

## Endosymbiotic Origin and Codon Bias of the Nuclear Gene for Chloroplast Glycerinaldehyde-3-Phosphate Dehydrogenase from Maize

Henner Brinkmann,<sup>1</sup> Pascal Martinez,<sup>1</sup> Françoise Quigley,<sup>1</sup> William Martin,<sup>2</sup> and Rüdiger Cerff<sup>1</sup>

<sup>1</sup> Laboratoire de Biologie Moléculaire Végétale, CNRS UA 1178, Université de Grenoble I, B.P. 68, F-38402 Saint Martin D'Hères Cedex, France

<sup>2</sup> Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, FRG

**Summary.** The nuclei of plant cells harbor genes for two types of glycerinaldehyde-3-phosphate dehydrogenases (GAPDH) displaying a sequence divergence corresponding to the prokaryote/eukaryote separation. This strongly supports the endosymbiotic theory of chloroplast evolution and in particular the gene transfer hypothesis suggesting that the gene for the chloroplast enzyme, initially located in the genome of the endosymbiotic chloroplast progenitor, was transferred during the course of evolution into the nuclear genome of the endosymbiotic host. Codon usage in the gene for chloroplast GAPDH of maize is radically different from that employed by present-day chloroplasts and from that of the cytosolic (glycolytic) enzyme from the same cell. This reveals the presence of subcellular selective pressures which appear to be involved in the optimization of gene expression in the economically important graminaceous monocots.

**Key words:** cDNAs — GAPDH evolutionary tree — Horizontal gene transfer — Coding strategies — Monocotyledons — Dicotyledons

