Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 7624–7628, December 1982
Biochemistry

Structural diversity and differential light control of mRNAs coding for angiosperm glyceraldehyde-3-phosphate dehydrogenases

(polyadenylylated mRNA/immunoprecipitation of *in vitro* precursors/subunit compositions/transit peptides/enzyme evolution)

RÜDIGER CERFF AND KLAUS KLOPPSTECH

Institut für Botanik, Universität Hannover, D-3000 Hannover 21, Federal Republic of Germany

Communicated by Winslow R. Briggs, August 18, 1982

ABSTRACT Subunits A and B of chloroplast glyceraldehyde-3-phosphate dehydrogenase are synthesized as higher molecular weight precursors when polyadenylylated mRNA from angiosperm seedlings is translated *in vitro* by wheat germ ribosomes. The *in vivo* levels of mRNA coding for these precursors are strongly light dependent, and the increase in translational activity stimulated by continuous white light, relative to dark-grown seedlings, is at least 5- to 10-fold for the seven plant species investigated. As opposed to this, light does not seem to change mRNA levels coding for cytosolic glyceraldehyde-3-phosphate dehydrogenase, and the polypeptides synthesized *in vitro* have the same size as the authentic subunits. In addition, precursors of the chloroplast enzyme were identified for 12 different angiosperm species and compared with their respective subunits synthesized *in vivo*. The patterns of the *in vitro* and *in vivo* products correlate in several major characteristics. They both display a remarkable interspecific heterogeneity with respect to size and number of polypeptides. The peptide extensions of the enzyme precursors calculated from these data vary between 4,000 and 12,000 daltons and seem to fall into three major size classes. The present data demonstrate that chloroplast glyceraldehyde-3-phosphate dehydrogenase, like its cytosolic counterpart, is encoded in the nucleus. Yet, the two dehydrogenases are controlled differently at both the ontogenetic and phylogenetic levels. They follow separate biosynthetic pathways with respect to light regulation, post-translational processing, and transport and also exhibit different evolutionary rates. The fast evolutionary change observed for the chloroplast enzyme contrasts sharply with the conservative structure and sequence of the cytosolic enzyme.

Chloroplast and cytosolic glyceraldehyde-3-phosphate dehydrogenases (GAPDHs) of higher plants are marker proteins for important aspects of chloroplast evolution and biogenesis. Although descendants of a common phylogenetic ancestor (1, 2), they differ in several structural features. The cytosolic NAD-specific dehydrogenase is a single homotetramer like the corresponding enzymes from other sources. Chloroplast NADP-dependent dehydrogenase is composed of two major isoenzymes A_2B_2 (isoenzyme 1) and A_4 (isoenzyme 2); subunits A and B are distinguished by slightly different molecular weights (A $\lesssim$ B) (3). The primary structures of subunits A and B are similar but differ considerably from the subunit structure of the cytosolic enzyme (1). The two dehydrogenases also differ in their evolutionary rates, as shown by a recent electrophoretic survey of the enzymes from 12 different angiosperm species (4). Whereas the cytosolic enzyme is an extremely conservative protein, the chloroplast enzyme seems to change rapidly during evolution.

This differential control of the two dehydrogenases is not restricted to their evolutionary rates but also can be observed at the ontogenetic level. It has long been known that formation of the chloroplast dehydrogenase activity during seedling development is dependent on light and phytochrome, whereas the activity of the cytosolic enzyme remains comparatively unaffected (5–9). In the study reported here we undertook to elucidate whether the appearance of mRNA for the chloroplast enzyme is under light control and, if so, how this light effect compares with the mRNA levels of the cytosolic enzyme.

Furthermore, by characterizing the enzyme-specific mRNAs we hoped to settle the longstanding dispute about whether or not the chloroplast dehydrogenase is encoded in the nucleus. A definite and unambiguous answer to this question can only be expected from direct molecular data: If the subunits of the chloroplast enzyme are nuclear gene products, their mRNAs should be polyadenylylated and would be expected to code for polypeptide precursors of considerably larger size than the authentic subunits (for review, see refs. 10–13).

A previous electrophoretic survey (4) demonstrated that the subunits of the chloroplast enzyme are remarkably heterogeneous with respect to size and number within a single plant species and between different plant species. As a third goal of the present investigation we attempted to clarify whether this subunit heterogeneity is due to heterogeneity of the primary translation products or to differential processing *in vivo* or to both.

