Expression of Glucose-6-phosphate Dehydrogenase and 6-Phosphogluconate Dehydrogenase Isoform Genes in Suspension-Cultured *Arabidopsis thaliana* Cells

Yuling Yin and Hiroshi Ashihara*

Department of Biological Sciences, Graduate School of Humanities and Sciences, Ochanomizu University, Tokyo, 112-8610, Japan. Fax: +81-3-59 78-53 58.
E-mail: ashihara.hiroshi@ocha.ac.jp

* Author for correspondence and reprint requests

Z. Naturforsch. **63c**, 713–720 (2008); received April 7/May 21, 2008

The activities of glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44) were found to increase in suspension-cultured *Arabidopsis thaliana* cells after 10-day-old stationary phase cells were transferred to fresh Murashige-Skoog medium. The activities of these enzymes peaked early in the exponential growth stage of the culture (day 4) and then decreased gradually. The transcript levels of six isoform genes of G6PDH (*AtG6PD1* to *AtG6PD6*) and three of 6PGDH (*At6PGD1* to *At6PGD3*) were monitored during the culture. Two distinct transcript accumulation patterns were observed. In type A, the level of transcripts increased rapidly one day after the cells were inoculated into the fresh medium, and then remained almost constant until the culture reached its stationary phase (day 7). In type B, the transcripts were accumulated transiently at the first day after cell inoculation, then promptly decreased. We also investigated the effect of phosphate (Pi)-starvation and recovery on the expression of these genes. For this, the early stationary phase cultures (day 7) were transferred to fresh Pi-free culture medium. During 7 days of phosphate starvation, no growth of cultures was observed, and the transcript levels of all G6PDH and 6PGDH isoform genes were reduced, apart from one G6PDH isoform gene, *AtG6PD5*, which was continuously expressed throughout Pi-starvation. Compared to the reduction of almost all isoform genes of G6PDH in Pi-starved cultures, the reduction of 6PGDH genes was less severe. We discuss the localization and possible role of individual isoform genes of G6PDH and 6PGDH in connection with published databases.

Key words: Dehydrogenase, Pentose Phosphate Pathway, *Arabidopsis thaliana*

Introduction

The oxidative pentose phosphate (PP) pathway is one of the most important pathways in plant carbohydrate catabolism (Copeland and Turner, 1987; Turner and Turner, 1980). One role of this pathway is the generation of NADPH for the use in nitrate reduction and in various reduction reactions including biosynthesis of fatty acids, amino acids and many secondary metabolites (Agrawal and Canvin, 1971; Emes and Fowler, 1979; Pryke and ap Rees, 1977; Sarkissian and Fowler, 1974). The pathway also provides building blocks for biosyntheses (Ashihara and Komamine, 1975; Ashihara and Matsumura, 1977), especially ribose-5-phosphate for the biosynthesis of nucleotide-related compounds and erythrose-4-phosphate for the biosynthesis of aromatic compounds; these compounds are also provided by the non-oxidative segment of the pathway (Hirose and Ashihara, 1984). Glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44) are involved in the oxidative steps of the pathway, and are committed enzymes for the control of the activity of the PP pathway (Ashihara and Komamine, 1974a, 1976; Turner and Turner, 1980).

In earlier papers, we reported the regulation and function of the PP pathway in plant cells, using suspension-cultured *Catharanthus roseus* cells. Studies include changes in the activity of the pathway during growth (Kanamori *et al.*, 1979), the effect of short- and long-term phosphate (Pi)-starvation on this pathway (Li and Ashihara, 1990; Nagano and Ashihara, 1993), the relation between the PP pathway and nucleotide synthesis (Hirose and Ashihara, 1984), and properties of 6PGDH (Ashihara *et al.*, 1989; Ishida and Ashihara, 1993).

Genome sequences of *Arabidopsis thaliana* are fully determined (The Arabidopsis Genome Initiative, 2000), so that further investigation of this topic at the gene expression level uses suspension-cultured *A. thaliana* cells. In the present study, we used a similar culture system of *A. thaliana*, and monitored changes in the activity of G6PDH and 6PGDH in cell extracts and the expression of genes of various isoforms of these enzymes during the culture. The effect of long Pi-starvation and of re-addition of Pi upon the activity and expression of these genes was also examined. Based on the results, we will discuss the role of the isoforms of G6PDH and 6PGDH.

