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Isolation of a cDNA Encoding a Chloroplast Triosephosphate Isomerase from Strawberry

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Abstract

A cDNA clone encoding chloroplast triosephosphate isomerase (TPI-cp) was isolated from strawberry fruit cDNA library. Sequence analyses indicated that the cDNA contains an open reading frame of 314 amino acids (33.5 kDa) composed of a transit peptide (59 amino acids) in amino terminal region and mature protein (255 amino acids). The existence of transit peptide in the deduced amino acid sequence implies that it encodes a chloroplast isoform. The protein sequence is more similar to other plant chloroplast isoforms than cytosolic isoforms. RNA blot analysis indicated that its expression is ubiquitous in examined five tissues, flowers, leaves, petioles, roots and fruits, and shows differential pattern according to fruit ripening. Genomic DNA blot analysis showed that *TPI-cp* is encoded by multiple genes in strawberry. Through sequence comparison and phylogenetic tree construction, *TPI-cp* is distinctively grouped into dicot and chloroplast isoforms.

Introduction

Triosephosphate isomerase (TPI, D-glyceraldehyde-3-phosphate ketol isomerase; EC5.3.1.1) is known to play an essential role in various metabolic pathways, including the glycolytic pathway, gluconeogenesis, fatty acid biosynthesis, the pentosephosphate cycle, and the Calvin cycle. It catalyses the rapid interconversion of D-glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. A homodimeric enzyme composed of 27 kDa subunits, TPI has been found ubiquitously in prokaryotes and eukaryotes. Each subunit has an eight-stranded α/β-barrel structure and is highly conserved in structure and sequence across both prokaryotes and eukaryotes. Three-dimensional structures have been reported in human (Mande et al., 1994), chicken (Banner et al., 1975), yeast (Lolis et al., 1990), trypanosome (Wierenga et al., 1987), *Escherichia* (Kishan et al., 1994), *Leishmania* (Williams et al., 1999), *Plasmodium* (Velanker et al., 1997), *Pyrococcus* (Bell et al., 1998), and *Bacillus* (Delboni et al., 1995).

Higher plants and *Euglena* have two distinct TPI forms, cytosolic and chloroplastic isoforms, which have slightly different characteristics such as primary structure, molecular weight, and isoelectric point, but both are encoded by nuclear DNA (Henze et al., 1994; Kurzok and Feierabend, 1984; Kurzok and Feierabend, 1984; Schmidt et al., 1995).

Biochemical studies for TPI of higher plants at the protein level have been done from several sources, including rye (Kurzok and Feierabend, 1984; Kurzok and Feierabend, 1984), spinach, celery and lettuce (Pichersky and Gottlieb, 1984). cDNAs for cytosolic isoforms have been isolated from maize (Marchionni and Gilbert, 1986), rice (Xu and Hall, 1993), *Arabidopsis* (Shih, 1994), rye (Schmidt et al., 1995) and spinach (Henze et al., 1994), and those for chloroplast isoforms from rye (Schmidt et al., 1995) and spinach (Henze et al., 1994). The genomic structure of the cytosolic *TPI* isoform has been characterized from maize (Marchionni and Gilbert, 1986) and rice (Zhang and Chinnappa, 1994), and that of chloroplast isoform from *Arabidopsis* (GenBank ac-

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cession number AC006264). Analysis of the genomic structure reveals a remarkable conservation of intron position between plant and animal ones. Sequence comparison for TPI from different species indicated that chloroplast TPI, except for transit sequence, is more similar to cytosolic isoform(s) of eukaryotes than to eubacterial enzymes, and it suggested that both isoforms are derived from a common ancestor (Feierabend et al., 1990; Henze et al., 1994; Schmidt et al., 1995).

Although many cDNAs encoding both TPI isoforms were isolated, the expression was examined only in the cytosolic isoform of rice, which is expressed in all vegetative tissues as a single transcript (Xu and Hall, 1993). It is presumed that the expression pattern of the chloroplast isoform would be different from that of the cytosolic isoform because of its different compartmentalization. Although cDNAs encoding chloroplast isoforms from rye and spinach were isolated as single genes (Henze et al., 1994; Schmidt et al., 1995), the expression patterns have not been investigated.

