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Microsequencing and cDNA cloning of the Calvin cycle/OPPP enzyme ribose-5-phosphate isomerase (EC 5.3.1.6) from spinach chloroplasts

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Abstract

Ribose-5-phosphate isomerase (RPI) catalyses the interconversion of ribose-5-phosphate and ribulose-5-phosphate in the reductive and oxidative pentose phosphate pathways in plants. RPI from spinach chloroplasts was purified and microsequenced. Via PCR with degenerate primers designed against microsequenced peptides, a hybridisation probe was obtained and used to isolate several cDNA clones which encode RPI. The nuclear-encoded 239 amino acid mature RPI subunit has a predicted size of 25.3 kDa and is translated as a cytosolic precursor possessing a 50 amino acid transit peptide. The processing site of the transit peptide was identified from protein sequence data. Spinach leaves possess only one type of homodimeric RPI enzyme which is localized in chloroplasts and is encoded by a single nuclear gene. Molecular characterization of RPI supports the view that a single amphibolic RPI enzyme functions in the oxidative and reductive pentose phosphate pathways of spinach plastids.

Abbreviations: RPI, ribose-5-phosphate isomerase; OPPP, oxidative pentose phosphate pathway; CNBr, cyanogen bromide; R5P, ribose-5-phosphate; Ru5P, ribulose-5-phosphate

Introduction

Despite the obvious importance of the Calvin cycle for photoautotrophic matabolism, many questions concerning the biochemistry of its regulation are still unresolved [16, 42, 49]. In photosynthetic proteobacteria, genes for enzymes of the pathway are organized in operons and are coordinately expressed through transcriptional activation [5, 18, 45]. In higher plants, the study of coordinated gene regulation for the pathway *in toto* has been intractable due to the lack of mo-

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number L43068.

lecular probes for all enzymes of the cycle. Reports of the initial molecular characterization of Calvin cycle enzymes from plants have appeared steadily over the years [23, 29–31, 34, 35, 40]; and with the recent cloning of several transketolases from the resurrection plant *Craterostigma* [4] and ribulose-5-phosphate 3-epimerase from spinach [33], ribose-5-phosphate isomerase (RPI, EC 5.3.1.6) has become the last of the Calvin cycle enzymes for which molecular probes have not been isolated from any photosynthetic eukaryote.

RPI catalyses the interconversion of D-ribose-5-phosphate and D-ribulose-5-phosphate in the reductive and oxidative pentose phosphate pathways (OPPP) and thus plays an important role in primary metabolism of both photosynthetic and non-photosynthetic organisms [48]. Early investigations [21] indicated that RPI occurs as a single, chloroplast-localized enzyme in spinach, sugar beet and *Elodea*. Rutner [36] purified the enzyme from spinach leaves 2800-fold and suggested that it exists as a single chloroplast species with a native size of 57 kDa and a K_m of 46 μM for R5P. Later reports indicated the existence of an additional cytosolic isoenzyme for RPI [1]. Such reports suggesting the existence of cytosolic isoenzymes for other activities common to the OPPP and Calvin cycle led *inter alia* [28] to the widely held view that plants in general possess a complete OPPP in both the cytosol and the chloroplast. However a recent critical re-examination of OPPP/Calvin cycle compartmentlization revealed that enzymes common to the OPPP and Calvin cycle occur, within the limits of detection, as a single chloroplast species, at least in spinach leaves [41]. Spinach RPI thus has a dual (amphiholic) function as it supplies both the oxidative and reductive PPPs in chloroplasts with isomeric intermediates needed to regenerate glucose-6-phosphate and ribulose-1,5-bisphosphate, respectively. Yet, as summarized in Fig. 1, metabolite flux across RPI differs in these pathways.

In contrast to the Calvin cycle, the OPPP can

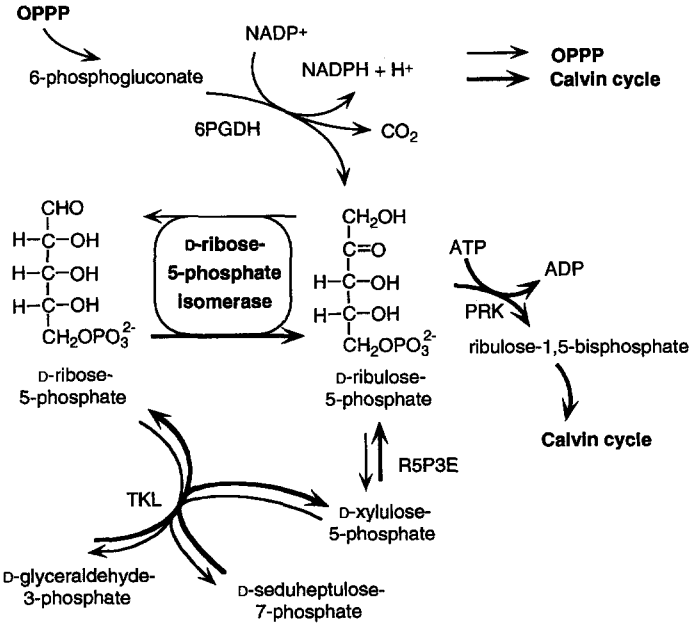

Fig. 1. Role of ribose-5-phosphate isomerase in spinach leaves. OPPP reactions are designated with thin arrows, Calvin cycle reactions with heavy arrows. 6GPDH, 6-phosphogluconate dehydrogenase; TKL, transketolase; R53PE, ribulose-5-phosphate 3-epimerase; PRK, phosphoribulokinase.

