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Histidine biosynthesis in plants

Review Article

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Summary. The study of histidine metabolism has never been at the forefront of interest in plant systems despite the significant role that the analysis of this pathway has played in development of the field of molecular genetics in microbes. With the advent of methods to analyze plant gene function by complementation of microbial auxotrophic mutants and the complete analysis of plant genome sequences, strides have been made in deciphering the histidine pathway in plants. The studies point to a complex evolutionary origin of genes for histidine biosynthesis. Gene regulation studies have indicated novel regulatory networks involving histidine. In addition, physiological studies have indicated novel functions for histidine in plants as chelators and transporters of metal ions. Recent investigations have revealed intriguing connections of histidine in plant reproduction. The exciting new information suggests that the study of plant histidine biosynthesis has finally begun to flower.

Keywords: Histidine – Biosynthesis – Plants

Abbreviations: PRPP, phosphoribosylpyrophosphate; PRATP, N′-5′-phosphoribosyl-ATP; ATP-PRT, N′-5′-phosphoribosyl-ATP transferase; PR-AMP, N′-5′-phosphoribosyl-AMP; 5′-ProFAR or BBMII, N′-[(5′-phosphoribosyl)-formimino]5-aminoimidazole-4-carboxamide-ribonucleotide; PRA-PH, phosphoribosyl ATP pyrophosphohydrolase; PRA-CH, phosphoribosyl-AMP cyclohydrolase; 5′-PRFAR or BBMIII, N′-[(5′-phosphoribulosyl)-formimino]-5-aminoimidazole-4-carboxamide-ribonucleotide; IGP, imidazoleglycerol-phosphate; AICAR, 5′-amino-4-carboxamide ribonucleotide; IGPD, imidazoleglycerol-phosphate dehydratase; IAP, imidazole acetolphosphate; HOL-P, L-histidinolphosphate; HOL, L-histidinol; HAL, L-histidinal; AIR, aminoimidazole ribotide; HMP, hydroxymethylpyrimidine; THF, tetrahydrofolate

Introduction

Studies of the histidine biosynthesis pathway in prokaryotes and lower eukaryotes have uncovered many fundamental mechanisms in biology. For example, the histidine system was of the utmost importance in development of the operon theory (Ames et al., 1960, 1963; Hartman, 1956) and in defining the mechanisms controlling operon expression (Anton, 1968; Fink et al., 1967; Silbert et al., 1966). This pathway is also considered a cornerstone in the foundation and evolving concepts on the evolution of biosynthetic pathways and modern cell biology (Alifano et al., 1996).

In plants, however, the study of histidine was undeservedly neglected until the 1990s. This was ascribed partially to the difficult biochemistry involved in this pathway and the inability to attack the problem by a genetic approach owing to the lack of auxotrophic mutants in higher-plant systems (Miflin, 1980). Recent progress in molecular biology techniques has revealed that many of the enzymatic steps of histidine biosynthesis in some plants are accounted for by single genes, which is in contrast to the extensive gene redundancy found in other plant amino acid biosynthesis pathways (Radwansky and Last, 1995). Thus, histidine biosynthesis has revealed itself to be an attractive target for isolation and analysis of plant amino acid auxotrophs. The enzymes in histidine biosynthesis pathway are also targets for herbicide discovery since this metabolic pathway is not present in animals. Finally, histidine itself was found to play an important role in regulation of biosynthesis of other unrelated amino acids, in chelation and transport of metal ions, and in plant reproduction and growth.

The histidine biosynthesis pathway

Through genetic analysis of histidine biosynthesis in microbes, coupled with genomic annotation of a multitude of prokaryotic and eukaryotic species, it has become clear

