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Review

# Vitamin B biosynthesis in plants

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**Abstract**

The vitamin B complex comprises water-soluble enzyme cofactors and their derivatives that are essential contributors to diverse metabolic processes in plants as well as in animals and microorganisms. Seven vitamins form this complex: $B_1$ (thiamin (**1**)), $B_2$ (riboflavin (**2**)), $B_3$ (niacin (**3**)), $B_5$ (pantothenic acid (**4**)), $B_6$ (pyridoxine, pyridoxal (**5**), and pyridoxamine), $B_8$ (biotin (**6**)), and $B_9$ (folate (**7**)). All seven B vitamins are required in the human diet for proper nutrition because humans lack enzymes to synthesize these compounds *de novo*. This review aims to summarize the present knowledge of vitamin B biosynthesis in plants.


*Keywords:* Thiamin; Riboflavin; Niacin; Pantothenic acid; Pyridoxine; Pyridoxal; Pyridoxamine; Biotin; Folate

**Contents**



## 1. Introduction

The importance of the vitamin B complex in the metabolism of plants and other organisms, as well as in human nutrition and health, has long been recognized. Because B vitamins (Fig. 1) are essential participants in numerous metabolic processes, knowledge of their biosynthesis is central to understanding plant metabolism. Besides their well-known role as cofactors in enzyme-catalyzed reactions, some B vitamins have other distinctive functions in plants. For example, thiamin (**1**) treatment was shown to induce systemic acquired resistance in plants (Ahn et al., 2005, 2007), riboflavin (**2**) treatment was shown to

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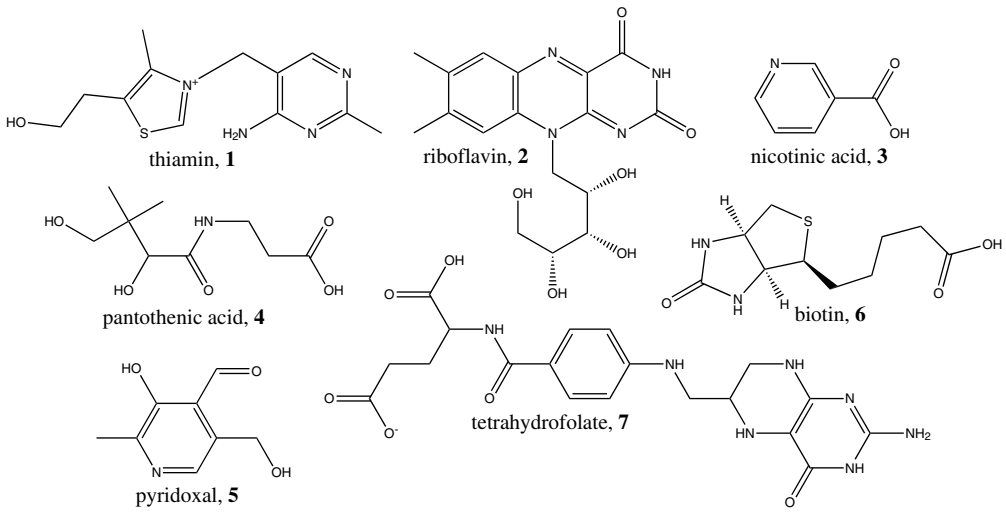


Fig. 1. Chemical structures of B vitamins.

protect plants from infections (Aver'yanov et al., 2000; Taheri and Höfte, 2006), and vitamin $B_6$ was shown to contribute to photoprotection as well as tolerance to osmotic and oxidative stresses in plants (Chen and Xiong, 2005; Titiz et al., 2006). Regulation of protein expression through a riboswitch, present in the mRNA of an enzyme in the thiamin (**1**) biosynthesis pathway, is a recently discovered role of thiamin pyrophosphate (Thore et al., 2006). B vitamins are also important in human nutrition because humans cannot synthesize these compounds *de novo*. Thus, knowledge of the enzymes that participate in vitamin biosynthesis in plants could be used to enrich recombinant plant tissues with vitamins for improved human nutrition. In addition, because humans lack most enzymes required for vitamin biosynthesis, the inhibitors of those enzymes might be effective as herbicides, with little effect on human metabolism.

The first studies of vitamin B biosynthesis in plants focused solely on thiamin (**1**) and niacin (**3**). These studies started in the 1930s and 1940s, concurrently with efforts to understand the biochemical causes of beriberi and pellagra, the two human diseases caused, respectively, by severe deficiency of thiamin (**1**) and niacin (**3**). Despite this early start, the biosynthesis of thiamin (**1**) and niacin (**3**) is incompletely understood, and most enzymes and genes of the thiamin (**1**) and niacin (**3**) biosynthesis pathways have yet to be identified and characterized in plants.

The pathways leading to pantothenic acid (**4**) and pyridoxal (**5**) biosynthesis in plants are better understood. Nonetheless, the precursor of β-alanine, required for pantothenic acid (**4**) biosynthesis, is still unknown. Also, the genes for some enzymes of the $B_6$ salvage pathway have yet to be discovered. These studies have been accomplished during the last two decades. Well understood are the pathways of riboflavin (**2**), biotin (**6**), and tetrahydrofolate (**7**) biosynthesis in plants. All the intermediates in the pathways leading to these three vitamins are known, and most of the required enzymes and genes have been identified and characterized. This review provides a summary of the research that contributed to the present knowledge of vitamin B biosynthesis in plants, and points out those steps that have yet to be characterized in detail.

## 2. Vitamin $B_1$-thiamin

Thiamin (**1**), the precursor of the cofactor thiamin diphosphate (**8**) (Fig. 2), is required by numerous enzymes participating in the metabolism of carbohydrates and amino acids (Jordan, 2003; Settembre et al., 2003; Nosaka, 2006). Humans can synthesize thiamin diphosphate (**8**) from thiamin (**1**) but cannot synthesize thiamin (**1**) *de novo*, thus requiring this vitamin in the diet. Thiamin mono- (**9**) and diphosphate (**8**) are important dietary sources of vitamin $B_1$ because enzymes in the human intestine hydrolyze these derivatives to thiamin (**1**), which is then absorbed (Said and Mohammed, 2006). Severe deficiency of vitamin $B_1$ causes the disease beriberi in humans (Lonsdale, 2006).

Thiamin (**1**) biosynthesis is still incompletely understood. In plants, yeast, and bacteria, 4-methyl-5-β-hydroxyethylthiazole phosphate (HET-P, **10**) and 2-methyl-4-amino-5-hydroxymethylpyrimidine diphosphate (HMP-PP, **11**) are condensed and phosphorylated to afford the cofactor thiamin diphosphate (**8**) (Fig. 2) (Kim et al., 1998; Park et al., 2003; Dorrestein et al., 2004; Lawhorn et al., 2004a,b; Begley, 2006; Nosaka, 2006).

Two HET-P (**10**) biosynthesis pathways are known. Bacteria synthesize HET-P (**10**) from 1-deoxy-D-xylulose-5-phosphate, L-cysteine (**12**), and glycine (**13**) or L-tyrosine in an intricate reaction catalyzed by a multi-enzyme complex (Park et al., 2003; Settembre et al., 2003; Dorrestein et al., 2004; Lawhorn et al., 2004a; Begley, 2006). Yeast cells synthesize HET-P (**10**) from L-cysteine (**12**), glycine (**13**), and an unidentified metabolite (possibly $NAD^+$ (**14**)). A single enzyme (thiazole synthase, THI4) catalyzing