Materials and Methods

Plant material

Suspension-cultured cells of *Arabidopsis thaliana*, accession Columbia (strain T87), were obtained from the Experimental Plant Division of the Riken Bioresource Center, Tsukuba, Japan. For the growth experiments, the cells were subcultured at 10-day intervals in complete MS medium (Murashige and Skoog, 1962) containing 1 μM naphthaleneacetic acid and 1.5% sucrose. For the preparation of Pi-deficient cultures, 5 ml of the suspension from 7-day-old cultures were transferred to 45 ml aliquots of the Pi-free culture medium in 300-ml Erlenmeyer flasks. The flasks were then incubated on a horizontal rotary shaker (120 strokes min^{-1}, 80 mm amplitude) at 25 °C in the dark.

Determination of G6PDH and 6PGDH activity

A. thaliana cells were harvested and washed with distilled water by vacuum filtration through a layer of Miracloth (Calbiochem, La Jolla, CA, USA) on a Buchner funnel. The washed cells (ca. 1 g fresh weight) were homogenized in a glass homogenizer with 10 volumes of extraction medium consisting of 50 mM tris (hydroxymethyl) aminomethane (Tris)-HCl buffer (pH 7.5), 2 mM NaEDTA, and 2.5% (w/v) polyvinylpolypyrrolidone. The homogenates were centrifuged at 12,000 × *g* for 20 min at 2 °C. A portion of the supernatant (2.5 ml) was desalted on a pre-packed Sephadex G-25 column (PD-10, GE Healthcare Bioscience Ltd., Tokyo, Japan) which had been equilibrated with the extraction buffer.

The activities of G6PDH and 6PGDH were determined by the method of Glock and McLean (1953). The reaction mixture contained 25 mM Tris-HCl buffer (pH 7.5), 0.2 mM $NADP^+$, 15 mM $MgCl_2$, and either 2 mM G6P plus 0.5 mM 6PG or 0.5 mM 6PG, and finally the enzyme preparation. The G6PDH activity was calculated as the difference between the activity measured with 0.5 mM 6PG and that measured in the presence of 2 mM G6P plus 0.5 mM 6PG.

Semi-quantitative RT-PCR

Total RNA was extracted from cells of *A. thaliana* at various stages of growth. The samples (ca. 1 g) were ground to a powder in liquid nitrogen, and transferred to a 15-ml tube containing one volume of extraction buffer (0.1 M Tris-HCl, pH 8.0; 0.1 M EDTA; 0.1 M LiCl; 1% SDS) preheated at 60 °C. Following the addition of one volume of citrate buffer (0.1 M, pH 4.3)-saturated phenol (preheated at 60 °C) and one volume of chloroform/isoamyl alcohol (24:1; Sigma-Aldrich Co., St. Louis, MO, USA), the sample was mixed and centrifuged. The aqueous phase was precipitated with one volume of phenol/chloroform/isoamyl alcohol (25:24:1), then mixed and centrifuged. The aqueous phase was precipitated with one volume of chloroform/isoamyl alcohol (24:1), then mixed and centrifuged. The aqueous phase was precipitated with one volume of 4 M LiCl overnight at 4 °C. After centrifuging, the resulting pellet was collected and rinsed in 70% ethanol. Following air-drying, the RNA preparation was re-suspended in diethylpyrocarbonate (DEPC)-treated water. The total RNAs obtained were further treated with DNase I (Promega, Madison, WI, USA). DNA-free total RNA was used for first strand cDNA synthesis. The reaction mixture (50 μl) contained 62.5 units of MuLv reverse transcriptase (Applied Biosystems, Foster City, CA, USA) and 1 μM oligo-d(T)16. The PCR reaction mixture (25 μl) contained 40 ng cDNA and 12.5 μl GoTaq Green mastermix (Invitrogen, Carlsbad, CA, USA). 25–35 cycles were carried out with the following program: 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min. The reaction product was visualized by UV light on 2% agarose gels stained with ethidium bromide, using a luminescent image analyzer (LAS-3000, Fuji Film, Tokyo, Japan).

The primer sequences of G6PDH isoform and actin genes used were the same as those described by Wakao and Benning (2005). The primers for 6PGDH isoform genes were designed using the

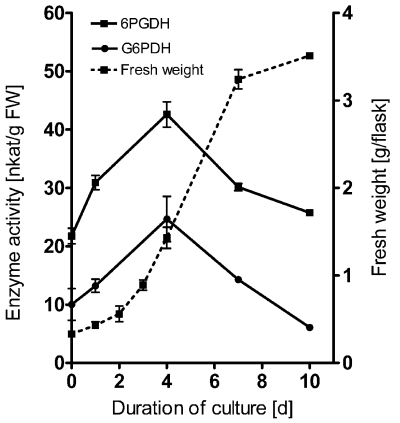

Fig. 1. Changes in fresh weight (FW) and activities of G6PDH and 6PGDH in suspension-cultured cells of *Arabidopsis thaliana* (strain T87) during culture. Enzyme activity is expressed as nkat per g FW. Mean values and SD are shown.

