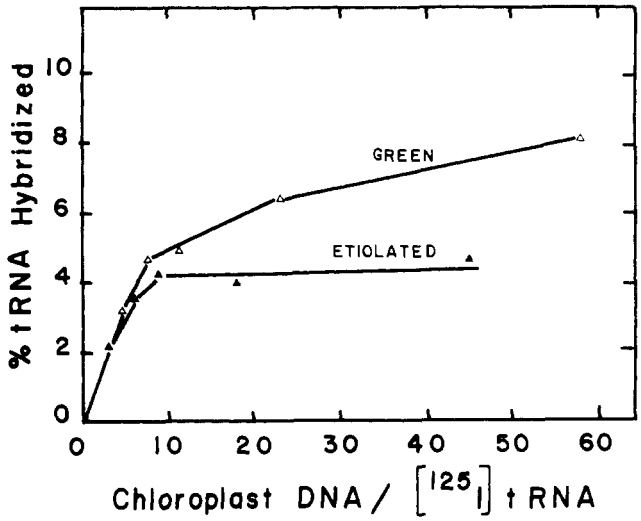

FIGURE 1: Hybridization of [^{125}I]tRNA to excess amounts of maize chloroplast DNA. Variable amounts of [^{125}I]tRNA prepared from green or etiolated maize seedlings were incubated with filters containing 19.8 μg of chloroplast DNA. The specific activity of the etiolated maize tRNA was 7.5×10^3 cpm/μg. The specific activity of the tRNA from green maize seedlings was adjusted to the same specific activity by addition of unlabeled tRNA to labeled green maize tRNA of slightly higher specific activity. The samples were incubated as described under Experimental Procedures for 20 h and the hybrids were purified as described by Nass and Buck (1970), including the ribonuclease T_1 treatment. The percentage of input tRNA forming stable hybrids with chloroplast DNA was plotted against the ratio of chloroplast DNA to [^{125}I]tRNA in the incubation mixture. Blank values averaged about 0.04% of input radioactivity. Hybridizations under these conditions are nearly complete, since in a second incubation with 19.8 μg of chloroplast hybridized only an additional 1% of the tRNA from green seedlings and 0.7% of the tRNA from etiolated seedlings were hybridized.

out in stoppered siliconized glass centrifuge tubes in a total volume of 50 μl. Up to 100 μg of tRNA was incubated at 60 °C in a solution of 0.1 M sodium acetate, pH 5.0, 5 μM potassium iodide; 0.1 mM thallic trichloride, and containing up to 1 mCi of carrier-free ^{125}I. After 10 min, the reaction was terminated by chilling to 0 °C and 1 ml of 0.1 M Tris-HCl (pH 8.5) was added. The sample was incubated for 30 min at 60 °C to destroy unstable intermediates. The ^{125}I-labeled tRNA was then chromatographed on a methylated albumin-kieselguhr column as described by Osawa and Sibatani (1967), which separated unbound ^{125}I from the tRNA. The radioactivity in [^{125}I]tRNA coeluted with unlabeled maize tRNA (assayed by ultraviolet absorbance), indicating that the radioiodination procedure did not extensively degrade tRNA. All tRNA fractions eluting from the column between 0.15 and 0.6 M NaCl were combined, and the labeled tRNA was precipitated by the addition of two volumes of ethanol. Typically, 85 μg of tRNA labeled to a specific activity of 10^4 cpm/μg was obtained from 100 μg of tRNA incubated with 0.5 mCi of ^{125}I. The hybridization specificity of RNA labeled to this specific activity should not be perturbed (Tereba and McCarthy, 1973).

Preparation of Maize Amino Acid-tRNA Ligases. Green maize leaves were harvested and homogenized as described by Bottomley et al. (1971). Chloroplasts in the homogenate were lysed by osmotic shock, the homogenate was centrifuged at 3000 rpm for 15 min in a Sorvall SS-34 rotor, and the pellet was discarded. The supernatant solution was centrifuged in a Spinco 30 rotor for 4.75 h at 30 000 rpm and the upper two-thirds of each tube was collected. tRNA was removed from the sample by chromatography on DEAE-cellulose as described by von Ehrenstein (1967). About 50 μg/ml of ligase mixture produced optimal rates of aminoacylation.