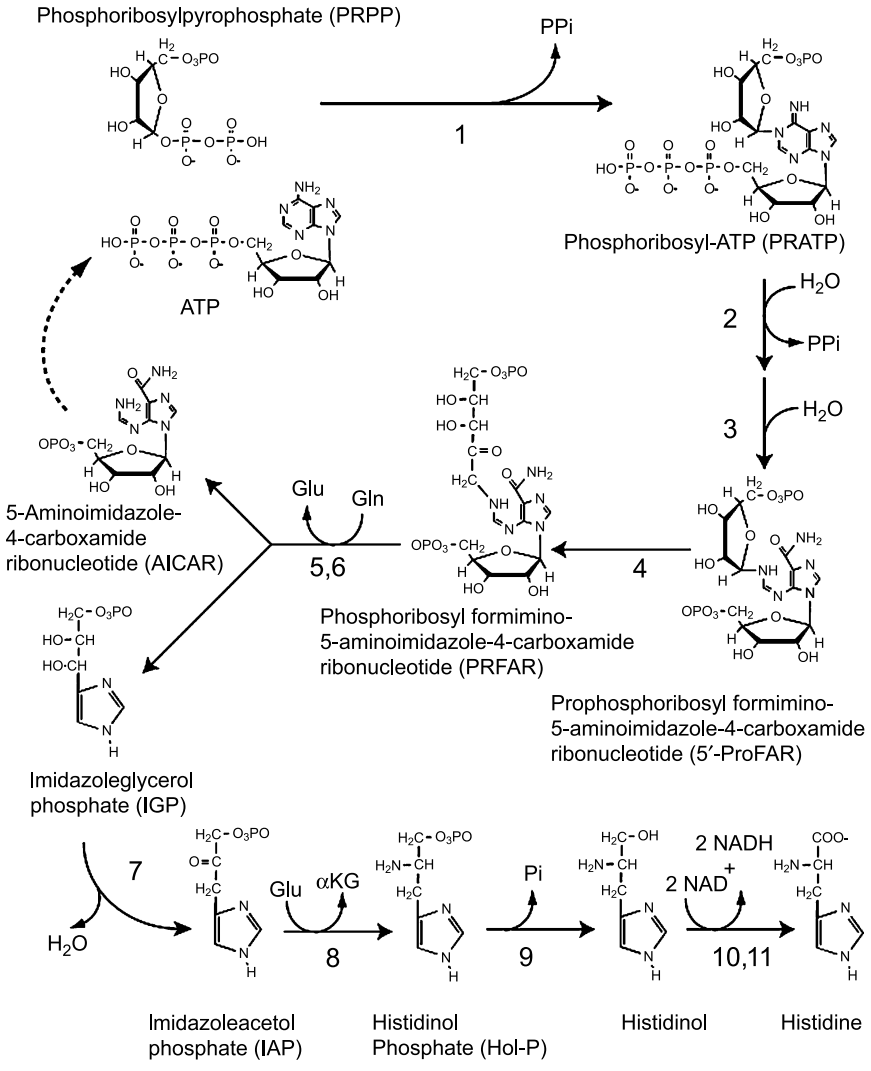

Fig. 1. Pathway of histidine biosynthesis. The reaction sequence catalyzed by (1) N′-5′-phosphoribosyl-ATP transferase, (2) phosphoribosyl ATP pyrophosphohydrolase, (3) phosphoribosyl-AMP cyclohydrolase, (4) BBMII isomerase, (5, 6) imidazoleglycerol-phosphate synthase, (7) imidazoleglycerol-phosphate dehydratase, (8) IAP aminotransferase, (9) histidinol phosphate phosphatase, (10, 11) histidinol dehydrogenase. Points of entry of glutamine (Gln), glutamate (Glu) and NAD^+ are also shown. The dotted arrow shows the entry of AICAR into the ATP recycling pathway

that a common pathway for histidine biosynthesis exists in plants and this pathway is depicted in Fig. 1. Histidine is formed through a series of 11 reactions beginning with the condensation of ATP and phosphoribosylpyrophosphate (PRPP) to form N′-5′-phosphoribosyl-ATP (PRATP) catalyzed by the N′-5′-phosphoribosyl-ATP transferase (ATP-PRT). PRATP is then hydrolyzed to N′-5′-phosphoribosyl-AMP (PR-AMP) followed by hydrolytic opening of the purine ring to produce an imidazole intermediate, N′-[(5′-phosphoribosyl)-formimino]5-aminoimidazole-4-carboxamide-ribonucleotide (abbreviated 5′-ProFAR or BBMII). These sequential reactions are catalyzed by phosphoribosyl ATP pyrophosphohydrolase (PRA-PH) and the phosphoribosyl-AMP cyclohydrolase (PRA-CH), respectively. The next step consists of an internal redox reaction, catalyzed by the BBMII isomerase, leading to production of the aminoketose N′-[(5′phosphoribulosyl)-formimino]-5-aminoimidazole-4-carboxamide-ribonucleotide (5′-PRFAR or BBMIII), which is then converted by the imidazoleglycerol-phosphate synthase (IGP synthase) in the presence of glutamine to 5′-amino 4-carboxamide ribonucleotide (AICAR) and imidazole glycerol phosphate (IGP). The latter compound is dehydrated by imidazoleglycerol-phosphate dehydratase (IGPD) to imidazole acetolphosphate (IAP). The followed reversible transamination reaction involving IAP and an amino group from glutamate is catalyzed by pyridoxal-phosphate-dependent IAP aminotransferase leading to the production of an α-ketoglutarate and L-histidinolphosphate (HOL-P). Latter, HOL-P is converted to L-histidinol (HOL) by histidinol phosphate phosphatase. Finally, L-histidinol dehydrogenase catalyzes two oxidations in which HOL is converted to L-histidine via of the intermediate L-histidinal (HAL).

