BIOSYNTHESIS OF 5-PHOSPHORIBOSYL-1-PYROPHOSPHATE IN PLANTS: A REVIEW

Hiroshi Ashihara[a]*

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5-Phosphoribosyl-1-pyrophosphate (PRPP) is an essential substrate for biosynthesis of nucleotides, tryptophan and histidine, functioning as the phosphoribosyl donor. PRPP is synthesised from ribose-5-phosphate and ATP by PRPP synthetase (ribose phosphate pyrophosphokinase, EC 2.7.6.1). In the present review, the occurrence and biosynthesis of PRPP in plant cells and tissues are summarized, and then the properties of two types of plant PRPP synthetases are described. In addition to the inorganic phosphate (Pi)-dependent PRPP synthetases (class I) which have similar properties to mammalian enzymes, plant specific Pi-independent PRPP synthetases (class II) have been discovered in plants. Finally, reports which show fluctuation of the PRPP synthetase activity accompanied by various physiological phenomena in plants and the transgenic plants which enhanced PRPP synthetase activity and relation to biotechnology are introduced.

* Corresponding Author
Fax: +81-3-5700-4225
E-Mail: ashihara.hiroshi@ocha.ac.jp
[a] Department of Biology, Ochanomizu University, Tokyo, Japan

Introduction

5-Phosphoribosyl-1-pyrophosphate (PRPP) is an essential substrate for several pathways including *de novo* and salvage biosynthesis of purine, pyrimidine and pyridine nucleotides as well as tryptophan and histidine biosynthesis.[1] The enzyme reactions which utilize PRPP as substrate are summarised in table 1. In plants, some natural products, such as caffeine and nicotine, are derived from nucleotides,[2,3] therefore, PRPP is also an important metabolite for the biosynthesis of these secondary metabolites. Nevertheless, compared with bacteria and animals, little attention has been given to PRPP in plants and research has been carried out by only a few groups. From 1970s, a group led by Ashihara at Ochanomizu University in Tokyo studied PRPP-related topics and showed the occurrence of PRPP and a partial characterization of PRPP synthetase.[4-6] They reported that PRPP synthetase activity is Pi-independent and this properties were different from the enzymes obtained from other organisms.[5-8] Cloning and characterization of plant PRPP synthetase genes started by Hove-Jensen's group at the University of Copenhagen in 1999.[9] Their excellent studies revealed that plants have two classes of PRPP synthetase, mammalian-like Pi-dependent PRPP synthetase and plant specific Pi-independent PRPP synthetase.[10] In the present review, the current status of research on PRPP synthesis in plants are described. The synthesis of PRPP in humans has been reviewed by Becker.[11]

Occurrence of PRPP in plant tissue and cells

There are several methods for the measurement of PRPP, but up to now only radiochemical methods are reliable for plant materials. The most sensitive and easy assay method of PRPP determination is based on the release of $^{14}CO_2$ from [carboxyl-^{14}C]orotic acid by the consecutive action of purified orotate phosphoribosyltransferase and orotidine-5′-monophosphate decarboxylase. The levels of PRPP in plant cells and tissues are summarized in table 2.

The PRPP levels in plant tissue were first reported by Ross and Murray[12] with germinating pea seeds using this radiochemical method. PRPP was undetectable in dry seeds, but the level in cotyledons increased rapidly during imbibition and attained a maximum (23 nmoles per a pair of cotyledons) 12 h after imbibition. Similar results were also reported with germinating seeds of black gram (*Phaseolus mungo*) using essentially the same radiochemical method of Ashihara and Kameyama.[13] The level of PRPP was extremely low in dry seeds (<0.03 nmol per a seed), but increased rapidly in cotyledons after imbibition, attaining maximum level (0.53 nmol per a pair of cotyledons) at 24 h after which it decreased. In contrast, the level in the embryonic axes increased with growth. At 96 h after imbibition, the level was 0.78 nmol per an axis. These results suggest that dry seeds contain very limited amounts of PRPP but it is synthesized during germination.

More detailed studies on PRPP and "PRPP availability" have been reported in cultured cells of *Catharanthus roseus* by Hirose and Ashihara.[4] In culture, four growth phases, (i) the lag phase (day 0−1), (ii) the cell division phase (day 1−4), (iii) the cell expansion phase (day 4−7) and (iv) the stationary phase (day 7−10), were recognized from several growth parameters.[14,15] The PRPP level varied between 0.41−2.2 nmol g^{-1} of fresh weight (FW) during culture and these values corresponded to 0.24−1.4 nmol per 10^7 cells. On both a cell number and a fresh weight basis, the PRPP content curve was seen to rise sharply during the first day, and, after a fall at day 2, increasing again to its maximum level at day 3, following which it then fell away for the remainder of the period.

In addition to the measurement of PRPP levels, cellular "availability of PRPP" was measured using intact *Catharanthus roseus* cells.

Table 1. PRPP-utilizing enzymes in plants.

Enzyme	EC number	Reaction
Purine nucleotide biosynthesis		
Phosphoribosylamine synthetase	2.4.2.14	Glutamine + PRPP → Phosphoribosylamine + PPi
Adenine phosphoribosyltransferase	2.4.2.7	Adenine + PRPP → AMP + PPi
Hypoxanthine-guanine phosphoribosyltransferase	2.4.2.8	Hypoxanthine + PRPP → IMP + PPi Guanine + PRPP → GMP + PPi
Xanthine phosphoribosyltransferase	2.2.2.22	Xanthine + PRPP → XMP + PPi
Pyrimidine nucleotide biosynthesis		
Orotate phosphoribosyltransferase	2.4.2.10	Orotate + PRPP → OMP + PPi
Uracil phosphoribosyltransferase	2.4.2.9	Uracil + PRPP → UMP + PPi
Pyridine nucleotide biosynthesis		
Quinolinate phosphoribosyltransferase	2.4.2.19	Quinolinate + PRPP → Nicotinate mononucleotide + PPi + CO_2
Nicotinate phosphoribosyltransferase	2.4.2.11	Nicotinate + PRPP → Nicotinate mononucleotide + PPi
Nicotinamide phosphoribosyltransferase*	2.4.2.12	Nicotinamide + PRPP → Nicotinamide mononucleotide + PPi
Tryptophan biosynthesis		
Anthranilate phosphoribosyltransferase	2.4.2.18	Anthranilate + PRPP →Phosphoribosylanthranilate + PPi
Histidine biosynthesis		
ATP phosphoribosyltransferase	2.4.2.17	ATP + PRPP → Phosphoribosyl-ATP + PPi

The "availability" was estimated from the rate of incorporation of exogenously supplied [8-^{14}C]adenine into nucleotides and nucleic acids in the cells.[4] This estimation methods was essentially the same used for Ehrlich ascites tumour cells by Henderson and Khoo,[16] although these investigators measured only incorporation of [8-^{14}C]adenine into nucleotides and did not include the incorporation into nucleic acids.