In this study, we present the isolation and characterization of a cDNA clone encoding *TPI-cp* from strawberry (*Fragaria* x *ananassa*). We compared the expression patterns of *TPI-cp* with respect to tissues and fruit development. Our data including sequence comparison and phylogenetic inference strongly support the evolution of both cytosolic and chloroplast *TPI* from a common ancestral gene.

Materials and Methods

Plant materials

Strawberry (*Fragaria* x *ananassa* Duch. cv. Yoho) was used throughout this work. Plants were cultivated under greenhouse conditions. Fully expanded leaves were harvested from mature plants, and petioles were also collected from the fully expanded leaves. Fully elongated roots and flowers were sampled from mature plants. Fruits were harvested at four stages of development determined by maturity and external fruit color. Developmental stages were as follows: small green (SG), 15 days after anthesis; mature green (MG), 25 days after anthesis; turning (1/2 red) (TR), 30 days after anthesis; and full red (FR), 35 days after anthesis. These materials was immediately frozen with liquid nitrogen, stored at 70℃, and then were used for isolation of total RNA.

Construction of a cDNA library and screening of TPI-cp cDNA.

Total RNA was extracted from ripe (turning stage) strawberry fruits using the hot phenol RNA isolation procedure as described by Verwoerd et al. (1989). Poly(A)$^+$ RNA was isolated by PolyATtract mRNA Isolation System III (Promega). A strawberry fruit cDNA library was constructed by using the Zap-cDNA synthesis and Gigapack® II gold cloning kits (Stratagene) according to the manufacturer's instruction manual.

On the basis of database comparison, we designed two degenerate primers as follows: TPI-5, 5'-CA(G/A)GT(I/C)AA(G/A)AG(I/C)TC(A/C)CT(A/T)AC(A/C/T); TPI-3, 5'-(A/G)CAIAC(A/G)TC(A/G)AA(I/C)GTTTT(T/C)CC, corresponding to QVKSSLT and GKTFDVC (Figure 1, 2), respectively, from the conserved region of three chloroplast TPI proteins of *Arabidopsis* (Genbank accession number AC006264), spinach (Henze et al., 1994) and rye (Schmidt et al., 1995). The library was screened with random-primed, [α-^{32}P]dCTP-labeled, 282-bp PCR product, putatively encoding strawberry TPI-cp, as a probe, by using standard plaque lift methods (Sambrook et al., 1989). After prehybridization for 1-2 hr at 42℃ in 30% formamide, 5x Denhardt's solution, 5x SSPE, and 100 μ g/mL denatured salmon sperm DNA, the filters were incubated with ^{32}P-labeled PCR products for 24 hr under the same conditions. The filters were washed twice in 2x SSC and 0.05 % SDS for 15 min at 42 ℃ and twice in 0.2x SSC and 0.1% SDS for 15 min at 68 ℃.

Nucleotide sequencing

Nucleotide sequencing using the dideoxy chain termination method (Sanger et al., 1977) was performed by using Sequenase 2.0 kit (United State Biochemical) for a double strand to avoid errors; that is, the sense strand with the Erase-a-Base® system (Promega) for serial deletion and the antisense strand with custom-made (DNA International) oligonucleotide primers for sequencing of internal sequences. Computer analyses for the nucleotide and amino acid sequences were done by PCGENE software (IntelliGenetics Inc., Release 6.60).