Metabolic links between the histidine and other biosynthetic pathways

The histidine biosynthesis pathway is integrated with a number of other metabolic pathways including purines, pyrimidines, pyridine nucleotides, folates, and tryptophan (Fig. 2). The connection between histidine and purine mononucleotides biosyntheses has long been recognized from genetic studies with bacteria and yeasts. One feature of this interaction is that PRPP, derived from the pentose phosphate pathway, is the initial substrate for histidine, purine, and pyrimidine biosynthesis. The other initial substrate, ATP, is the end product of purine biosynthesis. In total, histidine biosynthesis is an energy-expensive process, consuming 41 molecules of ATP for each molecule of histidine (Alifano et al., 1996). An additional point of interaction is that the side product of histidine biosynthesis, aminoimidazole carboxamide ribonucleotide (AICAR) serves as the precursor for a branch of adenine nucleotide biosynthesis known as the recycling pathway.

The metabolic interconnection between histidine and purine mononucleotides is also evidenced by regulatory cointegration between these pathways. Thus, expression of yeast *HIS1*, *HIS4*, and *HIS7* genes of histidine biosynthesis is activated by two transcription factors, Bas1p and Bas2p, that also regulate *ADE* gene expression (Arndt et al., 1987; Denis et al., 1998; Springer et al., 1996). Moreover, adenine was shown to repress the expression of yeast *HIS* genes encoding enzymes of the early steps of the pathway thereby preventing AICAR accumulation when sufficient adenine exists to meet the cellular demand for ATP (Arndt et al., 1987; Denis et al., 1998). The rate of histidine synthesis may also influence purine biosynthesis by controlling the size of the PRPP pool (Pendyala and Wellman, 1975).

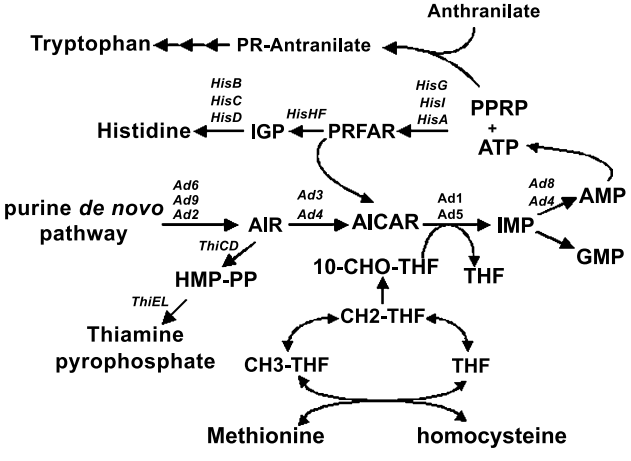

Fig. 2. Schematic representation of metabolic connections between histidine, purine, folate, tryptophan and methionine metabolism. Gene names are italicized. The following abbreviations are used: PPRP, phosphoribosylpyrophosphate; PRFAR, N′-[(5′-phosphoribulosyl)-formimino]-5-aminoimidazole-4 carboxamide-ribonucleotide; IGP, imidazoleglycerol phosphate; AICAR, 5′-amino 4-carboxamide ribonucleotide; AIR, aminoimidazole ribotide; THF, tetrahydrofolate; CHO-THF, formyl-tetrahydrofolate; CH-THF, methenyl-tetrahydrofolate; HMP, hydroxymethylpyrimidine

Genetic studies of purine mononucleotide mutants of *Salmonella enterica* revealed that increased flux through the histidine biosynthetic pathway resulted in accumulation of AICAR negatively affected the conversion of aminoimidazole ribotide (AIR) into hydroxymethylpyrimidine (HMP), an intermediate of thiamine biosynthesis. This finding pointed to regulatory interaction between the histidine and thiamine biosynthesis pathways (Allen et al., 2002) (Fig. 2).

Accumulation of AICAR seems also to interfere with methionine biosynthesis. The methionine auxotrophy of a triple *fau1ade16ade17* yeast mutant, lacking methenyl THF synthetase and two isozymes utilizing AICAR for the production of THF, was suppressed by additional combined mutations in both *HIS4* and *ADE2*, which act by blocking all the pathways responsible for AICAR biosynthesis (Holmes and Appling, 2002). These observations raise the intriguing possibility of a metabolic link between the histidine and methionine biosynthetic pathways (Fig. 2).

Another metabolic interconnection exists between histidine and tryptophan biosynthesis pathways through the common intermediate PRPP. The tryptophan biosynthetic branch requires PRPP for the production of N-phosphorybosyl anthranilate from anthranilate (Pendyala and Wellman, 1975) (Fig. 2). In addition, tryptophan-mediated control of histidine biosynthetic enzymes was experimentally shown in *Neuspora crassa* (Carsiotis and Jones, 1974).

