Number of tRNA genes in wheat chloroplast DNA

Two different genes for valine tRNAs

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Wheat chloroplast $tRNA_{total}$ $tRNA^{Val}$ *gene*

1. INTRODUCTION

Chloroplast DNAs (ctDNAs) from various plants show considerable sequence homologies [1,2] and a similar arrangement of two repeats of rRNA gene clusters separated by small and large unique regions [2,3], pea [4], broad bean [5] and chickpea [3] being single cluster exceptions. The size of these DNAs ranges only from 119 [3] to 182 kb [6]. Against the background of this overall similarity there are, however, differences in the size of the 16 S–23 S spacer [7,8] in the positions of restriction sites even among plants of the same genus [9–11], in the arrangements of the conserved sequences [9] and in the variable capacity of coding for tRNAs. In pea chloroplast DNA 40 tRNA genes were estimated [12]: 21 in spinach [13], 26 in Euglena [14,15] and 40–45 4 S RNA genes were found in duckweed [6]. The chloroplast genome of maize, the only representative of cereals so far investigated with this perspective, has 20–26 tRNA genes [16]. No information was available on the number of chloroplast tRNA genes in wheat, although detailed studies were recently made on the location of structural genes for several proteins on the wheat ctDNA [17–19]. Here, we estimated the number of total tRNA genes in the wheat chloroplast genome, and in particular the number of genes coding for valine tRNAs. These tRNAs were of interest because previously we observed 3 $tRNA^{Val}$ species in wheat chloroplasts [20] while most of the other plants were shown to contain only 1 or 2 valine isoacceptors [21].

2. MATERIALS AND METHODS

Wheat (*Triticum vulgare* cv. Aria) was obtained from the Plant Breeding Station (Nagradowice). Wheat germ was a commercial product from Mill Works (Poznań), BSA, agarose and cellulase were from Serva, 2-mercaptoethanol from LOBA-Chemie, acrylamide, *N,N'*-methylenebisacrylamide and Tris were from BDH, D-mannitol from Fluka AG and DNase from Millipore. RNase was from Worthington, DEAE-cellulose, protease were from Sigma, nitrocellulose filters from Schleicher and Schüll, Sephacryl S-1000 from Pharmacia. ^{14}C-Labelled amino acids were purchased from UVVVR (Prague), Biofluor was purchased from NEN and $Na^{125}I$ was from the Institute of Nuclear Research (Swierk). Nuclear wheat DNA was a kind gift of Z. Kulińska from this laboratory. Other chemicals were obtained from P.O.Ch. (Gliwice).

Wheat chloroplasts were isolated from 10-day old seedlings grown at room temperature and kept overnight in the dark. Green parts of the seedlings were cut into 3–5 mm pieces and incubated with

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1.5% cellulase in 0.4 M mannitol, 1% BSA (pH 5.6) for 24 h at 4°C [22]. Further procedures were as in [23].

Transfer RNA was isolated from the chloroplasts as in [23] and separated by two-dimensional gel electrophoresis [24] run at room temperature. Individual spots were cut out and tRNAs were eluted from the minced gel with 0.5 M ammonium acetate (pH 4.5), 10 mM $MgCl_2$, 1 mM EDTA and 0.1% SDS [25], and identified by aminoacylation using chloroplast and/or *Escherichia coli* aminoacyl-tRNA synthetases. Wheat chloroplast and embryo tRNA and aminoacyl-tRNA synthetases were prepared as in [26].

DNA was isolated from wheat chloroplasts as described in [27] and purified either by gel filtration on Sephacryl S-1000 or by CsCl gradient centrifugation. On 1% agarose gel electrophoresis in 0.06 M Tris–borate, 1 mM EDTA buffer (pH 8.3) it gave only one band with a low mobility.

Radioiodination of tRNA was carried out as in [28]. Concentrations of the ^{125}I-labelled tRNAs were measured by a modification [29] of the ethidium bromide–fluorimetric method described for DNA [30]. Radioactivity was measured in a Beckman LS-100 scintillation counter using Biofluor, a high-efficiency scintillation liquid for γ-emitters. Specific radioactivities of the labelled tRNAs were in the range of $1-9 \times 10^6$ cpm/μg.