Northern blot analysis

Total RNA was isolated from four tissues (leaves, petioles, roots and flowers) and fruits in four developmental stages (SG, MG, TR and FR). The harvest stages of each material were described above. The RNA was fractionated on denaturing agarose (1.0%) gel. After the transfer to nylon membrane (Hybond-N from Amersham), filters were prehybridized at 42℃ for 1-2 hr in 50% formamide, 5x

SSPE, 5x Denhardt's solution, 0.1% SDS, and 100 μg/mL denatured salmon sperm DNA. The probe was *Eco*RI-*Eco*RV fragment (363-bp) of *TPI-cp* cDNA, labeled with [α-^{32}P]dCTP. The hybridization with [α-^{32}P]dCTP-labeled probe was done overnight in prehybridization buffer. The filters were washed twice at the room temperature for 10 min in 2x SSC and 0.1% SDS, once at 65℃ for 15 min in 1x SSC and 0.1% SDS, and twice at 65℃ for 15 min in 0.1x SSC and 0.1% SDS. After the filters (Hybond-N from Amersham) were stripped off probe according to the manufacturer's instruction, the same blot was hybridized with partial cDNA of 18s rRNA labeled with [α-^{32}P]dCTP.

Genomic DNA blot analysis

Genomic DNA was isolated from young leaves of strawberry plants by the method of Dellaporta (Dellaporta et al., 1983). Genomic DNA was digested with *Eco*RI, *Hin*dIII, *Xba*I, or *Eco*RV, separated on 0.7% agarose gels, and then blotted onto a Hybond-N (Amersham). Hybridization and washing of filters were done as described in northern blot analysis.

Results and Discussion

Isolation of a TPI-cp cDNA clone from strawberry

A cDNA clone encoding the full coding region of TPI-cp was isolated from strawberry fruit cDNA library. The nucleotide and deduced amino acid sequences of a *TPI-cp* cDNA (GenBank accession number: AF257322) are presented in Figure 1. The 1047-bp cDNA contains an ORF of 945-bp, a 9-bp 5' UTR and a 93-bp 3' UTR. The predicted amino acid length and protein size are 314 residues and about 33.5 kDa, respectively. In the 3' UTR, two putative polyadenylation signals, AATAAA or AACAAA, are found (Figure 1, boxed). The amino terminal region composed of 59 amino acids is serine-rich and presumed as a transit peptide by its comparison with the amino terminal sequence of mature chloroplast TPI from other plants such as spinach (Henze et al., 1994), rye (Schmidt et al., 1995), and lettuce (Pichersky and Gottlieb, 1984). Consequently, mature TPI-cp may consist of 255 amino acids and has a molecular weight of about 27 kDa.

Mature TPI-cp contains three cystein residues, Cys-73 conserved in plant TPI, Cys-185 in all reported TPI and Cys-202 only in chloroplast TPI (Figure 1, boxed; Figure 2, shaded). Two cystein residues forming a disulfide bridge in rye (Schmidt et al., 1995) are not found in TPI-cp. Cys-202, located in the middle of α-helix 5, is known as a modified residue in rye. In plant TPI, differences in amino acid number between cytosol and chloroplast isoforms are found in the amino terminal and carboxy terminal regions. In addition, two gaps preceding Asp-95 and Asp-97 in the chloroplast isoforms are observed in a region between α-helix 1 and β-strand 2. Several conserved residues related to functional significance are found as follows: the catalytic residues (Asn-70, Lys-72, His-153, Glu-224), the residues involved in interaction with phosphate moiety (Gly-232, Tyr-267, Ser-270, Gly-291, Gly-292), the residues in accession of the substrate during catalysis (at positions 225-230), the residues involved in conformational changes (Glu-188, Tyr-223, Trp-227, Ser-270), the residues in the dimerization of the subunits in the dimeric enzyme (at positions 131-137, Cys-73, Glu-156) (Banner et al., 1975; Lolis et al., 1990; Mande et al., 1994; Wierenga et al., 1987; Wierenga et al., 1992).

Comparison between TPI-cp and other TPI sequences, and phylogenetic relationships