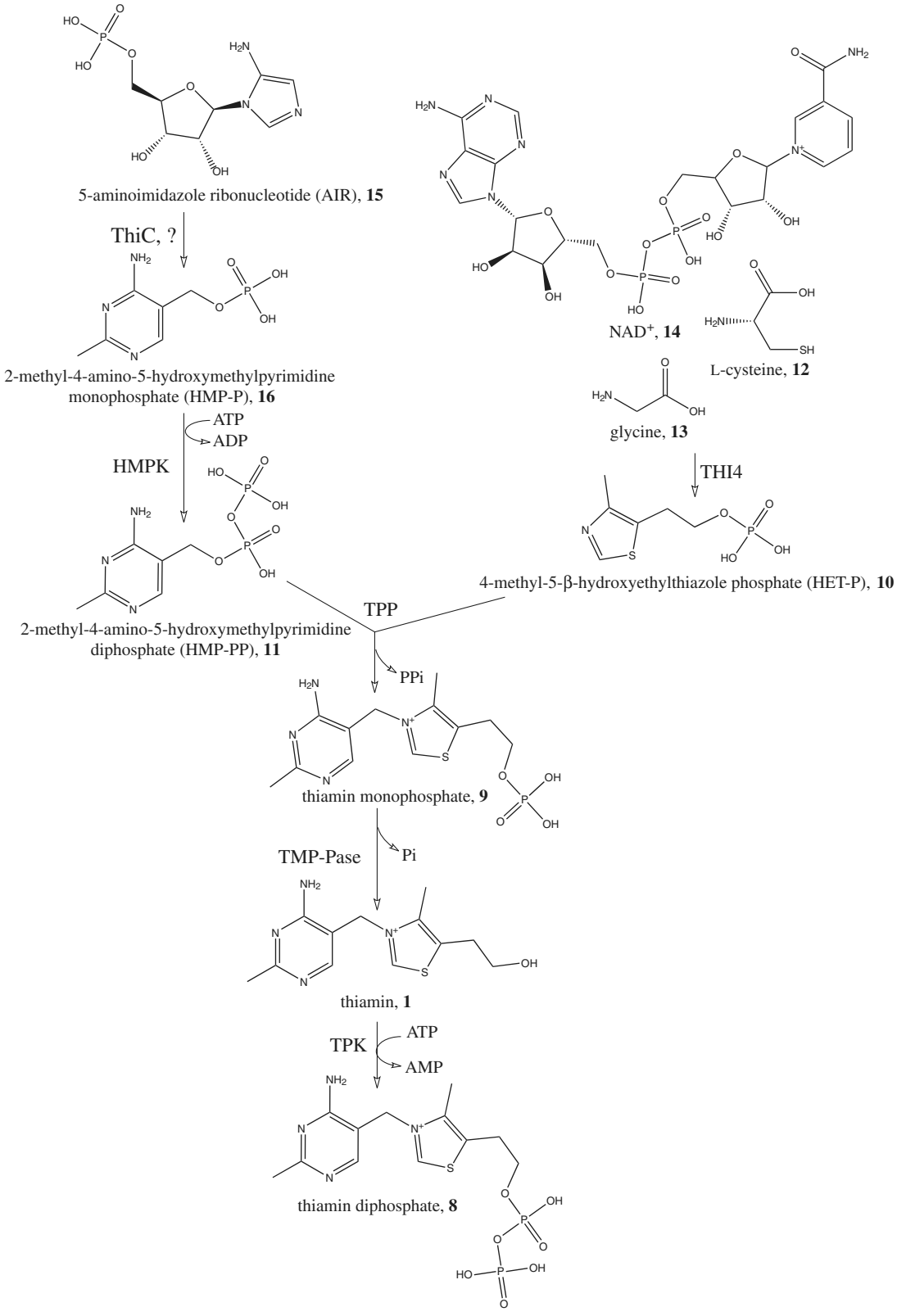


Fig. 2. Proposed pathway for thiamin diphosphate (**8**) biosynthesis in plants. THI4, thiazole synthase; ThiC, an enzyme required for HMP-P (**16**) biosynthesis from AIR (**15**); HMPK, hydroxymethylpyrimidine kinase; TPP, thiamin phosphate pyrophosphorylase; TMP-Pase, thiamin phosphate phosphatase; TPK, thiamin pyrophosphokinase; ?, unknown enzyme(s) required for HMP-P (**16**) biosynthesis from AIR (**15**).

HET-P (**10**) formation in yeast has to date been identified (Chatterjee et al., 2006; Nosaka, 2006); it remains to be determined whether additional enzymes are required for product formation in this organism. Plants probably synthesize HET-P (**10**) *via* the yeast pathway (see below) (Fig. 2).

Two HMP-PP (**11**) biosynthesis pathways are also known. Bacteria synthesize HMP-PP (**11**) from 5-aminoimidazole ribonucleotide (AIR, **15**) (Settembre et al., 2003; Lawhorn et al., 2004a,b). The *thiC* gene product and an additional unidentified protein catalyze the rearrangement of AIR (**15**) to HMP-P (**16**) in *Escherichia coli* (Lawhorn et al., 2004b); the *thiD* gene product then phosphorylates HMP-P (**16**) to HMP-PP (**11**) (Lawhorn et al., 2004a). Yeast cells synthesize HMP-PP (**11**) from pyridoxine and L-histidine; the enzymes and genes of this pathway remain to be identified and characterized (Zeidler et al., 2003; Nosaka, 2006). Plants apparently synthesize HMP-PP (**11**) *via* the bacterial pathway (see below) (Fig. 2).

Thiamin (**1**) was first isolated as an anti-beriberi substance from rice polishings (Jansen and Donath, 1926). Successful chemical synthesis and structure determination of thiamin (**1**) (Williams, 1936; Williams and Cline, 1936; Cline et al., 1937) paved the way for studies of its biosynthesis *in vivo*. The first studies of thiamin (**1**) biosynthesis *in vivo* were done with plant roots grown in tissue culture, requiring thiamin (**1**) in the growth medium (Bonner, 1937, 1938; Robbins and Bartley, 1937b). A finding that HET-P (**10**) can replace thiamin (**1**) in a tissue culture medium used to grow tomato roots suggested that plants synthesize thiamin (**1**) by condensing the precursors of the pyrimidine and thiazole moieties (Robbins and Bartley, 1937a). This suggestion was soon confirmed by a study in pea roots (Bonner and Buchman, 1938).

Further progress in understanding the final steps of thiamin (**1**) biosynthesis in plants was sparked by studies showing that microorganisms condense HET-P (**10**) and HMP-PP (**11**) to thiamin monophosphate (**9**) (Camiener and Brown, 1960; Leder, 1961; Nose et al., 1961). The first *in vitro* evidence for thiamin (**1**) biosynthesis activity in plants came from a finding that a crude homogenate from radish seedlings synthesizes thiamin (**1**) from HET and HMP (Kawasaki et al., 1963). Two studies (Mitsuda et al., 1970b, 1971) showed that plant protein extracts require ATP and $Mg^{2+}$ to catalyze synthesis of thiamin monophosphate (**9**) when at least one of the two precursors is not phosphorylated, supporting the view that the phosphorylated pyrimidine and thiazole precursors are condensed to thiamin monophosphate (**9**).

The gene encoding a bifunctional enzyme, catalyzing phosphorylation of HMP-P (**16**) (HMP-P kinase) and condensation of HET-P (**10**) and HMP-PP (**11**) to thiamin monophosphate (**9**) (thiamin phosphate pyrophosphorylase, TMP-PPase), was recently cloned from *Brassica napus*; notably, this finding represents the first assignment of a catalytic function to a thiamin (**1**) biosynthetic enzyme in plants (Kim et al., 1998). The *B. napus* enzyme (BTH1) contains an N-terminal extension with characteristics of an organellar targeting peptide. Subcellular localization of BTH1 has not been reported; prediction programs assign it to mitochondria using TargetP and to plastids using ChloroP. Two studies (Mitsuda et al., 1975a,b) added evidence that thiamin monophosphate (**9**) is dephosphorylated to thiamin (**1**) before being pyrophosphorylated to thiamin diphosphate (**8**), as in yeast; in the latter study (Mitsuda et al., 1975b), thiamin pyrophosphokinase was purified from parsley leaf.

The first study of plant HET-P (**10**) biosynthesis reported that spinach chloroplasts can synthesize this thiamin precursor from L-cysteine (**12**), tyrosine, and 1-deoxy-D-*threo*-2-pentulose (or from pyruvate and glyceraldehyde-3-phosphate) (Julliard and Douce, 1991). This study led to the proposition that plants synthesize HET-P (**10**) in plastids *via* the bacterial pathway. Two recent lines of evidence, however, suggest the yeast pathway for HET-P (**10**) biosynthesis in plants from L-cysteine (**12**), glycine (**13**), and an unidentified metabolite (possibly $NAD^+$ (**14**)). First, *Zea mays* (Belanger et al., 1995), *Alnus glutinosa* (Ribeiro et al., 1996), *Arabidopsis thaliana* (Machado et al., 1996), and *Oryza sativa* (Wang et al., 2006) contain sequence homologs of the yeast thiazole (HET-P) synthase (THI4). Second, thiamin (**1**) content is lower in transgenic rice plants with reduced expression of the putative HET-P synthase than in wild-type control plants (Wang et al., 2006). Experimental evidence supports localization of HET-P (**10**) biosynthesis in plastids *via* the yeast pathway, as putative HET-P synthases from *Z. mays* (Belanger et al., 1995) and *A. thaliana* (Chabregas et al., 2001) were detected in these organelles using immunogold labeling. HET-P synthase was also detected in mitochondria in *A. thaliana* (Chabregas et al., 2001, 2003), suggesting these organelles as another site of HET-P (**10**) biosynthesis in some plants.