generate excess erythrose-4-phosphate for the shikimate pathway without being depleted of intermediates [16, 41], and up to 60% of plant dry weight can consist of shikimate-derived compounds [26]. Little is known about the mechanisms of metabolite partioning between Calvin cycle and OPPP and, with few exceptions [4, 20, 33], enzymes of the OPPP in plants have been neglected at the molecular level. The first efforts devoted to cloning of RPI were those of Hove-Jensen and Maigaard [24], who characterized one of the two genes for RPI in *Escherichia coli*. More recently, a gene encoding enzymatically active RPI from mouse was characterized through exon trapping by virtue of its proximity to the immunoglobulin κ gene [3]. Hitherto, isolation of clones for the coding sequence of ribose-5-phosphate isomerase have not been reported from any plant source. Here we describe the characterization and cloning of spinach chloroplast ribose-5-phosphate isomerase.

Material and methods

Enzyme purification

Spinach chloroplast ribose-5-phosphate isomerase was purified from a commercially available preparation from Sigma by ion exchange chromatography on DEAE-Fractogel equilibrated with 10 mM potassium phosphate, pH 7.5, 10 mM EDTA, and 2-mercaptoethanol. After extensive dialysis against column buffer proteins were applied to the resin and eluted by a 0 to 0.3 M KCl gradient in column buffer. Fractions with PRI activity were collected, concentrated by dialysis against polyethylene glycol 20000 and then against column buffer.

Native molecular mass determination

The native size of proteins was estimated by HPLC gel filtration at 1.0 ml/min on Bio-Sil TSK 250 equilibrated in 50 mM Na_2SO_4, 20 mM NH_2PO_4, pH 6.8. Standards were β-galactosidase (465 kDa), IgG (150 kDa), Fab (50 kDa) and myoglobin (17 kDa). Native molecular masses were interpolated from the plot of retention time versus log M_r.

Enzyme assays

As described [41], ribose-5-phosphate isomerase was assayed in 40 mM potassium phosphate, 7.4, 5 mM $MgCl_2$, 0.5 units each of ribulose-5-phosphate epimerase and transketolase 1 unit each of triosephosphate isomerase and glycerol-3-phosphate dehydrogenase, 240 μM NADH, and 10 mM ribose-5-phosphate.

Protein sequencing

Purified protein was digested with endoproteinase LysC or cyanogen bromide as described by Eckerskorn and Lottspeich [12]. Peptides were separated on a 2 μm × 125 mm Supersher 60 RP select B column (Merck) at a flow rate of 200 μl/min in a 1%/min gradient of 0.1% (v/v) trifluoroacetic acid (TFA) in water to 0.1% (v/v) TFA in acetonitrile. Peptides were sequenced by amino-terminal degradation [13] in an automatic Porton 3600 Sequencer (Beckman) and amino acids were identified in a Microbore HPLC System Gold (Beckman).

Isolation of an RPI hybridization probe

The degenerate oligonucleotides 5′-AARATGGTNGARGCNGC-3′ and 5′-TTCCARCARAAYTGNAC-3′ were synthesized against the protein motifs (K)MVEAA and VQFCWK determined from protein sequencing. These were used in a PCR reaction containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 50 μM of each dNTP, 1.6 μM of each primer, 0.02 U:μl *Taq* polymerase (Perkin-Elmer) and 8 ng/μl of double-stranded spinach cDNA in a total volume of 25 μl. Thirty-five cycles of 1 min at 92 °C, 1 min at 45 °C and 1 min at 72 °C were per-

formed. The $MgCl_2$ concentration in this reaction was critical. The 120 bp amplification product was used as a substrate for reamplification under the same conditions, the product of which was purified by ultrafiltration on Microcon 30 devices (Amicon), filled in with Klenow polymerase, phosphorylated with T_4 kinase as described [39], purified by ultrafiltration on Microcon 30 devices and ligated into *Hinc*II-cut pBluescriptSK+ (Stratagene) and transformed into *E. coli* nm522 to yield the plasmid pPCRrpi1. The sequence pPCRrpi1 was determined by the dideoxy method to confirm the identity of the amplified product. The 140 bp *Cla*I-*Xho*I fragment of pPCRrpi1 was isolated by gel electrophoresis and DE52 ion-exchange chromatography as a hybridisation probe for cDNA screening.

cDNA cloning

The spinach cDNA library in nm1149 [32] previously described by Henze *et al.* [23] was used. 40000 freshly plated recombinant cDNA clones (unamplified) were plated on *E. coli* POP13 and screened by plaque hybridization on 82 mm nitrocellulose filters (Gelman Sciences) at 60 °C in 3× SSPE, 0.1% (w/v) SDS, 0.02% (w/v) PVP, 0.02% (w/v) Ficoll-400, and 50 μg/ml calf thymus DNA containing 10 ng/ml of the 140 bp *Cla*I-*Xho*I fragment of pPCRrpi1 random-labelled [14] to 2×10^7 cpm/μg. Filters were washed for 60 min at 60 °C in 2× SSPE, 0.1% (w/v) SDS and autoradiographed overnight on XAR films. *Not*I inserts of three positively hybridizing cDNAs were subcloned into pBluescriptSK plasmids (Stratagene) to yield plasmids pRI1, pRI12 and pRI22, respectively, the terminal sequences were determined by the dideoxy method. The insert of pRI12 was sequenced on both strands using synthetic primers.