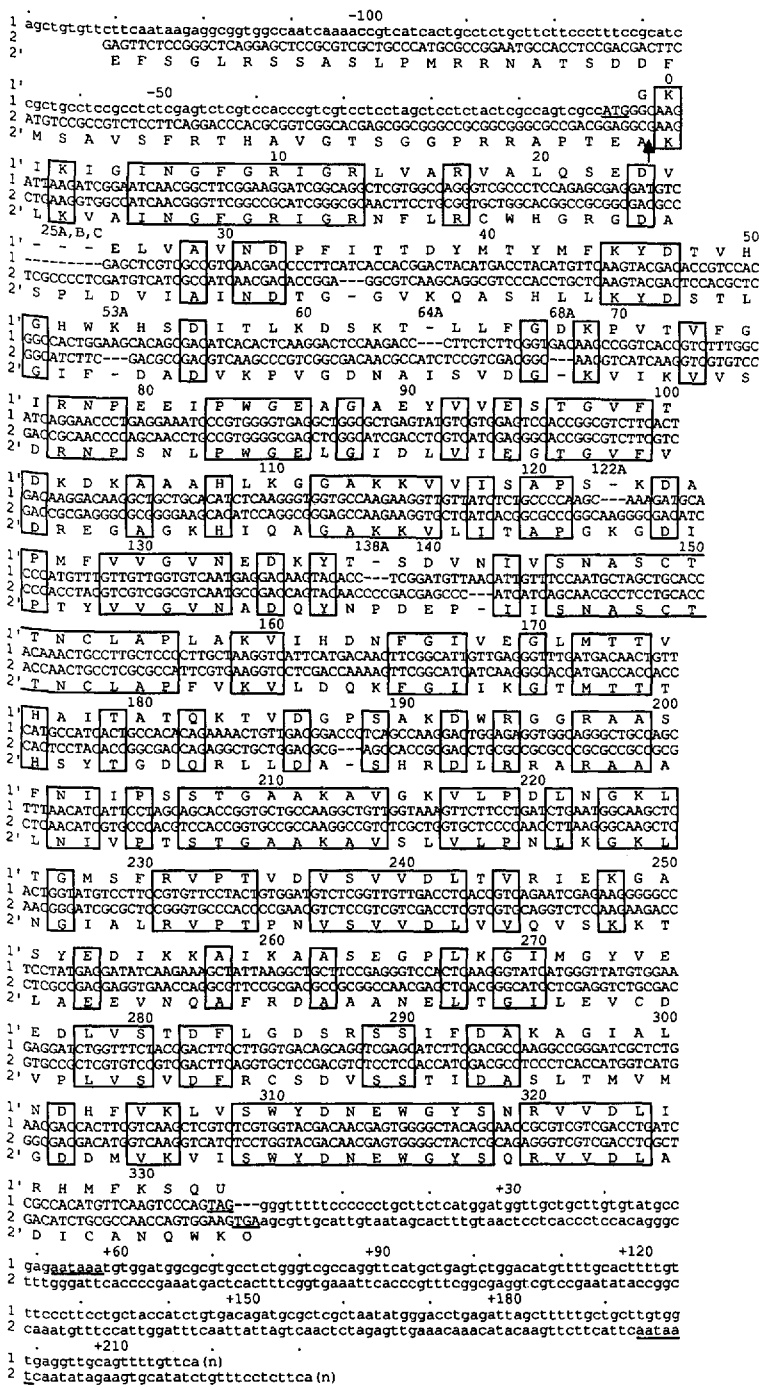
### Introduction

Glycerinaldehyde-3-phosphate dehydrogenase (GAPDH) was the first enzyme for which sequence data of the Calvin cycle/glycolysis homologues (Weeden 1981) became available. The recent anal-

ysis of partial sequences from mustard (Martin and Cerff 1986) and subsequently from tobacco (Shih et al. 1986) showed that the photosynthetic enzyme of higher plants, i.e., its catalytic subunit (Cerff 1982a, Cerff et al. 1986), is more similar to the GAPDH of thermophilic bacteria than it is to the glycolytic enzyme encoded within the same nucleus, thereby providing initial evidence in support of the gene transfer hypothesis for the origin of this nucleus-encoded chloroplast enzyme. Here we report the first comparison of complete sequences for both mature polypeptides of chloroplast and cytosolic GAPDH from a single plant species, *Zea mays*. The two enzymes share only 45% of their amino acid residues and show strikingly different codon choice patterns, with chloroplast GAPDH using 97% G or C in the third base position.

### Materials and Methods

The maize cDNA library used in the present study had previously been constructed at the Max-Planck-Institut für Züchtungsforschung at Cologne in the laboratory of H. Saedler by cloning the cDNA into the EcoRI site of the lambda vector NM 1149 (Schwarz-Sommer et al. 1987). The library was screened by plaque hybridization with the heterologous probes from mustard pS198c (cytosolic GAPDH) and pS84b (chloroplast GAPDH, subunit A) (Martin and Cerff 1986). The positive maize clones pZm9 (cytosolic GAPDH) and pZm57 (chloroplast GAPDH) were subcloned into the EcoRI site of pUC9 and submitted to DNA sequence analysis. For clone pZm57 the chemical degradation method of Maxam and Gilbert (1977) was used. For clone pZm9 Sanger's enzymatic technique (Sanger et al. 1977) was employed in combination with the use of the single-stranded DNA bacteriophage M13 (Vieira and Messing 1982).



**Fig. 1.** Nucleotide and deduced amino acid sequences of cDNAs for cytosolic (clone pZm9, lines 1, 1') and chloroplast GAPDH (clone pZm57, lines 2, 2') from maize. Amino acid residues of the mature subunits are numbered according to Harris and Waters (1976) and regions of homologies between the two enzymes are boxed. The initiation and termination codons and the presumptive polyadenylation signals aataaa and aataat in the trailers are underlined. The arrow indicates the presumptive processing site of the chloroplast GAPDH precursor corresponding to the amino terminus determined for the mustard enzyme (Cerff and Witt 1983). To obtain the best alignment of the two corresponding coding regions and the most probable positions of the insertions and deletions within the NADP-binding domain, the chloroplast sequence was fitted into the general GAPDH matrix previously established (Martin and Cerff 1986) by maximizing sequence identities and conservative replacements within non-identical stretches.