Biochemistry of HMP-PP (**11**) biosynthesis in plants has yet to be investigated. Note, however, that *A. thaliana* and other plant species contain sequence homologs of the pyrimidine biosynthetic enzyme ThiC from *E. coli*, suggesting that HMP-PP (**11**) is synthesized from AIR (**15**), as in bacteria (Fig. 2). In the absence of experimental evidence, TargetP and ChloroP predict the *A. thaliana* ThiC homolog to be in plastids.

## 3. Vitamin $B_2$-riboflavin

Riboflavin (**2**) (Fig. 3) is the precursor of FMN (**17**) and FAD (**18**), the cofactors for scores of enzymes in all organisms. Mitochondrial electron transport, photosynthesis, fatty acid oxidation, and metabolism of vitamins $B_6$, $B_{12}$ and folates are among the vital processes in which these two flavins participate. Humans can synthesize FMN (**17**) and FAD (**18**) from riboflavin (**2**) but cannot synthesize riboflavin (**2**) *de novo*, thus requiring this vitamin in the diet. FMN (**17**) and FAD (**18**) are important dietary sources of riboflavin (**2**) because phosphatases in the human intestine hydrolyze FMN (**17**) and FAD (**18**) to riboflavin (**2**), which is then absorbed (Powers, 2003; Said and Mohammed, 2006). Deficiency of vitamin $B_2$ has been linked to cancer, cardiovascular disease, anemia, and various neurological and developmental disorders in humans and experimental animals (Powers, 2003).

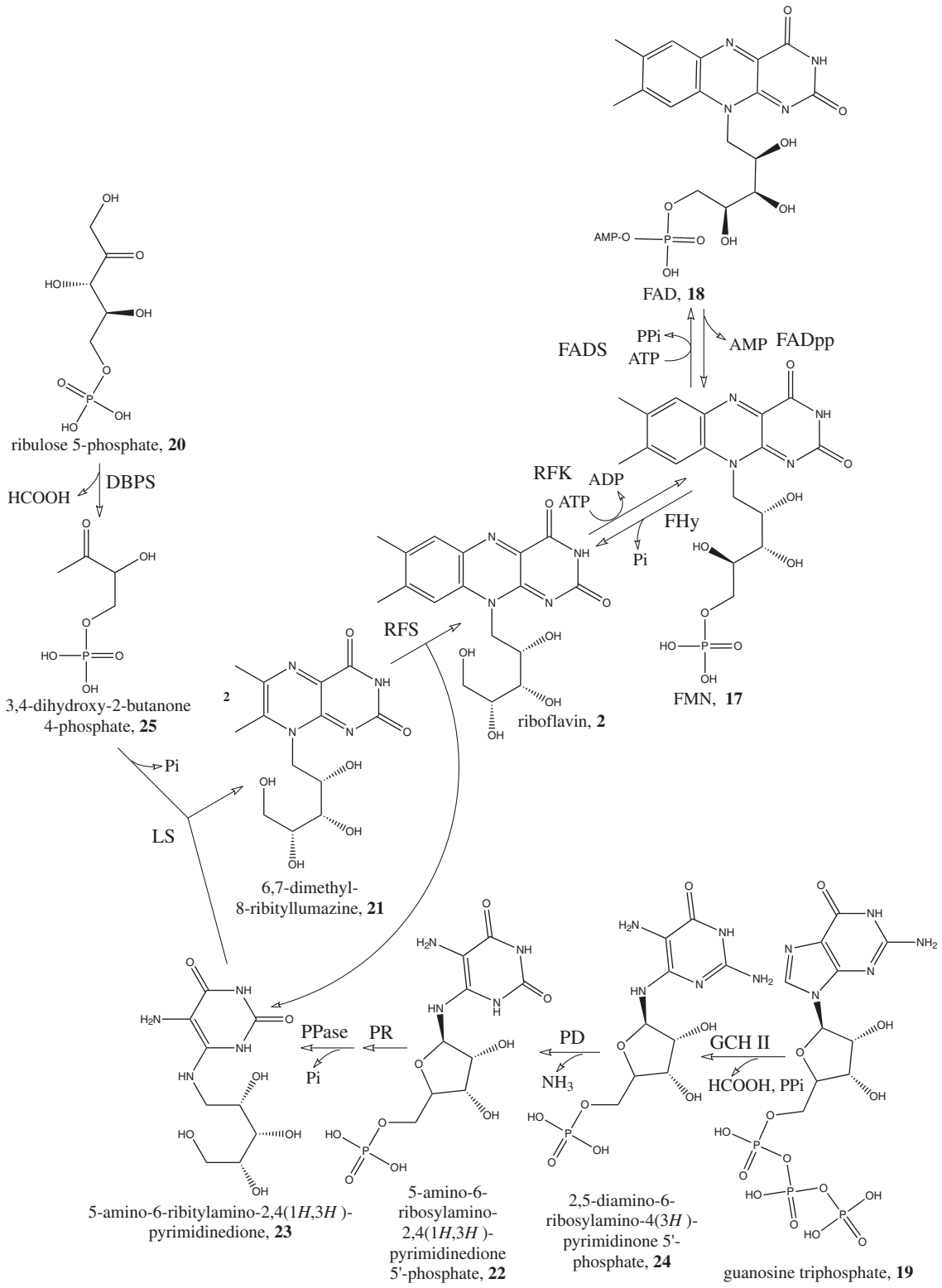


Fig. 3. Biosynthesis of riboflavin (**2**), FMN (**17**), and FAD (**18**) in plants. GCH II, GTP cyclohydrolase II; DBPS, 3,4-dihydroxy-2-butanone 4-phosphate synthase; PD, 2,5-diamino-6-ribosylamino-4(3*H*)-pyrimidinone 5′-phosphate deaminase; PR, 5-amino-6-ribosylamino-2,4(1*H*,3*H*)-pyrimidinedione 5′-phosphate reductase; LS, lumazine synthase; RFS, riboflavin synthase; RFK, riboflavin kinase; FHy, FMN hydrolase; FADS, FAD synthetase; FADpp, FAD pyrophosphatase; PPase, phosphatase.

The riboflavin (**2**) biosynthesis pathway is nearly identical in plants, yeast, and bacteria (Bacher et al., 2000, 2001). In all these organisms, riboflavin (**2**) is first synthesized from GTP (**19**) and ribulose 5-phosphate (**20**), and then phosphorylated to FMN (**17**) and adenylated to FAD (**18**) (Fig. 3). Discoveries of the precursors, the reaction

order, and the enzymes of the riboflavin (**2**) biosynthesis pathway, accomplished by studies in microorganisms, were recently reviewed in detail (Bacher et al., 2000, 2001).

The riboflavin (**2**) biosynthesis pathway has been amply studied in yeast and bacteria (Bacher et al., 2000, 2001). In plants, little information was available until recently. The activity converting 6,7-dimethyl-8-ribityllumazine (**21**) to riboflavin (**2**) was studied in leaves (Mitsuda et al., 1961a,b, 1963b) and the responsible enzyme was partially purified from spinach (Mitsuda et al., 1970a) in the early studies. The genes for GTP cyclohydrolase II–3,4-dihydroxy-2-butanone 4-phosphate synthase (Herz et al., 2000), 2,5-diamino-6-ribosylamino-4(3*H*)-pyrimidinone 5′-phosphate deaminase (Fischer et al., 2004), lumazine synthase (Jordan et al., 1999), and riboflavin synthase (Fig. 3) (Fischer et al., 2005) have recently been cloned from plants based on sequence similarity to their microbial homologs, providing strong evidence that riboflavin (**2**) biosynthesis proceeds through the same steps in plants, yeast, and bacteria. Nothing is yet known about the enzymes that reduce 5-amino-6-ribosylamino-2,4(1*H*,3*H*)-pyrimidinedione 5′-phosphate (**22**) to 5-amino-6-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione 5′-phosphate, and dephosphorylate the latter compound to 5-amino-6-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione (**23**) (Fig. 4). All known enzymes of the riboflavin (**2**) biosynthesis pathway reside in plastids based on experimental and bioinformatic evidence (Jordan et al., 1999; Herz et al., 2000; Fischer et al., 2004, 2005).