Primer 3 program (http://frodo.wi.mit. edu/cgi-bin/primer3/primer3 www.cgi).

The primers of 6PGD used in this work were as follows: *At6PGD1* (At1g64190): 5′-CCAAAACCTCGCCTTAAACA-3′ and 5′-GGCTCCATGTATTCGGAGAA-3′; *At6PGD2* (At3g02360): 5′-CTCTCTTGATGCGAGGTTCC-3′ and 5′-ATCCCCACCCCTTTTCTATG-3′; *At6PGD3* (At5g41670): 5′-TTATCAGGGCGGTTTTCTTG-3′ and 5′-TAGCACGCCTGTAAGTGTCG-3′.

Results

Changes in the activities of G6PDH and 6PGDH during growth

The growth of the suspension culture of *A. thaliana* and changes in the activity of G6PDH and 6PGDH during culture are shown in Fig. 1. Approx. two days after the cell transfer to fresh medium, the fresh weight of the cultures increased exponentially and plateaued after seven days. The activities of G6PDH and 6PGDH expressed as nkat per g fresh weight (FW) increased rapidly just after the inoculation of 10-day-old cells into the fresh medium. The activities increased up to day 4, then decreased to almost the same value as the initial values at day 10.

Changes in the expression of genes encoding G6PDH and 6PGDH during culture

The *A. thaliana* database records six genes (*G6PD1–G6PD6*), encoding G6PDH, and three genes (*6PGD1–6PGD3*), encoding 6PGDH (Table I). Using these gene sequences, we examined changes in the transcript levels of these genes in *A. thaliana* cells during the culture as shown in Fig. 2. The results indicate that transcript levels change during growth of the cell cultures. For G6PDH genes, the levels of transcripts of all genes except *AtG6PD4* were low at day 0 and increased markedly at day 1, then gradually decreased. Two

Table I. Isoforms of G6PDH and 6PGDH in *Arabidopsis thaliana.*

Gene	Locus	Localization	Gene expression[a]					
			Day 0	Day 1	Day 4	Day 7	Type	Pi-Defiency
G6PD1	At5g35790	Plastid[b]	L	H	H	H	A	L
G6PD2	At5g13110	Plastid[b]	L	H	L	L	B	L
G6PD3	At1g24280	Plastid[b]	L	H	H	H	A	L
G6PD4	At1g09420	Plastid[b]						
G6PD5	At3g27300	Cytosol[b]	L	H	H	H	A	Stable
G6PD6	At5g40760	Cytosol[b]	L	H	L	L	B	L
6PGD1	At1g64190	Em[c, d]	L	H	H	H	A	L
6PGD2	At3g02360	Em[c, d]	L	H	L	L	B	SL
6PGD3	At5g41670	Mitochondria[c]	L	H	H	H	A	SL

[a] L, low; H, high; SL, slightly low.
[b] Wakao and Benning (2005).
[c] The Institute for Genome Research, http://www.arabidopsis.org/servlets/TairObject?
[d] EM, endomembrane system.