The amino acid sequence of *TPI-cp* showed 84%,

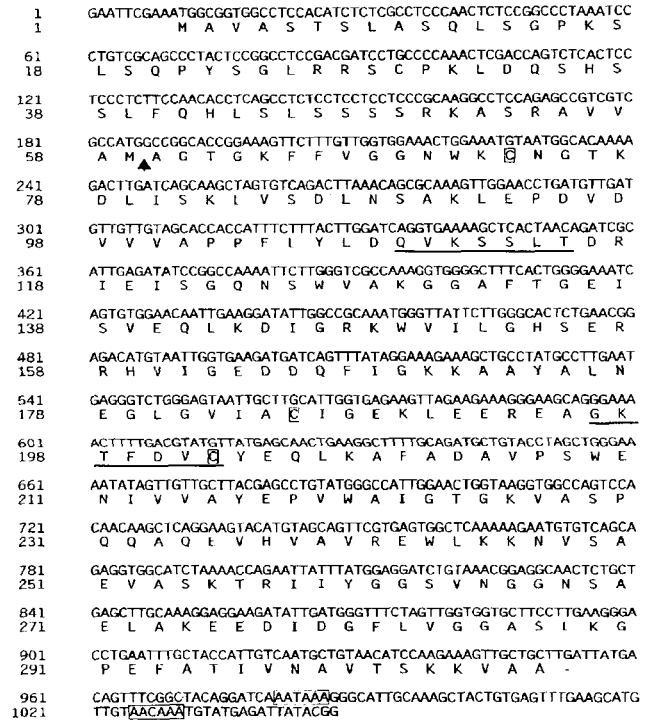

Figure 1. Nucleotide sequence and deduced amino acid sequence for the *TPI-cp* clone isolated from strawberry fruit. The cystein residues (Cys-73, Cys-185, Cys-202) and putative polyadenylation signals are boxed. The sequences corresponding to two primers designed for PCR are underlined. Arrow represents the putative cleavage site for the transit peptide.

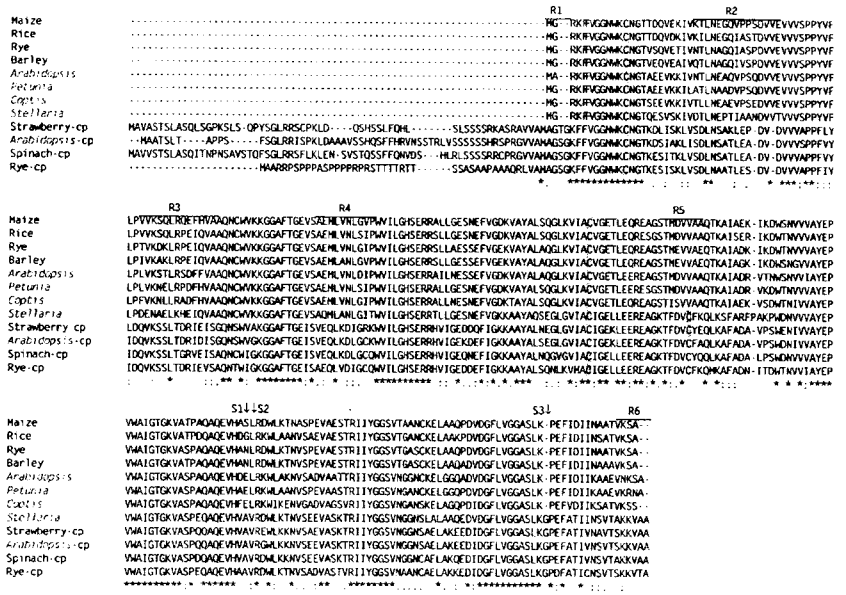

Figure 2. Alignment of *TPI* amino acid sequences. Alignment is performed with the deduced amino acid sequences of *TPI* from various plants, such as *Arabidopsis* (GenBank accession number AC006264 for chloroplast isoform, *Arabidopsis*-cp; U02949 for cytosolic isoform) (Shih, 1994), spinach (Spinach-cp, accession number L36387) (Henze et al., 1994), rye (accession number Z 32521 for chloroplast isoform, Rye-cp; Z26875 for cytosolic isoform) (Schmidt et al., 1995), *Stellaria* (accession number S70730) (Zhang and Chinnappa, 1994), maize (accession number L00371) (Marchionni and Gilbert, 1986), rice (accession number M 87064) (Xu and Hall, 1993), *Coptis* (accession number J04121) (Okada et al., 1989), barley (accession number U83414), *Petunia* (accession number X83227) (Ben-Nissan and Weiss, 1995), and strawberry (Strawberry-cp corresponding to TPI-cp, the cDNA clone of this work). The alignment was generated by Clustal W (version 1.60) (Thompson et al., 1994). An asterisk indicates the identical amino acids. Gaps were introduced to maximize identities, represented by a dash. The regions (R1-R6) and sites (S1-S3) with different sequences between chloroplast and cytosolic isoforms are represented by lines and arrows, respectively. Conservative cystein residues are shaded.