Genomic Southern blots

A 25 μg portion of spinach DNA was digested with the appropriate enzyme, electrophoresed and transferred to Hybond-N (Amersham) as described [23]. Filters were prehybridized at 68 °C in 6× SSPE, 0.1% (w/v) SDS, 0.02% (w/v) PVP, 0.02% (w/v) Ficoll-400 containing 50 μg/ml calf thymus DNA. Hybridization was carried out overnight at 68 °C in 3× SSPE, 0.1% (w/v) SDS, 0.02% (w/v) PVP, 0.02% (w/v) Ficoll-400, 50 μg/ml calf thymus DNA containing 10 ng/ml of hybridization probe. The hybridisation probe was the complete 1.2 kb *Eco*RI fragment of pRI12 random-labelled to 3×10^7 cpm/μg with α-^{32}P-dCTP. Filters were washed for 60 min at 68 °C in 2× SSPE, 0.1% (w/v) SDS and autoradiographed overnight on XAR films.

Other molecular methods

Standard DNA manipulations and SDS-PAGE were performed as described [39]. Oligonucleotides were synthesized with the Pharmacia machine. Sequence analysis was performed with the GCG Package [11] and as previously described [33].

Results

Purification and cloning of RPI from spinach chloroplasts

The final purified preparation of ribose-5-phosphate isomerase from spinach chloroplasts contained one major band of ca 24 kDa (Fig. 2). Gel filtration revealed a native M_r of ca. 60 kDa for the purified enzyme. A 100 μg portion of protein were subjected to proteolysis and CNBr cleavage, the HPLC-separated products of which were sequenced. Peptides determined from cyanogen bromide cleavage are given in Table 1. The identical amino termini of peptides 1 and 4 from CNBr and LsyC cleavages, respectively, provided identification of the amino terminus of the mature subunit. Peptides 2, 5, 6 and 7 overlapped to provide a 40 residue contiguous sequence, termed peptide 2–7. Using degenerate primers constructed against the N- and C-termini of peptide

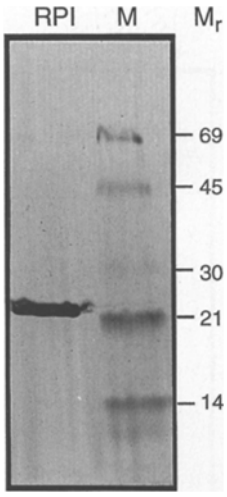

Fig. 2. Silver-stained SDS-PAGE gel of purified chloroplast RPI from spinach. 1 μg of protein was loaded per lane. RPI, ribose-5-phosphate isomerase; M, marker. Sizes of molecular weight standards (kDa) are indicated.

Table 1. Microsequenced peptides from spinach chloroplast RPI. Residues in parentheses indicate unsequenced amino acids expected to precede the given peptide.

Cyanogen bromide
1. SPTPVLTQDDLKKLAAEKAVDSVK
2. (M)VEAASDKFIVVVDDTKLVDGLGGS
3. (M)ASEVIIAGKTGVSVKT
Endopeptidase LysC
4. SPTPVLTQDDLK
5. (K)MVEAASDK
6. (K)FIVVVDDTK
7. (K)LVDGLGGSRLAMPVEVVQFCWK
8. (K)RTAEQAASLGIPLSVLDDHPRIDLAIDGADEVDPDLN
9. (K)NIVGIPTSK
10. (K)YNLK
11. (K)XRLQEIFK

2–7 in a PCR reaction with spinach cDNA as a substrate, we obtained a 120 bp amplification product which as subcloned and shown to encode the expected peptide. This insert was isolated and used to screen the cDNA library. From 40000 recombinants we obtained eight positively hybridizing clones. Three of these contained inserts of the length anticipated (ca 1.1 kb) for full-size clones encoding the ca 24 kDa mature subunit. The *Not*I inserts of these were subcloned and their terminal sequences proved to be identical with pRI12, the sequence of which is shown in Fig. 3.

pRI12 encodes an open reading frame of 870 bp and contains all peptides sequences determined from the purified, active enzyme. The N-terminus of the native 239 amino acid mature subunit is preceded by a 50 amino acid long, serine-rich transit peptide. The predicted size of the mature subunit is 25.3 kDa, similar to that determined by SDS-PAGE (Fig. 2). The 223 bp long 3′-untranslated region appears to contain multiple polyadenylation signals, as suggested by the A_{10} stretch found in pRI22 (Fig. 3).