This paper describes the *in vitro* synthesis of cytosolic and chloroplast GAPDH from polyadenylylated mRNA of light- and dark-grown angiosperm seedlings. Furthermore, the *in vitro* precursors of the chloroplast enzyme are identified for 12 different angiosperm species and are compared with their respective subunits synthesized *in vivo*.

MATERIALS AND METHODS

Growth of Plants. Seedlings of 12 different angiosperm species were grown on moist compost at 20–25°C for 4–12 days in absolute darkness or under continuous white light from sowing onward.

Isolation of Enzymes. Purification of chloroplast GAPDHs from light-grown seedlings was performed essentially as described (4, 14).

Purification of Antisera. Monospecific antisera against cytosolic and chloroplast GAPDH (isoenzyme 1) from white mustard were prepared by H. Mossmann (Max-Planck-Institut für Immunbiologie, Freiburg) and purified by ammonium sulfate precipitation (1). The IgG fraction reacting with the chloroplast

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; preGAPDH, precursors of chloroplast glyceraldehyde-3-phosphate dehydrogenase; preA, preB, precursors of subunits A and B of chloroplast GAPDH; preS, precursor of small subunit of ribulose-1,5-bisphosphate carboxylase; preLHCP, precursor of the light harvesting chlorophyll *a/b*–protein complex.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

enzyme was further purified by affinity chromatography on immobilized chloroplast GAPDH (isoenzyme 1 from white mustard) coupled to CNBr-activated Sepharose 4B according to the procedures described by the manufacturer (Pharmacia).

Isolation of Poly(A)$^+$mRNA. The procedures were essentially the same as those described by Kloppstech and Schweiger (15). Total RNA was extracted from 5 g of shoot apices (4–12 days old) from dark- and light-grown seedlings and the poly(A)-containing fraction was purified by adsorption on oligo(dT)-cellulose (type 2, Collaborative Research, Waltham, MA).

***In Vitro* Translation and Product Analysis.** Protein synthesis was carried out *in vitro* in a cell-free wheat germ system programmed with poly(A)$^+$RNA or total RNA according to Roberts and Paterson (16). GAPDH-specific primary translation products were immunoprecipitated by an indirect method using *Staphylococcus aureus* cells (17). Dodecyl sulfate electrophoresis of immunoprecipitates and enzymes was performed on gradient polyacrylamide slab gels according to Neville (18). Radioactive gels were prepared for fluorography by impregnation with EN^{3}HANCE solution (New England Nuclear Chemicals, Dreieich, Federal Republic of Germany).

RESULTS

Differential Light Regulation of Specific mRNAs. Poly(A)$^+$RNA was isolated from 4-day-old *Sinapis alba* seedlings grown under the following light regimens: (*i*) 4 days dark; (*ii*) 3 days dark plus 24 hr white light; (*iii*) 4 days white light. Equal amounts of poly(A)$^+$mRNA were translated in a protein-synthesizing wheat germ system *in vitro*, and the translation mixture was divided into two aliquots. Each aliquot was treated with one of the two monospecific antisera raised against the cytosolic and the chloroplast enzyme, respectively. The immunoprecipitates were separated by dodecyl sulfate/polyacrylamide slab gel electrophoresis and visualized by fluorography.

Lanes 1–3 in Fig. 1 show the *in vitro* translation products of the cytosolic and the chloroplast enzyme from *S. alba* for light programs *i–iii*, respectively. Light does not seem to influence the mRNA levels coding for the cytosolic enzyme because similar amounts of *in vitro* products were found for all three light regimes. The immunoprecipitates migrate as a single band (or very close doublet) of M_r 39,000, which is the molecular weight of the authentic subunit (1, 3). *In vitro* synthesis of the tomato enzyme (lane 4, Fig. 1*A*) also led to a single polypeptide that may be slightly larger than the mustard enzyme.