80% and 79% identity with those of the chloroplast *TPI* cDNAs from *Arabidopsis* (GenBank accession number AC006264), spinach (Henze et al., 1994) and rye (Schmidt et al., 1995), respectively. However, the N-terminal regions, containing a transit peptide, show no significant similarity. The average identity with cytosolic TPI of plants is 61%, except for *Stellaria* (70%) (Zhang and Chinnappa, 1994). Figure 2 shows the similarity of the primary structure of TPI-cp, corresponding to Strawberry-cp, to those of plant chloroplast and cytosolic *TPI* genes. The amino acid residues with important function, as described above, are conserved in both isoforms. However, particular regions (R1-R6) or sites (S1-S3) with different sequences between both isoforms are also observed, but their significance, if any, is obscure, except for R1 (the cleavage site of the transit peptide) (Pichersky and Gottlieb, 1984). These variable sequences might be less important regions or sites in enzyme activity than conserved portions.

The phylogenetic tree of the *TPI* is presented in Figure 3. For the chloroplast isoforms, only the sequence of the mature protein was used for comparison. The results showed that *TPI-cp* corresponding to Strawberry-cp is more closely related to chloroplast isoforms than to cytosolic isoforms, as shown in Figure 2. Furthermore, both chloroplast and cytosolic isoforms are branched into dicot isoforms of *Arabidopsis* (Shih, 1994), spinach (Henze et al., 1994), *Petunia* (Ben-Nissan and Weiss, 1995) and *Coptis* (Okada et al., 1989), and monocot isoforms of maize (Marchionni and Gilbert, 1986), rice (Xu and Hall, 1993), barley (GenBank accession number U 83414) and rye (Schmidt et al., 1995), except into cytosolic isoform of *Stellaria* (Zhang and Chinnappa, 1994). These results support that both isoforms of plants were derived from a common ancestor. However, in order to confirm this hypothesis, it is necessary to identify a *TPI* gene from cyanobacteria, an anticipated photosynthetic endosymbiont, or to isolate a new *TPI* gene with bridging sequence between cytosolic and chloroplast isoforms in the phlyogenetic tree. Comparing *TPI-cp* to other plant *TPI* isoforms, we presumed that that the cytosolic *TPI* gene of *Stellaria* may be a candidate for the latter.