For hybridization experiments 1-μg samples of ctDNA were immobilized on 8-mm nitrocellulose filters and incubated in 2 × SSC and 33% formamide at 37°C for 48 h with different concentrations of the labelled tRNAs. The filters were washed twice for 10 min each time with the same solution and incubated in 2 × SSC with RNase A, 20 μg/ml for 20 min at 37°C, again washed twice with 2 × SSC and counted in Biofluor. The results were corrected for the background counts taken from hybridization of cytoplasmic tRNA to ctDNA or of ct-tRNAs to nuclear DNA.

3. RESULTS AND DISCUSSION

Like tRNA isolated from chloroplasts of other plants [21], wheat ct-tRNA separates by two-dimensional gel electrophoresis into 30–34 spots (fig.1). Three of them were identified by aminoacylation as valine tRNAs ($tRNA_{1-3}^{Val}$) and were eluted from the gel, radioiodinated and

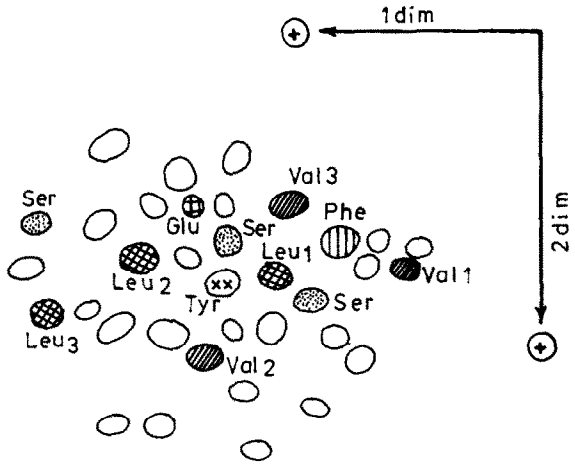

Fig.1. Two-dimensional gel electrophoresis of wheat chloroplast tRNA. First dimension was in 10%, second dimension in 20% polyacrylamide. About 10 A_{260} units of ct-tRNA were applied on the gel. The separated tRNAs were visualized in UV on a fluorescing screen. The positions of the spots were marked on a UV-transparent foil overimposed on the gel. tRNAs eluted from individual spots were dialysed against 10 mM Tris–HCl, 1 mM $MgCl_2$ (pH 7.5), made up to a standard volume (1 ml), and 50-μl portions of every eluate were used for aminoacylations with the following ^{14}C-labelled amino acids: Glu, Leu, Phe, Ser, Tyr, and Val. Each aminoacylation was carried out in the presence of 19 other cold amino acids. Valine was accepted by tRNAs eluted from 3 spots, denoted as $tRNA_{1-3}^{Val}$. Eluate from no other spot accepted valine and the valine spots did not accept other amino acids. From the counts given on aminoacylation by valine tRNAs it was calculated that they were present in about equimolar quantities.

hybridized to ctDNA at various tRNA/DNA ratios. The results are shown in fig.2. When the tRNA/DNA ratio exceeded 0.12:1, further increase in $tRNA_1^{Val}$ or $tRNA_2^{Val}$ concentrations in the hybridization mixture did not cause any increase in the amount of tRNA bound to the filters, indicating that the binding sites on DNA had been saturated with tRNA. Assuming wheat ctDNA and ct-tRNA to be 89 MDa [17] and 25 kDa, respectively, and knowing the specific radioactivity of tRNA, it was calculated that in wheat ctDNA there is one gene for each of these two tRNAs. These two tRNAs could, however, be the product of the same gene and differ only by post-transcriptional modifications. To check this possibility, increasing