Preparation of Labeled Aminoacyl-tRNA. Whole tRNA from either green or etiolated maize seedlings was incubated at 30 °C for 10 min in 2 ml of a solution containing: 100 mM Tricine-KOH (pH 7.5), 10 mM $MgCl_2$, 10 mM KCl, 1 mM ATP, 0.1 mM CTP, 7 mM mercaptoethanol, 25 μg/ml of tRNA, 50 μg/ml of maize amino acid-tRNA ligase mixture, and about 10^8 cpm of amino acids. The composition of the amino acid mixture is presented in Table I. The reaction was stopped by addition of 2 ml of 0 °C 1 M sodium acetate (pH 4.5) and the mixture was deproteinized by extraction with an equal volume of 90% phenol-10% *m*-cresol (adjusted to pH 4.5 with acetic acid). The labeled aminoacyl-tRNA was precipitated with ethanol, dissolved in 10 mM sodium acetate (pH 4.5), and chromatographed on a 45 cm^3 column of Sephadex G-50-80 to remove unreacted amino acids.

DNA-tRNA Hybridization Conditions. Filters containing immobilized DNA were prepared as described by Gillespie (1968). The DNA content of the filters was assayed by the A_{260} released by incubating the filters for 30 min at 37 °C in 1 ml of 25 mM Tricine-KOH (pH 7.0)-10 mM $MgCl_2$-50 μg/ml of deoxyribonuclease. [^{125}I]tRNA was hybridized with DNA as described by Gillespie (1968) in 2 ml of a solution containing 4 × SSC and 40% formamide, at 37 °C. Labeled aminoacyl-tRNAs were hydridized to DNA as described by Nass and Buck (1970).

Analysis of Labeled Aminoacyl-tRNAs. Labeled aminoacyl-tRNAs, either alone or hybridized to DNA, were incubated in 25 mM ammonium hydroxide for 1 h at 37 °C. The amino acids discharged from the tRNA by this alkaline hydrolysis were desalted on a Dowex 50W-X8 column, dissolved in 50 mM HCl, and separated by column chromatography in an updated Beckman 120 C automatic amino acid analyzer. Labeled amino acids were identified by their position of elution with unlabeled standard amino acids (detected by ninhydrin assay).

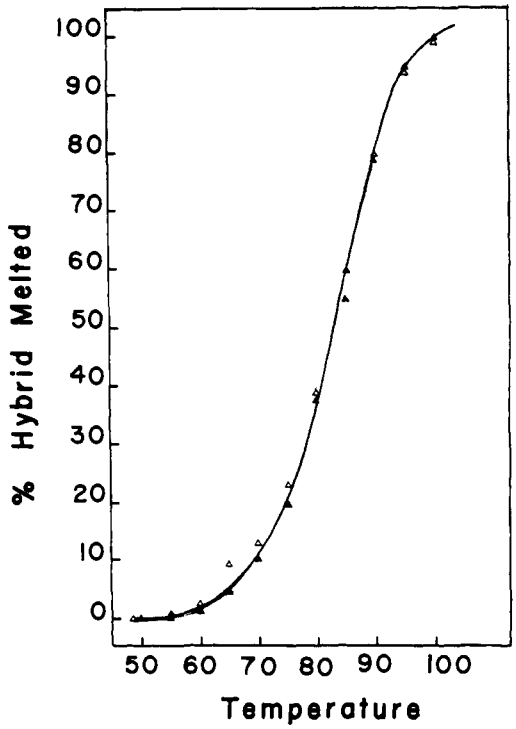

FIGURE 2: Thermal denaturation plot of [^{125}I]tRNA-chloroplast DNA hybrids. Filters containing [^{125}I]tRNA-chloroplast DNA hybrids were immersed in 1 × SSC at 50 °C for 10 min. The temperature was raised in increments of about 5 °C while the filter equilibrated to the new temperature for 10 min. The dissociated RNA was withdrawn with a Pasteur pipet and the procedure was repeated up to a temperature of 100 °C. The cumulative radioactivity released was plotted against temperature. (△) Three filters, each containing 19.8 μg of immobilized chloroplast DNA and 0.13 μg of hybridized [^{125}I]tRNA isolated from green maize seedlings, were incubated as described above. The specific activity of the hybridized tRNA was 7.5 × 10^3 cpm/μg. (▲) Three filters, each containing 19.8 μg of immobilized chloroplast DNA and 0.07 μg of hybridized [^{125}I]tRNA isolated from etiolated maize seedlings, were incubated as described above. The specific activity of the hybridized tRNA was 7.5 × 10^3 cpm/μg.