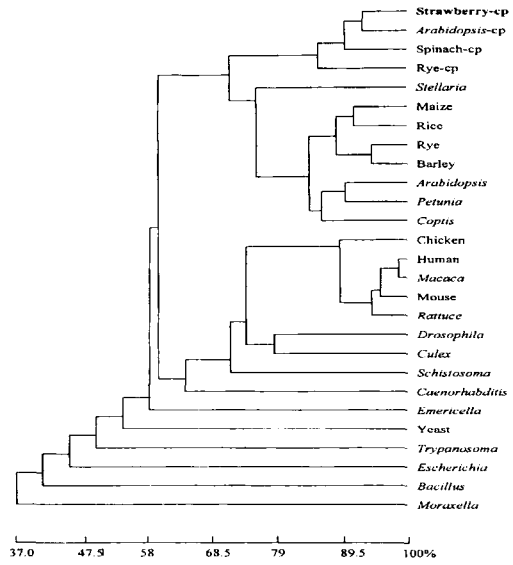

Figure 3. Evolutionary relatedness of the strawberry *TPI-cp* to other *TPI*. Phylogenetic analysis is based on the deduced amino acid sequences of *TPI* from the plants mentioned in Figure 2 and other organisms, including chicken (accession number M11314), human (accession number X69723), *Macaca* (accession number M37572), mouse (accession number AC002397), *Rattuce* (accession number L36250), *Drosophila* (accession number X57576), Culex (accession number L 07390), *Caenorhabditis* (accession number U23081), *Schitosoma* (accession number U50847), *Emericella* (accession number D10019), yeast (accession number Z49209), *Bacillus* (accession number X66129), *Escherichia* (accession number AE000466), trypanosome (accession number X03921), and *Moraxella* (accession number X66130). The tree was generated by UPGMA (unweighted pair-group method). The scale on the bottom shows similarity between two clusters (Clustal W version 1.60) (Thompson et al., 1994). The N-terminal regions corresponding to transit peptides of chloroplast isoforms were not calculated.

Expression of TPI-cp in strawberry

To determine the expression patterns of strawberry *TPI-cp* gene, we carried out northern blot analysis as shown in Figure 4. Total RNAs were isolated from several tissues and fruits in four developmental stages. To avoid cross-hybridization with cytosolic isoform, we washed the filter with high stringency. Also the 363-bp *Eco*RI-*Eco*RV fragment of *TPI-cp* cDNA was used as probe DNA, whose sequence doesn't show sequence similarity to cytosolic isoforms of other plants. A transcript is expressed in all four tissues as well as in fruits. The order of expression level is flowers, leaves, petioles, and roots. The signal intensity from flowers was about 3- to 5-fold stronger than that from roots. It was expected that the transcript would be detected in all tissues due to its essential role in several metabolic pathways, as described in rice. However, the expression pattern of strawberry *TPI-cp* is different from that of the cytosolic isoform of rice whose signal intensities from roots and culums were 2- to 3-fold stronger than from leaves.

TPI-cp in strawberry displayed a differential expression pattern depending on the stages of fruit development. The expression levels are hardly different in SG to TR stages and then sharply increased in FR stage. The increase in FR stage might be related to physiological changes during the fruit ripening that requires more energy. Fruit ripening is characterized by the differentiation of chloroplast to chromoplast and involves compositional, structural, and metabolic changes including fatty acid degradation (Biale, 1964). It is presumed that the glycolytic and fatty acid biosynthetic pathways, TPI-involving metabolic pathways, are important to produce energy and meet the degradation of fatty acids.

TPI-cp is encoded by multiple genes in strawberry

The genomic DNA isolated from strawberry leaves was digested with *Eco*RI, *Hin*dIII, *Xba*I, or *Eco*RV, and then separated on 0.7% agarose gels. As shown in Figure 5, the digested DNA fragments were hybridized with the *TPI-cp* cDNA probe and washed at high stringency (0.1SSPE and 0.1% SDS, 68℃). The genomic blot pattern showed 4-7 bands in each DNA digestion. *Fragaria* x *ananassa*, the plant used in this study, is a hybrid of two parents, *Fragaria virginiana* and *Fragaria chiloensis* (Bringhurst. 1990). It has a polyploidic genome, which makes it difficult to interpret the number of *TPI-cp* genes per chromosome set. The multiple bands might result

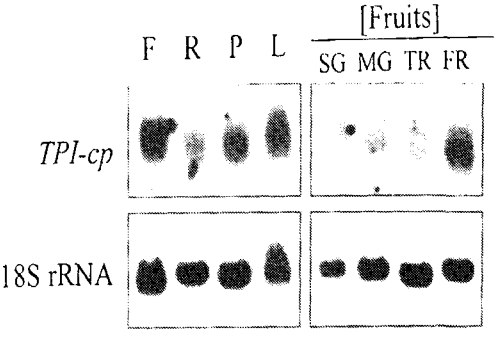

Figure 4. Northern blot analysis of ***TPI-cp*** gene expression in various tissues of strawberry plants. Total RNAs (20μg/lane) were separated onto a 1.2% formaldehyde gel, transferred to nylon membranes, and hybridized with probe. The same blot was hybridized with an 18s rDNA probe. Symbols are represented as follows: F, flowers; R, roots; P, petioles; L, leaves; SG, small green fruits; MG, mature green fruits; TR, turning (1/2 red) fruits; FR, full red fruits.