Little is known about the enzymes that synthesize and hydrolyze FMN (**17**) and FAD (**18**) (Fig. 3) in plants. Riboflavin kinase and FAD synthetase activities were detected in various species (Giri et al., 1957, 1958, 1960; Mitsuda et al., 1963a,c, 1965b; Sadasivam and Shanmugasundaram, 1966; Sobhanaditya and Rao, 1981), and a riboflavin kinase was purified from mung bean (Sobhanaditya and Rao, 1981). These enzymes were not fully characterized nor were the respective genes cloned. A bifunctional riboflavin kinase–FMN hydrolase unique to plants was recently described; bioinformatic evidence suggests a cytosolic enzyme (Sandoval and Roje, 2005). Early studies with spinach suggested that other enzymes hydrolyzing FMN (**17**) to riboflavin (**2**) and inorganic phosphate exist in plants (Mitsuda et al., 1965b, 1970c). Enzymes hydrolyzing FAD (**18**) to FMN (**17**) and AMP also exist in plants (Kornberg and Pricer, 1950; Kumar et al., 1965; Ravindranath and Rao, 1969; Mitsuda et al., 1970d; Balakrishnan et al., 1977), but the corresponding genes have yet to be cloned. Subcellular localization of FMN- and FAD-hydrolyzing enzymes in plants remains to be explored.

## 4. Vitamin $B_3$-niacin

Niacin (nicotinic acid, **3**) is a metabolic product of the cofactors $NAD^+$ (**14**) and $NADP^+$. In some plants, this compound is also a precursor of pyridine alkaloids such as nicotine, trigonelline, and ricinine (Noctor et al., 2006). Severe deficiency of niacin (**3**) causes the human disease pellagra (Smith et al., 1937; Cervantes-Laurean et al., 1999). The pyridine ring of $NAD^+$ (**14**) is synthesized *de novo* from L-tryptophan in animals (Rongvaux et al., 2003), from L-aspartate (**26**) in plants (Fig. 4) (Noctor et al., 2006), and from L-tryptophan or L-aspartate (**26**) in bacteria (Kurnasov et al., 2003). Controversy still exists as to whether grasses can synthesize nicotinic acid (**3**) *de novo* from L-tryptophan despite lack of support from genomic evidence (Katoh et al., 2006; Noctor et al., 2006). Because nicotinic acid (**3**) can be recycled to $NAD^+$ (**14**) *via* the pyridine nucleotide cycle, also known as the salvage pathway, this metabolite is a vitamin for animals that cannot synthesize L-tryptophan *de novo*.

The dietary importance of vitamin $B_3$ was first shown by using nicotinic acid (**3**) as a dietary supplement to cure the canine disease black tongue, which is caused by the maize-rich, pellagra-inducing diet (Elvehjem et al., 2002). The nicotinic acid (**3**) treatment was also effective in human patients suffering from pellagra (Kohn, 1938), and in pigs previously fed with the pellagra-inducing diet (Chick et al., 1938). The first evidence for L-tryptophan as the precursor of nicotinic acid (**3**) in animals came from a study showing that this amino acid can replace dietary nicotinic acid (**3**) (Krehl et al., 1945). Recognition that tryptophan is the precursor of nicotinic acid (**3**) in mammals and fungi spurred research seeking to determine if plants synthesize nicotinic acid (**3**) from L-tryptophan, too (Gustafson, 1949; Nason, 1949, 1950; Aronoff, 1956; Henderson et al., 1959; Waller and Nakazawa, 1963; Leete, 1965; Arditti, 1967; Arditti and Tarr, 1979; Tarr and Arditti, 1982).

The existence of a different route for the biosynthesis of nicotinic acid (**3**) was proposed on two grounds: first, that L-tryptophan is not a precursor of nicotinic acid (**3**) in *E. coli* and *Bacillus subtilis* (Yanofsky, 1954); second, that the probable precursors of nicotinic acid (**3**) in *E. coli* are a 4-carbon dicarboxylic acid and either glycerol or its metabolite (Ortega and Brown, 1960). Incorporation of glycerol and L-aspartic acid (**26**) into the pyridine ring of nicotine in tobacco (Griffith et al., 1962; Jackanicz and Byerrum, 1966) suggested that plants and bacteria synthesize nicotinic acid (**3**) *via* similar pathways. Quinolinic acid (**27**), previously postulated as an intermediate in the biosynthesis of nicotinic acid (**3**) from L-tryptophan (Henderson, 1949), also appeared to be a precursor of nicotinic acid (**3**) in plants and *E. coli* (Andreoli et al., 1963; Hadwiger et al., 1963).

Supporting L-aspartate (**26**) as the precursor of quinolinic acid (**27**) in plants, aspartate oxidase activity was detected in cotton callus (Hosokawa et al., 1983). The genes for aspartate oxidase and quinolinate synthase, the two enzymes required for quinolinic acid (**27**) synthesis from aspartic acid (**26**), have recently been cloned from *Arabidopsis* (Katoh et al., 2006). Both enzymes reside in plastids and are essential for plant growth (Katoh et al., 2006). Although a preliminary report claimed the existence of a tryptophan-based pathway for the synthesis of quino-

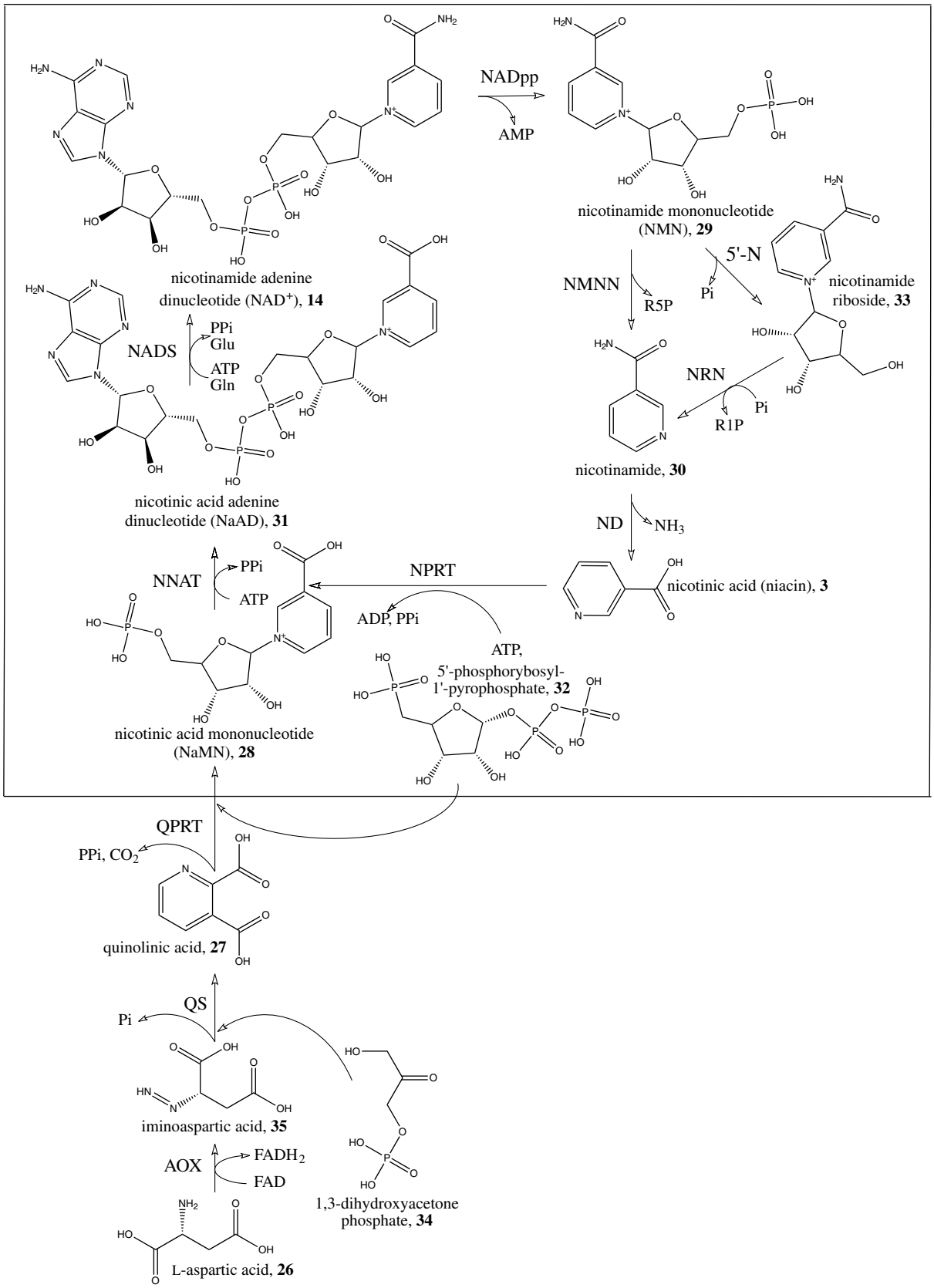


Fig. 4. Biosynthesis of $NAD^+$ (**14**) and proposed reactions of the pyridine nucleotide cycle in plants. AOX, aspartate oxidase; QS, quinolinate synthase; QPRT, quinolinate phosphoribosyltransferase; NNAT, NaMN adenyltransferase; NADS, NAD synthetase; NADpp, NAD pyrophosphatase; NMNN, NMN nucleosidase; 5′-N, 5′-nucleotidase; NRN, nicotinamide riboside nucleosidase; ND, nicotinamide deamidase; NPRT, nicotinate phosphoribosyltransferase. Proposed reactions of the pyridine nucleotide cycle are boxed.

linic acid (**27**) in grasses, these sequences were later removed in the annotation process (Noctor et al., 2006). In summary, current evidence supports quinolinic acid (**27**) synthesis from aspartic acid (**26**) in plants.