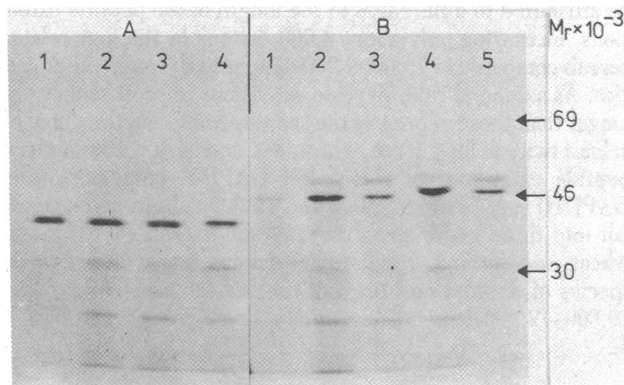

FIG. 1. Fluorogram of immunoprecipitates, showing light effects on the mRNA levels coding for cytosolic (*A*) and chloroplast (*B*) GAPDH. Poly(A)$^+$RNA was isolated from dark- and light-grown seedlings and translated by wheat germ ribosomes. The *in vitro* products of the two enzymes were immunoprecipitated from aliquots of identical translation mixtures. Lanes: 1–3 in *A* and *B*, mRNA from 4-day-old mustard seedlings grown 4 days in the dark (1), 3 days dark and 24 hr white light (2), or 4 days white light (3); 4 in *A* and *B*, mRNA from white light-grown tomato seedlings; 5 in *B*, mRNA from white light-grown pea seedlings. Dodecyl sulfate electrophoresis was performed on gradient polyacrylamide slab gels (7.5–18%) according to Neville (18).

In contrast to this, subunits A and B of the chloroplast enzyme were synthesized as apparent precursors (lanes 1–3, Fig. 1*B*) of considerably larger size than the authentic subunits (see below, Fig. 3, and Table 1). Appreciable amounts of precursors were synthesized only in the presence of mRNA from light-grown seedlings; a 24-hr light period prior to mRNA isolation seems to be more effective than continuous white light from sowing onward (compare lanes 2 and 3 in Fig. 1*B*). Lanes 4 and 5 show the *in vitro* precursors of the chloroplast enzyme from tomato and pea as synthesized from poly(A)$^+$mRNA of light-grown seedlings (see below).

Although light treatment resulted in a dramatic increase of mRNA species coding for the chloroplast enzyme, there was always some detectable immunoprecipitate from translation products of mRNA extracted from dark-grown plants (lane 1, Fig. 1*B*). To be sure that we are dealing with a quantitative rather than a qualitative effect, we investigated the light influence in six other plant species. These seedlings were grown in either absolute darkness or continuous white light from sowing onward. To eliminate the possibility of short-term light effects, the dark-grown seedlings were also harvested in absolute darkness and frozen in liquid nitrogen immediately afterward. The dark-grown seedlings of all species (tomato, cucumber, pumpkin, bean, and wheat) except the pea seem to contain at least traces of active mRNA coding for the precursors of the chloroplast enzyme (Fig. 2).

Structural Diversity of Chloroplast GAPDH Precursors. One of the most puzzling properties of the chloroplast enzyme is its interspecies heterogeneity in subunit size and number (1, 3, 4). It appears from Fig. 2 that this may at least partially be due to heterogeneity at the level of the primary translation products. To clarify this question we identified the precursors of the chloroplast enzyme for 12 different angiosperm species and compared them with their respective subunits synthesized *in vivo* (Fig. 3). The enzymes were purified from all 12 plant species by means of our simple three-step isolation procedure (4, 14). Each *in vivo* enzyme contains a major type A subunit and minor amounts of one or two type B subunits of slightly larger M_r (A ≤ B) (Fig. 3*B*). The apparent dominance of subunit A is due to the fact that the patterns represent the total enzyme, which is a mixture of two major isoenzymes, A_2B_2 and A_4 (1, 3). The enzyme preparations were checked for purity by electro-

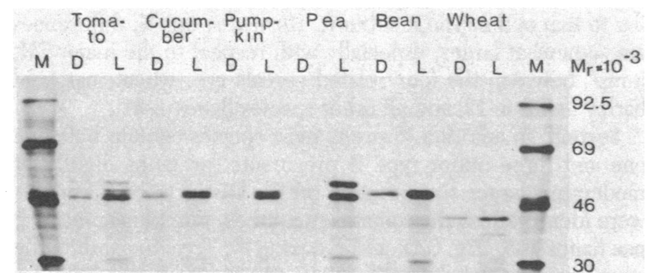

FIG. 2. Fluorogram of immunoprecipitates, showing interspecies comparison of light effects on levels of mRNA coding for chloroplast GAPDH. Lanes: D and L, mRNA from dark- and white light-grown seedlings, respectively; M, radioactive marker proteins (from top to bottom) phosphorylase *b* (M_r 92,500), bovine serum albumin (M_r 69,000), ovalbumin (M_r 46,000), and carbonic anhydrase (M_r 30,000). For all other details, see Fig. 1.