A single gene in spinach for ribose-5-phosphate isomerase

The ca. 200 bp terminal sequences of pRI1 and pRI22 reveal no nucleotide substitutions relative to pRI12, indicating that these represent transcripts from the same gene. A Southern blot of spinach DNA hybridized at low stringency to the complete 1.1 kb insert of pRI12 reveals a very simple pattern (Fig. 4). The presence of only a single band in *Kpn*I and *Xho*I digests, and only two bands in the *Sac*I digest suggest that ribose-5-phosphate isomerase is either encoded by a single gene in spinach which contains a *Sac*I site in an intron, or that two genes exist, possibly allelic variants. This is congruent with previous findings demonstrating only a single gene for several other Calvin cycle enzymes in spinach: fructose-1,6-bisphosphate aldolase [34], chloroplast triosephosphate isomerase [23], ribulose-5-phosphate 3-epimerase [33] and transketolase (unpublished data). In wheat and its relatives, several enzymes of the Calvin cycle were also found to be encoded as by one copy per halpoid genome, whereas others, such as the small subunit of Rubisco (*rbcS*), were found in multiple copies per haploid genome [7]. Several copies of *rbcS* also exist in potato [47], petunia [46], pea [9], *Mesembryanthemum* [10] and spinach (unpublished data).

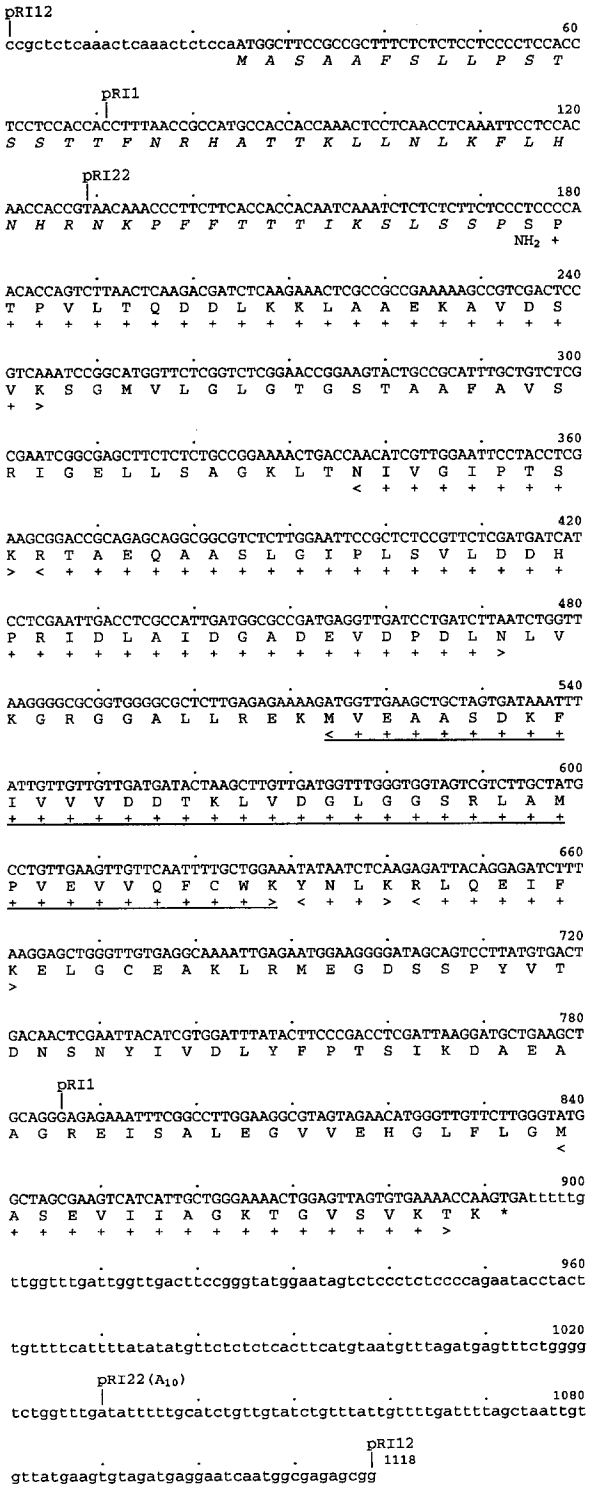

Fig. 3. Sequence of cDNA clones for chloroplast ribose-5-phosphate isomerase from spinach. Terminal nucleotides of clones pRI12, pRI1, and pRI22 (underlined) are indicated (accession number L43068). The transit peptide is shown in italics, the amino terminus of the mature subunit is indicated. Regions sequenced from the isolated protein are indicated with < + + >. The peptide from which the PCR probe was generated is underlined. Non-coding regions are shown in lower case.

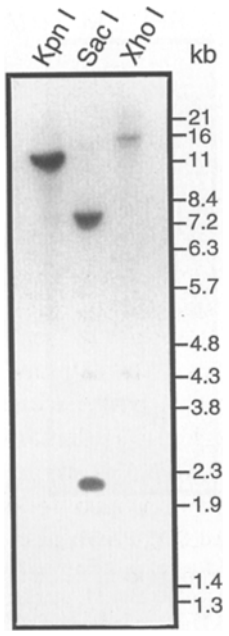

Fig. 4. Southern blot of genomic DNA from spinach probed with the region encoding the mature subunit of RPI. 25 μg of spinach DNA was loaded per lane. Lane 1, *Kpn*I; lane 2, *Sac*I; lane 3, *Xho*I. Sizes of molecular weight standards (kb) are indicated. The film was exposed for 2 days at −80 °C.