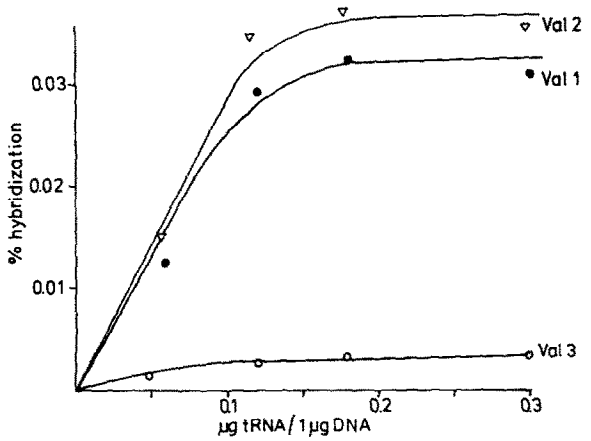

Fig.2. Saturation hybridization of wheat chloroplast ^{125}I-labelled $tRNA_1^{Val}$ (●—●), $tRNA_2^{Val}$ (▽—▽), and $tRNA_3^{Val}$ (○—○) with ctDNA. The quantity of ctDNA involved in the hybrid formation is indicated on the ordinate as percentage hybridization, while the quantity of tRNA used in the hybridization mixture is shown on the abscissa as tRNA/DNA ratio. One µg of ctDNA was used for each hybridization. The volume of the hybridization mixtures was 0.5 ml.

amounts of $tRNA_1^{Val}$ were added to hybridization mixtures that contained saturating amounts of $tRNA_2^{Val}$ (or vice versa). In every case the radioactivity bound to the filters was the sum of counts measured for individually hybridized $tRNA_1^{Val}$ and $tRNA_2^{Val}$, indicating that each of these tRNAs was coded for by a separate gene.

The extent of hybridization of $tRNA_3^{Val}$ to the ctDNA was about 10-times smaller than that of $tRNA_1^{Val}$ or $tRNA_2^{Val}$. The simplest explanation of this result would be that $tRNA_3^{Val}$ represented only a cytoplasmic contamination of ct-tRNA. However, its hybridization to nuclear DNA was no better than to the ctDNA. Alternatively, its low hybridization to ctDNA could be explained, assuming that it is derived from a gene split by a rather long intron. Long intervening sequences were recently reported for chloroplast genes in maize coding for $tRNA^{Ile}$, $tRNA^{Ala}$ [8] and $tRNA^{Leu}$ [31].

Fig.3 shows the curves of saturation hybridizations of total ct-tRNA to the ctDNA. At tRNA/DNA ratios of 4 or higher a plateau of saturation had been reached. At the plateau 0.97% (± 0.09%) of ctDNA formed hybrids with ct-tRNA, which is equivalent to 35 (± 3) tRNA genes

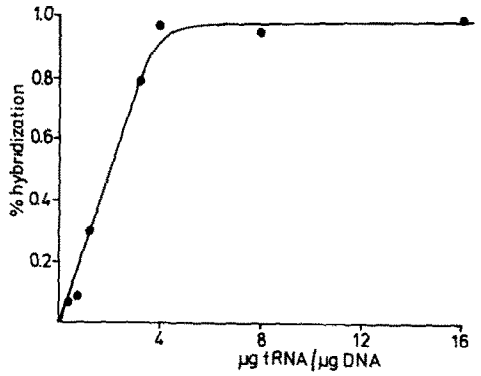

Fig.3. Saturation hybridization of wheat total ^{125}I-labelled tRNA (spec. act. 1.45 × 10^6 cpm/µg tRNA) with wheat ctDNA.

in the wheat chloroplast genome. This value obviously was not affected by tRNAs which, like $tRNA_3^{Val}$, did not hybridize to ctDNA even if they were contained in the hybridizing mixture. The estimated number of tRNA genes is therefore the minimal value.

Chloroplast DNAs of higher plants code for a complete set of tRNAs [2]. In the wheat chloroplasts 35 tRNA genes were found. If the number of tRNA genes in wheat ctDNA is compared with the 34 spots yielded by total ct-tRNA on two-dimensional gel electrophoresis, it seems that the coding capacity of ctDNA in wheat is large enough to provide a separate gene for every isoaccepting chloroplast tRNA.

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REFERENCES

[1] Lamppa, G.K. and Bendich, A.J. (1979) Plant Physiol. 63, 660–668.
[2] Bogorad, L. (1981) J. Cell Biol. 91, 256–270.