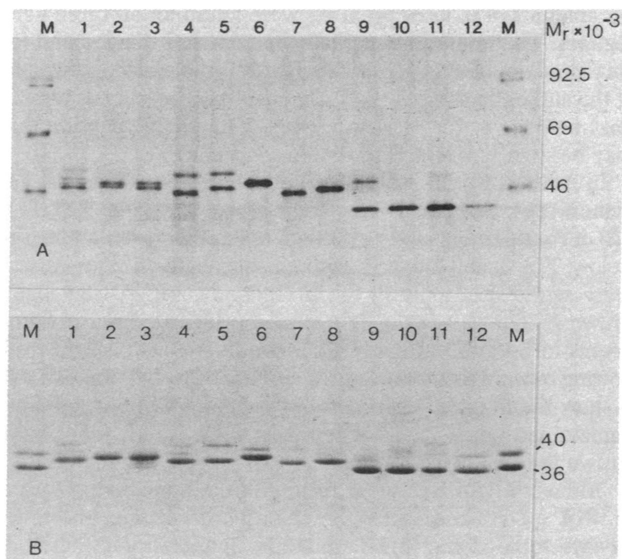

FIG. 3. Comparison of *in vitro* precursors (*A*) and *in vivo* subunits (*B*) of chloroplast GAPDH from 12 different angiosperm species. Lanes: M, markers (see Fig. 2); 1, tomato; 2, cucumber; 3, pumpkin; 4, mustard; 5, pea; 6, bean; 7, maize; 8, sorghum; 9, rye; 10, wheat; 11, oat; and 12, barley. (*A*) Fluorogram of immunoprecipitates synthesized *in vitro*. (*B*) Subunit polypeptides synthesized *in vivo* and stained with Coomassie brilliant blue. Dodecyl sulfate electrophoresis was performed on gradient (7.5–18%) polyacrylamide slab gels.

phoresis under nondenaturing conditions and subsequent side-by-side staining for proteins and enzyme activity, respectively (data not shown). Activity staining was performed by means of a modified tetrazolium test of high sensitivity (for details, see ref. 4).

In addition, the polypeptide patterns shown in Fig. 3*B* are reproducible and species specific (see figure 3 in ref. 4) and also can be duplicated by immune replication (19) (data not shown). Traces of impurities or proteolytic degradation products can be seen in lane 3 (two faint bands just below the major subunit) and lanes 10–12 (one or two faint bands of M_r considerably higher than the subunits). Close inspection of the original gel revealed that also the enzymes from cucumber and pumpkin each contain a type B subunit. This minor band (see lanes 2 and 3, Fig. 3*B*) migrates only slightly slower than the major type A subunit and therefore was overlooked in a previous investigation (4).

The patterns of the *in vitro* translation products (Fig. 3*A*) resemble those of the *in vivo* subunits (Fig. 3*B*) in several respects. First, they all contain a major type A precursor (preA) as a fast band, which displays an interspecies M_r variation similar to that of the type A subunit. However, the M_r differences are somewhat larger, especially with respect to the major "M_r jump" between the four related cereals rye, wheat, oat, and barley (lanes 9–12) and all other species (lanes 1–8).

Second, in addition to preA, most species contain between one and three minor type B precursors (preB) of slightly or moderately larger size (preA $\lesssim$ preB). Distinct preB products were identified for the tomato, cucumber, pumpkin, mustard, pea (lanes 1–5, Fig. 3*A*), and rye (lane 9). Tomato exhibits the most complex pattern with three distinct preB products for "light" mRNA, at least two of which also seem to be present in translation mixtures programmed with "dark" mRNA (Fig. 2). Cucumber and pumpkin are special in that, like the respective subunits of these species, the preA and preB products migrate as narrow doublets. Rather faint preB bands, but clearly visible on overexposed fluorograms, always were found for the five cereals sorghum, rye, wheat, oat, and barley (lanes 8–12, Fig. 3*A*), whereas no preB products could be identified for bean (lane 6) and maize (lane 7). The rather broad bands of the bean and maize precursors in Fig. 3*A* (lanes 6 and 7) are due to overexposure of the film. A doublet structure can be excluded because electrophoretic experiments with lower protein loads yielded sharp single bands for these species (see Fig. 2, lane D under "Bean").