The frequency of RPI-hybridizing recombinants among cDNA clones studied indicates an abundance of this mRNA in light-grown seedlings of 0.02% of poly(A)$^+$ mRNA. RPI mRNA in this tissue is thus about five-fold less abundant than that for the A subunit of Calvin cycle GAPDH in most green tissues [25], of roughly equal abundance as ribulose-5-phosphate 3-epimerase [33] but about two-fold higher than that for Calvin cycle triosephosphate isomerase [23].

Discussion

Clones encoding functional RPI

Hove-Jensen and Maigaard [24] cloned one of the two RPI genes in *E. coli* (*rpiA*) via complementation and demonstrated enzymatic activity of the *rpiA*-encoded product; Apel *et al.* [3] cloned a gene for enzymatically active RPI in mouse via exon trapping in their search for functional genes located downstream of the immunoglobulin κ locus. In both cases, function of the cloned gene product was demonstrated by overexpression in *E. coli*. We used the purification-microsequencing approach to clone PRI from spinach. In a previous study, we found no evidence for the existence of chloroplast-cytosol isoenzymes for ribose-5-phosphate isomerase in spinach leaves [41], but rather detected a single chloroplast-localized enzyme. Instead of purifing the chloroplast enzyme from scratch, as we did for triosephosphate isomerase [23], we simply subjected the commercially available spinach chloroplast RPI from Sigma to an additional ion-exchange column to obtain an electrophoretically homogenous, enzymatically active protein suitable for microsequencing. The amino acid sequences determined directly were identical to those deduced from the complete cDNA sequence. Database searching revealed that RPI-related sequences from *Caenorhabditis elegans*, *Haemophilus influenzae* and *Synechocystis* PCC6803 with ca. 36% amino acid identity to the spinach chloroplast enzyme have been deposited in GenBank (accession numbers U10438, U32729 and D64002 respectively), but the function of those sequences, if any, is unknown.

An alignment of amino acid sequences for the functional spinach, mouse and *E. coli* enzymes in the context of other RPI-related sequences from other organisms reveals a modest degree of conservation (36–40%) in most comparisons and a more or less uniform distribution of conserved residues (Fig. 5a). Recognizable amino acid identity between these enzymes suggests that their genes share a common ancestry. This finding is not trivial since 'class I' and 'class II' forms exist for several enzymes of primary metabolism, i.e. fructose-1,6-bisphosphate aldolase [34], Rubisco [8, 17], glutamin synthase [27] and GAPDH [6] which possess little or no amino acid identity. A phylogenetic tree for these sequences reveals that the spinach nuclear gene for chloroplast RPI is not significantly more closely related to its cyanobacterial homologue than it is to eukaryotic homologues. Due to the small number of sequences for comparison, it is currently not possible to discern whether the nuclear gene for this Calvin cycle enzyme is a descendant of its nuclear counterpart in nonphotosynthetic eukaryotes, as in the case of chloroplast isoenzymes of TPI [23, 40] and aldolase [34], or whether it was transferred from an ancestral organellar genome to its current location in the nucleus, as in the case of PGK [6] and GAPDH [22]. Further sequence studies should, in time, reveal the evolutionary origin of plant RPI genes.

Subunit structure of spinach chloroplast RPI

The subunit size of ca. 24 kDa found for spinach RPI in SDS-PAGE is in agreement with predicted M_r of the mature subunit derived from the cDNA sequence (25.3 kDa). The native M_r of ca. 60 kDa reported here is slightly higher than the value of 53 kDa previously determined for spinach RPI by Rutner [36], but taken together, the data indicate that the active enzyme of spinach chloroplasts is homodimeric, as is the case for its *E. coli* homologue [24].

Amphibolic function of RPI

In plants, ribose-5-phosphate isomerase participates in the Calvin cycle and the OPPP. The lack of detectable RPI activity in the spinach cytosol [41] and the absence of detectable RPI isoenzymes in spinach leaves [36, 41] indicate that a single, amphibolic RPI enzyme participates in both pathways in chloroplasts of spinach leaves, although the flux of substrate through the reaction is opposite in the Calvin cycle and the OPPP