Table 2. PRPP levels in plant cell and tissue.

Sample	Content (nmol)	Method	Ref. No.
Pea (germinating seeds)	0−23 cotyledon^{-1}	RI	12
Black gram (germinating seeds)	0.03−0.5 cotyledon^{-1} 0.08−0.8 embryonic axis^{-1}	RI	13
Catharanthus roseus(cultured cells)	0.4−2.2 g^{-1} FW	RI	4, 8
Maize(endosperm)	Not detectable	SP	17
Arum maculatum(club)	Not detectable	SP	17
Arabidopsis thaliana	Not detectable	SP	18

RI: radioisotopic analysis; SP: spectrophotometrical analysis

The rates of "PRPP availability" were 33−125 nmol h^{-1} g^{-1} FW, values corresponding to 20−68 nmol h^{-1} 10^{7}cells^{-1}. The "PRPP availability" for nucleotide synthesis increased rapidly during the lag phase of cell culture, decreased at the early cell division phase (day 2) and then gradually increased accompanied by cell development until day 7 and then decreased. The lowest rate was observed in the cells at the stationary phase (day 10). The "PRPP availability", i.e., rate of adenine nucleotide formation from adenine was always much higher than the PRPP content during the entire culture period.[4] These results suggest that the cellular pool size of PRPP is small, but its turnover PRPP in plant cells is rapid.

In connection to a study on concentration of inorganic pyrophosphate (PPi) in plant cells, Dancer and Rees[17] attempted to measure PRPP level in clubs of the spadices of *Arum maculatum* and the developing endosperm of *Zea mays*, but they could not find detectable amounts of the enzyme. It is obvious that the level of PPi (30−60 nmol g^{-1} FW) seems to be much higher than that of PRPP, therefore, PPi content released from PRPP during extraction is negligible. However, the difficulties of measuring PRPP in this study may be due to the use of spectrometric assay with a relatively low sensitivity. The limit of detection corresponded to a value of 3 nmol g^{-1} FW.[17] Koslowsky et al.[18] also mentioned that several attempts were made to measure PRPP contents in seedlings of *Arabidopsis thaliana* and *Nicotiana tabacum* cultures. However, no reliable results were obtained. This appears to be also due to the limited sensitivity of the non-radiochemical assay. The radiochemical methods used by Ross and Murray[12] and Hirose and Ashihara[4] have also been also used with animal cells and in various clinical investigations.[19,20]

PRPP biosynthesis in plants

PRPP is synthesized from ribose-5-phosphate and ATP by PRPP synthetase (EC 2.7.6.1, ATP: D-ribose-5-phosphate diphosphotransferase) (Figure 1). In plants, ribose-5-phosphate, a substrate of PRPP synthetase, is an intermediate of the photosynthetic carbon reduction cycle (Calvin-Benson-Bassham cycle) as well as the oxidative pentose phosphate (PP) pathway.[21] Therefore, ribose-5-phosphate is generated both in chloroplasts and in cytosol of plant cells.

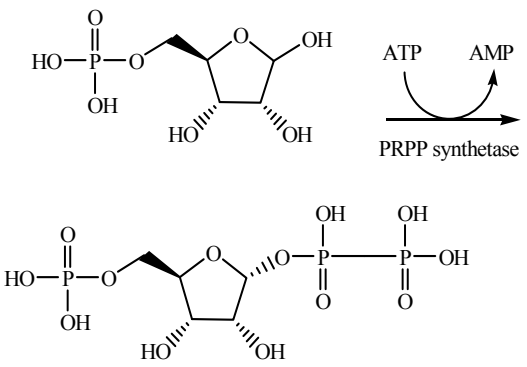

Figure 1. Reaction of the 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase.

The relationship between the PP pathway and PRPP biosynthesis has been reported in non-photosynthetic plant tissues and cells where the function of the photosynthetic carbon reduction cycle is negligible. In these tissues, carbohydrate metabolism starts from sucrose or starch and after conversion to hexose phosphate, namely glucose-6-phosphate or fructose-6-phosphate, they are metabolised in the PP pathway and ribose-5-phosphate is formed (Figure 2).

There are two distinct branches in the PP pathway; one is the oxidative branch in which NADPH is generated by two dehydrogenases and these steps are physiologically irreversible (steps 1−2 in Figure 2). The second is a non-oxidative branch catalysed by transketolase, transaldolase, ribose-5-phosphate isomerase and ribulose-5-phosphate epimerase. These reactions are reversible.[22] Ribose-5-phosphate is produced from both the oxidative (steps 1−3 in Figure 2) and/or the non-oxidative branches (steps 5−6 in Figure 2).

Levels of PRPP and intermediates of the PP pathway has been measured in hypocotyls of etiolated black gram seedlings[22] and cultured *Catharanthus roseus* cells[4,23] (Table 3). As shown in table 3, the pool size of PRPP was smaller than any metabolites of the PP pathway. PRPP content (2 nmol g-1 FW) was 15 times lower than the content of ribose-5-phosphate (30 nmol g^{-1} FW) and more than 100 times lower than that of glucose-6-phosphate (276 nmol g^{-1} FW). Similarly, the PRPP content of *Catharanthus roseus* cells (1 nmol g^{-1} FW) is more than 300 times lower than the level of glucose-6-phosphate content (337 nmol g^{-1} FW).

Profiles of activity of enzymes involved in the PP pathway and the PRPP synthetase have been investigated in the hypocotyls of etiolated black gram seedlings representing different stages of differentiation (Table 4). Compared with the enzyme activity of the PP pathway, PRPP synthetase activity (1−22 pkat g^{-1} FW) was extremely low. The activities of the enzymes of the PP pathway and PRPP synthetase were higher in the immature part (Part I), but the relative amounts of the enzymes were fairly constant in more differentiated tissues (Parts III and V). The enzyme profile data suggested that activity of the enzymes of non-oxidative branch of the pathway, namely, transketolase and transaldolase, was lower than those of oxidative branch.