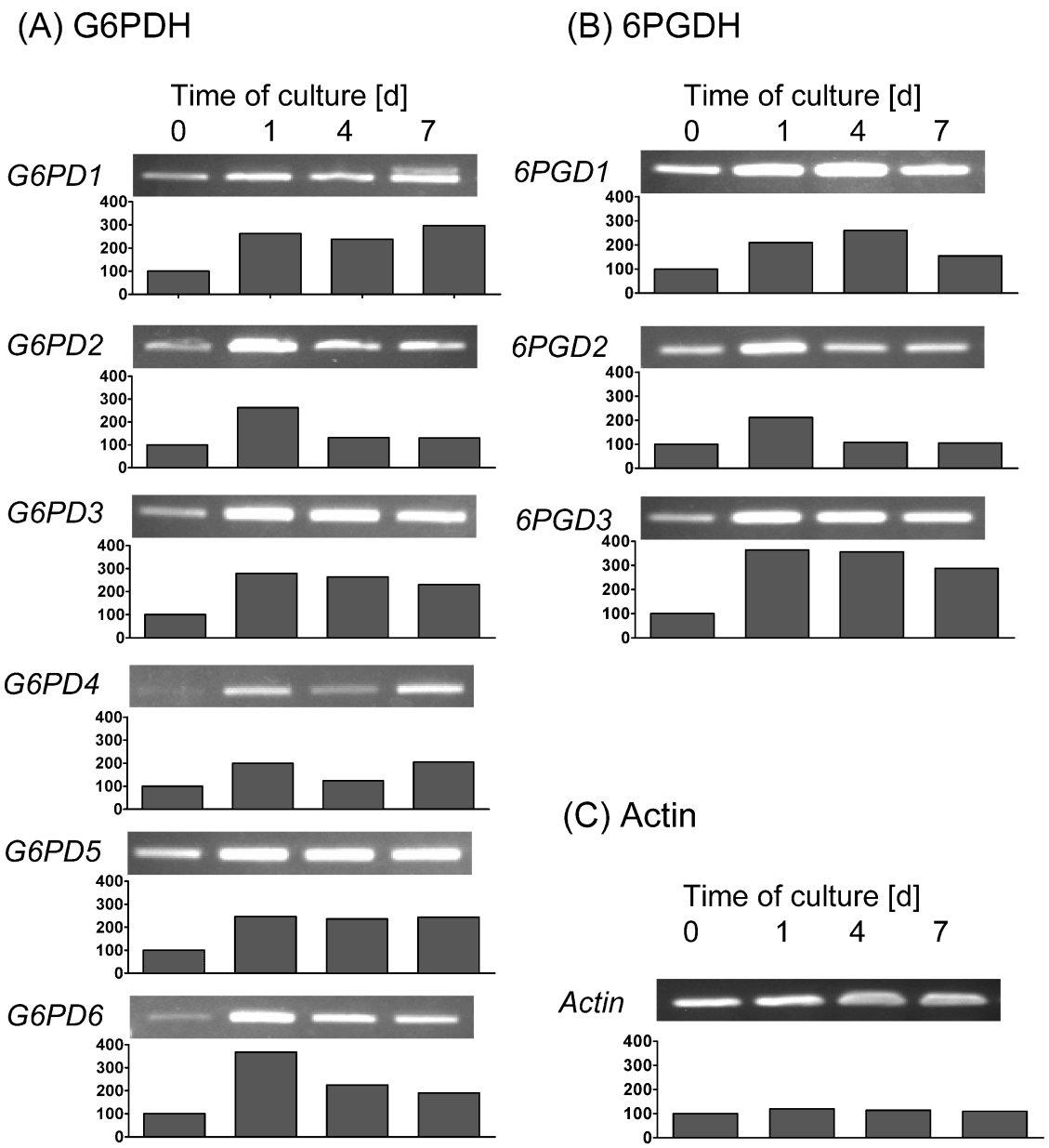

Fig. 2. Expression of genes encoding isoforms of (A) G6PDH and (B) 6PGDH in suspension-cultured *A. thaliana* cells at different growth stages. The results were obtained by 26 cycles of PCR amplification. (C) Transcripts of *actin* are also shown as standard. The bar graphs show relative intensities, expressed as % of the sample at the start (day 0).

different patterns of change were found after day 1, designated as types A and B. In type A isoform genes, the transcript levels were almost constant up to the early stationary phase (day 7) and then decreased. In contrast, in type B genes, the levels decreased immediately from day 1. Changes in the pattern of transcript levels of 4 of 5 G6PDH isoform genes were involved in these two types, but the pattern of *AtG6PD4* was different from the others. In intact plants of *A. thaliana*, *AtG6PD4*

does not encode for a functional G6PDH (Wakao and Benning, 2005).

The levels of transcripts of the three isoform genes encoding 6PGDH are also shown in Fig. 2. The transcript level of the three 6PGDH isoform genes also increased one day after transfer of cells to fresh medium. The changing pattern of the levels of *At6PGD1* and *At6PGD3* were similar to type A of G6PDH. The pattern of *At6PGD2* resembled type B isoforms.

Changes in the activities of G6PDH and 6PGDH during Pi-starvation and recovery

Fig. 3A shows the culture system for this experiment. The early stationary phase (7-day-old) cells of a stock culture of *A. thaliana* were transferred to fresh MS medium containing 1.25 mM Pi (+ Pi medium) for seven days. To prepare Pi-deficient cells, the 7-day-old cells grown in the complete MS medium were transferred to the fresh Pi-free MS medium. During Pi-deficiency, no growth of cell cultures was observed. When Pi was added to the Pi-deficient culture, the cells began to grow with no lag interval (Fig. 3A). The cells appeared to be resting during Pi-starvation, and re-started growth as soon as Pi was supplied to the culture medium. The effect of Pi on the growth of *A. thaliana* cells is similar to that observed for *C. roseus* (Nagano and Ashihara, 1993; Yin *et al.*, 2007). The activities of G6PDH and 6PGDH, respectively, decreased to 42% and 67% of their initial activities after seven days of Pi-starvation. After one day of Pi-recovery, these activities had increased to their initial level (Fig. 3B).