**Results and Discussion**

*Evidence for a Horizontal Transfer of GAPDH Genes*

In Fig. 1 the complementary DNAs encoding the complete sequences of the catalytic subunits of cytosolic (clone pZm9, lines 1 and 1') and chloroplast GAPDH (clone pZm57, lines 2 and 2') from maize are aligned. The coding region of clone pZm9 com-

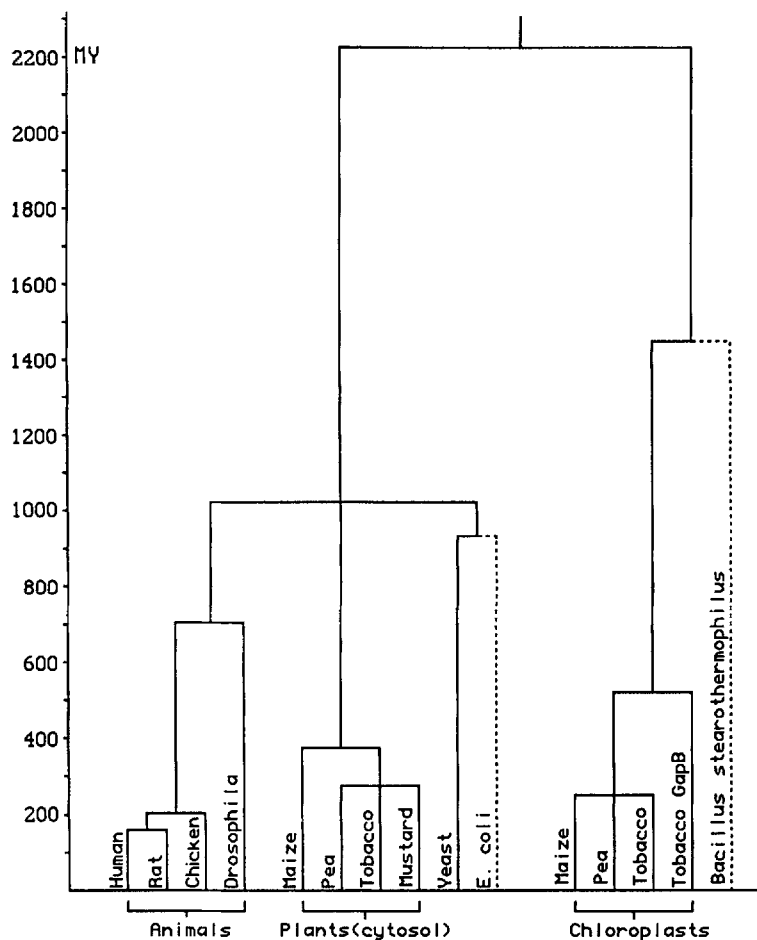
prises 338 codons, including start and stop codons, and is followed by a trailer of 217 nucleotides plus a polyA tail. The trailer contains a conventional polyadenylation signal aataaa which, however, is located 165 nucleotides upstream of the polyadenylation site (underlined in Fig. 1).

Clone pZm57 (chloroplast GAPDH, lines 2 and 2' in Fig. 1) starts within the sequence coding for the transit peptide 46 codons upstream of the presumptive processing site. It extends 230 nucleotides

### A Identities Matrix of Plant GAPDH Sequences

Cytosol					Chloroplast				
	1	2	3	4		1	2	3	4
1. Maize					1. Maize				
2. Pea	83				2. Pea	90			
3. Tobacco	83	88			3. Tobacco GapA	91	89		
4. Mustard	85	88	89		4. Tobacco GapB	81	79	79	
5. E. coli	65	64	63	64	5. Bacillus	58	57	56	57

### B GAPDH Evolutionary Tree



**Fig. 2.** The evolution of GAPDH enzymes. The GAPDH evolutionary tree (B) is based on the sequence differences calculated from the previously published identities matrix (Martin and Cerff 1986) and that shown in Fig. 2A. These differences were transformed into PAM units (accepted point mutations per 100 residues) by correcting for superimposed mutations according to Dayhoff (1972). The approximate divergence times were calculated by dividing the half PAM values separating two particular sequences with the unit evolutionary rate determined for the animal GAPDH enzymes (2.2 PAM per 100 million years, Dayhoff 1978). For sources of sequence information in Fig. 2A see Table 2 and text.

3' to the termination codon, has a polyA tail, and 35 nucleotides upstream of the latter the putative polyadenylation signal aataat. The aminoterminal of the purified chloroplast GAPDH from mustard starts with methionine at position 0 (Cerff and Witt 1983). This corresponds to lysine in the maize enzyme leaving 337 amino acids for the polypeptide chain of the mature subunit.