Determination of Radioactivity. Samples containing ^{125}I were counted in a Picker Liquimat Model 110 γ-ray counter. Samples containing ^{3}H or ^{14}C were counted in a Packard Model 3375 scintillation counter with Aquasol, and corrected for quenching by the addition of internal standards.

Miscellaneous Instrumentation. Salt concentrations were determined using a YSI Model 31 conductivity bridge. Ultraviolet absorbance was determined with a Gilford Model 2000 ultraviolet spectrophotometer. RNA concentrations were calculated assuming that a 1 mg/ml solution of RNA has an absorbance of 22.5 at 250 nm. Filtrations were performed on a New Brunswick Model DNA-10 Membrane Filtration Apparatus.

Results

Detection of Plastid tRNAs in Whole Maize tRNA Preparations. The percentage of plastid tRNA in whole leaf tRNA was determined by hybridizing chloroplast DNA to whole [^{125}I]tRNAs. As shown in Figure 1, a maximum of about 4% of the tRNA from etiolated leaves hybridized to excess amounts of chloroplast DNA, while 8% or more of the tRNA from green leaves can hybridize to chloroplast DNA. When incubated for the same time and under the same conditions, a much smaller fraction (1.5% of the input) of the tRNA from green maize leaves hybridized to 20 μg of whole corn DNA. This slow rate of hybridization to whole corn DNA was expected, since sequences encoding tRNAs in plant nuclear DNAs are relatively dilute (Williams and Williams, 1970). The results obtained in hybridizations with purified chloroplast DNA are, therefore, not greatly affected by small amounts of contaminating nuclear DNA in chloroplast DNA preparations.

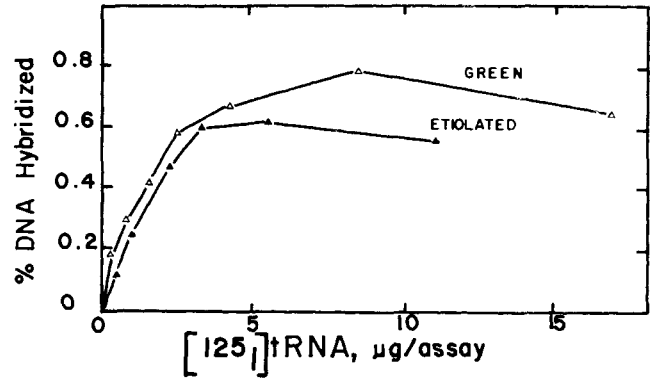

FIGURE 3: Percentage of maize chloroplast DNA complementary to [^{125}I]tRNA. The indicated amounts of [^{125}I]tRNAs (preparation utilized in Figure 1) were incubated with filters containing 10.2 μg of chloroplast DNA as described under Experimental Procedures, with an incubation time of 17 h. The amount of hybridized DNA was determined from the radioactivity of hybridized tRNA. Blank values determined with plain filters averaged about 0.04% of input radioactivity.

Both preparations of tRNA formed tRNA-chloroplast DNA hybrids that melted sharply with a T_m of 83 °C (Figure 2). Therefore, the increased amount of green leaf tRNA that hybridized to chloroplast DNA is not likely to be an artifact created by tRNAs that hybridized with poor specificity to chloroplast DNA.

Amounts of Chloroplast DNA Complementary to tRNA. The amount of chloroplast DNA complementary to tRNA was determined by hybridizing excessive amounts of [^{125}I]tRNA to chloroplast DNA. As shown in Figure 3, a maximum of 0.60% of chloroplast DNA hybridized to [^{125}I]tRNA prepared from etiolated seedlings. About 0.75% of chloroplast DNA hybridized to the corresponding tRNA prepared from green seedlings. The melting curves of these hybrids were essentially identical to the curve shown in Figure 2. The difference in amounts of chloroplast DNA hybridizing to the two tRNA preparations may not be significant, since the specific radioactivity of the individual hybridized [^{125}I]tRNAs may vary from the mean.