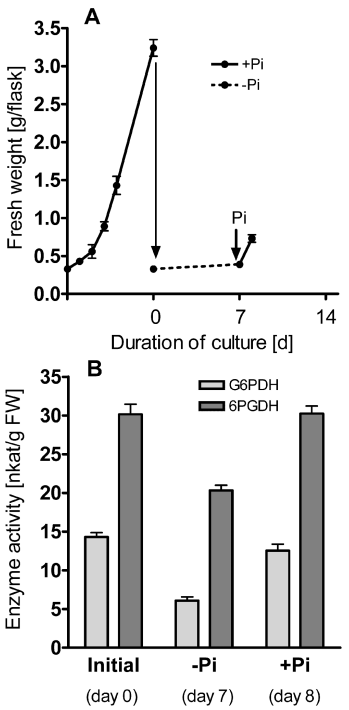

Fig. 3. Effect of Pi-starvation and recovery on (A) the growth and (B) the activities of G6PDH and 6PGDH in cultured *A. thaliana* cells. The early stationary cells (7 days old) were transferred to the complete MS medium. Seven days after culture, a portion of the cell culture was transferred to fresh MS medium, excluding 1.25 mM Pi (day 0), and was further cultured for 7 days (day 7). After that, 250 μl of 250 mM Pi (final concentration was 1.25 mM) were added, and the cells were cultured for one day (day 8). Mean values and SD are shown.

Changes in the expression of G6PD and 6PGD genes during Pi-starvation and recovery

Gene expression of all isoform genes of G6PDH, except *AtG6PD5*, was reduced seven days after the cells had been transferred to the Pi-free medium (Fig. 4). The transcript level of *AtG6PD1* was greatly reduced in Pi-deficient cells. One of the cytosolic isoforms, *AtG6PD5* was expressed at its initial level even in the Pi-deficient cells. The transcript level of *AtG6PD4* did not increase after addition of Pi. As mentioned above, this gene does not encode for a functional G6PDH (Wakao and Benning, 2005). The transcript level of three isoforms of 6PGDH decreased during Pi-starvation, although compared to most isoform genes of G6PDH the rate of reduction of three *At6PGD* was not so severe. The levels increased one day after addition to Pi (Fig. 4).

Discussion

G6PDH and 6PGDH are important enzymes in the PP pathway for the generation of NADPH, especially in non-photosynthetic tissues (Copeland and Turner, 1987; Turner and Turner, 1980). Localization of isoforms of these enzymes in cytosol and plastids has been clearly demonstrated (Tobin and Bowsher, 2005). The activities of G6PDH and 6PGDH are inhibited by NADPH and some metabolites (Ashihara and Fowler, 1979; Ashihara and Komamine, 1974b; Lendzian and Bassham, 1975), and plastidic isoforms of G6PDH are inactivated by thioredoxin reduction (Lendzian, 1980; Scheibe and Anderson, 1981).