Maize chloroplast GAPDH shows 58% sequence similarity to the enzyme from *Bacillus stearothermophilus* (alignment not shown) but shares only 45% of its amino acids (54% of its nucleotides) with its glycolytic counterpart (amino acids boxed in Fig. 1).

This indicates a very distant relationship between the two maize enzymes and suggests that they may have diverged as early as the pro- and eukaryotic lineages. The sequences of chloroplast and cytosolic GAPDHs from maize, pea (Brinkmann and Cerff, publication in preparation), tobacco (Shih et al. 1986), and mustard (cytosolic GAPDH only, Martin and Cerff 1986) together with some sequences from animals, bacteria, and yeast have been used to construct the GAPDH evolutionary tree shown in Fig. 2B (for details see legend of Fig. 2B). This tree clearly demonstrates that chloroplast and cytosolic GAPDH diverged long before the plant-animal division pre-

**Table 1.** Codon usage of cytosolic (a) and chloroplast (b) GAPDH from maize

	a	b		a	b		a	b		a	b
Phe-TTT	3	0	Ser-TCT	2	0	Tyr-TAT	3	0	Cys-TGT	0	0
Phe-TTC	11	11	Ser-TCC	7	20	Tyr-TAC	6	6	Cys-TGC	2	6
Leu-TTA	0	0	Ser-TCA	1	0	ter-TAA	—	—	ter-TGA	—	1
Leu-TTG	1	0	Ser-TCG	4	4	ter-TAG	1	—	Trp-TGG	5	5
Leu-CTT	5	1	Pro-CCT	4	0	His-CAT	3	0	Arg-CGT	1	0
Leu-CTC	10	20	Pro-CCC	4	11	His-CAC	5	6	Arg-CGC	2	11
Leu-CTA	0	0	Pro-CCA	2	1	Gln-CAA	0	1	Arg-CGA	0	0
Leu-CTG	4	9	Pro-CCG	2	5	Gln-CAG	3	8	Arg-CGG	0	5
Ile-ATT	6	0	Thr-ACT	6	0	Asn-AAT	3	2	Ser-AGT	0	0
Ile-ATC	16	22	Thr-ACC	11	17	Asn-AAC	10	17	Ser-AGC	10	6
Ile-ATA	0	0	Thr-ACA	4	0	Lys-AAA	4	0	Arg-AGA	2	0
Met-ATG	8	7	Thr-ACG	1	6	Lys-AAG	24	21	Arg-AGG	6	5
Val-GTT	15	0	Ala-GCT	14	1	Asp-GAT	7	1	Gly-GGT	14	1
Val-GTC	17	30	Ala-GCC	13	21	Asp-GAC	19	31	Gly-GGC	11	24
Val-GTA	0	0	Ala-GCA	2	0	Glu-GAA	2	0	Gly-GGA	3	2
Val-GTG	4	11	Ala-GCG	0	14	Glu-GAG	13	11	Gly-GGG	2	6

cluding the possibility that chloroplast GAPDH originated by a gene duplication event in the early plant eukaryote. Thus, either animals have lost the gene or plants have acquired it horizontally from the progenitors of chloroplasts after their divergence from animals. The similarity of the chloroplast enzyme to that of *B. stearothermophilus* clearly supports the latter alternative in agreement with the endosymbiotic theory of chloroplast evolution.

The evolutionary tree in Fig. 2B reveals three other major points of interest. First, the enzyme of *Escherichia coli* (Branlant and Branlant 1985) belongs to the eukaryotic part of the tree, which has previously been interpreted in terms of a possible gene transfer event in the opposite, eukaryote to prokaryote, direction (for details see Martin and Cerff 1986). Second, in dicotyledonous (dicot) plants cytosolic and chloroplast GAPDHs seem to evolve at similar rates as shown by the sequence similarities of 88 and 89% for the comparisons tobacco cytosol/pea cytosol and tobacco chloroplast/pea chloroplast, respectively (see values boxed by dashed lines in Fig. 2A). With respect to cytosolic GAPDH it is surprising that 11–17% sequence divergence between maize, pea, tobacco, and mustard (see boxed values in the left half of Fig. 2A) does not lead to significant charge differences at the native enzyme level, as shown by our previous zymogram analysis of cytosolic and chloroplast GAPDHs from higher plants (Cerff 1982b). Third, in maize, as opposed to dicot species, the evolutionary rates of chloroplast and cytosolic GAPDH differ considerably, the chloroplast enzyme being more conserved (90/91% positional identity with pea/tobacco) than its cytosolic counterpart (83/83/85% positional identity with pea/tobacco/mustard, see values boxed by continuous lines in Fig. 2A). This probably reflects a slowdown