However, activity of the oxidative branch enzyme, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, is regulated by the NADPH/$NADP^+$ ratio and other effectors strictly *in planta*.[24-26]

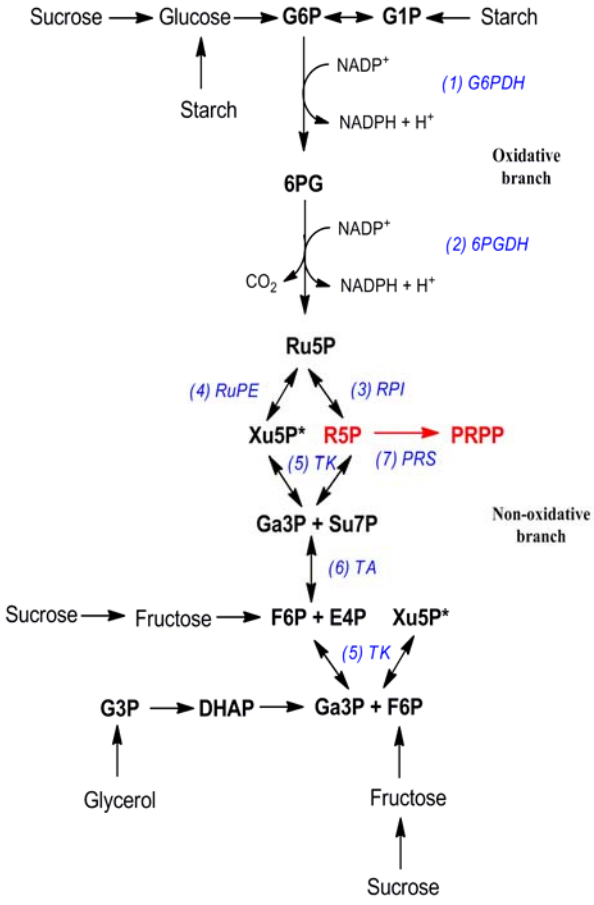

Figure 2. The oxidative pentose phosphate (PP) pathway and 5-phosphoribosyl-1-pyrophosphate (PRPP) synthesis. Enzymes and the EC numbers are as follows: (1) glucose-6-phosphate dehydrogenase (G6PDH) [EC:1.1.1.49];(2) 6-phosphogluconate dehydrogenase (6PGDH) [EC:1.1.1.44]; (3)ribose-5-phosphate isomerase (RPI) [EC:5.3.1.6];(4) ribulose-5-phosphate 3-epimerase (RuPE) [EC:5.1.3.1]; (5)transketolase (TK) [EC:2.2.1.1]; (6) transaldolase (TA) [EC:2.2.1.2]; (7) PRPP synthetase (PRS) [EC:2.7.6.1]. Abbreviations of metabolites: DHAP, dihydroxyacetone phosphate; E4P, erythorose-4-phosphate; F6P, fructose-6-phosphate; G1P, glucose-1-phosphate; G3P, glycerol-3-phosphate; G6P, glucose-6-phosphate; Ga3P, glyceraldehyde-3-phosphate; R5P, ribose-5-phosphate; Ru5P, ribulose-5-phosphate; Su7P, sedoheptulose-7-phosphate; Xu5P, xylulose-5-phosphate

Interrelationship between the PP pathway and PRPP synthesis has been investigated in cultured cells of *Catharanthus roseus*.[27] Theoretically, ribose 5-phosphate for PRPP synthesis is supplied both from the oxidative and the non-oxidative branch of the PP pathway. The relative contribution of these branches to ribose 5-phosphate synthesis for PRPP production has been estimated by monitoring exogenous adenine induced increases in AMP synthesis. Exogenously supplied 0.1 mM adenine stimulated 15−31 fold AMP synthesis which was used to produce PRPP in the cultured cells.

However, $^{14}CO_2$ released from [1-^{14}C]glucose at 6-phosphogluconate dehydrogenase step (step 2 in figure 2) and incorporation of [6-^{14}C]glucose into nucleotides were not accelerated by the adenine treatment. In contrast, incorporation of [2-^{14}C]glycerol into nucleotides via PRPP increased with the adenine treatment (see Figure 2). No significant increase in nucleotide synthesis was caused by methylene blue, a stimulator of the oxidative branch of the PP pathway (steps 1 and 2 in Figure 2).[27] From these results it has been concluded that the oxidative branch of the PP pathway is not an essential contributor in the supply of ribose 5-phosphate for PRPP synthesis in plant cells.

Table 3. Comparison of the levels of PRPP and metabolites involved in the pentose phosphate pathway in plants.

Metabolites (nmol g^{-1} FW)	**Black gram hypocotyls[a]**	***Catharanthus roseus* cells[b]**
PRPP	2	1
Glucose-6-phosphate	276	337
6-Phosphogluconate	3	–
Ribulose-5-phosphate	4	–
Xylulose-5-phosphate	3	–
Ribose-5-phosphate	30	–
Erythrose-4-phosphate	12	–
Sedohepturase-7-phosphate	30	–
Fructose-6-phosphate	61	58
Glyceraldehyde-3-phosphate	5	10
Dihydroxyacetone-phosphate	5	20
Fructose-1,6-bisphosphate	8	32
ATP	54	160
Pi	–	5680

[a]Part III of hypocotyl (the segment 15−25 mm from the hypocotyl tip); [b]Four-day-old (cell-division phase) cultured cells. The data were taken from Ashihara and Komamine[22] and from Kubota and Ashihara.[23]

Table 4. Comparison of the activity of PRPP synthetase and enzymesinvolved in the PP pathway in different parts of black gram hypocotyls.