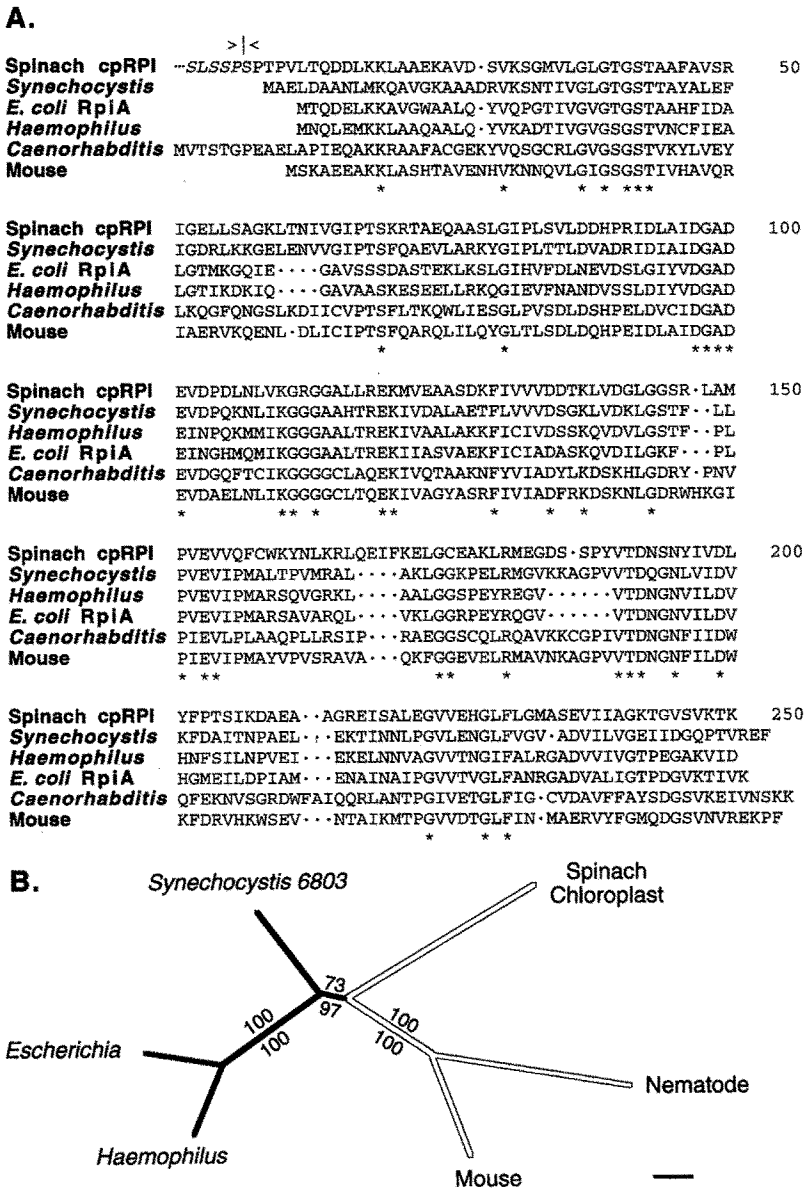

Fig. 5. Comparison of ribose-5-phosphate isomerase sequences. A. Alignment of sequences of functional ribose-5-phosphate isomerase enzymes (spinach chloroplast, *E. coli* and mouse) with similar sequences from *Haemophilus*, *Synechocystis* PCC6803 and *Caenorhabditis* (sources of sequences given in the text). Strictly conserved residues are indicated by an asterisk. The processing site of the spinach transit peptide (italics) is indicated by >|<. Gaps are indicated as dots. B. Phylogenetic tree for the same sequences constructed using the neighborjoining method [38] for Dayhoff matrix distances of PHYLIP [15]. Numbers above branches indicate the bootstrap proportion for 100 NJ trees using the same distance estimation, numbers below branches indicate the bootstrap proportion for 100 parsimony trees using PROTPARS [15]. The scale bar indicates 0.1 substitutions per site. Sequences encoded in eukaryotic nuclei are borne on open branches, those encoded in bacterial genomes are borne on solid branches.

(Fig. 1). This finding bears upon models which could explain differential metabolite flux through these pathways. Several independent reports have indicated that RPI may be associated in multi-

protein complexes with other enzymes of the Calvin cycle. RPI has been found to be associated with phosphoribulokinase (PRK) [19, 37, 43, 44] and Anderson [2] has detected an increased reaction rate for PRK in the presence of RPI, suggesting channelling of substrate in the Calvin cycle direction. Furthermore, spinach leaf RPI was reported to be associated in even larger complexes of up to five Calvin cycle enzymes [19, 44]. If such complexes are of physiological relevance in spinach leaves, the finding that this organ possesses only a single ribose-5-phosphate isomerase enzyme raises questions concerning the balance and regulation of Calvin cycle/OPPP function. In order to supply erythrose-4-phosphate for the shikimate pathway [16, 41], RPI of the OPPP would either need to exist in molar excess relative to other Calvin cycle enzymes, assuring the presence of sufficient uncomplexed RPI. Alternatively, the formation of such complexes may be reversible and conceivably regulated in order to satisfy the metabolic requirements of different organs under different environmental and developmental conditions. Further studies with antibodies against spinach chloroplast RPI are needed to address these questions.