of chloroplast GAPDH evolution in maize rather than a corresponding acceleration of the evolutionary rate of cytosolic GAPDH. This interpretation is based on our observation (Table 1) that in maize the chloroplast enzyme is subjected to extremely selective constraints at the level of codon utilization.

#### Codon Bias and Gene Expression in Cereals

It is shown in Table 1 that the codon choice pattern of maize chloroplast GAPDH is highly skewed. The gene uses only 38 amino acid triplets as compared to 51 of the gene for the cytosolic enzyme. This difference is absent in the three other GAPDH pairs from pea (Brinkmann and Cerff, unpublished), mustard (Martin and Cerff 1986), and tobacco (Shih et al. 1986), which use between 50 and 59 different codons (not shown). The codon bias of maize chloroplast GAPDH is expressed as a 97% preference for G (28%) or C (69%) at the third base position. In contrast, cytosolic GAPDH shows 67% third position G+C (20% G and 47% C).

To obtain a more general impression of angiosperm coding strategies, the third position G+C percentage was analyzed for 27 nuclear genes or mRNAs from cereals and dicot plants as well as for some chloroplast genes from two plant species (Table 2). The lowest G+C percentage in the triplet third base position (around 30%) is found for chloroplast genes of both cereals and dicot species (maize and tobacco, see Table 2). With respect to nuclear genes, however, the two taxa exhibit clearly different coding strategies. While in cereals G+C values range between roughly 40 and 100% in each species (see Table 2), values in dicot plants are rather homogeneous, the largest span being found in soybean with 39 and 61% G+C for leghemoglobin and the

**Table 2.** G+C percentage at the third positions of codons in genes from higher plants. Values in parentheses refer to the G+C percentage within noncoding regions of individual genes (introns and flanking sequences). Coding sequences that are not full length with respect to the mature protein are marked with an asterisk.

Plant	% G+C	Genes or mRNAs	Expression induced by/in/during	EMBL codes and references
Maize	30	3 chloroplast genes		CHZMATBE, CHZMO2; 1, 2
	43 (37)	Zein	Seed development	ZMZE01; 3
	49 (42)	Triosephosphate isomerase		4
	52	Adenine nucleotide translocator		ZMANT1; 5
	57 (38)	Actin		ZMACT1; 6
	64 (41)	Alcohol dehydrogenase		ZMADH1S; 7
	67	Cytosolic GAPDH		This paper
	67 (44)	Sucrose synthase	Seed development	ZMSUCS2; 8
	78	Glutelin 2	Seed development	9
	84	Chloroplast PEP carboxylase	Light/phytochrome	ZMPEPCR; 10
	84 (45)	A1-locus enzyme	Seed development	11
	93 (53)	Waxy-locus enzyme	Seed development	12
	94	Bronze-locus enzyme	Seed development	13
	95 (52)	Histones H3-H4	Cell cycle/S-phase	14
	97 (45)	Small subunit RuBCase	Light/phytochrome	15
97	Chloroplast GAPDH	Light/phytochrome	This paper	
Wheat	40 (35)	Gliadin	Seed development	TAGLIAA; 16
	41 (41)	HMW-glutenin	Seed development	TAGLUIDG; 17
	54*	HMW gluten	Seed development	TAHGO1; 18
	87*	Small subunit RuBPCase	Light/phytochrome	TARUB2; 19
	97 (52)	H3-H4 histones	Cell cycle/S-phase	TAHIO1, TAHIO2; 20, 21
Barley	38 (41)	B-hordein	Seed development	HVB1HORG; 22
	59*	Cytosolic GAPDH		23
	89	$\alpha$ -amylase	Giberellic acid	HVAMYA; 24
	91	UDP-glucose SGT	Seed development	25
	100*	H3-histone	Cell cycle/S-phase	26
Pea	43	Chloroplast GAPDH	Light/phytochrome	27
	44 (33)	Small subunit RuBPCase	Light/phytochrome	PSRCO1; 28
	46 (29)	Light harvesting protein	Light/phytochrome	PSCAB80; 29
	46	Cytosolic GAPDH		27
Mustard	41*	Chloroplast GAPDH	Light/phytochrome	30
	55	Cytosolic GAPDH		30
Tobacco	31	6 chloroplast genes		31
	38	Chloroplast GAPDH (Gap B)	Light/phytochrome	32
	44*	Cytosolic GAPDH (Gap C)		32
	50	Chloroplast GAPDH (Gap A)	Light/phytochrome	32
	52 (32)	Small subunit RuBPCase	Light/phytochrome	NTRUBSS; 33
Soybean	39 (24)	Leghemoglobin	Root nodules	GMGLO3; 34
	47	7S seed storage protein	Seed development	GM7SAA; 35
	49 (30)	Heat shock protein (hs 6871)	Heat	GMHSP2; 36
	61 (33)	Small subunit RuBPCase	Light/phytochrome	GMRUBP; 37