Table 1. M_r estimates of *in vitro* precursors, *in vivo* subunits, and peptide extensions of chloroplast GAPDH from 12 different angiosperm species.

	$M_r \times 10^{-3}$					
	Precursors*		Subunits		Peptide extensions	
	preA	preB	A	B	preA	preB
1. Tomato	47	48/50/54	38	40/43	9	8–11
2. Cucumber	48	49	39	40	9	9
3. Pumpkin	48	49	39	40	9	9
4. Mustard	45	52	38	40/42	7	10–12
5. Pea	47	53	38	42	9	11
6. Bean	49	—	40	41	9	—
7. Maize	45	—	38	41	7	—
8. Sorghum	46	(49)	38	40/41	8	8–9
9. Rye	40	(46)/49	36	39/40	4	7/9
10. Wheat	40	(46)/(50)	36	40/41	4	6/9
11. Oat	40	(51)	36	40/43	4	8–11
12. Barley	40	(47)/(49)	36	40	4	7–9

The estimates are based on the electrophoretic data of Fig. 3. Peptide extensions were calculated as M_r differences (preA minus A) and (preB minus B), respectively.

* Values in parentheses represent faint preB bands; however, these were clearly discernible on overexposed fluorograms.

The approximate M_r values of all *in vitro* and *in vivo* polypeptides are listed in Table 1 together with the values for the peptide extensions calculated from these data for preA and preB. These calculations are based on the assumption that subunits A and B are derived from preA and preB, respectively (see *Discussion*). The major difference between the M_r of the preA of the four related cereals rye, wheat, oat, and barley (40,000) and that of all other species (45,000–49,000) largely can be attributed to differences in the length of the peptide extensions, measuring only about 4,000 daltons in the four related cereals compared to 7,000–9,000 daltons in the eight other species. As a general rule, peptide extensions of preB seem to be longer than those of preA of the same species, and they may be at least twice as long in rye, wheat, oat, and barley. In summary, peptide extensions of chloroplast GAPDH precursors (preGAPDH) vary between 4,000 and 12,000 daltons and seem to fall into three major size classes: 4,000 daltons (preA of rye, wheat, oat, barley), 7,000–9,000 daltons (preA of first eight species of Table 1 and preB of rye, wheat, oat, barley), and 10,000–12,000 daltons (some preB of first eight species of Table 1).

DISCUSSION

Chloroplast and cytosolic GAPDHs are controlled differently at both the phylogenetic and ontogenetic levels. The possibility that the genes for the two enzymes are located in separate compartments can now be definitely excluded. Like its cytosolic counterpart, chloroplast GAPDH is encoded in the nucleus. This conclusion is based on our findings that the enzyme subunits are translated *in vitro* as higher molecular weight precursors from mRNA containing poly(A), as shown previously for other nucleus encoded chloroplast proteins—small subunit of ribulose-1,5-bisphosphate carboxylase (20–24), light-harvesting

chlorophyll *a/b* complex (25, 26), plastocyanin (27, 28), and ferredoxin-$NADP^+$ oxidoreductase (28). Because most chloroplast proteins are encoded in the nucleus, the elucidation of their biosynthetic pathways and the underlying control mechanisms on the one hand and their phylogenetic origin and evolution on the other will contribute decisively to our understanding of chloroplast biogenesis and evolution. The present results bear on both of these aspects.

Differential Light Regulation of Specific mRNAs. The present findings demonstrate that the two dehydrogenases follow separate biosynthetic pathways with respect to light control and post-translational processing and transport. Differential light control over mRNA levels *in vivo* is clearly indicated by the results shown in Fig. 1 (the primary translation products of the two dehydrogenases were immunoprecipitated from identical translation mixtures primed with mRNA from dark- and light-grown mustard seedlings). The development of chloroplast dehydrogenase activity in mustard seedlings (8, 9) can now be interpreted in terms of a light-stimulated *de novo* enzyme synthesis whereas the synthesis of the cytosolic dehydrogenase appears to be largely unaffected by light.