Plant orthologs of histidine biosynthesis enzymes

Recent progress in plant genomics has permitted the identification of genes that account for 10 of the 11 reactions needed for histidine biosynthesis. Of these, all have been functionally characterized from either *Arabidopsis thaliana* and/or other plant species. The present review focuses on the *Arabidopsis* genome because the pathway has been studied in the greatest detail in this species. As shown in Fig. 3, *Arabidopsis* contains 11 orthologs of histidine biosynthesis genes scattered across all 5 of its chromosomes. Recently, *Arabidopsis* histidine biosynthesis gene products have been registered at TAIR (http://arabidopsis.org/jsp/processor/genesymbol/symbol_main.jsp) as the HISN symbol names followed by a number indicating the step catalyzed in sequential order in histidine biosynthesis

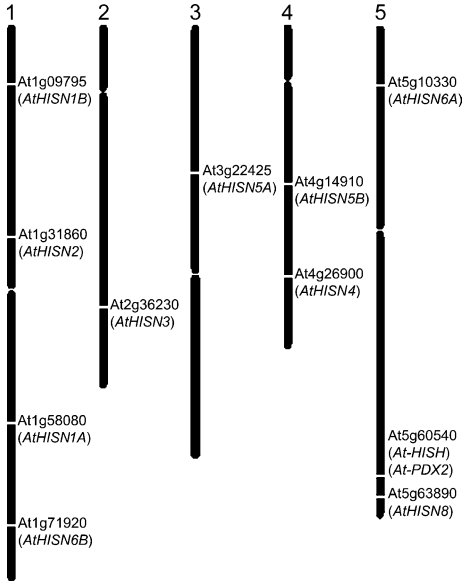

Fig. 3. Distribution of the histidine biosynthesis genes in *Arabidopsis* genome. Eleven orthologs of histidine biosynthesis genes are given in white lines and their accession numbers are also shown

pathway and then a letter indicating a specific gene in the case of gene families (Table 1).

Since histidine biosynthesis is an extremely energy-consuming process, the compartmentalization of the pathway likely reflects the source of the energy supply. Indeed, on the basis of accumulated evidences from analysis of the structure of plant histidine biosynthesis genes isolated to date, it appears that each of the enzymes contains an N-terminal extension corresponding to a chloroplast transit peptide and suggesting that the entire histidine biosynthesis pathway is contained within the chloroplast (Fujimori and Ohta, 1998a, b; Fujimori et al., 1998; Ohta et al., 2000; Tada et al., 1994; El Malki et al., 1998; Ingle et al., 2005). Interestingly, detailed analysis of the structural organization of the histidine biosynthesis gene encoding *Arabidopsis* PRA-PH/PRA-CH (Fujimori and Ohta, 1998), IGP synthase (Brilli and Fani, 2004a) and IGPD enzymes revealed a pattern in which the chloroplast transit peptide is separated by an intron from the domain corresponding to the mature enzymes (Fig. 4). Such an organization is thought to arise from fusion of an exon encoding a chloroplast transit peptide with the exons encoding the catalytic segment of the protein during evolution. The same organization has been reported for several nuclear genes encoding chloroplastic proteins (Wolter et al., 1988; Gantt et al., 1991).

ATP phosphoribosyltransferase

In all species that have been examined, ATP-PRT is a monofunctional enzyme. In *Escherichia coli* and in *Saccharomyces cerevisiae*, it is encoded by the *hisG* and *HIS1* genes, respectively. The first plant HISN1 cDNA to be cloned was isolated from *Thlaspi goesingense* (THG1 or THISN1, accession nr. AF003347) by functional complementation of an *E. coli hisG* mutant. Moreover, genomic DNA analysis suggested the presence of at least one further *HISN1* gene in this species (Persans et al., 1999). The predicted amino acid sequence derived from the THISN1 cDNA showed 29% and 26% identity to the *E. coli* HisG and *S. cerevisiae* HIS1 protein sequences, respectively. Later, Ohta et al. (2000) characterized two isoforms of ATP-PRT from *Arabidopsis*, AtHISN1A and AtHISN1B, by cloning cDNAs capable of complementing an *S. cerevisiae HIS1* mutant. These cDNAs were then found to correspond to two genes located on *Arabidopsis* chromosome 1 (At1g58080 and At1g09795 [Fig. 3]).

Table 1. Histidine biosynthesis gene products in bacteria, yeasts, and plants

Bacterial gene product symbols	Yeast gene product symbols	Plant gene product symbols	Arabidopsis HISN gene locus	Enzyme activity
HisG	HIS1	HISN1A	At1g58080	phosphoribosyltransferase
		HISN1B	At1g09795	
HisIE	HIS4	HISN2	At1g31860	cyclohydrolase, pyrophosphohydrolase
HisA	HIS6	HISN3	At2g36230	BBMII isomerase
HisHF	HIS7	HISN4	At4g26900	amidotransferase/cyclase
HisB	HIS3	HISN5A	At3g22425	dehydratase
		HISN5B	At4g14910	
HisC	HIS5	HISN6A	At5g10330	aminotransferase
		HISN6B	At1g71920	
HisB	HIS2	HISN7		phosphatase
HisD	HIS4	HISN8	At5g63890	dehydrogenase