*Abbreviations:* PEP, phosphoenolpyruvate; RuBPCase, ribulose biphosphate carboxylase; waxy-locus enzyme = UDP-glucose SGT, UDP-glucose starch glycosyl transferase; bronze-locus enzyme, UDP-glucose flavonol-3-O-glycosyl transferase. This analysis has been performed by screening the EMBL Nucleotide Sequence Data Library and the sequences of some recent papers with the DNA sequencing program developed by Greaves and Ware (University of Bristol, unpublished) and adapted to the MULTICS computer of the University of Grenoble. Sources of sequence information: 1, Kribbers et al. 1982; 2, McIntosh et al. 1980; 3, Pedersen et al. 1982; 4, Marchionni and Gilbert 1986; 5, Baker and Leaver 1985; 6, Shah et al. 1983; 7, Dennis et al. 1984; 8, Werr et al. 1985; 9, Prat et al. 1985; 10, Izui et al. 1986; 11, Schwarz-Sommer et al. 1987; 12, Klösgen et al. 1986; 13, D. Furtek, personal communication; 14, Chaubet et al. 1986; 15, Lebrun et al. 1987; 16, Anderson et al. 1984; 17, Thompson et al. 1985; 18, Forde et al. 1983; 19, Smith et al. 1983; 20, Tabata et al. 1983; 21, Tabata et al. 1984; 22, Forde et al. 1985; 23, Chojecki 1986a; 24, Rogers and Milliman 1983; 25, Rhoades et al. 1986; 26, Chojecki 1986b; 27, Brinkmann and Cerff, unpublished; 28, Coruzzi et al. 1984; 29, Cashmore 1984; 30, Martin and Cerff 1986; 31, Maruyama et al. 1986; 32, Shih et al. 1986; 33, Mazur and Chui 1985; 34, Hyldig-Nielsen et al. 1982; 35, Schuler et al. 1982; 36, Schoeffl et al. 1984; 37, Berry-Lowe et al. 1982

small subunit of ribulose biphosphate carboxylase, respectively. These findings complement and extend a recent correspondence analysis by Boudraa (1987; for methodological details see Grantham 1980 and references therein) of the codon choice patterns of chloroplast (17 sequences) and nuclear (20 sequences) mRNA sequences from higher plants. This analysis showed a segregation of the two mRNA classes along the horizontal axis which, according to the present data, is due to their overall difference in G+C content at the degenerate codon position. However, while the chloroplast sequences, because of their low and homogeneous G+C content (see Table 2 and Maruyama et al. 1986), form a rather compact cluster, the nuclear mRNAs are horizontally scattered (see Boudraa 1987) and, in view of the present findings, may be separated into two horizontal subclasses: one including all dicot sequences and the "relaxed" monocotyledonous (monocot) sequences maize zein, wheat gliadin, and maize actin (40, 43, and 57% G+C, see Table 2), and the other comprising the highly biased monocot sequences alpha-amylase and histone H3 from barley (89 and 100% G+C) and, interestingly, the sequence prethaumatin (90% G+C, not shown in Table 2) from *Thaumatococcus danielli* (Edens et al. 1982), a member of the monocot plant family Marantaceae (Zingiberales, Liliidae). The high third position G+C content of 90% for prethaumatin, the only noncereal monocot sequence presently available (EMBL release 9, 1986), presumably indicates that the coding strategies of cereals investigated here are a characteristic feature of monocot angiosperms in general.