Enzyme activity (nkat g^{-1} FW)	**Parts of hypocotyls[a]**		
	Part I	**Part III**	**Part V**
PRPP synthetase	0.022	0.004	0.001
Glucose-6-phosphate dehydrogenase	5.2	1.1	1.1
6-Phosphogluconate dehydrogenase	4.3	1.0	0.9
Ribose-5-phosphate isomerase	74.0	22.3	21.1
Ribulose-5-phosphate epimerase	1.5	0.2	0.2
Transketolase	0.4	0.1	0.1
Transaldolase	0.3	0.1	0.2
Glucose-6-phosphate isomerase	20.6	2.7	1.6

[a]Part I, apical 5 mm of a hypocotyl including a hook, Part III, the segment 15−25 mm from the tip, and Part V, the segment 45−55 mm from the tip. Based on data from Ashihara and Komamine.[22]

In plants, the main functions of the PP pathway are (i) supply of NADPH for the biosynthesis as a reducing power and (ii) supply of sugar substrates for biosynthesis. If the tissues needs high reducing power, the oxidative branch of the PP pathway is stimulated and ribose-5-phosphate is supplied. In contrast, no marked regulatory mechanism is present in the non-oxidative blanch of the PP pathway.[22]

Properties of PRPP synthetase from plants

Plant PRPP synthetase has been characterised using native enzymes since 1970s and it has been shown that the Pi-independent properties are distinct from bacterial and mammalian PRPP synthetase for which Pi is essential for their activity.[11,28] Recent recombinant enzyme studies revealed that both Pi-dependent (class I) and Pi-independent PRPP synthetase (class II) occur in plants. In this review, the properties of native enzymes are summarized first, and then discussed in the context of the recently revealed isozymes of plant PRPP synthetase from recombinant enzymes (Table 5).

Properties of native PRPP synthetase from plants

There are four reports on native PRPP synthetase from plant sources (Table 5). Ashihara and Komamine[7] first examined the kinetic and regulatory properties using crude enzyme preparation (35-50 % ammonium sulphate participating fraction) of etiolated black gram hypocotyls using an assay method based on Kornberg et al.[29] The apparent Km for ATP and R5P were, respectively, 180 μM and 14 μM. Mg^{2+} was essential for the enzyme activity. About 50 % inhibition of the activity was caused by 1 mM of ADP, GDP or AMP. In contrast to enzymes from other sources, such as human erythrocytes, inhibition of the plant enzyme by $NADP^+$ and NADPH was negligible. Pi was not necessary for the activity of black gram PRPP synthetase, and Pi inhibited the activity at concentrations >5 mM.

Ashihara[5] partially purified PRPP synthetase from spinach leaves by fractionation with ammonium sulphate, DEAE-cellulose column chromatography, and ultrafiltration. Spinach PRPP synthetase required divalent cations for activity. The highest activity was found with Mg^{2+}. While Mn^{2+} or Co^{2+} can replace Mg^{2+} to a limited extent. As with black gram PRPP synthetase,[7] Pi was not required for the activity but serves as an inhibitor. Among the various nucleoside triphosphate tested, ATP was the best pyrophosphoryl donor for the reaction, but inosine 5′-triphosphate (ITP) could also act as a donor although the rate was about one-third of that with ATP. The pH optimum of the enzyme was 7.6. The Km values for ATP, ribose-5-P and Mg^{2+} at the optimal pH were 36 μM, 10 μM, and 1.0 mM, respectively. The subcellular distribution of spinach PRPP synthetase was also investigated. A homogenate of spinach leaves was fractionated by differential centrifugation into four fractions containing chiefly nuclei (600 g pellet), chloroplasts (1200 g pellet), mitochondria (12,000 g pellet), and soluble supernatant. More than 95 % of the activity was found in the supernatant fraction and the specific activity in this fraction was more than ten times higher than in the other fractions. The results suggest that the bulk of the PRPP synthetase activity is located in the cytosol.

Using the partially purified spinach PRPP synthetase, the regulation of the activity of this enzyme, especially the effect of the "energy charge", was investigated.[6] The concept of "energy charge" has been proposed by Atkinson.[30] "Adenylate energy charge", which is calculated by the relative concentrations of ATP, ADP and AMP, i.e., ([ATP]+1/2 [ADP]) / ([ATP]+[ADP]+[AMP]) is an index used to measure the energy status of cells.

Enzymes of ATP-generating, catabolic pathways are inhibited by a high energy charge. On the other hand, anabolic enzymes utilizing ATP for biosynthetic purposes are less active at a low energy charge values and are more active at a high energy charge values.[30]

By using partially purified enzymes from *Escherichia coli*, Atkinson and Fall[31] and Klungsøyr et al.[32] showed that the activity of PRPP synthetase was dependent upon the "adenylate energy charge" in the assay mixture as predicted for an anabolic enzyme.

The activity of spinach PRPP synthetase appears to be dependent upon the "adenylate energy charge" as shown with the enzyme from *Escherichia coli*. However, the response curve of the spinach PRPP synthetase reaction to the "energy charge" is less steep than that obtained from *E. coli* enzyme. The curve is linear at high "energy charge" values. This suggests that the "energy charge" control of spinach PRPP synthetase is weaker compared to that of *E. coli* enzyme.[6]

Table 5. Properties of the native and recombinant PRPP synthases from plants.

Enzyme source	Iso-zyme	Purification	Molecular mass (kDa)	Pi-requirement (Class)	K_m (μM)		Inhibitor	Localization	Ref. No.
					R5P	ATP			
Black gram hypocotyls[a]		crude		No (Class II)	14	180	Pi, ADP, GDP	cytosol	7
Spinach leaves[a]		10-fold		No (Class II)	10	36	Pi NMP, NDP	cytosol	5,6
Rubber tree latex[a]		380-fold	200 (57 x 4)	Dependent on ribose-5-P conc.	40	200	no effect	cytosol	33
Bryopsis sp.[a]		crude		No (Class II?)			Pi	cytosol chloroplasts	36
Arabidopsis thaliana[b]	PRS1		38	Yes (Class I)					9
	PRS2		34	Yes (Class I)					
	PRS3		36	No (Class II)					
	PRS4		38	No (Class II)					
Spinach[b]	PRS1		37	Yes (Class I)			ADP[c]	chloroplasts	10
	PRS2		43	Yes (Class I)			ADP[c]	chloroplasts	10
	PRS3	homogeneity	45	No (Class II)	110	170	ADP[d], GDP[d]	mitochondria	10,34
	PRS4	homogeneity	~110 (35 x 3)	No (Class II)	48	77	ADP[d]	cytosol	10,35
Sugarcane[b]	PRS4			No (Class II)			Pi		40

[a]native enzyme; [b]recombinant enzyme; [c]allosteric inhibitor; [d]competitive inhibitor. Abbreviations: NMN, nucleoside triphosphates; NDP, nucleoside monophosphate.