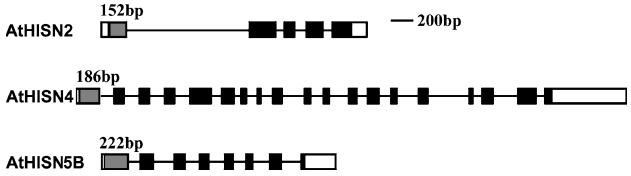

Fig. 4. Structural organization of histidine biosynthesis genes. The structure of *Arabidopsis* HISN2, HISN4, and HISN5B mRNAs are represented. Exons are given in black boxes and introns in black lines. White boxes represent untranslated 5′ and 3′ regions of the corresponding mRNAs. First exons encoding chloroplast transit peptides given in gray. The lengths of the HISN2, HISN4, and HISN5B chloroplast transit peptides are 50, 60, and 74 amino acids, respectively, which are correlated with lengths of exons in basepairs that are also shown

Recently, a number of new plant *HISN1* orthologous genes have been added to the nucleotide sequence databases. Two full-length HISN1A and HISN1B cDNAs were cloned from *Alyssum montanum* (accession nr. AY570537 and AY570536) and *Alyssum lesbiacum* (AY570529 and AY570528 [Ingle et al., 2005]). DNA gel blot analysis indicated that two *HISN1* genes in the *A. lesbiacum* genome exist as single copies just as in *Arabidopsis* (Ingle et al., 2005). A DNA fragment from the partially sequenced chromosome 3 of *Oryza sativa* revealed at least one orthologous gene encoding the ATP-PRT enzyme. The predicted protein sequence derived from the putative rice HISN1 cDNA (accession nr. AAN05502) is 70% and 72% identical in to the *Arabidopsis* HISN1A and HISN1B proteins, respectively (data not shown). Only a single gene has been identified to date in *Zea mays* (AY112299 [Ingle et al., 2005]).

Overall, the plant HISN1 orthologs are well conserved and cluster together as indicated in the neighbor-joining tree showing the relationship of orthologous HISN1 protein sequences from plants and microbes (Fig. 5). Indeed, comparison of the primary structures of the HISN1A protein with its corresponding counterpart (HISN1B) from *A. thaliana*, *A. lesbiacum*, or *A. montanum* revealed an overall 74.6%, 81%, or 84% amino acid identity, respectively. Interestingly, the amino acid identities among the *Arabidopsis* and *Alyssum* HISN1A or HISN1B protein sequences were greater than the identities between HISN1A and HISN1B within each species. The existence of two distinct HISN1 forms is also confirmed by the neighbor-joining tree, in which plant HISN1A and HISN1B orthologs cluster into two subgroups (Fig. 5). These observations indicate that the two plant HISN1 forms diverged by gene duplication before the speciation of *Arabidopsis* and *Allysum* and so they should be considered paralogs (Ingle et al., 2005). The fact that recombinant *Arabidopsis* HISN1B protein is less stable during its purification from crude bacterial cell extract compared with AtHISN1B further suggests that the two forms may have distinct biochemical properties (Ohta et al., 2000).