Large differences in G+C content at the degenerate position of codons have previously been reported for genes of birds and mammals (for review see Ikemura 1985). The original work of Bernardi et al. (1985) and the subsequent investigations by Bernardi and Bernardi (1986) and Aota and Ikemura (1986) suggests that in warm-blooded vertebrates these differences are a consequence of the overall mosaic structure of the genome. According to these findings genes of birds and mammals are embedded in "isochores" (Bernardi et al. 1985), long DNA segments (>300 kilobases) characterized by different G+C levels and fairly homogeneous base compositions. This means that the G+C content at the third base position of a particular mammal gene reflects the G+C level of the surrounding isochore, which comprises introns, flanking sequences, and "spacer DNA." For the plant genes shown in Table 2 the total G+C percentage of introns and flanking regions, where available, are given in parentheses, yet show little if any correlation to third base position values. This observation indicates that the differences in codon choice patterns in monocot genes are maintained independently of the G+C

content of the surrounding portions of the genome, in contrast to the situation found in birds and mammals. Since the natural rate of silent substitutions will continuously strive towards randomization of nucleotide frequencies at the third base position (Kimura 1982), an extreme bias for particular nucleotides in certain genes clearly represents the results of selection acting at a level below that of amino acid substitution.

For the cereal genes in Table 2, a rough correlation between increasing codon bias and gene expressivity is observed. The selective mechanism responsible for codon bias, therefore, seems to relate third position G+C preference with the tendency of a gene to show strong expression in response to endogenous (cell cycle, developmental, hormonal) and exogenous stimuli (such as morphogenetic light mediated by the photoreceptor phytochrome; e.g., chloroplast GAPDH, see Cerff and Kloppstech 1982). Since no such correlation is found for dicot plants, the selective pressure yielding codon bias has either evolved in (graminaceous) monocots or has been lost in dicots since the divergence of these angiosperm classes. Also the cereal storage protein genes (zein, hordein, gliadin) do not appear to conform to the trend just described. However, since storage proteins are encoded by multigene families, their strong expression during seed development may depend on high endogenous levels of multiple mRNAs rather than on the high translational activity of individual mRNA species (see below).

Since it is clear that a broad spectrum of codon bias does exist for cereal genes, one would like to know what selective process is involved. One possibility is that RNA processing efficiency or RNA secondary structure and stability are positively influenced by a high G+C content at the third base position. Although RNA secondary structure and stability can surely not be excluded as points where selection can become effective (see Bernardi and Bernardi 1986), "processability" seems rather improbable as a major pressure since the maize gene for UDP-glucose starch glycosyl transferase (the waxy-locus enzyme) with 13 introns and a polyadenylated mRNA (Klößgen et al. 1986) possesses about the same third position G+C as the maize genes for histones H3 and H4 (93 vs 95%), genes that boast neither polyadenylation nor introns (Tabata et al. 1983, 1984; Chaubet et al. 1986).

A tempting explanation for the occurrence of strongly preferred codons in highly expressed monocot genes is the presence of distinct classes of major isoaccepting tRNAs with anticodons best adapted to only a subset of possible synonymous codons. The existence of such a coding strategy has been demonstrated for *E. coli* (Gouy and Gautier 1982; Grosjean and Fiers 1982; Ikemura 1985) and yeast