The activity of spinach PRPP synthetase was inhibited by nucleoside 5′-monophosphates and nucleoside 5′-diphosphates examined. ~50 % inhibition was found at 1 mM.[6] The enzymes are also inhibited by nucleoside 5′-triphosphates, but the rate of inhibition is slight. Similar inhibition was also detected in PRPP synthetase from black gram seeds and a moss, *Atrichum undulatum*.[5-7] The activity of the enzyme is not influenced by plant growth regulators, such as indole-3-acetic acid, 2,4-dichlorophenoxyacetic acid, giberellic acid, kinetin and 3′,5′-cyclic AMP.[6]

PRPP synthetase from latex of the rubber tree (*Hevea brasiliensis*) was purified to apparent homogeneity by sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) by Gallois et al.[33] The apparent molecular weight of non-denatured protein estimated by gel filtration was ~200,000. A single band corresponding to a molecular weight ~57,000 was detected after SDS-PAGE. The enzyme seemed to be present as a homo-tetramer. The Km values for ribose-5-phosphate and ATP are 40 μM and 200 μM, respectively. Mg^{2+} is essential for the activity and optimum pH is 7.5. In contrast to spinach PRPP synthetase,[5,6] ITP did not act as a pyrophosphate group donor and nucleoside mono- and diphosphates had no notable effect on the rubber latex PRPP synthetase.[33] Effect of Pi on the activity of the latex PRPP synthetase was influenced by the concentration of ribose-5-phosphate; in the presence of a low concentration (20 μM), Pi strongly inhibits the activity, while of a high concentration of ribose-5-phosphate (1 mM), Pi acts as a positive allosteric effector of this enzyme (Hill's coefficient, 2.3).

Genes and recombinant enzymes of plant PRPP synthetase

Hove-Jensen and co-workers performed the cloning and sequencing of four *Arabidopsis thaliana* PRPP synthase-encoding cDNAs which they expressed in *Escherichia coli*.[9] The four cDNAs were designated *PRS1*, *PRS2*, *PRS3* and *PRS4* and their gene products PRPP synthetase isozymes PRS1, PRS2, PRS3 and PRS4, respectively (Table 5). In addition, the *Arabidopsis thaliana* genome sequencing project has revealed a fifth member of this new gene family designated *PRS5*. The deduced amino acid sequences of PRPP synthetase isozymes, PRS1 and PRS2 are 96 % similar, whereas the similarity of the gene product of *PRS5* with PRS1 and PRS2 is 81 % and 88 %, respectively. The

deduced amino acid sequences of isozymes PRS3 and PRS4 are 71 % similar. In contrast, the similarity of PRS1, PRS2 or the gene product of *PRS5* with PRS 3 or PRS4 is only 21 % to 26 %. Pi is required for the maximum activity of PRS1 and PRS2. In contrast, the activity of PRS3 and PRS4 is Pi-independent. PRPP synthase isozymes, PRS1 and PRS2 resemble the PRPP synthetase obtained from *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis* and mammalian organisms (class I). In contrast, isozymes, PRS3 and PRS4 appeared to be a novel "plant-specific" enzymes (class II). This is based on both their low sequence similarity and apparent phylogenetic divergence from the bacterial and mammalian PRPP synthases.

Comparison of class I and class II PRPP synthetase

Subcellular localization of four isozymes and characterization of class II PRPP synthetase was investigated in spinach by Krath and Hove-Jensen.[10,34,35] The distinction between the two types is based on their enzymatic properties i.e., dependence on Pi for activity, allosteric regulation, and specificity for pyrophosphoryl donor. Thus, the activity and stability of type I PRPP synthases is dependent on Pi, whereas class II PRPP synthetases are independent of Pi (Figure 3A). Enzymes of type I are inhibited allosterically by purine ribonucleoside diphosphates, whereas class II enzymes are not (Figure 3B). Finally, class I enzymes use ATP or, in some instances, dATP, whereas class II enzymes have much broader specificity, accepting dATP, GTP, CTP, or UTP in addition to ATP.

Subcellular localization of PRPP synthetase

PRPP synthase isozymes, PRS2 and PRS3 contained 76 and 87-amino acid extensions, respectively, at their *N*-terminal ends in comparison with other PRS. PRS2 was synthesized *in vitro* and shown to be imported and processed by pea chloroplasts. Amino acid sequence analysis indicated that PRS3 may be transported to mitochondria and that PRS4 may be located in the cytosol (Table 5).

Occurrence of PRPP synthetase activity in the different compartments have been already demonstrated by the traditional biochemical subcellular fractionation. It has been reported that PRPP synthetase occurred mainly in cytosol (>96 % of total activity) of spinach leaves[5] and in chloroplasts (45 % of total activity) and cytosol (54 % of total activity) of fronds of *Bryopsis sp.*[36] The latter material was chosen because subcellular components of *Bryopsis sp.* were easily squeezed out from the algal fronds without any requirement for homogenization, thus, it is suitable for isolating intact organelles.[37] The occurrence of PRPP synthase activity in mitochondria (27 % of total activity) and cytosol (59 % of total activity) has been reported in heterotrophically grown *Catharanthus roseus* cells.[38] Le Floc'h and Lafleuriel[39] also reported the presence of PRPP synthetase in mitochondria in Jerusalem artichoke tubers and suggested that adenine is recycled for the synthesis of purine nucleotides within mitochondria.

Characterization of plant specific class II PRPP synthetase

Detailed characterization of Pi-independent class II PRPP synthetase, namely PRS3 and PRS4, has been reported in spinach by Krath and Hove-Jensen.[34,35] A recombinant form of PRS3 resembling the presumed mature enzyme has been synthesized in an *Escherichia coli* strain in which the endogenous PRPP synthase gene was deleted, and has been purified to near homogeneity. The activity of PRS3 is independent of Pi, and the enzyme is inhibited by nucleoside 5′-diphosphates in a purely competitive manner, which indicates a lack of allosteric inhibition by these compounds. The spinach PRS 3 shows an unusual low specificity toward pyrophosphoryl donors by accepting dATP, GTP, CTP, and UTP in addition to ATP. The kinetic mechanism of the enzyme is an ordered steady state Bi Bi mechanism with Km values for ATP and ribose-5-phosphate are 170 and 110 μM, respectively. The enzyme has an absolute requirement for Mg^{2+}, and maximal activity is obtained at pH 7.6.[34]

Recombinant spinach PRS 4 was also synthesized in *Escherichia coli* and purified to near homogeneity.[35] Similar to PRS3, the activity of the enzyme is independent of Pi; it is inhibited by ADP in a competitive manner, indicating a lack of an allosteric site; and it accepts ATP, dATP, GTP, CTP, and UTP as pyrophosphoryl donors. Km values for ATP and R5P are 77 and 48 μM, respectively. Gel filtration reveals a molecular mass of the enzyme of ∼110 kD, which is consistent with a homotrimer. The properties of isozyme 4 is similar to those of isozyme 3, but some differences are present. Isozyme 3 shows substrate inhibition with GTP and hyperbolic saturation kinetics with UTP, a similar result to that observed with the PRS4. Although both isozymes were inhibited in a competitive manner by ADP, only the PRS3 was inhibited by GDP.