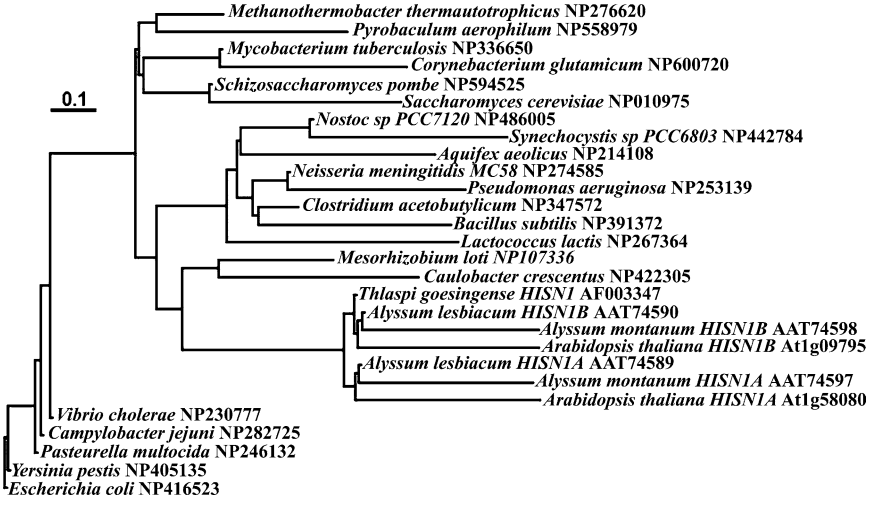

Fig. 5. Phylogenetic tree of HisG orthologs. Plant HisG orthologs were identified in NCBI database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi). Queries are chosen from the Clusters of Orthologous Groups (COG) database (http://www.ncbi.nlm.nih.gov/COG/). Phylogenetic analysis was carried out on protein sequences using CLUSTALW web program (http://www.ebi.ac.uk/clustalw) and the phylogenetic tree was constructed using the TreeTop web program (http://www.genebee.msu.su/services/phtree_reduced.html) with the topological algorithm. Taxa are represented as follows: archaea (*M. thermautotrophicus* and *P. aerophilum*), actinobacteria (*M. tuberculosis* and *C. glutamicum*), Eukaryota (*S. pombe* and *S. cerevisiae*), cyanobacteria (*Synchocystis* and *Nostoc* sp.), bacteria and proteobacteria (*A. aeolicus* to *C. crescentus* and *V. cholerae* to *E. coli*), Viridiplantae (*T. goesigense* to *A. thaliana*)

Phosphoribosyl-ATP phosphohydrolase and phosphoribosyl-AMP cyclohydrolase

PRA-PH and PRA-CH exist on a single polypeptide encoded by the *hisI* gene in *E. coli*. In some microbes such as *Methanococcus vannielii* (Beckler and Reeve, 1986) and *Methanococcus maripaludis* the two catalytic activities reside on different polypeptides (e.g., encoded by loci MMP0280 [hisI] and MMP0051 [hisE]). In yeast, PRA-PH and PRA-CH activities exist on a trifunctional enzyme (HIS4) in which the amino terminus is homologous with *E. coli* HisI and the carboxyl terminus is a histidinol dehydrogenase domain. Histidinol dehyrdogenase catalyzes the last reaction of the histidine biosynthesis pathway. In plants, histidinol dehydrogenase has been shown to be encoded by a separate gene (Nagai and Scheidegger, 1991).

A single full-length cDNA encoding a bifunctional protein with both PRA-PH and PRA-CH activities was cloned from *Arabidopsis* (AtHISN2, accession nr. NM102923) by functional complementation of an *E. coli hisI* mutant lacking PRA-CH activity (Fujimori and Ohta, 1998). *AtHISN2* is derived from a single gene located on chromosome 1 (At1g31860 [Fig. 3]). Fujimori and Ohta (1998) showed that when the PRA-CH and PRA-PH domains of the AtHISN2 protein were expressed separately, they retained the individual catalytic activities, implying that the two domains are functionally independent. The two domains are separated by a linker region that does not show significant homology with either HisIE or HIS4 proteins (Fujimori and Ohta, 1998a). One ortholog of AtHISN2 was identified on chromosome 1 of *Oryza sativa* (accession nr. BAD81115 [Sasaki et al., 2002]). The predicted amino acid sequence of this protein showed 68% identity to the AtHISN2.