[3] Chu, N.M. and Tewari, K.K. (1982) Mol. Gen. Genet. 186, 23–32.
[4] Chu, N.M., Oishi, K.K. and Tewari, K.K. (1981) Plasmid 6, 279–292.
[5] Koller, B. and Delius, H. (1980) Mol. Gen. Genet. 178, 261–269.
[6] Groot, G.S.P. and Van Harten-Loosbroek, N. (1981) Curr. Genet. 4, 187–190.
[7] Allet, B. and Rochaix, J.D. (1979) Cell 18, 55–60.
[8] Koch, W., Edwards, K. and Kössel, H. (1981) Cell 25, 203–213.
[9] Schiller, B., Herrmann, R.G. and Melchers, G. (1982) Mol. Gen. Genet. 186, 453–459.
[10] Gordon, K.H.J., Crouse, E.J., Bohnert, H.J. and Herrmann, R.G. (1982) Theor. Appl. Genet. 61, 373–384.
[11] Gordon, K.H.J., Crouse, E.J., Bohnert, H.J. and Herrmann, R.G. (1981) Theor. Appl. Genet. 59, 281–296.
[12] Meeker, R. and Tewari, K.K. (1980) Biochemistry 19, 5973–5981.
[13] Driesel, A.J., Crouse, E.J., Gordon, K., Bohnert, H.J., Herrmann, R.G., Steinmetz, A., Mubumbila, M., Keller, M., Burkard, G. and Weil, J.H. (1979) Gene 6, 285–306.
[14] Schwartzbach, S.D., Hecker, L.I. and Barnett, W.E. (1976) Proc. Natl. Acad. Sci. USA 73, 1984–1988.
[15] Mubumbila, M., Burkard, G., Keller, M., Steinmetz, A., Crouse, E.J. and Weil, J.H. (1980) Biochim. Biophys. Acta 609, 31–39.
[16] Haff, L.A. and Bogorad, L. (1976) Biochemistry 15, 4105–4109.
[17] Bowman, C.M., Koller, B., Delius, H. and Dyer, T.A. (1981) Mol. Gen. Genet. 183, 93–101.
[18] Koller, B., Delius, H. and Dyer, T.A. (1982) Eur. J. Biochem. 122, 17–23.
[19] Howe, C.J., Bowman, C.M., Dyer, T.A. and Gray, J.C. (1982) Mol. Gen. Genet. 186, 525–530.
[20] Karwowska, U., Goździcka-Józefiak, A., Janowicz, Z., Rudzińska, M. and Augustyniak, J. (1980) in: Biological Implications of Protein–Nucleic Acid Interactions (Augustyniak, J. ed) pp.429–437, A. Mickiewicz Univ. Press, Poznań–Elsevier, Amsterdam, New York.
[21] Weil, J.H. and Parthier, B. (1982) in: Encyclopedia of Plant Physiology new ser. vol.14 A Nucleic Acids and Proteins in Plants I (Boulter, D. and Parthier, B. eds) pp.65–112, Springer, Berlin, New York.
[22] Karimov, M., Lysenko, A.M. and Vinetsky, P.Yu. (1978) Biokhimia 43, 597–601.
[23] Karwowska, U., Goździcka-Józefiak, A. and Augustyniak, J. (1979) Acta Biochim. Polon. 26, 319–326.
[24] Fradin, A., Gruhl, H. and Feldman, H. (1975) FEBS Lett. 50, 185–189.
[25] Maxam, A.M. and Gilbert, W. (1980) Methods Enzymol. 65, 499–560.
[26] Rudzińska, M., Goździcka-Józefiak, A., Karwowska, U. and Augustyniak, J. (1980) Acta Biochim. Polon. 27, 309–319.
[27] Bohnert, H.J. and Crouse, E.J. (1981) P.M.B. News Lett. 11, 70–72.
[28] Commerford, S.L. (1971) Biochemistry 10, 1993–1999.
[29] Bujałowski, W., Goździcka-Józefiak, A. and Augustyniak, J. (1983) submitted.
[30] Blackburn, M.J., Andrews, T.M. and Watts, R.W.E. (1973) Anal. Biochem. 51, 1–10.
[31] Steinmetz, A., Gubbins, E.J. and Bogorad, L. (1982) Nucleic Acids Res. 10, 3027–3037.