The biosynthesis of nicotinate mononucleotide (NaMN, **28**) from quinolinic acid (**27**), the next step *en route* to nicotinic acid (**3**), is catalyzed by the enzyme quinolinate phosphoribosyltransferase (Fig. 4). This enzyme has been purified from castor bean (Mann and Byerrum, 1974), and the cDNAs complementing the *E. coli* mutant of quinolinate phosphoribosyltransferase have been isolated from *Nicotiana rustica* and *N. tabacum* (Sinclair et al., 2000). The cDNAs from these two *Nicotiana* species encode proteins containing N-terminal extensions with characteristics of mitochondrial targeting signals.

The pyridine nucleotide cycle (Fig. 4) completes the vitamin $B_3$ metabolic network in plants and comprises the biosynthesis of $NAD^+$ (**14**) from NaMN (**28**), the degradation of $NAD^+$ (**14**) to nicotinic acid (**3**), and the recycling of nicotinic acid (**3**) to $NAD^+$ (**14**). This cycle remains poorly investigated in plants. NAD pyrophosphatase, catalyzing hydrolysis of $NAD^+$ (**14**) to nicotinamide mononucleotide (NMN, **29**), is the only enzyme of the pyridine nucleotide cycle that has to date been purified from plants (Kornberg, 1948, 1950). Conversion of nicotinamide (**30**) to nicotinic acid (**3**), of nicotinic acid (**3**) to $NAD^+$ (**14**), and of $NAD^+$ (**14**) to nicotinamide (**30**) was described in *Ricinus communis* (Waller et al., 1966); and incorporation of nicotinamide (**30**) and nicotinic acid (**3**) into $NAD^+$ (**14**) was shown in *Hordeum vulgare* (Ryrie and Scott, 1969). Both studies built upon earlier research with mammalian tissues (Handler and Klein, 1942; Leder and Handler, 1951; Preiss and Handler, 1957, 1958a,b). Recently, conversion of nicotinic acid (**3**) to $NAD^+$ (**14**), NMN (**29**), and nicotinamide (**30**) was described in *Picea glauca* and *Catharantus roseus* (Ashihara et al., 2005). These studies collectively suggest that 6–8 enzymes participate in the pyridine nucleotide cycle in plants (Fig. 4) (Ashihara et al., 2005; Zheng et al., 2005; Noctor et al., 2006). These enzymes are NaMN adenyltransferase, NAD synthetase, NAD pyrophosphatase, NMN nucleosidase, 5′-nucleotidase, nicotinamide riboside nucleosidase, nicotinamide deamidase, and nicotinate phosphoribosyltransferase (Fig. 4). Subcellular localization of these plant enzymes remains to be explored.

## 5. Vitamin $B_5$-pantothenic acid

Pantothenate (**4**) (Fig. 5) is the precursor of coenzyme A and the acyl carrier protein. No major health disorders associated with pantothenate (**4**) deficiency are known to occur in humans, as deficiency of this vitamin is uncommon. The pantothenate (**4**) biosynthesis pathway in *E. coli* is well understood (Webb et al., 2004). This pathway consists of two branches. In the first branch, β-alanine (**36**) is synthesized from L-aspartate; in the second, 2-ketopantoate (**37**) is synthesized from α-ketoisovalerate (**38**), and then reduced to pantoate (**39**). Pantoate (**39**) and β-alanine (**36**) are next condensed to pantothenate (**4**). Pantothenate (**4**) biosynthesis in plants (Fig. 5) appears to proceed through the same intermediates as in bacteria, although the source of β-alanine (**36**) is unclear (Raman and Rathinasabapathi, 2004; Webb et al., 2004; Coxon et al., 2005).

Identification of a *Datura innoxia* pantothenate (**4**) auxotroph was the first study examining pantothenate (**4**) biosynthesis in plants (Savage et al., 1979). The ability of the mutant plants to grow on 2-ketopantoate (**37**) and pantoate (**39**) as well as on pantothenate (**4**) provided the first indication that the pantothenate (**4**) biosynthesis pathway in plants is similar to that in bacteria (Sahi et al., 1988). Another indication came from isotope labeling experiments showing incorporation of the radiolabel from L-[$^{14}$C]valine into α-ketoisovalerate (**38**), ketopantoyl lactone, and pantoyl lactone (Jones et al., 1994).

Sequence similarity to previously characterized bacterial enzymes (Webb et al., 2004) facilitated the gene cloning of pantothenate synthases from *Lotus japonicus* and *O. sativa*; the recombinant *L. japonicus* enzyme has been purified and biochemically characterized (Genschel et al., 1999). The absence of an organellar targeting signal in pantothenate

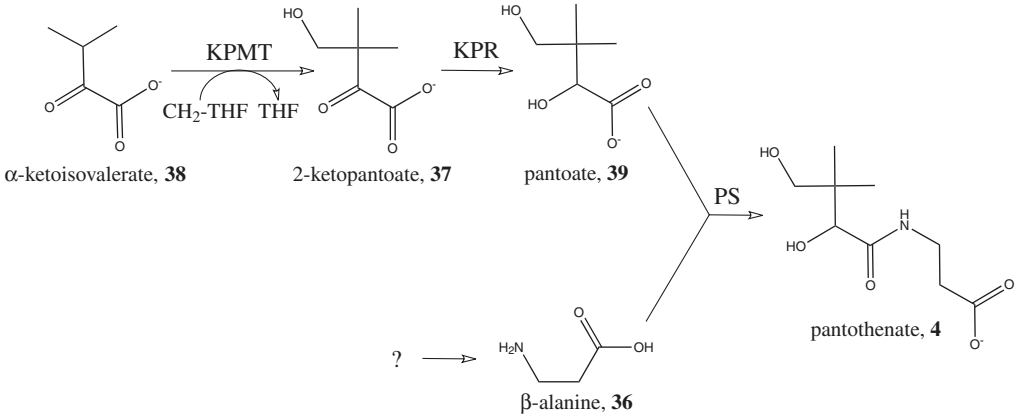


Fig. 5. Biosynthesis of pantothenic acid (**4**) in plants. KPMT, ketopantoate hydroxymethyltransferase; KPR, ketopantoate reductase; PS, pantothenate synthase; ?, the unknown precursor(s) of β-alanine (**36**).

synthases from *L. japonicus* and *O. sativa* suggested that, in plants, the last step in pantothenate (**4**) biosynthesis occurs in the cytosol. Confocal microscopy later localized the GFP-fused pantothenate synthase from *A. thaliana* in the cytosol (Ottenhof et al., 2004).

Sequence similarity to bacterial enzymes also facilitated the identification of the two genes for ketopantoate hydroxymethyltransferases from *A. thaliana*; confocal microscopy localized the GFP-fused enzymes in mitochondria (Ottenhof et al., 2004). Evidence of ketopantoate hydroxymethyltransferase activity in purified mitochondria from pea and *Arabidopsis* (Ottenhof et al., 2004) added support to the view that 2-ketopantoate (**37**) is synthesized in these organelles in plants. Ketopantoate reductase has yet to be characterized in plants, although a candidate for this enzyme from *Arabidopsis* is being investigated (Coxon et al., 2005). Synthesis of β-alanine (**36**) in plants remains to be elucidated. Possible precursors include uracil, spermidine, and propionate (Raman and Rathinasabapathi, 2004; Coxon et al., 2005).

## 6. Vitamin $B_6$-pyridoxine, pyridoxal, pyridoxamine

Pyridoxine (**40**), pyridoxal (**5**), and pyridoxamine (**41**) (Fig. 5) are derivatives of the cofactor pyridoxal 5′-phosphate (PLP, **42**), required for numerous enzymatic reactions predominantly in amino acid metabolism. Human cells can synthesize PLP (**42**) from these three vitamers *via* the $B_6$ salvage pathway (Fig. 6) but cannot synthesize PLP (**42**) *de novo*, thus requiring vitamin $B_6$ in the diet. The 5′-phosphorylated derivatives of pyridoxine (**40**), pyridoxal (**5**), and pyridoxamine (**41**) are dietary sources of vitamin $B_6$ because phosphatases in the human intestine dephosphorylate these compounds before absorption (Said and Mohammed, 2006). Vitamin $B_6$ deficiency affects the general population in industrialized and developing countries alike (Bates et al., 1999a,b; Ronnenberg et al., 2000; Setiawan et al., 2000) and is associated with an increased risk of cardiovascular disease, stroke, and certain types of cancer (Robinson et al., 1998; Ames, 1999; Friso et al., 2004; Lin et al., 2006).