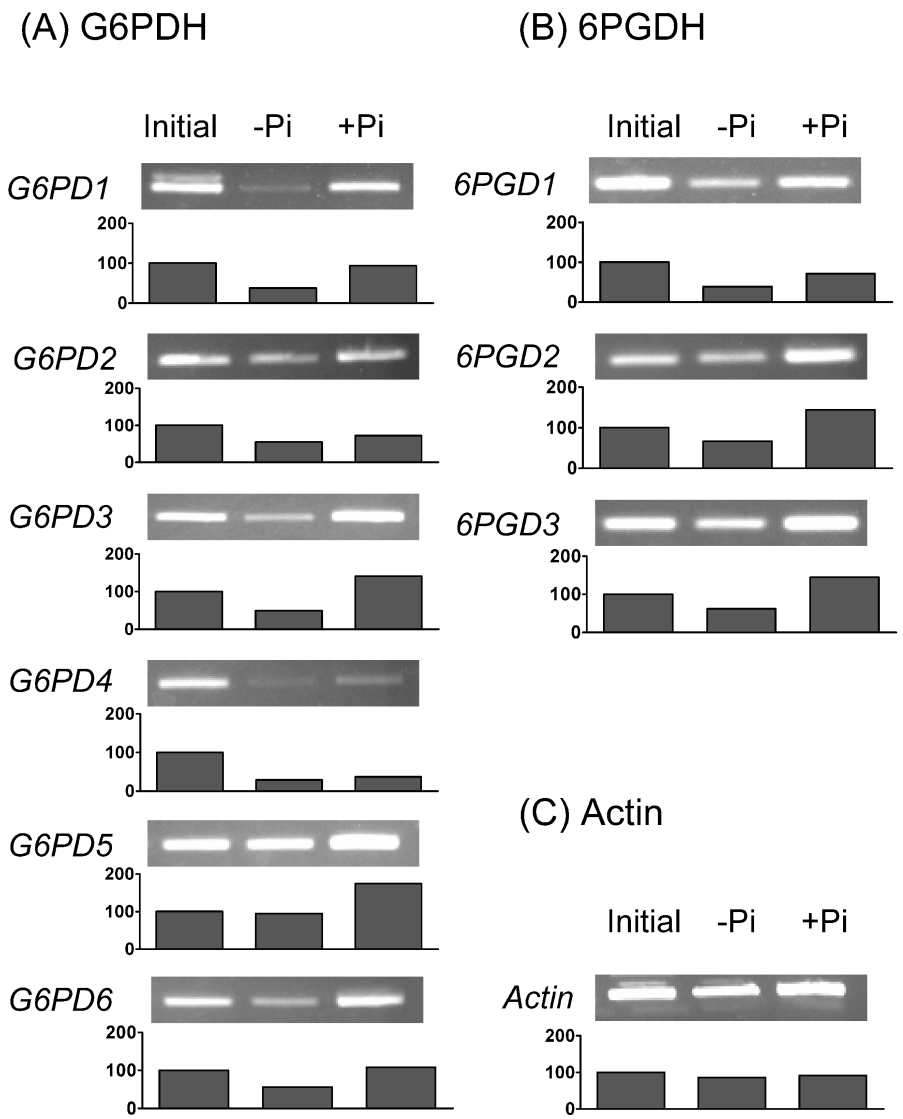

Fig. 4. Expression of genes encoding isoforms of (A) G6PDH and (B) 6PGDH in *A. thaliana* cells (Pi-deficient and during recovery). The results were obtained by 26 cycles of PCR amplification. (C) Transcripts of *actin* are also shown as standard. The bar graphs show relative intensities, expressed as % of the sample at the start (day 0).

Recently, genomic information on G6PDH and 6PGDH in *A. thaliana* has been obtained. Furthermore, localization of isoforms of G6PDH was estimated from the presence or absence of transit peptides, and the kinetic properties of each isoform were determined using recombinant enzymes (Table I, Wakao and Benning, 2005).

We found that the changing profiles of the activity of G6PDH and 6PGDH during the growth of *A. thaliana* cell cultures are similar to observations on a suspension culture of *C. roseus* (Ishida and Ashihara, 1993), although the rate of increase in the *C. roseus* cultures after inoculation was much greater. Three isoforms of G6PDH, namely AtG6PD1, AtG6PD2 and AtG6PD3, are plastidic enzymes, and two isoforms, AtG6PD5 and AtG6PD6, are cytosolic enzymes (Table I). Among plastidic isoforms, the levels of *AtG6PD2* and *AtG6PD3* transcripts were greater than for *AtG6PD1* transcripts. In tissue-specific expression

studies it was found that *AtG6PD2* and *AtG6PD3* are expressed mainly in roots and grouped as P2 isoforms (Tobin and Bowsher, 2005), but *AtG6PD1*, which is involved in the P1 isoform, was expressed only in above-ground parts (leaves, buds and flowers) of *A. thaliana* (Wakao and Benning, 2005). Our suspension culture of *A. thaliana* was grown heterotrophically, so that the expression of *AtG6PD1* was probably lower than those of *AtG6PD2* and *AtG6PD3*.

The expression of plastidic *AtG6PD3* and cytosolic *AtG6PD5* is almost constant during the lag, exponential and early stationary growth phases (type A). In contrast, plastidic *AtG6PD2* and cytosolic *AtG6PD6* are exclusively expressed on the first day after cell transfer to new medium (type B). The different expression pattern for the latter group of genes suggests that transcriptional control is involved in controlling the PP pathway. It is not known what kind of inducers are involved in the expression of type B genes. A possible candidate in the fresh medium is NO_3^-. Redinbaugh and Campbell (1998) reported that accumulation of transcripts of plastidic isoforms of 6PGDH was transiently increased in maize roots with KNO_3 treatment.

In contrast to G6PDH, intracellular localization of 6PGDH from the genome database (Table I) is not consistent with biochemical data. The Institute for Genomic Research (Tair) database (http://www.arabidopsis.org/) suggests that At6PGD1 and At6PGD2 are located in the endomembrane system, and At6PGD3 is located in mitochondria. However, localization of 6PGDH isoforms in plastids and the cytosol is well established by the isolation of organelles (Ashihara and Fowler, 1979; Debnam and Emes, 1999; Emes *et al.*, 1979; Ishida and Ashihara, 1993; Krepinsky *et al.*, 2001; Redinbaugh and Campbell, 1998). Consequently, the localization of 6PGDH isoforms which encode *At6PGD1* to *At6PGD3* is still unknown. As with G6PDH genes, two different expression patterns were also found.