Light- or phytochrome-mediated stimulation of the translational activity of specific mRNA sequences have previously been demonstrated for the precursor of the small subunit of ribulose-1,5-bisphosphate carboxylase (preS) in *Lemna gibba* (29, 30) and cucumber (31) and for the precursor of the light-harvesting chlorophyll *a/b*–protein complex (preLHCP) in *L. gibba* (30), barley (25, 32), and pea (33). The present interspecies survey (Fig. 2) clearly shows that we are dealing with a quantitative rather than a qualitative phenomenon, and this is consistent with previous studies demonstrating the presence of chloroplast GAPDH and ribulose-1,5-bisphosphate carboxylase in dark-grown seedlings (6–8, 31, 34). However, the magnitude of the light effect may vary considerably from species to species, which emphasizes the importance of interspecies comparisons if general conclusions are to be drawn. For instance, large amounts of mRNA for preGAPDH have been extracted reproducibly from light-grown pea and bean seedlings, whereas in dark-grown seedlings of these species an appreciable translational activity for preGAPDH can only be found in the bean (up to 20% of the "light" value; see Fig. 2).

Light may affect mRNA levels via enhanced transcription or post-transcriptional processing or by stabilizing mRNAs against degradation. A light-induced polyadenylylation seems unlikely because similar light effects can be observed when translation mixtures are programmed with total RNA. This has been reported for the mRNAs of preLHCP (33) and preS (35) from peas and now is confirmed by us for preGAPDH from mustard (data not shown). Recent studies (35) using cloned hybridization probes have shown that light stimulates the accumulation of preS transcripts in both the nucleus and cytoplasm of pea leaves. With similar techniques, light- and phytochrome-induced increases have also been demonstrated for the mRNA sequences of the chloroplast encoded "photogene" in maize (36) and *S. alba* (37).

Structural Diversity, an Evolutionary *Leitmotiv* of Chloroplast GAPDH. A unique aspect of the present study is that structural and functional conclusions are based on a broad interspecies survey. The interspecific heterogeneity of the enzyme can now be analyzed with respect to its *in vivo* subunits, its *in vitro* precursors, and their apparent NH_2-terminal extensions. We do not believe that artifactual proteolysis has a major influence on the heterogeneity of the *in vivo* and *in vitro* patterns shown in Fig. 3 for the following reasons. The smallest polypeptide in each pattern always represents the major product showing similar interspecies M_r variations *in vivo* and *in vitro*. Furthermore, it should be remembered that the major *in vivo* product is an active enzyme subunit and, at the same time, a stoichiometric component of two isoenzymes, A_2B_2 and A_4 (1, 3). Therefore, it seems reasonable to assume that the type A *in vitro* products are the true precursors of the type A subunits. The situation is somewhat more complex with respect to the type B products where correlations between *in vivo* and *in vitro* polypeptides seem to be less stringent. However, type B polypeptides are always less abundant and slightly larger than type A polypeptides, which makes it seem likely that at least some if not most type B subunits are derived from preBs. This is almost certainly the case for rye, wheat, oat, and barley, for which type B subunits seem to be too large to be derived from preAs (Table 1).

Although a one precursor–one subunit relationship may hold in many cases, some type B subunits may arise from differential processing. This is indicated in all cases where the number of enzyme subunits exceeds the number of primary translation products synthesized *in vitro* (e.g., mustard, bean, maize, sorghum; see Fig. 3 and Table 1). Whether or not this putative differential processing is related to post-translational transport may be clarified by *in vitro* uptake experiments (28, 38). On the other hand we cannot exclude the possibility that certain type B precursors were not discovered by our techniques because of low antigenic crossreactivity with our purified antiserum raised against the mustard enzyme. Examples for this methodological problem are the three cereal species wheat, oat, and barley, for which we always find distinct type B subunits but only trace amounts of apparent preBs (compare lanes 10 to 12 in Fig. 3).

An important question that emerges from our work is whether the preBs are encoded by separate messengers or whether they are run-through translation products (39) of a single messenger coding for both preA and preB. This question cannot be answered definitely without the use of suitable hybridization probes. However, because it seems likely that preA and preB differ in their NH_2-terminal extensions (see below and Table 1), whereas run-through translation would only affect the COOH terminus, we favor the interpretation that the heterogeneity of the primary translation products is mainly due to structural diversity of multiple mRNAs.