Two pathways for PLP (**42**) biosynthesis *de novo* are known. Plants synthesize PLP (**42**) from glutamine (**43**), ribose 5-phosphate (**44**) or ribulose 5-phosphate (**20**), and dihydroxyacetone phosphate (**45**) or glyceraldehyde 3-phosphate (**46**) (Fig. 6) (Tambasco-Studart et al., 2005; Tanaka et al., 2005). This pathway is also known to exist in fungi (Dong et al., 2004; Tanaka et al., 2005), a metazoan (Tanaka et al., 2005), a protozoan (Wrenger et al., 2005), archaebacteria (Tanaka et al., 2005), and eubacteria (Belitsky, 2004; Tanaka et al., 2005). A two-protein PLP

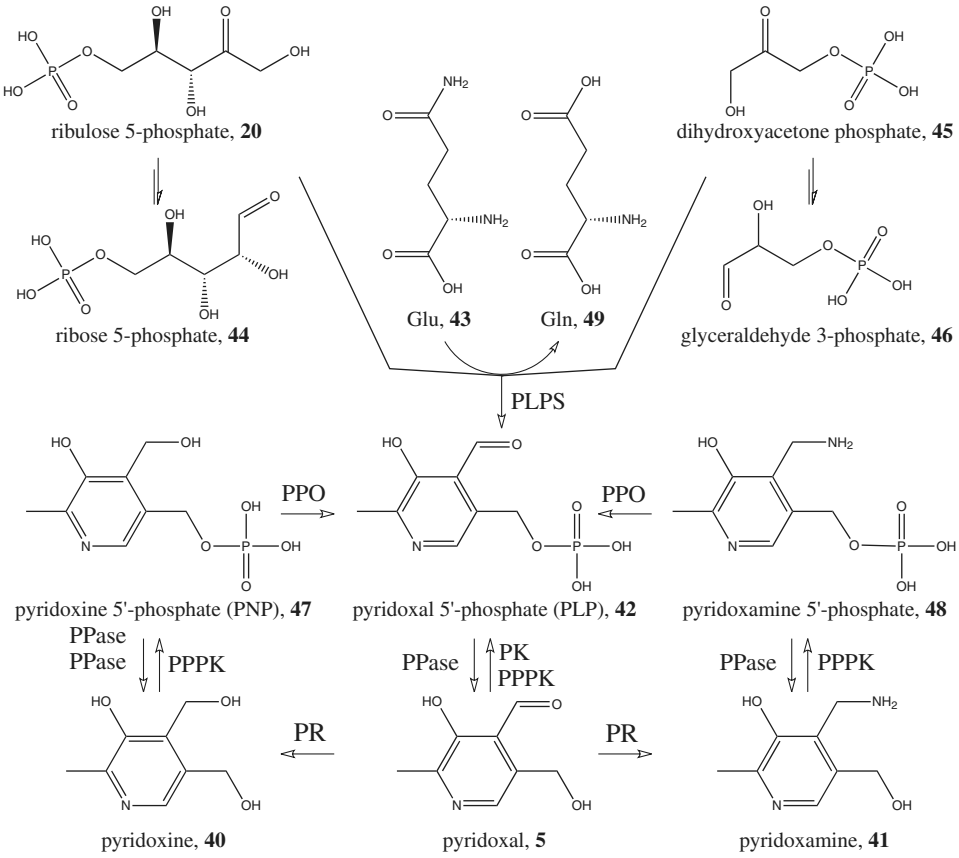


Fig. 6. *De novo* and salvage pathways for PLP (**42**) biosynthesis in plants. PLPS, PLP synthase; PPO, pyridoxine (pyridoxamine) 5′-phosphate oxidase; PK, pyridoxal kinase; PPPK, pyridoxine (pyridoxal, pyridoxamine) kinase; Ppase, phosphatase(s).

synthase complex catalyzes PLP (**42**) biosynthesis in these organisms (Belitsky, 2004; Dong et al., 2004; Burns et al., 2005; Tambasco-Studart et al., 2005). Unique to the eubacterial lineage is synthesis of pyridoxine 5′-phosphate (PNP, **47**) from 3-hydroxy-4-phosphohydroxy-α-ketobutyrate, glutamate (**43**), and 1-deoxy-D-xylulose-5-phosphate. PNP (**47**) is then oxidized to PLP (**42**) *via* the salvage pathway, which exists in all organisms (Tanaka et al., 2005).

Investigation of PLP (**42**) biosynthesis in plants began only recently. The genes for the two proteins that constitute the PLP synthase complex, PDX1 and PDX2, were recently identified in *A. thaliana*; corresponding sequences from *Cercospora nicotianae* were used as an *in silico* probe (Tambasco-Studart et al., 2005). Functional complementation of a *Saccharomyces cerevisiae snz1* mutant and enzyme activity assays with recombinantly expressed proteins showed, first, that two copies of the *PDX1* gene, *AtPDX1.1* and *AtPDX1.3*, encode functional proteins; second, that a third copy, *AtPDX1.2*, apparently encodes an inactive protein (Tambasco-Studart et al., 2005). Functional complementation of an *S. cerevisiae snz1* mutant using *AtPDX1.3* cDNA was confirmed independently (Chen and Xiong, 2005).

The GFP-fused PDX1.1–3 and PDX2 proteins from *A. thaliana* were localized in the cytosol by confocal microscopy (Tambasco-Studart et al., 2005); PDX1.3 likely exists in association with cellular membranes (Chen and Xiong, 2005). A null mutation in the gene encoding PDX1.1 or PDX1.3 results in impaired growth and development (Chen and Xiong, 2005; Tambasco-Studart et al., 2005; Titiz et al., 2006; Wagner et al., 2006); these two null mutations are lethal when combined (Titiz et al., 2006). A null mutation in the gene encoding PDX2 is also lethal (Tambasco-Studart et al., 2005).

A pyridoxal kinase, catalyzing phosphorylation of pyridoxal (**5**) to PLP (**42**), was the first plant enzyme of the salvage pathway to be identified and characterized (Lum et al., 2002; Shi and Zhu, 2002). The gene for this enzyme apparently encodes two transcripts in *A. thaliana*, both predicted to encode cytosolic proteins (Shi and Zhu, 2002). A null mutation in the gene encoding the pyridoxal kinase causes a defect in root hair development as well as increased sensitivity to salt in *A. thaliana* (Shi and Zhu, 2002; Shi et al., 2002). A gene for a pyridoxine (pyridoxamine) 5′-phosphate oxidase has also been cloned from *A. thaliana*, and has been functionally overexpressed in *E. coli* (Sang et al., 2007). This gene encodes a protein carrying an N-terminal extension with characteristics of a plastidial targeting signal (Sang et al., 2007).

## 7. Vitamin $B_8$ or H-biotin

Biotin (**6**) is the cofactor for a small group of enzymes that catalyze carboxylation, decarboxylation, and transcarboxylation reactions in carbohydrate and fatty acid metabolism. Deficiency of this vitamin is rare in humans,

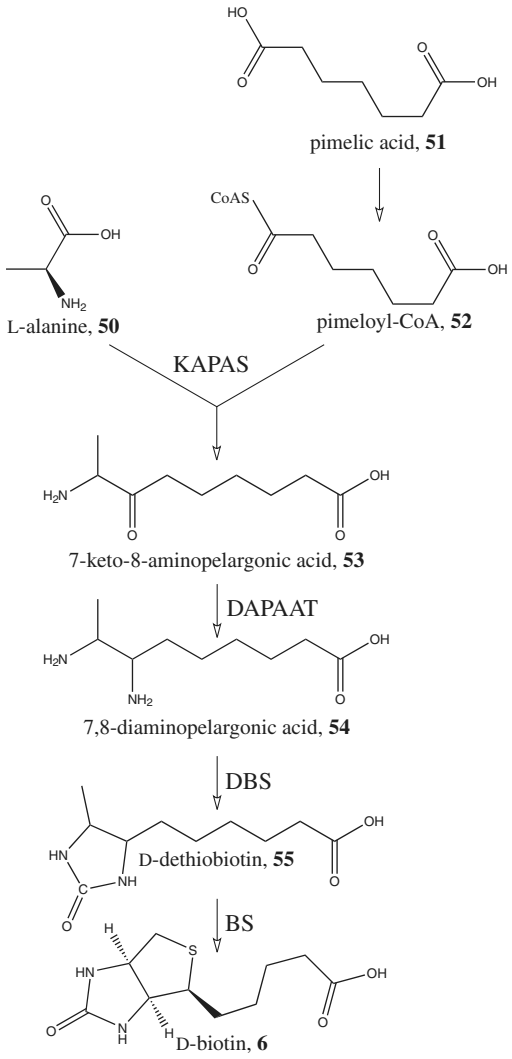


Fig. 7. Biosynthesis of biotin (**6**) in plants. KAPAS, 7-keto-8-aminopelargonate synthase; DAPAS, 7,8-diaminopelargonate synthase; DBS, dethiobiotin synthase; BS, biotin synthase.

but can be induced in special circumstances: in individuals with inborn errors of biotin (**6**) metabolism, in individuals taking certain medications, and in some women during pregnancy (McMahon, 2002; Said, 2002). In both plants and bacteria, biotin (**6**) biosynthesis (Fig. 7) proceeds from L-alanine (**50**) and pimelic acid (**51**) through the pathway intermediates pimeloyl-CoA (**52**), 7-keto-8-aminopelargonic acid (**53**), 7,8-diaminopelargonic acid (**54**), and D-dethiobiotin (**55**) (Alban et al., 2000; Streit and Entcheva, 2003).