(Bennetzen and Hall 1982; Ikemura and Ozeki 1982), where the more heavily a gene is expressed the more extreme its codon bias is, and the codons preferred correspond to the most abundant tRNA species in each isoaccepting subset. Whether such is the case for (graminaceous) monocots remains to be shown. Data for nuclear tRNAs in plants are currently scarce (for a compilation see Sprinzl et al. 1985). The prediction is that when such data become available for cereals, the anticodons of the major species will correspond to those triplets having G or C at the third position. It is interesting in this connection that the highly expressed genes in cereals do not avoid "strong" codons (G/C G/C G/C), in contrast to those in bacteria (for review see Grosjean and Fiers 1982) and yeast (Bennetzen and Hall 1982). In fact, they clearly select for them whenever possible, as shown by the codon families for proline (CCX), alanine (GCX), arginine (CGX), and glycine (GGX) in Table 1 (chloroplast GAPDH, columns b). The gene for maize chloroplast GAPDH is representative in this respect for the other cereal genes with a high G+C content in the third base position (see Table 2). This extends the previous observations by Wells et al. (1986) for the highly expressed histone H3 and actin genes of higher animals, which also show this tendency, although to a lesser extent. Hence, the present data and those of Wells et al. (1986) seem difficult to reconcile with the hypothesis of Grosjean and Fiers (1982) that codons with intermediate binding energies ("intermediate codons"), preferentially used by highly expressed genes of prokaryotes and yeast, are more efficiently translated than "strong" and "weak" codons. The studies of Sharp and Li (1986) and of Holm (1986) suggest that the codon bias of a particular gene does not actively modulate but rather passively reflects its level of expression. This seems reasonable since changes in gene expression may evolve much easier by modifying a single component of the expression system, such as promoter strength (Sharp and Li 1986). The preference of codons recognized by the most abundant tRNAs in *E. coli* genes encoding abundant proteins is explained by Sharp and Li (1986) on the basis of selection against codons that might impair the efficiency of (rapid) translation, and by Holm (1986) in terms of constraints on translation accuracy and on the cost of proofreading at the level of tRNA acylation. If these explanations are correct and if they also apply to the codon bias of genes from monocot angiosperms, the present contribution raises the intriguing question of how dicot plants could have escaped these selection pressures. The striking absence of a marked codon bias in dicot genes is highlighted by a recent publication (Chaboute et al. 1987) showing that in the dicot plant *Arabidopsis thaliana* even the H3 and H4 histone genes, usually G/C-rich in all

higher eukaryotes (Wells et al. 1986 and Table 2), use only 54% G+C in the third base position.

#### *Splicing of Transcripts from Highly Biased Cereal Genes in Transgenic Tobacco*

Where the manipulative transfer of genes across the monocot-dicot boundary is concerned, the different coding strategies within these two classes of angiosperms could be of considerable importance. Keith and Chua (1986), for example, have recently shown that the gene encoding the small subunit of ribulose biphosphate carboxylase from wheat is inefficiently spliced in transgenic tobacco plants and the spliced and unspliced products are polyadenylated at multiple novel sites in the wheat 3' flanking region. In contrast, the introns of the corresponding pea gene are excised efficiently in tobacco suggesting some incompatibility between monocot and dicot splicing mechanisms. It is possible that unknown differences between the intron sequences of monocot and dicot plants play a discriminative role. However, the G+C content of the exons may be equally important. As shown in Table 2, the G+C content at the third base position is 88% in wheat and only 44 and 55% in pea and tobacco small subunit genes, respectively. It seems possible, therefore, that this high G+C percentage may favor secondary structures of wheat primary transcripts that can neither be efficiently spliced nor correctly polyadenylated in tobacco. It should be possible to test this hypothesis by introducing into tobacco a hybrid small subunit gene containing the intron from wheat and the exons from pea and vice versa. In addition, since the splicing apparatus from cereals can cope with large differences in the G+C content of exons, one might expect that primary transcripts from dicot genes can be efficiently spliced in cereals, a testable hypothesis in light of the currently available cereal transformation systems (Fromm et al. 1985, 1986; Lörz et al. 1985; Potrykus et al. 1985; Cocking and Davey 1987).

The amino acid sequence data presented here and elsewhere (Martin and Cerff 1986; Shih et al. 1986) suggest strongly that early in the evolution of plants chloroplasts still possessed the gene for the photosynthetic GAPDH enzyme, but during the course of geologic time relinquished it to the nucleus by an intracellular gene transfer process. With respect to the notably low G+C content at the third base position for present-day chloroplast genes (see Table 2 and Maruyama et al. 1986; see also Morris and Herrmann 1984), the extreme to which the maize chloroplast GAPDH gene has evolved at the third base position in response to selective pressure within the nucleus is striking. By observing the patterns of nature that have evolved in the optimization of

gene expression subsequent to the transfer of genes between subcellular compartments, we should be able to draw conclusions that allow us to optimize the expression of genes transferred between species.

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