Although the location of the extra sequence in chloroplast preGAPDH has not been shown, it is likely that it is present as an NH_2-terminal extension as demonstrated for the preS of *Chlamydomonas* (40). This peptide extension has been termed "transit peptide" because it is thought to play a role during post-translational transport of the precursor through the chloroplast envelope (23, 24, 26, 28, 38, 40, 41) (for reviews, see refs. 10–13). The peptide extensions of chloroplast preGAPDH are remarkable in several respects.

First, they are exceptionally long. In the first eight species in Table 1, they are 7,000–9,000 daltons in preA and may be as long as 11,000–12,000 daltons in preB, which is about twice the size of the peptide extensions usually found for the chloroplast proteins preS and preLHCP [4,000–6,000 daltons (20–26, 29, 30)]. Longer presequences have been found for the precursors of plastocyanin (15,000 daltons) and ferredoxin-$NADP^+$ oxidoreductase (8,000 daltons) in peas (28) and for preS of cucumber (11,000 daltons) (31).

Second, peptide extensions of chloroplast preGAPDH display a remarkable heterogeneity in length between different species and presumably also between preA and preB within a single species. They vary between 4,000 and 12,000 daltons, corresponding to 36–110 amino acids and seem to fall into three major size classes that are 3,000–4,000 daltons apart. In view of these findings it is not surprising that no sequence homology

was found between preS presequences of *Chlamydomonas* (40) and pea (42). This high degree of structural heterogeneity of GAPDH presequences would seem to support the view that the NH_2 terminus is probably not the recognition site on the entering polypeptide chain (24). A recent review (13) proposed that presequences of organellar proteins serve to maintain the polypeptide released from the ribosome in a configuration, in which a true signal, to which other features of the polypeptide may contribute, is recognizable by the organelle surface receptors. Removal of this NH_2-terminal segment after post-translational transport would lead to the functional configuration and, at the same time, may "trap" the protein inside the organelle.

A prominent feature of chloroplast GAPDH is its unusual fast evolutionary rate which contrasts sharply with the high degree of evolutionary conservation of the cytosolic enzyme (4). The subunit patterns in Fig. 3*B* clearly suggest the presence of interspecies structure differences also outside the "hypervariable" region of the peptide extension. The subunits also seem to differ significantly in sequence between distantly related angiosperm species as shown (1) for chloroplast GAPDHs of mustard and barley on the basis of antigenic crossreactivity, tryptic peptides, and amino acid compositions. On the other hand, enzymes of the same taxonomic group show structural similarities, as demonstrated in the present study for the graminean subfamily Festucoideae (rye, wheat, oat, and barley, lanes 9–12, Fig. 3) and the family Cucurbitaceae (cucumber and pumpkin, lanes 2 and 3, Fig. 3). From an enzymological standpoint it is especially interesting that chloroplast GAPDH is composed of two separate subunits (43). The functional role of subunit B remains to be elucidated.

Previous (4) and present results contradict the Gottlieb hypothesis (44) which suggests that chloroplast enzymes in general may be less variable than their cytosolic counterparts. An interesting example of this reverse situation are the phosphoglucose isomerases from the chloroplast and cytoplasm of higher plants (45); however, generalizations remain premature until more (iso)enzyme pairs have been examined.

We thank E. Campbell for skillful technical assistance and M. Viro for help with the mRNA preparations. In its initial phase the present investigation benefited from generous support by C. J. Leaver (Edinburgh) and G. Schatz (Basel). We also thank D. von Wettstein (Copenhagen) for valuable suggestions. This research was sponsored by the Deutsche Forschungsgemeinschaft (Ce 1/10 and Kl 401/1).