Incorporation of the radiolabel from [$^3$H]pimelic acid into pimeloyl-CoA (**52**), 7-keto-8-aminopelargonic acid (**53**), 7,8-diaminopelargonic acid (**54**), and D-dethiobiotin (**55**) in lavender cell cultures first suggested that biotin (**6**) biosynthesis in plants proceeds through the same intermediates as in bacteria (Baldet et al., 1993). Consistently, the gene for 7,8-diaminopelargonate aminotransferase from *E. coli* complemented the *bio1* mutant of *A. thaliana* (Patton et al., 1996). This embryo-lethal mutant containing

almost no biotin (**6**) in seeds (Shellhammer and Meinke, 1990) requires biotin (**6**) or D-dethiobiotin (**55**) added to the growth medium (Schneider et al., 1989).

Biotin synthase from *A. thaliana* was the first plant enzyme of the biotin (**6**) biosynthesis pathway to be studied in some detail. This enzyme catalyzes a mechanistically complex, albeit still incompletely understood, insertion of a sulfur atom between the two 5′-deoxyadenosyl radical-activated carbon atoms (Lotierzo et al., 2005). The gene for biotin synthase from *A. thaliana* was identified based on sequence similarity to the bacterial orthologs, its function was confirmed by complementation of a biotin synthase deficient strain of *E. coli* (Weaver et al., 1996), and its mitochondrial localization was determined using antibodies raised to the recombinant protein (Baldet et al., 1997).

Biochemical characterization of the recombinant biotin synthase from *A. thaliana* suggested that biotin (**6**) biosynthesis from D-dethiobiotin (**55**) *in vitro* requires the presence of yet-to-be-identified factors from plant mitochondria (Picciocchi et al., 2001). These factors were subsequently identified as the mitochondrial proteins adrenodoxin, adrenodoxin reductase, and cysteine desulfurase. These proteins constitute the components of a mitochondrial electron transfer chain (Picciocchi et al., 2003). Consistent with the need for accessory mitochondrial proteins in catalysis, the gene for the cytosol-targeted recombinant biotin synthase failed to rescue the previously isolated (Patton et al., 1998) *bio2* mutant of *A. thaliana* (Arnal et al., 2006).

A gene for 7-keto-8-aminopelargonic acid (KAPA) synthase from *A. thaliana* has also been cloned, and the encoded enzyme has been functionally overexpressed in *E. coli* (Pinon et al., 2005). KAPA synthase from *A. thaliana* was localized in the cytosol by confocal microscopy of the GFP-fused enzyme and by Western blotting (Pinon et al., 2005). Sequence homologs of the bacterial 7,8-diaminopelargonic acid (DAPA) aminotransferase and dethiobiotin synthase exist in the *A. thaliana* genome (Pinon et al., 2005). The putative DAPA aminotransferase from *A. thaliana* appears to be cytosolic; the putative dethiobiotin synthase has an N-terminal extension with characteristics of a mitochondrial targeting signal (Pinon et al., 2005). Thus, the first two reactions of the biotin (**6**) biosynthesis pathway appear to be cytosolic, the last two reactions appear to be mitochondrial in plants.

## 8. Vitamin $B_9$-folate

Folate is a generic term for the cofactor 5,6,7,8-tetrahydrofolate (THF, **7**) and its one-carbon ($C_1$) derivatives. These compounds participate in the biosynthesis of purines, thymidylate, pantothenate (**4**), formyl-Met-tRNA, and methionine. Folate (**7**) deficiency, common even in many industrialized countries, has been linked to birth defects, cardiovascular disease, cancer, and other health disorders (Lucock, 2000; Lucock and Daskalakis, 2000; Scott et al., 2000). THF (**7**) biosynthesis in plants and microorganisms proceeds in two branches (Fig. 8) (Hanson and Gregory, 2002; Basset et al., 2005). 6-Hydroxymethyl-7,8-dihydropterin pyrophosphate (HMDHP-PP, **56**) and *p*-aminobenzoate (*p*ABA, **57**) are first synthesized from the precursors GTP (**19**) and chorismate (**58**), respectively. Second, HMDHP-PP (**56**) and *p*ABA (**57**) are condensed to 7,8-dihydropteroate (**59**), which is then glutamylated and reduced to afford THF (**7**).

Folic acid (Latin, folium-leaf) was first isolated from spinach leaves as a factor that stimulates growth of *Streptococcus lactis* and *Lactobacillus casei* (Mitchell et al., 1941). Following an early demonstration of enzymatic THF (**7**) biosynthesis in leaves (Mitsuda et al., 1965a), GTP (**19**) was identified as the precursor of the pteridine moiety (Mitsuda et al., 1966). Other early studies of THF (**7**) biosynthesis in plants focused on the last three steps of the pathway. These studies identified three enzymes in the THF (**7**) biosynthesis pathway: 7,8-dihydropteroate synthase catalyzing biosynthesis of 7,8-dihydropteroate (**59**) from HMDHP-PP (**56**) and *p*ABA (**57**) (Iwai and Okinaka, 1968; Iwai et al., 1968; Okinaka and Iwai, 1970a,b,c); 7,8-dihydrofolate synthase catalyzing biosynthesis of 7,8-dihydrofolate (**60**) from 7,8-dihydropteroate (**59**) (Iwai et al., 1968; Ikeda and Iwai, 1970); and 7,8-dihydrofolate reductase catalyzing reduction of 7,8-dihydrofolate (**60**) to THF (**7**) (Suzuki and Iwai, 1970; Reddy and Rao, 1976).

Native dihydropteroate synthases have been purified from pea mitochondria (Rébeillé et al., 1997) and *Arabidopsis* leaves (Prabhu et al., 1997). The cDNA for the enzyme from pea mitochondria encodes the bifunctional 7,8-dihydropteroate synthase–6-hydroxymethyl-7,8-dihydropterin kinase, also catalyzing biosynthesis of HMDHP-PP (**56**) from 6-hydroxymethyl-7,8-dihydropterin (**61**) (Rébeillé et al., 1997; Mouillon et al., 2002). A previous study found this bifunctional enzyme in pea seedlings (Okinaka and Iwai, 1970b). A cDNA for dihydrofolate synthase, catalyzing the next step of THF (**7**) biosynthesis, has been cloned from *Arabidopsis*; confocal microscopy localized the GFP-fused N-terminus of this enzyme in mitochondria (Ravanel et al., 2001).

Studies of native 7,8-dihydrofolate reductases in soybean (Reddy and Rao, 1976; Ratnam et al., 1987), carrot (Albani et al., 1985; Toth et al., 1987; Lazar et al., 1989; Cella et al., 1991), sunflower (Mohammad et al., 1989), and the green alga *Scenedesmus obliquus* (Bachmann and Follmann, 1987) described multiple forms of this enzyme, differing in quaternary structure and in biochemical properties. A detailed review of this work is available (Cella and Parisi, 1993). All plants investigated to date contain the bifunctional dihydrofolate reductase–thymidylate synthase (Cella and Parisi, 1993; Luo et al., 1993; Balestrazzi et al., 1995; Wang et al., 1995; Neuburger et al., 1996). Some plants may also contain a monofunctional dihydrofolate reductase (Ratnam et al., 1987).