Acknowledgements

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References

1. Anderson LE: Chloroplast and cytoplasmic enzymes. III. Pea leaf ribose 5-phosphate isomerases. Biochim Biophys Acta 235: 507–512 (1971).
2. Anderson LE: Ribose-5-phosphate isomerase and ribulose-5-phosphate kinase show apparent specificity for a specific ribulose 5-phosphate species. FEBS Lett 212: 45–48 (1987).
3. Apel TW, Scherer A, Adachi T, Auch D, Ayane M, Reth M: The ribose-5-phosphate isomerase-encoding gene is located immediately downstream from that encoding murine immunoglobulin κ. Gene 156: 191–197 (1995).
4. Bernacchia G, Schwall G, Lottspeich F, Salamini F, Bartels D: The transketolase gene family of the resurrection plant *Craterostigma plantagineum*: differential expression during the rehydration phase. EMBO J 14: 610–618 (1995).
5. Bowien B, Bednarski R, Kusian B, Windhövel U, Freter A, Schäferjohann J, Yoo J-G: Genetic regulation of CO_2 assimilation in chemoautotrophs. In: Murrel JC, Kelley DP (eds) Microbial Growth on C1 Compounds, pp. 481–491. Intercept Scientific, Andover, UK (1993).
6. Brinkmann H, Martin W: Higher plant chloroplast and cytosolic 3-phosphoglycerate kinases: a case of endosymbiotic gene replacement. Plant Mol Biol 30: 65–75 (1996).
7. Chao S, Raines CA, Longstaff M, Sharp PJ, Gale MD, Dyer TA: Chromosomal location and copy number in wheat and some of its close relatives of genes for enzymes involved in photosynthesis. Mol Gen Genet 218: 423–430 (1989).
8. Chen J-H, Gibson JL, McCue LA, Tabita FR: Identification, expression, and deduced primary structure of transketolase and other enzymes encoded within the form II carbon dioxide fixation operon of *Rhodobacter sphaeroides*. J Biol Chem 266: 20447–20452 (1991).
9. Coruzzi G, Broglie R, Edwards C, Chua NH: Tissue-specific and light-regulated expression of a pea nuclear gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. EMBO J 3: 1671–1679 (1984).
10. DeRocher EJ, Quigley F, Mache R, Bohnert HJ: The six genes of the Rubisco small subunit multigene family from *Mesembryanthemum crystallinum*, a facultative CAM plant. Mol Gen Genet 239: 450–462 (1993).
11. Devereux J, Haeberli P, Smithies O: A comprehensive set of sequence analysis programs for the VAX. Nucl Acids Res 12: 387–395 (1984).
12. Eckerskorn C, Lottspeich F: Internal amino acid sequence analysis of proteins separated by gel electrophoresis after tryptic digestion in polyacrylamide matrix. Chromatographia 28: 92–94 (1989).
13. Edman P, Begg G: A protein sequenator. Eur J Biochem 1: 80–91 (1967).
14. Feinberg AP, Vogelstein B: A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137: 266–267 (1984).
15. Felsenstein J: PHYLIP: Phylogeny Inference Package (version 3.2). Cladistics 5: 164–166 (1989).
16. Geiger DR, Servaites JC: Diurnal regulation of photosynthetic carbon metabolism in C3 plants. Annu Rev Plant Physiol Plant Mol Biol 45: 235–256 (1994).
17. Gibson JL, Falcone DL, Tabita FR: Nucleotide sequence, transcriptional analysis and expression of genes endoded within the form I CO2 fixation operon of *Rhodobacter sphaeroides*. J Biol Chem 266: 14646–14653 (1991).
18. Gibson JL, Tabita FR: Nucleotide sequence and functional analysis of CbbR, a positive regulator of the Calvin