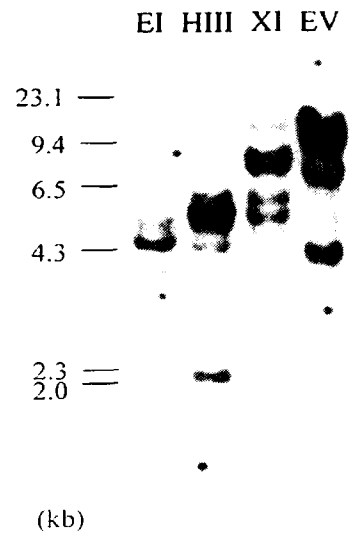

Figure 5. Genomic blot analysis of *TPI-cp*. Genomic DNA (10g) was digested with *Eco*RI (EI), *Hin*dIII (HIII), *Xba*I (XI), and *Eco*RV (EV) for each DNA sample. Size markers (kb) are indicated on the left. The blot was hybridized with the isolated *TPI-cp* cDNA probe labeled with [α-^{32}P]dCTP.

from multiple alleles, as shown in our previous report on the cytosolic ascorbate peroxidase (Kim and Chung, 1998). To confirm the number, it may be necessary to isolate several *TPI-cp* clones from the genomic library and to compare their nucleotide sequences. The existence of multiple genes was also observed for the cytosolic *TPI* of maize (Marchionni, 1986) and *Stellaria* (Zhang and Chinnappa, 1994).

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References

Banner BW, Bloomer AC, Petsko GA, Phillips DC, Pogson CI, Wilson IA, Corran PH, Furth AJ, Milman JD, Offord RE, Priddle JD, Waley SG (1975) Structure of chicken muscle triose phosphate isomerase determined crystallographically at 2.5Å resolution. Nature 255: 609-614.

Bell GS, Russel RJ, Kohlhoff M, Hensel R, Danson MJ, Hough DW, Taylor GL (1998) Preliminary crystallographic studies of triosephosphate isomerase (TIM) from the hyperthermophilic Archeon *Pyrococcus woesei*. Acta Crystallogr D Biol Crystallogr 54: 1419-1421.

Ben-Nissan G, Weiss D (1995) Developmental and hormonal regulation of triosephosphate isomerase gene in *Petunia corollas*. J Plant Physiol 147: 58-62.

Biale JB (1964) Growth, maturation, and senescence in fruits. Science 146: 880-888.

Bringhurst RS (1990) Cytogenetics and evolution in American *Fragaria*. HortScience 25: 879881.

Delboni LF, Mande SC, Rentier-Delrue F, Mainfroid V, Turley S, Vellieux FM, Martial JA, Hol WG (1995) Crystal structure of recombinant triosephosphate isomerase from *Bacillus stearothermophilus*. An analysis of potential thermostability factors in six isomerases with known three-dimensional structures points to the importance of hydrophobic interactions. Protein Sci 4: 2594-2604.

Dellaporta SL, Wood J, Hicks JBA (1983) Plant DNA minipreparation: Version II. Plant Mol Biol Rep 1: 19.

Feierabend J, Kurzok HG, Schmidt M (1990) Genetic and evolution of chloroplast isozymes of triosephosphate isomerase. Prog Clin Biol Res 344: 665-682.

Henze K, Schnarrenberger C, Kellermann J, Martin W (1994) Chloroplast and cytosolic triosephosphate isomerases from spinach: purification, microsequencing and cDNA cloning of the chloroplast enzyme. Plant Mol Biol 26: 1961-1973.

Kim IJ, Chung WI (1998) Isolation of genomic DNA containing a cytosolic ascorbate peroxidase gne (*ApxSC*) from the strawberry (*Fragaria* x *ananassa*). Biosci Biotechnol Biochem 62: 1358-1363.

Kishan R, Zeelen JP, Noble ME, Borchert TV, Mainfroid V, Goraj K, Martial JA, Wierenga RK (1994) Modular mutagenesis of a TIM-barrel enzyme: the crystal structure of a chimeric *E. coli* TIM having the eighth beta alpha-unit replaced by the equivalent unit of chicken TIM. Protein Eng 7: 945-951.