Preparation of In Vitro Labeled Amino Acyl-tRNA. tRNA preparations were incubated with a mixture of labeled amino acids and maize amino acid-tRNA ligases, as described under Experimental Procedures. After the labeled aminoacyl-tRNA was separated from unreacted amino acids, the amino acids were discharged from the tRNA and subjected to amino acid analysis. Table II illustrates the composition of the aminoacyl-tRNAs synthesized from total maize tRNA. The molar percentage of each aminoacyl-tRNA was determined from the radioactivity in each amino acid and the data in Table I assuming a molecular weight of 25 000/tRNA. With these assumptions, we calculated that nearly 1 mole of amino acid (±20%) was esterified per mole of tRNA. Reliably detectable amounts of aminoacyl-tRNA were detected for all amino acids tested except cysteine, glutamine, and methionine.

Hybridization of Labeled Maize Amino Acyl-tRNA to Chloroplast DNA. Previous experiments (such as shown in Figure 1) indicated that most chloroplast-DNA hybridizable tRNAs would hybridize to chloroplast DNA upon incubation

TABLE II: Composition of in Vitro Labeled Total Maize Aminoacyl-tRNA.[a]

Aminoacyl-tRNA	Mol % of Aminoacyl-tRNA	
	Green	Etiolated
Ala	0.79	0.48
Arg	4.98	5.59
Asn	ND[c]	8.01
Asp	1.42	1.47
Cy-SH	ND[c]	ND[c]
Glu	1.85	3.18
Gln	ND[c]	ND[c]
Gly	11.7	3.23
His	0.92	0.91
Ile	1.04	3.05
Leu	3.63	5.43
Lys	1.89	1.31
Met	ND[c]	ND[c]
Phe	3.21	2.37
Pro	5.67	4.85
Ser[b]		
Thr[b]	26.6	17.2
Trp	0.22	0.26
Tyr	9.1	24.2
Val	27.0	18.5

[a] Total maize tRNA prepared from green or etiolated maize seedlings was charged with amino acids in vitro as described under Experimental Procedures. Each purified preparation (0.5 μg) was deacylated and the amino acids from the aminoacyl-tRNA were separated on an amino acid analyzer as described under Experimental Procedures. Each preparation contained a total of about 5×10^4 cpm (4×10^{-11} mol) of amino acid per μg of tRNA. [b] Serine and threonine were not resolved. The corresponding value represents the combined cpm of the two amino acids divided by the average of their specific activities (which differ by 25%). [c] ND, not detected (less than 50 cpm above blank values). Values represent results of duplicate experiments; these differed by less than 25%.

TABLE III: Labeled Maize Aminoacyl-tRNA Hybridized to Chloroplast DNA.[a]

Aminoacyl-tRNA	Mol Hybridized ($\times 10^{13}$)		Ratio Green/ Etiolated	Lower Limit of Detection (mol $\times 10^{13}$)
	Green	Etiolated		
Ala	0.90	0.74	1.2	0.064
Arg	1.12	0.84	1.3	0.042
Asn	0.58	1.0		0.76
Asp	0.58	1.0	0.56	0.074
Cy-SH	ND[b]	ND[b]		9.2
Glu	0.46	0.86	0.53	0.050
Gln	ND[b]	ND[b]		16.8
Gly	7.3	5.2	1.41	0.44
His	1.2	0.90	1.3	0.40
Ile	0.50	0.34	1.5	0.018
Leu	0.58	0.34	1.7	0.019
Lys	1.7	ND[b]		0.24
Met	ND[b]	ND[b]		3.08
Phe	4.86	2.66	1.8	0.14
Pro	2.3	3.0	0.77	0.28
Ser	4.2	3.3	1.3	0.38
Thr	10	4.6	2.2	0.66
Trp	0.48	0.36	1.3	0.022
Tyr	12	6.0	2.0	2.0
Val	43	18	2.4	0.78
Totals	92.0	49.0	1.9	