Purification, crystallization and preliminary crystallographic analysis of the sugar cane recombinant PRPP synthetase has been performed by Napolitano et al.[40] The recombinant enzyme contains 328 amino acids with a molecular mass of 36.6 kDa. Sculaccio et al.[41] characterized a sugarcane *prs* gene from the Sugar Cane Expressed Sequence Tag Genome Project. This gene contains a 984-bp open reading frame encoding a 328-amino acid protein. The predicted amino acid sequence has 77 % and 78 % amino acid sequence identity to *Arabidopsis thaliana* and spinach PRS4, respectively. The assignment of sugarcane PRS as a class II PRPP synthetase is verified following enzyme assay and phylogenetic reconstruction of PRS homologues. The results indicate that the maximum activity is achieved in the absence of Pi, and a linear decrease in activity is observed with higher concentrations of Pi. Contrast to from spinach PRS4,[10] Pi inhibits the catalytic reaction.

The reported properties of native PRPP synthetases in etiolated hypocotyls of black gram,[7] heterotrophically cultured *Catharanthus roseus* cells,[8] spinach leaves,[5] *Bryopsis* fronds[36] and rubber tree latex[33] are similar to PRS4 of spinach recombinant enzyme. These enzymes are mainly located in the cytosol and possess Pi-independent activity. Except for *Bryopsis* fronds and spinach leaves, materials used in these previous studies have no distinct chloroplasts. Therefore, chloroplast-type isozymes of PRPP synthetase appeared to be missing. The PRPP synthetase activity in the

Bryopsis chloroplast extracts was inhibited ~50 % by 25 mM Pi, thus this is not the class I enzyme shown in spinach chloroplasts. This discrepancy might be due to the difference in plant species, i.e., *Bryopsis* (green algae) and spinach (vascular plant).

In the case of native spinach enzyme, the existence of chloroplast PRS1 and PRS2 might have been overlooked probably because of their low activity and/or the low stability of chloroplast isozymes in extracts.[5] In fact, in contrast to spinach leaves, Yin and Ashihara[42] found that Pi (20 mM) stimulated PRPP synthetase activity in *Arabidopsis thaliana* cells cultured in the light. Consequently, both Pi-dependent and Pi-independent isoenzymes are present in the extracts from higher plant cells. The estimated molecular mass of the subunit of rubber tree latex enzyme (57 k Da) [33] is different from PRS3 and PRS4 (35 kDa) in molecular size (Table 5). Therefore, PRPP synthetase molecules in the latex seems to be different from the enzyme in plant cells and tissues.

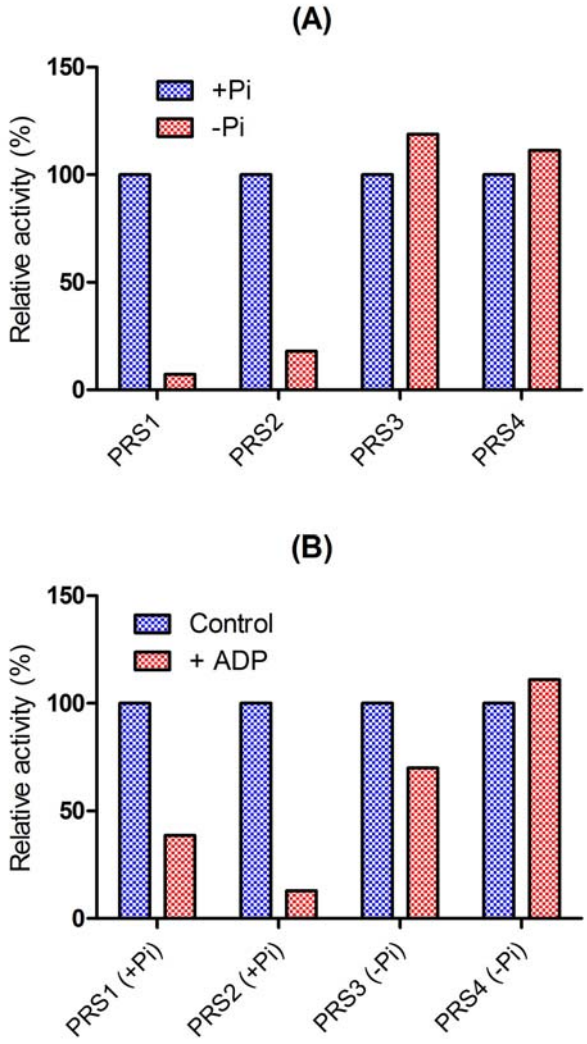

Figure 3. Comparison of relative activity of isozymes of spinach recombinant PRPP synthetase. (A) Effect of presence or absence of 50 mM Pi; (B) Effect of 1 mM ADP. PRPP synthetase activity was determined in the presence of 5 mM ribose-5-phosphate, 5 mM $MgCl_2$ and 3 mM (A) or 1 mM (B) ATP. The relative activity is expressed as % of the velocity in the presence of 50 mM Pi (A) or % of these in the absence of 1 mM ADP (B). Based on original data of Krath and Hove-Jensen.[10]

Fluctuation of level of PRPP synthetase activity in plant cells and organs

It has been reported that PRPP synthetase activity fluctuated in parallel with various physiological events of plants. Dry seeds of black gram possessed a significant level of PRPP synthetase activity and its level was maintained during early phase of seed germination and then decreased.[13] A similar pattern of fluctuation was found in the PRPP utilised enzymes, orotate phosphoribosyltransferase and uracil phosphoribosyltransferase, which, respectively, participate in the *de novo* and the salvage pathways of pyrimidine biosynthesis.[13] Ashihara and Kameyama[13] suggested that PRPP synthetase and PRPP utilizing enzymes that participate in the pyrimidine nucleotide biosynthesis are present at adequate levels in cotyledons of dry seeds and become functional after hydration of the enzyme proteins by imbibition.