1. Cerff, R. & Chambers, S. E. (1979) *J. Biol. Chem.* **254**, 6094–6098.
2. Cornish-Bowden, A. (1980) *Anal. Biochem.* **105**, 233–238.
3. Cerff, R. (1979) *Eur. J. Biochem.* **94**, 243–247.
4. Cerff, R. (1982) *Eur. J. Biochem.* **126**, 513–515.
5. Hageman, R. H. & Arnon, D. I. (1955) *Arch. Biochem. Biophys.* **57**, 421–436.
6. Marcus, A. (1960) *Plant Physiol.* **35**, 126–128.
7. Ireland, H. M. M. & Bradbeer, J. W. (1971) *Planta* **96**, 254–261.
8. Cerff, R. (1973) *Plant Physiol.* **51**, 76–81.
9. Cerff, R. (1974) *Plant Physiol.* **54**, 100–104.
10. Chua, N.-H. & Schmidt, G. W. (1979) *J. Cell Biol.* **81**, 461–483.
11. Kreil, G. (1981) *Annu. Rev. Biochem.* **50**, 317–348.
12. Ellis, R. J. (1981) *Annu. Rev. Plant Physiol.* **32**, 111–137.
13. Sabatini, D. D., Kreibich, G., Morimoto, T. & Adesnik, M. (1982) *J. Cell Biol.* **92**, 1–22.
14. Cerff, R. (1982) in *Methods in Chloroplast Molecular Biology*, eds. Edelman, M., Hallick, R. B. & Chua, N.-H. (Elsevier/North-Holland, Amsterdam), in press.
15. Kloppstech, K. & Schweiger, H. G. (1976) *Cytobiologie* **13**, 394–400.
16. Roberts, B. E. & Paterson, B. M. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2330–2334.
17. Kessler, S. W. (1975) *J. Immunol.* **115**, 1617–1624.
18. Neville, D. M. (1971) *J. Biol. Chem.* **246**, 6328–6334.
19. Burnette, W. N. (1981) *Anal. Biochem.* **112**, 195–203.
20. Roy, H., Terenna, B. & Cheong, L. C. (1977) *Plant Physiol.* **60**, 532–537.
21. Dobberstein, B., Blobel, G. & Chua, N.-H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1082–1085.
22. Cashmore, A. R., Broadhurst, M. K. & Gray, R. E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 655–659.
23. Chua, N.-H. & Schmidt, G. W. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 6110–6114.
24. Highfield, P. E. & Ellis, R. J. (1978) *Nature (London)* **271**, 420–424.
25. Apel, K. & Kloppstech, K. (1978) *Eur. J. Biochem.* **85**, 581–588.
26. Schmidt, G. W., Bartlett, S., Grossman, A. R., Cashmore, A. R. & Chua, N.-H. (1981) *J. Cell Biol.* **91**, 468–478.
27. Bohner, H., Böhme, H. & Böger, P. (1981) *FEBS Lett.* **131**, 386–388.
28. Grossman, A. R., Bartlett, S. G., Schmidt, G. W., Mullet, J. E. & Chua, N.-H. (1982) *J. Biol. Chem.* **257**, 1558–1563.
29. Tobin, E. M. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 4749–4753.
30. Tobin, E. M. (1981) *Plant Mol. Biol.* **1**, 35–51.
31. Walden, R. & Leaver, C. J. (1981) *Plant Physiol.* **67**, 1090–1096.
32. Apel, K. (1979) *Eur. J. Biochem.* **97**, 183–188.
33. Cuming, A. C. & Bennet, J. (1981) *Eur. J. Biochem.* **118**, 71–80.
34. Brüning, K., Drumm, H. & Mohr, H. (1975) *Biochem. Physiol. Pflanz.* **168**, 141–156.
35. Smith, S. M. & Ellis, R. J. (1981) *J. Mol. Appl. Genet.* **1**, 127–137.
36. Bedbrook, J. R., Link, G., Coen, D. M., Bogorad, L. & Rich, A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3060–3064.
37. Link, G. (1982) *Planta* **154**, 81–86.
38. Grossman, A., Bartlett, S. G. & Chua, N.-H. (1980) *Nature (London)* **285**, 625–628.
39. Ogawa, K. & Kaji, A. (1975) *Biochim. Biophys. Acta* **402**, 288–296.
40. Schmidt, G. W., Devillers-Thiery, A., Desruisseaux, H., Blobel, G. & Chua, N.-H. (1979) *J. Cell Biol.* **83**, 615–622.
41. Smith, S. M. & Ellis, R. J. (1979) *Nature (London)* **278**, 662–664.
42. Bedbrook, J. R., Smith, S. M. & Ellis, R. J. (1980) *Nature (London)* **287**, 692–697.
43. Harris, J. I. & Waters, M. (1976) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), Vol. 13, pp. 1–48.
44. Gottlieb, L. D. (1982) *Science* **216**, 373–380.
45. Gottlieb, L. D. & Weeden, N. F. (1981) *Evolution* **35**, 1019–1022.