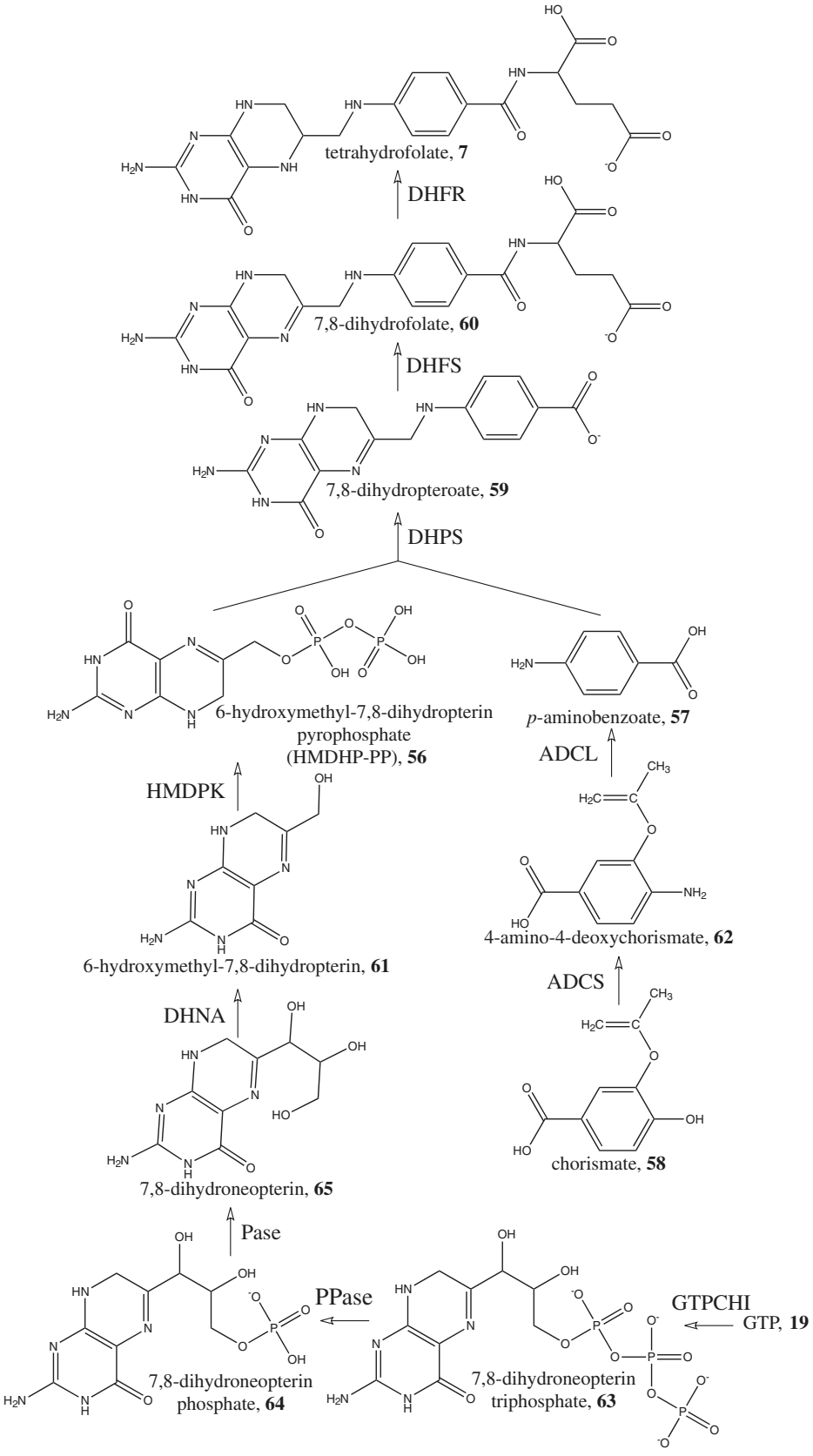


Fig. 8. Biosynthesis of tetrahydrofolate (**7**) in plants. GTPCHI, GTP cyclohydrolase I; PPase, 7,8-dihydroneopterin triphosphate pyrophosphohydrolase; DHNA, DHN aldolase; HMDPK, 6-hydroxymethyl-7,8-dihydropterin kinase; Pase, phosphatase catalyzing the removal of a phosphate from 7,8-dihydroneopterin phosphate (**64**); ADCS, 4-amino-4-deoxychorismate synthase; ADCL, 4-amino-4-deoxychorismate lyase; DHPS, 7,8-dihydropteroate synthase; DHFS, 7,8-dihydrofolate synthase; DHFR, 7,8-dihydrofolate reductase.

Enzyme assays using protein extracts from mitochondria, chloroplasts, nuclei, and the cytosol showed that dihydrofolate reductase activity occurs primarily in mitochondria in pea leaves (Neuburger et al., 1996). Dihydrofolate reductase in these organelles is most likely required for THF (**7**) biosynthesis *de novo*. Immunogold labeling detected dihydrofolate reductase in plastids in carrot (Luo et al., 1997). The role of dihydrofolate reductase in these organelles is probably regeneration of 7,8-dihydrofolate (**60**) produced by the activity of the plastidial thymidylate synthase (Luo et al., 1997).

The genes for the two enzymes of the *p*ABA branch in THF (**7**) biosynthesis, 4-amino-4-deoxychorismate synthase catalyzing biosynthesis of 4-amino-4-deoxychorismate (**62**) from chorismate (**58**) (Basset et al., 2004a; Sahr et al., 2006) and 4-amino-4-deoxychorismate lyase catalyzing biosynthesis of *p*ABA (**57**) from 4-amino-4-deoxychorismate (**62**) (Basset et al., 2004b), were recently cloned, and the encoded proteins were functionally overexpressed in *E. coli*. The recombinant 4-amino-4-deoxychorismate synthase was purified and biochemically characterized (Sahr et al., 2006). Confocal microscopy of the GFP-fused proteins localized 4-amino-4-deoxychorismate synthase and lyase in plastids (Basset et al., 2004a,b).

The enzymes and genes of the pteridine branch in THF (**7**) biosynthesis were also recently identified. Four enzymes constitute this branch: GTP cyclohydrolase I, catalyzing biosynthesis of 7,8-dihydroneopterin triphosphate (**63**) from GTP (**19**) (Basset et al., 2002); a pyrophosphatase, catalyzing hydrolysis of 7,8-dihydroneopterin triphosphate (**63**) to 7,8-dihydroneopterin phosphate (**64**) (Klaus et al., 2005); dihydroneopterin aldolase, catalyzing biosynthesis of 6-hydroxymethyl-7,8-dihydropterin (**61**) from 7,8-dihydroneopterin (**65**) (Goyer et al., 2004), and 6-hydroxymethyl-7,8-dihydropterin kinase, a bifunctional protein fused to a 7,8-dihydropteroate synthase (Rébeillé et al., 1997) described earlier. Lack of putative organellar targeting peptides suggested that GTP cyclohydrolase I, dihydroneopterin triphosphate pyrophosphohydrolase, and dihydroneopterin aldolase reside in the cytosol. The bifunctional 6-hydroxymethyl-7,8-dihydropterin kinase–7,8-dihydropteroate synthase resides in mitochondria (Rébeillé et al., 1997). The only yet unresolved step of the THF (**7**) biosynthesis pathway is the removal of a phosphate group from 7,8-dihydroneopterin phosphate (**64**), probably catalyzed by a non-specific phosphatase (Fig. 8) (Basset et al., 2005).

## 9. Concluding remarks

Early understanding of B vitamin biosynthesis primarily came from studies in yeast and bacteria, which were facilitated by the availability of mutant strains deficient in essential pathway genes. Rapid advances in understanding of the pathways and underlying enzymes for B vitamin biosynthesis in plants occurred during the last two decades, as a result of the growing availability of genome and EST sequence data. Although the pathways for B vitamin biosynthesis in plants were found to share overall similarity to those previously found in yeast and bacteria, they were also found to have unique, unexpected features. For example, both precursors of thiamin monophosphate (**9**), HET-P (**10**) and HMP-PP (**11**), are synthesized through different pathways in yeast and bacteria (Settembre et al., 2003). Surprisingly, plants appear to synthesize HET-P (**10**) *via* the yeast pathway and HMP-PP (**11**) *via* the bacterial pathway. As another example, a bifunctional enzyme with riboflavin kinase and FMN hydrolase activities is present only in the plant kingdom (Sandoval and Roje, 2005). Lastly, the β-alanine (**36**) biosynthesis pathway in plants may be different from those in yeast and bacteria (Raman and Rathinasabapathi, 2004; Coxon et al., 2005). These plant-specific discoveries testify the rewards of investigating B vitamin biosynthesis in plants. Considering the growing interest in vitamin B biosynthesis, a complete knowledge of the steps involved in these pathways can soon be expected, thereby contributing to fundamental understanding of plant metabolism. This knowledge will also enable the use of genes encoding vitamin B biosynthetic enzymes for agriculture and biotechnology.

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