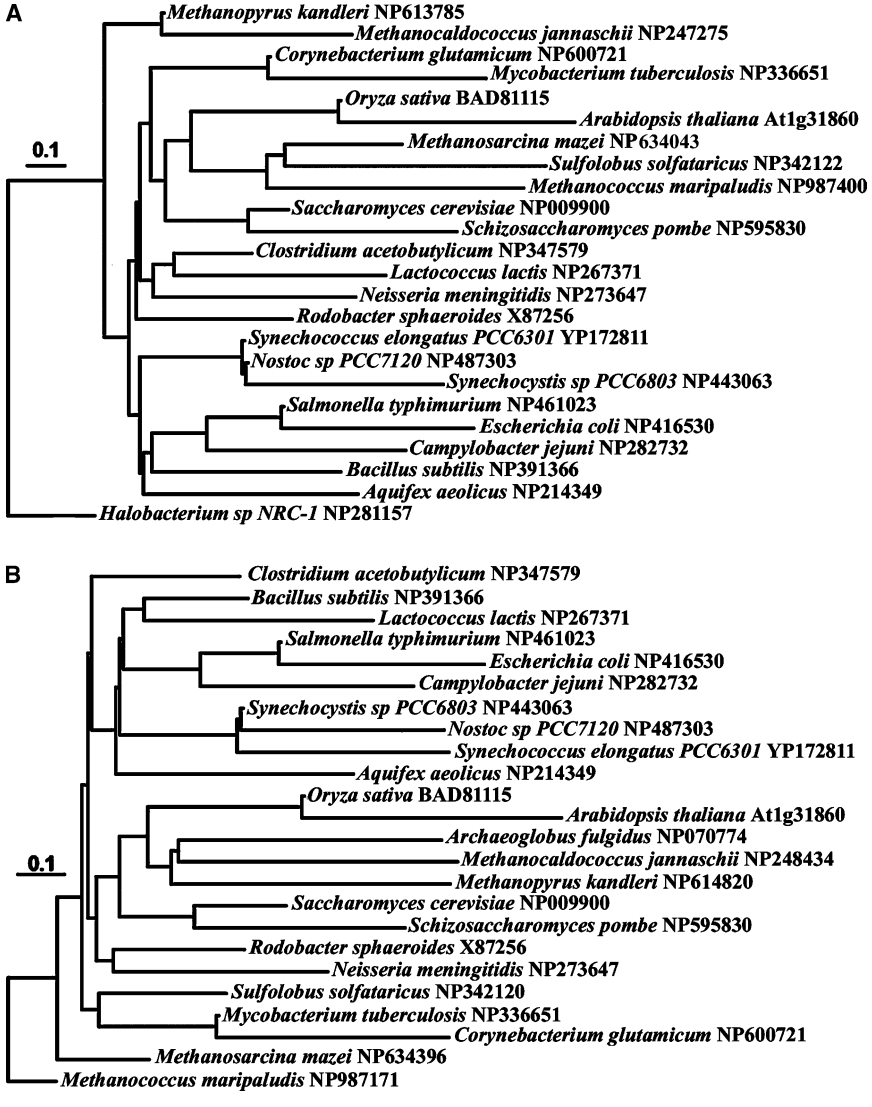

Fig. 6. Phylogenetic tree of HisI and HisE orthologs. Archaeal HisI (**A**) and HisE (**B**) orthologs were compared to protein sequences of bacterial HisI and plant HISN2 as well as yeast HIS4 orthologs as explained for Fig. 5. Taxa are represented as follows: archaea (*M. kandleri*, *M. jannaschii*, *M. mazei*, *S. solfataricus*, *M. maripaludis*, *A. fulgidus*, and *Halobacterium* sp.), actinobacteria (*M. tuberculosis* and *C. glutamicum*), Viridiplantae (*A. thaliana* and *O. sativa*), Eukaryota (*S. pombe* and *S. cerevisiae*), bacteria and proteobacteria (*C. acetobutylicum*, *B. subtilis*, *L. lactis*, *S. typhimurium*, *E. coli*, *C. jejuni*, *A. aeolicus*, *R. sphaeroides*, *N. meningitidis*), cyanobacteria (*Synchocystis* sp. and *Nostoc* sp.)

With the aim of determining the evolutionary origin of plant HISN2 orthologs, HisI amino acid sequences from divergent origins, including members of the Archaea, cyanobacteria, yeasts, and plants were compared (Fig. 5). Since two activities of the bifunctional HisI polypeptide are encoded by separate genes in members of the Archaea (HisI and HisE [Fani et al., 1993; Beckler and Reeve, 1986; Bult et al., 1996]), these protein sequences were compared separately with the HisI proteins of other species. In both neighbor-joining trees (Fig. 6A, B) plant HISN2 orthologs are combined with archaeal HisI and HisE proteins, while most of the microbial HisI and cyanobacterial HisIE orthologs clustered together. BLASTP probing of AtHISN2 with microbial HisI orthologs revealed similarity ranged between 30 and 37%. AtHISN2 also showed very poor homology to HisIE orthologs from cyanobacteria species (17%).

BBMII isomerase

BBMII isomerase is a monofunctional enzyme in all species that have been examined. In *E. coli* and yeasts it is encoded by the *hisA* and *HIS6* genes, respectively. Interestingly, *E. coli* HisA and yeast HIS6 share only 20% amino acid sequence identity, indicating that these enzymes diverged significantly during evolution. The first demonstration of the existence of a plant BBMII isomerase was the isolation of a full-length cDNA from *Arabidopsis* (AtHISN3, accession nr. AB195273) by functional complementation of an *E. coli hisA* mutant (Fujimori et al., 1998). The complementing cDNA could be traced to a locus on chromosome 2 (At2g36230) and it represents the only hisA/HIS6 ortholog in this species. Another plant HISN3 ortholog was recently annotated in the rice genome corresponding to locus Os05g33260. The primary structure of the putative rice HISN3 protein sequence (accession nr. AAT85143) comprises 147 amino acids compared with 305 amino acids for the *Arabidopsis* protein. Whether this is an annotation error is uncertain. Even so, the rice and *Arabidopsis* proteins share 74% sequence identity. A neighbor-joining tree showing the relationship of divergent HISN3 (Fig. 6) indicates the close relationship of the eukaryotic orthologs and the divergence of eukaryotic and microbial orthologs. This result demonstrates that plant HISN3 shares a more recent evolutionary lineage with heterotrophic eukaryotes than with the more distantly related enzymes from cyanobacteria, suggesting that the origin of plant HISN3 may not be through the phyletic ancestor of chloroplasts. Indeed, the divergence of plant HISN3 from the cyanobacterial *hisA* orthologs is as great as the divergence from the available heterotrophic bacterial and archaeal *hisA* orthologs. Despite the limited sequence similarity, it is interesting to note that *AtHISN3* was able to complement the *E. coli hisA* mutant. This result suggests that the concept of substrate channeling, postulated to explain the multiple fusions that have occurred in the evolution of histidine biosynthetic pathway genes, is not a prerequisite for functioning of the BBMII isomerase step.