Using cultured *C. roseus* cells, we have examined the effect of Pi-starvation and recovery on the carbohydrate metabolism, including the PP pathway (Li and Ashihara, 1990; Nagano and Ashihara, 1993). A synchronous cell division system of *C. roseus* was originally established using the double phosphate-starvation method (Amino *et al.*, 1983), and a simpler phosphate-starvation system was used in our previous studies (Yin *et al.*, 2007). In the present study, we used this same system for the culture of *A. thaliana* and obtained almost the same response of Pi-deficiency and recovery.

In *C. roseus* cells, we found that the ratio of the production of $^{14}CO_2$ from [6-^{14}C]glucose and from [1-^{14}C]glucose (*i.e.*, C6/C1 ratio) was extremely low in the Pi-starved cells (0.35), compared with the ratio for 1.25 mM Pi-fed cells (0.67) (Nagano and Ashihara, 1993). In Pi-starved cells, the enhanced operation of the PP pathway for the catabolism of carbohydrate may contribute to the supply of NADPH for enhancement of the secondary metabolism. Accumulation of phenolic compounds has been found in Pi-starved *C. roseus* cells (Ukaji and Ashihara, 1986). Specific isoforms of AtG6PD5 for G6PDH, and possibly At6PG2 or At6PGD3 for 6PGDH, may be related to the operation of the PP pathway in Pi-starved cells.

In contrast to the secondary metabolism, such as caffeine biosynthesis (Li *et al.*, 2008), control of the PP pathway activity is not simply performed at the transcriptional level. Several isoforms of G6PDH and 6PGDH seem to act as constitutive enzymes; the activity of these enzymes may be strictly regulated according to cellular requirements of reducing power and building blocks for biosynthesis. Plastid isoforms are involved in the modification of the carbohydrate metabolism in dark-light environment. Control is mainly executed by fine tuning of the enzyme activity by the NADPH/$NADP^+$ ratio and feedback inhibition of some metabolites. For the plastid isoforms, redox modification of the cysteine residues of the enzymes is also involved in regulation.

Agrawal P. K. and Canvin D. T. (1971), The pentose phosphate pathway in relation to fat synthesis in the developing castor oil seed. Plant Physiol. **47**, 672–675.

Amino S., Fujimura T., and Komamine A. (1983), Synchrony induced by double phosphate starvation in a suspension culture of *Catharanthus roseus*. Physiol. Plant. **59**, 393–396.

Ashihara H. and Komamine A. (1974a), Enzyme and metabolite profiles of the pentose phosphate pathway in hypocotyls of *Phaseolus mungo* seedlings. Plant Sci. Lett. **2**, 331–337.

Ashihara H. and Komamine A. (1974b), Regulation of activity of some enzymes of the pentose phosphate pathway in *Phaseolus mungo*. Z. Pflanzenphysiol. **74**, 130–142.

Ashihara H. and Komamine A. (1975), The function of the pentose phosphate pathway in *Phaseolus mungo* hypocotyls. Phytochemistry **14**, 95–98.

Ashihara H. and Komamine A. (1976), Characterization and regulatory properties of glucose-6-phosphate dehydrogenase from black gram (*Phaseolus mungo*). Physiol. Plant. **36**, 52–59.

Ashihara H. and Matsumura H. (1977), Changes in the activity and the function of the pentose phosphate pathway during germination of black gram (*Phaseolus mungo*) seeds. Int. J. Biochem. **8**, 461–471.

Ashihara H. and Fowler M. W. (1979), 6-Phosphogluconate dehydrogenase species from roots of *Pisum sativum*. Int. J. Biochem. **10**, 675–681.

Ashihara H., Yamashita Y., Kubota K., and Ishida S. (1989), The effect of fructose-2,6-bisphosphate on 6-phosphogluconate dehydrogenase from cultured cells of *Catharanthus roseus*. J. Plant Physiol. **133**, 605–607.

Copeland L. and Turner J. F. (1987), The regulation of glycolysis and the pentose-phosphate pathway. In: The Biochemistry of Plants (Stumpf P. K. and Conn E. E., eds.). Academic Press, New York, pp. 107–125.

Debnam P. and Emes M. J. (1999), Subcellular distribution of enzymes of the oxidative pentose phosphate pathway in root and leaf tissues. J. Exp. Bot. **50**, 1653–1661.

Emes M. J. and Fowler M. W. (1979), Intracellular interactions between the pathways of carbohydrate oxidation and nitrate assimilation in plant roots. Planta **145**, 287–292.