Kurzok HG, Feierabend J (1984) Comparison of a cytosolic and a chloroplast triosephosphate isomerase isoenzyme from rye leaves. I. Purification and catalytic properties. Biochim Biophys Acta 788: 214-221.

Kurzok HG, Feierabend J (1984) Comparison of a cytosolic and a chloroplast triosephosphate isomerase isoenzyme from rye leaves. II. Molecular properties and phylogenetic relationships. Biochim Biophys Acta 788: 222-233.

Lolis E, Alber T, Davenport RC, Rose D, Hartman FC, Petsko GA (1990) Structure of yeast triosephosphate isomerase at 1.9-Å Resolution. Biochemistry 29: 6609-6618.

Mande S, Mainfroid V, Kalk KH, Goraj K, Martial JA, Hol WG (1994) Crystal structure of recombinant human triosephosphate isomerase at 2.8Å resolution. Triosephosphate isomerase-related human genetic disorders and comparison with the trypanosomal enzyme. Protein Sci 3: 810-821.

Marchionni M, Gilbert W (1986) The triosephosphate isomerase gene from maize: introns antedate the plant-animal divergence. Cell 46: 133-141.

Okada N, Koizumi N, Tanaka T, Ohkubo H, Nakanishi S, Yamada Y (1989) Isolation, sequence, and bacterial expression of a cDNA for (S)-tetrahydroberberine oxidase from cultured berberine-producing *Coptis japonica* cells. Proc Nat'l Acad Sci USA 86: 534-538.

Pichersky E, Gottlieb LD (1984) Plant triose phosphate isomerase isoenzymes: Purification, immunological and structural characterization and partial amino acid sequences. Plant Physiol 74: 340-347.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, Chap. 8.

Sanger F, Nicklen S, Coulson R (1977) DNA sequencing with chain termination inhibitors. Proc Nat'l Acad Sci USA 74: 5463-5467.

Schmidt M, Svendsen I, Feierabend J (1995) 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.

Shih MC (1994) Cloning and sequencing of a cDNA clone encoding the cytosolic triose-phosphate isomerase from *Arabidopsis thaliana*. Plant Physiol 104: 1103-1104.

Thompson JD, Higgins AG, Gibson TJ (1994) Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acid Res 22: 4673-4680.

Velanker SS, Ray SS, Gokhale RS, Hemalatha SS, Balaram H, Balaram P, Murthy MR (1997) Triosephosphate isomerase from *Plasmodium falciparum*: the crystal structure provides insights into antimalarial drug design. Structure 5: 751-761.

Verwoerd TC, Dekker BM, Hoekema A (1989) A small-scale procedure for the rapid isolation of plant RNAs. Nucleic Acid Res 17: 2362.

Wierenga RK, Borchert TV, Noble MEM (1992) Crystallographic binding studies with triosephosphate isomerases: conformational changes induced by substrate and substrate-analogue. FEBS Letters 307: 34-39.

Wierenga RK, Kalk KH, Hol GJ (1987) Structure determination of the glycosomal triosephosphate isomerase from *Trypanosoma brucei* brucei at 2.4 Å resolution. J Mol Biol 198: 109-121.

Williams JC, Zeelen JP, Neubauer G, Vreind G, Backmann J, Michels PA, Lambeir AM, Wierenga RK (1999) Structural and mutagenesis studies of leishmania triosephosphate isomerase: a point mutation can convert a mesophilic enzyme into a superstable enzyme without losing catalytic power. Protein Eng 12: 243-250.

Xu T, Hall TC (1993) Cytosolic triosephosphate isomerase is a single gene in rice. Plant Physiol 101: 683-687.

Xu Y, Harris-Haller LW, McCollum JC, Hardin SH, Hall TC (1993) Nuclear gene encoding cytosolic triosephosphate isomerase from rice (*Oryza sativa* L.). Plant Physiol 102: 697.

Zhang XH, Chinnappa CC (1994) Triose phosphate isomerase of *Stellaria longipes* (Caryophyllaceae). Genome 37: 148-156.