[a] Two micrograms of purified labeled aminoacyl-tRNA, synthesized from the total tRNA of green or etiolated maize seedlings, was incubated with filters containing 250 μg of maize chloroplast DNA, as described under Experimental Procedures. The amionacyl-tRNA that hybridized to the chloroplast DNA was purified and then deacylated, and the amino acids were separated on an amino acid analyzer. The lower limit of detection for each amino acid was set at 50 cpm above blank samples, except that twofold higher values were set for amino acid pairs not completely separated from one another. Values represent the average of duplicate experiments; differences between experiments were less than 25% for all amino acids. [b] ND, not detected (below lower limit of detection).

to $C_0T = 10$. Therefore, a mixture of aminoacylated tRNAs charged with radioactive amino acids was incubated to about this C_0t value with excess amounts of chloroplast DNA to determine which tRNAs might be coded for by the plastid genome. Comparisons were made between tRNA prepared from etiolated and green maize. About 2 μg of each labeled aminoacyl-tRNA preparation was incubated together with filters containing a total of 250 μg of chloroplast DNA, for 4 h at 33 °C in a solution of 4 × SSC–50% formamide (pH 4.2). Under these conditions, the aminoacyl-tRNAs were relatively stable, deacylating with an average half-life of 45 h. About 10% of the aminoacyl-tRNA prepared from green seedlings hybridized, while 5% of the corresponding aminoacyl-tRNA from etiolated seedlings hybridized (cf. Figure 1).

We examined the composition of the hybridized aminoacyl-tRNAs as described under Experimental Procedures, using an automated amino acid analyzer. As shown in Table III, green maize seedlings appeared to be relatively enriched for aminoacyl-tRNAs that hybridized to chloroplast DNA, with the exceptions of aspartate, glutamate, and proline. The data in Table III reflect only which aminoacyl-tRNAs reliably hybridized to chloroplast DNA. Since chloroplast DNA remained in excess throughout the incubation, the amount of tRNA that hybridized was not directly proportional to the number of cistrons for that tRNA. Instead, the amount of tRNA hybridized was more directly dependent on the concentration of each tRNA in the mixture.

Discussion

One of the major objectives of this research was to determine the numbers of types of tRNA coded by the plastid genome. Another goal was to establish whether these genes were expressed differently in green and etiolated seedlings. We examined tRNAs by base complementarity to chloroplast DNA. These tRNAs could have been synthesized within the plastid or elsewhere, but, in any event, the hybridization data show that these genes are present in the plastid genome. The complementary approach of examining isolated tRNAs from plastid preparations has proved valuable, but the interpretation of data from these experiments is subject to several limitations. Cytoplasmically synthesized tRNA is nearly always detected in plastid tRNA (Burkard et al., 1972). This may not always be artifactual, since tRNAs may be imported into organelles (Chiu et al., 1975). The possibility of export of tRNA from organelles has not been eliminated. Therefore, the detection or lack of detection of a tRNA in a plastid preparation is not good evidence of its site of synthesis.

Hybridizations with an excess of [^{125}I]tRNA indicated that about 0.60–0.75% of chloroplast DNA contained sequences complementary to tRNA. Since maize chloroplast DNA is known to be about 88×10^6 daltons (Thomas and Tewari, 1974) and that tRNAs average about 25 000 daltons, it can be calculated that maize chloroplast DNA contains 20–26 cistrons specifying tRNAs. The data from Table III further

indicate that at least 16 distinct aminoacyl-tRNAs hybridized to chloroplast DNA, requiring maize chloroplast DNA to contain at least 16 distinct tRNA cistrons. Since the aminoacyl-tRNA hybridization technique only measured total amounts of each set of isoaccepting tRNAs, the maximum number of hybridizable tRNAs could not be determined. However, the multiplicity of plastid tRNAs must be tightly restricted, since a maximum of 20–26 sites appeared to be complementary to [^{125}I]tRNA. Merrick and Dure (1972), among others, have already identified some isoaccepting tRNAs of plastid origin. Our data confirm the conclusions of Tewari and Wildman (1970) that about 0.75% of the genome of higher plant chloroplast DNA codes for tRNA. Our data indicate, furthermore, that at least 16 different amino acids can be charged onto these tRNAs. The amounts of most of these tRNAs increase during the greening process. The possibility remains open that the plastid genome specifies genes for an entire set of tRNAs, i.e., enough to support independent protein synthesis.