cycle operons of *Rhodobacter sphaeroides*. J Bact 175: 5778–5784.
19. Gontero B, Cardenas ML, Ricard J: A functional five-enzyme complex of chloroplasts involved in the Calvin cycle. Eur J Biochem 173: 437–443 (1988).
20. Graeve K, von Schaewen A, Scheibe R: Purification, characterization, and cDNA sequence of glucose-6-phosphate dehydrogenase from potato (*Solanum tuberosum* L.). Plant J 5: 353–361 (1994).
21. Heber U, Hallier UW, Hudson MA: Untersuchungen zur intrazellulären Verteilung von Enzymen und Substraten in der Blattzelle. II. Lokalisation von Enzymen des reduktiven und dem oxidativen Pentosephosphat-Zyklus in den Chloroplasten und Permeabilität der Chloroplasten-Membran gegenüber Metaboliten. Z Naturforsch 22b: 1200–1215 (1967).
22. Henze K, Badr A, Wettern M, Cerff R, Martin W: A nuclear gene of eubacterial origin in *Euglena gracilis* reflects cryptic endosymbioses during protist evolution. Proc Natl Acad Sci USA 92: 9122–9126 (1995).
23. Henze K, Schnarrenberger C, Kellermann J, Martin W: Chloroplast and cytosolic triosephosphate isomerase from spinach: purification, microsequencing and cDNA sequence of the chloroplast enzyme. Plant Mol Biol 26: 1961–1973 (1994).
24. Hove-Jensen B, Maigaard M: *Escherichia coli rpiA* gene encoding ribose phosphate isomerase A. J Bact 175: 5628–5635 (1993).
25. Jansson S, Meyer-Gauen G, Cerff R, Martin W: Nucleotide distribution in gymnosperm nuclear sequences suggest a model for GC-content change in land plant nuclear genomes. J Mol Evol 39: 34–46 (1994).
26. Jensen RA: The shikimate/arogenate pathway: Link between carbohydrate metabolism and secondary metabolism. Physiol Plant 66: 164–168 (1985).
27. Kumada Y, Benson DR, Hillemann D, Hosted TJ, Rochefort DA, Thompson CJ, Wohlleben W, Tateno Y: Evolution of the glutamin synthase gene, one of the oldest existing and functioning genes. Proc Natl Acad Sci USA 90: 3009–3013 (1993).
28. Latzko E, Gibbs M: Distribution and activity of enzymes of the reductive pentose phosphate cycle in spinach leaves and in chloroplasts isolated by different methods. Z Pflanzenphysiol 59: 184–194 (1968).
29. Longstaff M, Raines CA, McMorrow E, Bradbeer JW, Dyer TA: Wheat phosphoglycerate kinase: evidence for recombination between the chloroplastic and cytosolic enzymes. Nucl Acids Res 17: 6569–6580 (1989).
30. Martin W, Cerff R: Prokaryotic features of a nucleus encoded enzyme: cDNA sequences for chloroplast and cytosolic glyceraldehyde-3-phosphate dehydrogenases from mustard (*Sinapis alba*). Eur J Biochem 159: 323–331 (1986).
31. Milanez S, Mural RJ: Cloning and sequencing of cDNA encoding the mature form of phosphoribulokinase from spinach. Gene 66: 55–63 (1988).
32. Murray NE: Phage lambda and molecular cloning. In: Hendrix RW (ed) Lambda II, pp. 395–432. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1983).
33. Nowitzki U, Wyrich R, Westhoff P, Henze K, Schnarrenberger C, Martin W: Cloning of the amphibolic Calvin cycle/OPPP enzyme D-ribulose-5-phosphate 3-epimerase (E.C. 5.1.3.1) from spinach chloroplasts: functional and evolutionary aspects. Plant Mol Biol 29: 1279–1291 (1995).
34. Pelzer-Reith B, Penger A, Schnarrenberger C: Plant aldolase: cDNA and deduced amino-acid sequence of the chloroplast and cytosol enzyme from spinach. Plant Mol Biol 21: 331–340 (1993).
35. Raines CA, Lloyd JC, Willingham NM, Potts S, Dyer TA: cDNA and gene sequences of wheat chloroplast sedulose-1,7-bisphosphatase reveal homology with fructose-1,6-bisphosphatases. Eur J Biochem 205: 1053–1059 (1992).
36. Rutner AC: Spinach 5-phosphoribose isomerase. Purification and properties of the enzyme. Biochemistry 9: 178–184 (1970).
37. Saines JK, Harris GC: The association of ribulose-1,5-bisphosphate carboxylase with phosphoriboisomerase and phosphoribulokinase. Biochem Biophys Res Comm 139: 947–954 (1986).
38. Saitou N, Nei M: The neighbor-joining method: a new method for the reconstruction of phylogenetic trees. Mol Biol Evol 4: 406–425 (1987).
39. Sambrook J, Fritsch E, Maniatis T: Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989).
40. Schmidt M, Svendsen Ib, Feierabend J: Analysis of the primary structure of the chloroplast isozyme of triosephosphate isomerase from rye leaves by protein and cDNA sequencing indicates a eukaryotic origin of its gene. Biochim Biophys Acta 1261: 257–264 (1995).
41. Schnarrenberger C, Flechner A, Martin W: Enzymatic evidence indicating a complete oxidative pentose phosphate in the chloroplasts and an incomplete pathway in the cytosol of spinach leaves. Plant Physiol 108: 609–614 (1995).
42. Sharkey TD, Socias X, Loreto F: CO_2 effects on photosynthetic and product synthesis and feedback. In: Alscher GC, Wellburn AR (eds) Plant Responses to the Gaseous Environment, pp. 55–78. Chapman and Hall, London (1994).
43. Skrukrud CL, Anderson LE: Chloroplast phosphoribulokinase associates with yeast phosphoriboisomerase in the presence of substrate. FEBS Lett 280: 259–261 (1991).
44. Süss K-H, Arkona C, Manteuffel R, Adler K: Calvin cycle multienzyme complexes are bound to chloroplast thylakoid membranes of higher plants *in situ*. Proc Natl Acad Sci USA 90: 5514–5518 (1993).
45. Tabita FR, Gibson JL, Falcone DL, Wang X, Li L-A,

Read BA, Terlesky KC, Paoli GC: Current studies on the molecular biology and biochemistry of CO_2 fixation in phototrophic bacteria. In: Murrel JC, Kelley DP (eds) Microbial Growth on C1 Compounds, pp. 469–479. Intercept Scientific, Andover, UK (1993).

46. Tumer NE, Clark WG, Tabor GJ, Hironaka CM, Fraley RT, Shah DM: The genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase are expressed differentially in petunia leaves. Nucl Acids Res 14: 3325–42 (1986).
47. Wolter F, Fritz C, Willmitzer L, Schell J, Schreier P: *rbcS* genes in *Solanum tuberosum*: conservation of transit peptide and exon shuffling during evolution. Proc Natl Acad Sci USA 85: 846–850 (1988).
48. Wood T: The Pentose Phosphate Pathway. Academic Press, New York (1985).
49. Woodrow IE, Berry JA: Enzymatic regulation of photosynthetic CO_2 fixation in C3 plants. Annu Rev Plant Physiol Plant Mol Biol 39: 533–594 (1988).