Emes M. J., Ashihara H., and Fowler M. W. (1979), The influence of NO_3^- on particulate 6-phosphogluconate dehydrogenase activity in pea roots. FEBS Lett. **105**, 370–372.

Glock G. E. and McLean P. (1953), Further studies on the properties and assay of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver. Biochem. J. **55**, 400–408.

Hirose F. and Ashihara H. (1984), Interrelationship between the pentose phosphate pathway and nucleotide synthesis in cultured cells of *Catharanthus roseus*. Plant Sci. Lett. **35**, 123–126.

Ishida S. and Ashihara H. (1993), Isoenzymes of phosphogluconate dehydrogenase (decarboxylating) from suspension-cultured *Catharanthus roseus* cells. Phytochemistry **33**, 1307–1311.

Kanamori I., Ashihara H., and Komamine A. (1979), Changes in the activities of the pentose phosphate pathway and pyrimidine nucleotide biosynthesis during growth of *Vinca rosea* cells in suspension culture. Z. Pflanzenphysiol. **93**, 437–448.

Krepinsky K., Plaumann M., Martin W., and Schnarrenberger C. (2001), Purification and cloning of chloroplast 6-phosphogluconate dehydrogenase from spinach. Cyanobacterial genes for chloroplast and cytosolic isoenzymes encoded in eukaryotic chromosomes. Eur. J. Biochem. **268**, 2678–2686.

Lendzian K. J. (1980), Modulation of glucose-6-phosphate-dehydrogenase by NADPH, $NADP^+$ and dithiothreitol at variable NADPH-$NADP^+$ ratio in an illuminated reconstituted spinach (*Spinacia oleracea* L.) chloroplast system. Planta **148**, 1–6.

Lendzian K. J. and Bassham J. A. (1975), Regulation of glucose-6-phosphate dehydrogenase in spinach chloroplasts by ribulose 1,5-diphosphate and NADPH/$NADP^+$ ratios. Biochim. Biophys. Acta **396**, 260–275.

Li X.-N. and Ashihara H. (1990), Effect of inorganic phosphate on sugar catabolism by suspension-cultured *Catharanthus roseus*. Phytochemistry **29**, 497–500.

Li Y., Ogita S., Keya C. A., and Ashihara H. (2008), Expression of caffeine biosynthesis gene in tea (*Camellia sinensis*). Z. Naturforsch. **63c**, 267–270.

Murashige T. and Skoog F. (1962), A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**, 473–497.

Nagano M. and Ashihara H. (1993), Long-term phosphate starvation and respiratory metabolism in suspension-cultured *Catharanthus roseus* cells. Plant Cell Physiol. **34**, 1219–1228.

Pryke J. A. and ap Rees T. (1977), The pentose phosphate pathway as a source of NADPH for lignin synthesis. Phytochemistry **16**, 557–560.

Redinbaugh M. G. and Campbell H. (1998), Nitrate regulation of the oxidative pentose phosphate pathway in maize (*Zea mays* L.) root plastids: induction of 6-phosphogluconate dehydrogenase activity, protein and transcript levels. Plant Sci. **134**, 129–140.

Sarkissian G. S. and Fowler M. W. (1974), Interrelationship between nitrate assimilation and carbohydrate metabolism in plant roots. Planta **119**, 335–349.

Scheibe R. and Anderson L. E. (1981), Dark modulation of NADP-dependent malate-dehydrogenase and glucose-6-phosphate-dehydrogenase in the chloroplasts. Biochim. Biophys. Acta **636**, 58–64.

The Arabidopsis Genome Initiative (2000), Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature **408**, 796–815.

Tobin A. K. and Bowsher C. G. (2005), Nitrogen and carbon metabolism in plastids: evolution, integration, and coordination with reactions in the cytosol. Adv. Bot. Res. **42**, 113–165.

Turner J. F. and Turner D. H. (1980), The regulation of glycolysis and the pentose phosphate pathway. In: The Biochemistry of Plants (Stumpf P. K. and Conn E. E., eds.). Academic Press, New York, pp. 279–316.

Ukaji T. and Ashihara H. (1986), Changes in levels of cellular constituents in suspension culture of *Catharanthus roseus* associated with inorganic phosphate depletion. Z. Naturforsch. **41c**, 1045–1051.

Wakao S. and Benning C. (2005), Genome-wide analysis of glucose-6-phosphate dehydrogenases in *Arabidopsis*. Plant J. **41**, 243–256.

Yin Y., Shimano F., and Ashihara H. (2007), Involvement of rapid nucleotide synthesis in recovery from phosphate starvation of *Catharanthus roseus* cells. J. Exp. Bot. **58**, 1025–1033.