The experiments shown in Figure 1 and Table III indicate that green maize seedlings are enriched, on the whole, for plastid tRNAs compared with etiolated seedlings. The total amount of tRNA per plant was relatively constant during greening, suggesting that plastid tRNAs specifically increase during greening. However, as shown in Table III, a few plastid tRNAs apparently decrease in amount during greening. The changes in amounts could be caused by changes in numbers of isoaccepting tRNAs as well as changes in amounts of constant numbers of isoaccepting tRNA.

The data in Tables II and III represent indirect measurements of the amounts of tRNA. The ability of tRNA to be charged can be modified through methylation and other modifications (Sueoka and Kano-Sueoka, 1970), and these processes might be altered during greening. However, the ability of these tRNAs to be charged might be regarded as a biologically useful measurement.

Burkard et al. (1972) and Barnett et al. (1969) have reported increased levels of plastid tRNA upon greening. Merrick and Dure (1972) found no light-dependent increase in plastid tRNA in cotton seedlings. As discussed by Whitfeld (1973), the greening process is different in each plant, and is greatly dependent on other variables such as the maturity of the plant. Our data demonstrate that dramatic changes in levels of chloroplast DNA-hybridizable tRNAs do not occur during greening of young maize seedlings.

References

Barnett, W. E., Pennington, C. J., and Fairfield, S. A. (1969), *Proc. Natl. Acad. Sci. U.S.A. 63,* 1262.

Bock, R. M., and Cherayil, J. D. (1967), *Methods Enzymol. 12A,* 638.

Bogorad, L. (1967), *Dev. Biol. Suppl. 1,* 1.

Bottomley, W., Smith, H. J., and Bogorad, L. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 2412.

Burkard, G., Guillemaut, P., Steinmetz, A., and Weil, J. H. (1973), in Nitrogen Metabolism in Plants, Goodwin, T. W., and Smelle, R. M. S., Ed., London, The Biochemical Society, p. 43.

Burkard, G., Guillemaut, P., and Weil, J. H. (1970), *Biochim. Biophys. Acta 224,* 184.

Burkard, G., Vaultier, J. P., and Weil, J. H. (1972), *Phytochemistry 11,* 1351.

Chiu, N., Chiu, A., and Suyama, Y. (1975), *J. Mol. Biol. 99,* 37.

Gillespie, D. (1968), *Methods Enzymol. 12B,* 641.

Haff, L. A., and Bogorad, L. (1976), *Biochemistry,* the following paper in this issue.

Kolodner, R., and Tewari, K. K. (1972), *J. Biol. Chem. 247,* 6355.

Loening, U. E. (1969), *Biochem. J. 113,* 131.

Merrick, W. C., and Dure, L. S. (1972), *J. Biol. Chem. 247,* 7988.

Miura, K.-I. (1967), *Methods Enzymol. 12A,* 543.

Nass, M. M., and Buck C. A. (1970), *J. Mol. Biol. 54,* 187.

Osawa, S., and Sibatani, A. (1967), *Methods Enzymol. 12A,* 687.

Scherberg, N. H., and Refetoff, S. (1974), *J. Biol. Chem. 249,* 2143.

Schildkraut, C. L., Marmur, J., and Doty, P. (1962), *J. Mol. Biol. 4,* 430.

Sueoka, N., and Kano-Sueoka, T. (1970), *Prog. Nucleic Acid Res. Mol. Biol. 10,* 23.

Suyama, Y., and Bonner, W. D. (1966), *Plant Physiol. 41,* 383.

Tereba, A., and McCarthy, B. J. (1973), *Biochemistry 12,* 4675.

Tewari, K. K., and Wildman, S. G. (1970), *Symp. Soc. Exp. Biol. 24,* 147.

Thomas, J. R., and Tewari, K. K. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 3147.

Von Ehrenstein, G. (1967), *Methods Enzymol. 12A,* 588.

Whitfeld, P. R. (1973), in The Ribonucleic Acids, Stewart, P. R., and Letham, D. S., Ed., New York, N.Y., Springer-Verlag.

Williams, G. R., and Williams, A. S. (1970), *Biochem. Biophys. Res. Commun. 39,* 858.