The activity of PRPP synthetase has been investigated in connection with purine alkaloid biosynthesis. Biosynthesis of caffeine was found in the young leaves of tea (*Camellia sinensis*) harvested in the spring. The highest level of PRPP synthetase activity was found in young leaves in April accompanied by the highest activity of *N*-methyltransferases which participates in caffeine biosynthesis. The level of PRPP synthetase activity declined significantly to ~6 % of the maximum in June when caffeine biosynthesis had ended.[43] Since the purine skeletons of caffeine are supplied mainly by the *de novo* purine biosynthesis in young tea leaves,[44] supply of large amounts of PRPP seems to be required in young tea leaves. In addition to young leaves, caffeine biosynthesis occurs in stamens and petals of tea flower buds.[45] Relatively high activity of PRPP synthetase was also observed in these organs.[46]

The level of PRPP synthetase activity was measured in a study on pyrimidine metabolism during senescence of tobacco leaves.[47] The highest activity of PRPP synthetase was found in fully-expanded dark-green mature leaves. The level of PRPP synthetase activity was 76 % of the mature leaves in an intermediate stage of senescence (50 % of chlorophyll content was reduced), and then decreased markedly in fully senescent stage where >90 % chlorophyll was degraded. The activity of uracil phosphoribosyltransferase activity was also reduced significantly in this stage.

Marked changes in respiration and some enzyme activities including PRPP synthetase in the early stage of callus formation in a root tissue culture of carrot (*Daucus carota*) has been reported.[48] The respiratory rate increased in two phases during early stages of callus formation. In the first phase, active RNA synthesis occurred accompanied by increase in PRPP synthetase activity. The increase was inhibited by cycloheximide, indicating that *de novo* synthesis of the enzyme may occur immediately after culture. In the second phase, the activities of some respiratory enzymes, i.e., glucose-6-phosphate isomerase, ATP-dependent phosphofructokinase and succinate dehydrogenase increased. The results indicated initial synthesis of PRPP synthetase is important for initiation of callus formation from carrot-root slices.

Changes in activity of PRPP synthetase during growth of cultured plant cells have been reported in some plant species. Kanamori et al.[14] reported that PRPP synthetase activity increased during the lag phase and operated actively during cell division of *Catharanthus roseus* cells. The activity of the uracil salvage using PRPP for RNA synthesis increases during the lag phase. The activity then decreases at the later cell division phase. In contrast, the *de novo* pathway of pyrimidine nucleotide synthesis for RNA is developed at the cell division phase. The activity of PRPP synthetase and *de novo* and salvage pathways of pyrimidine nucleotide biosynthesis declined during the cell expansion and stationary phase. These results suggest that the activity of PRPP synthetase is closely linked to the nucleic acid synthesis. Essentially the same fluctuation of the activity of PRPP synthetase has been also observed during the growth of cultured cells of *Arabidopsis thaliana*[43] and white spruce (*Picea glauca*).[49]

PRPP synthetase activity was determined during an investigation of somatic embryogenesis of carrot cells.[50] The activity of PRPP synthetase in the embryogenic cells was higher than in the non-embryogenic cells. Simultaneously, activity of orotate phosphoribosyltransferase, a PRPP utilizing enzyme which participated in the *de novo* pyrimidine biosynthesis, was three times higher in the embryogenic cells. In contrast, no difference in the activity of uracil phosphoribosyltransferase which participated in uracil salvage was found in both cells. Therefore, PRPP in the embryogenic cells may be preferentially utilized for increased activity of *de novo* pyrimidine biosynthesis during embryogenesis of carrot cells.

PRPP synthetase activity was determined at various stages of somatic embryo development of white spruce cells.[51] White spruce somatic embryogenesis was divided into four distinct stages designated as stages I to IV characterized by a different developmental stage of the embryos. The embryogenic tissues were maintained with 2,4-dichlorophenoxyacetic acid (stage I). Embryogenic tissue was transferred into hormone-free medium for 7 days (stage II) and then into a liquid maturation medium containing abscisic acid for 7 days (stage III). After that, the embryogenic tissues were placed on a solid maturation medium with abscisic acid (stage IV). Mature somatic embryos characterised by developed cotyledons were obtained after 40 days of incubation in the dark. The activity of PRPP synthetase increased sharply at stage II. The activity decreased at the stage III to the first week of the stage IV, before declining at the late stage of embryo development (stage IV). Orotate phosphoribosyltransferase activity was high during the initial phases of embryo development, after which it gradually declined. Low activity of uracil phosphoribosyltransferase was detected throughout the developmental period. These results indicate that the fluctuation pattern of PRPP synthetase activity was different from those of PRPP utilizing enzymes involved in pyrimidine biosynthesis during embryo development of white spruce.

Desiccation is a natural process occurring during the late stages of seed maturation. Although desiccation is absent during the late developmental stages of somatic embryogenesis, a drying period followed by rehydration has been found to stimulate germination of somatic embryos of several species including white spruce.[52] The imposition of a partial drying treatment on mature white spruce somatic embryos is a necessary step for germination and embryo conversion into plantlets. Stasolla et al.[53] reported that the activity of PRPP synthetase almost doubled during this treatment. The activity of adenine phosphoribosyltransferase, an enzyme responsible for the salvage of adenine to adenine nucleotides also increased in dried embryos. In contrast, the activity of orotate phosphoribosyltransferase and uracil phosphoribosyltransferase did not significantly change as the embryos dried. The increased PRPP synthetase activity may contribute to the rapid turnover of adenine nucleotide synthesis during the drying process.

Germination of white spruce embryos was achieved by transferring the partially dried embryos mentioned above onto the germination medium. Germination occurred under light. Pyrimidine nucleotide metabolism was investigated during the initial stages of somatic embryo. The activity of PRPP synthetase and orotate phosphoribosyltransferase increased with the onset of germination, whereas activity of uracil phosphoribosyltransferase was low and remained almost constant during germination. [54]

Brassinolide-improved development of *Brassica napus* microspore-derived embryos is associated with increased activities of purine and pyrimidine salvage pathways. However, no marked fluctuations in the activity of PRPP synthetase was observed in embryos cultured under control conditions and in the presence of brassinolide or brassinazole.[55]

Effect of phosphate on the biosynthesis of PRPP

Pi is one of the major nutrients essential for plant growth. There are several reports which indicate that Pi is the most important nutrient which influences the growth and metabolism of cultured plant cells. Ashihara and co-workers reported that the intracellular level of ATP was increased by addition of Pi to cultures of *Catharanthus roseus*.[56,57] Ukaji and Ashihara[8] reported that the intracellular level of PRPP and the "availability of PRPP" increased markedly in the Pi-deficient cultures of *C. roseus* during the 24 h after the addition of Pi. The activity of Pi-independent PRPP synthetase increased ~20 % in this period.

In mammalian cells, there are several lines of evidence which indicated that Pi causes an increase in the net biosynthesis of nucleotides as a result of activation of Pi-dependent PRPP synthetase by Pi.[1,58,59] In contrast to mammalian cells, Pi seems to be not directly connected to the activation of PRPP synthesis in plant cells, because plant PRPP synthetase activity is not Pi-dependent. The most plausible mechanism of rapid PRPP synthesis in *Catharanthus roseus* caused by Pi involves increments in the availability of ATP, a substrate of PRPP synthetase, which is increased by increases in the rate of turnover of ATP by Pi. The high ratio of ATP/ADP also influences the activity of PRPP synthetase, because *Catharanthus* PRPP synthetase is inhibited by ADP.[8]

In photosynthetic plant cells, both Pi-dependent PRPP synthetase (class I) and Pi-independent PRPP synthetase (class II) are present. Yin and Ashihara[42] investigated the effect of Pi level on the two classes of PRPP synthetase. They first estimated the cellular phosphate level using ^{33}Pi

in suspension-cultured *Arabidopsis thaliana* cells. ^{33}Pi in the culture medium was taken up by the cells, and the concentration of Pi in cells increased up to 5.5 μmol g^{-1} fresh weight within 24 h once the cells were transferred to the fresh medium; its concentration then fell because of the conversion of Pi to organic compounds. In vitro activity of PRPP synthetase increased after inoculation and maintained a high activity until the early exponential phase of growth. The transcript levels of *PRS1* and *PRS2* encoding class I PRPP synthetase and PRS3 encoding class II enzymes increased rapidly after the cells were transferred to fresh Pi-containing culture medium and then remained almost constant during the early exponential growth phase. In contrast, constitutive expression of *PRS4* encoding cytosolic class II enzyme was observed during culture. During long-term Pi-starvation the transcript levels of *PRS1* and *PRS2* were reduced, but *PRS3* and *PRS4* were expressed continually during the Pi starvation. Pi-dependent PRPP synthetase activity was simultaneously reduced, but Pi-independent activity did not change. Expression of *PRS1* and *PRS2* and the activity of Pi-dependent enzyme grew to normal rates by 24 h after supply of Pi.

Hewitt et al.[60] reported effects of Pi limitation on expression of genes involved in pyrimidine biosynthesis in seedlings of *Arabidopsis thaliana*. The expression of genes encoding Pi-transporter and enzymes involved in the *de novo* and the salvage pyrimidine biosynthesis increased from 2 to 10-fold in response to Pi-starvation in shoots. Pi-limitation resulted in induction of *PRS2* encoding Pi-dependent PRPP synthetase 2 (PRS2). However, they suggest that the potential contribution of an increase in PRS2 enzyme activity to overall PRPP synthesis may be minimal at low intracellular concentrations of Pi and ATP. In contrast, *PRS3* encoding Pi-independent PRPP synthetase 3 (PRS3) constitutively expressed in root and shoot tissues. They concluded that PRS3 may play a novel role in providing PRPP to cellular metabolism under low P availability.

The expression profile of *PRS2* observed in the Pi-deficient *Arabidopsis thaliana* seedlings by Hewitt et al.[60] differs from that reported in cultured cells by Yin and Ashihara,[42] but the discrepancy may be due to the materials used. Constitutively expressed Pi-independent class II PRPP synthetase, PRS4, in the cultured cells and PRS3 in root and shoot tissues of *A. thaliana*, may be an adaptation to severe environments, including Pi deficiency.

Transgenic plants

Koslowsky et al.[18] reported that a higher biomass accumulation was achieved by increasing PRPP synthetase activity in transgenic *Arabidopsis thaliana* and *Nicotiana tabacum* plants. The filamentous fungus, *Ashbya gossypii* genes coding for either PRPP synthetase (PRS class I) or a mutated variant of the same gene which resembles a protein of PRS class II activity, were over-expressed under the control of a constitutive promoter. It was shown that increased PRPP synthetase activity in *A. thaliana* or *N. tabacum* leads to a substantial increase in fresh weight (1.1−1.3 fold) under standardized growth conditions. Growth enhancement was accompanied by changes in the amount of some sugars and other metabolites. The decreased sucrose content and the increased hexose-to-sucrose ratio correlated well with the increased PRS activity in both *A. thaliana* and *N. tabacum* expressing either the wild-type or mutant *PRS* gene.

The results obtained from these transgenic plants provide evidence that the supply of PRPP co-limits the growth rates. It is postulated that increased PRPP synthetase activity increases PRPP accessibility in the cytosol, which promotes nucleotide availability by enhancing nucleotide salvage processes. This study has implications for biotechnological strategies aiming to increase plant biomass as an alternative renewable energy source. Further studies are needed to confirm these results under field conditions and to investigate the impact on the total seed yield.

Concluding Remarks

PRPP is an essential phosphoribosyl donor for the biosynthesis of purine, pyrimidine and pyridine nucleotides, tryptophan, histidine and some secondary metabolites derived from these primary metabolites. Although cellular pool size of PRPP is very small, active PRPP synthesis has been demonstrated in plant cells.[4] Plants have unique Pi-independent PRPP synthetase activity which was predicted in crude enzyme extracts more than 40 years ago.[7] Recent molecular genetic studies have identified at least four PRPP synthetase isozymes which are classified as class I (Pi-dependent) and class II (Pi-independent) enzymes.[10] In this article on the current status of PRPP related topics in plant cells has been comprehensively reviewed, however it is obvious that numbers of citable references are limited and more in depth studies are needed to reveal the detailed mechanism underlying the biosynthesis and utilization of PRPP in plants. The presence of plant specific Pi-independent PRPP synthetase appears to be adapted to plant specific physiological and environmental conditions, and as a consequence this topic is potentially an attractive area of future plant biology research. In addition, transgenic crop plants which enhanced PRPP synthetase activity and have increased yields represent an interesting area for biotechnological strategies.

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