IGP synthase

In nature, significant differences exist in the structure of the enzyme catalyzing IGP synthesis. Two catalytic functions are required for this step, including a transfer of an amide from glutamine followed by a cyclization reaction.

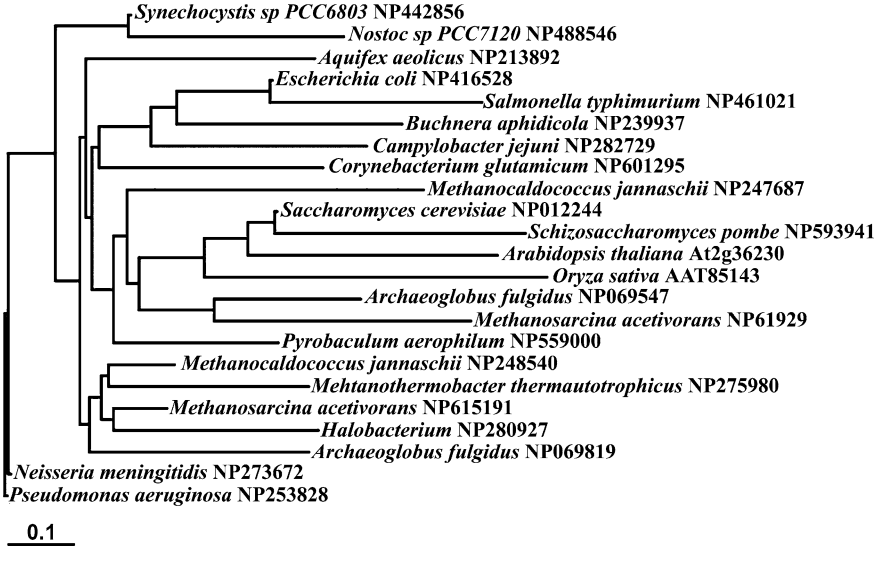

Fig. 7. Phylogenetic tree of HisA orthologs. Phylogenetic analysis of protein sequences of the corresponding archaeal and bacterial HisA, yeast HIS6 and plant HISN3 orthologs was carried out as explained for Fig. 5. Taxa are represented as follows: cyanobacteria (*Synchocystis* sp. and *Nostoc* sp.), bacteria and proteobacteria (*A. aeolicus* to *C. jejuni*, *N. meningitidis* and *P. aeruginosa*), actinobacteria (*C. glutamicum*), archaea (*M. jannaschii* and *A. fulgidus* NP069547 to *A. fulgidus* NP069819), Eukaryota (*S. pombe* and *S. cerevisiae*), Viridiplantae (*A. thaliana* and *O. sativa*)

In prokaryotic organisms, the glutamine amidotransferase is encoded by *hisH* and the cyclase is encoded by *hisF*. HisH and HisF form a stable complex with 1:1 stiochiometry (Klem and Davisson, 1993). In yeasts, a single gene (*HIS7*) encodes a bifunctional enzyme, which shows homology to HisH at its amino terminus and HisF at its carboxyl terminus. This gene has been postulated to have arisen from a gene fusion event (Brilli and Fani, 2004a).

The first demonstration of a bifunctional IGP synthase from plants was the isolation of an *Arabidopsis* cDNA (AtHISN4, accession nr. AB006210) capable of functionally complementing an *S. cerevisiae HIS7* mutant (Fujimori and Ohta, 1998). Moreover, the isolated cDNA was able to complement a *HIS7* deletion mutant of *S. cerevisiae*, lacking both amidotransferase and cyclase activities demonstrating that the AtHISN4 cDNA encodes a bifunctional enzyme. Analysis of the cDNA sequence confirmed that the *Arabidopsis* protein is similar to HIS7 in that it has the same bi-domain structure with the amidotransferase localized to the amino terminus and the cyclase at the carboxyl terminus. With the completion of the *Arabidopsis* genome sequence it became clear that the AtHISN4 cDNA was derived from a locus on chromosome 4 (At4g26900) representing the only HIS7-type bifunctional enzyme in *Arabidopsis*. A single *HISN4* gene has been identified in rice on chromosome 3 corresponding to locus Os03g15120.

Analysis of *HISN4* gene structure in members of the Eukaryota revealed conservation in domain organization with the amidotransferase moiety located upstream of the cyclase. This conservation suggests a common evolutionary origin of the eukaryotic IGP synthases and, perhaps, that this arrangement is advantageous for the enzymatic activity permitting the entry of PRFAR into IGP synthase catalytic site (Brilli and Fani, 2004a). Comparison of the eukaryotic HIS7/HISN4 sequences and their prokaryotic

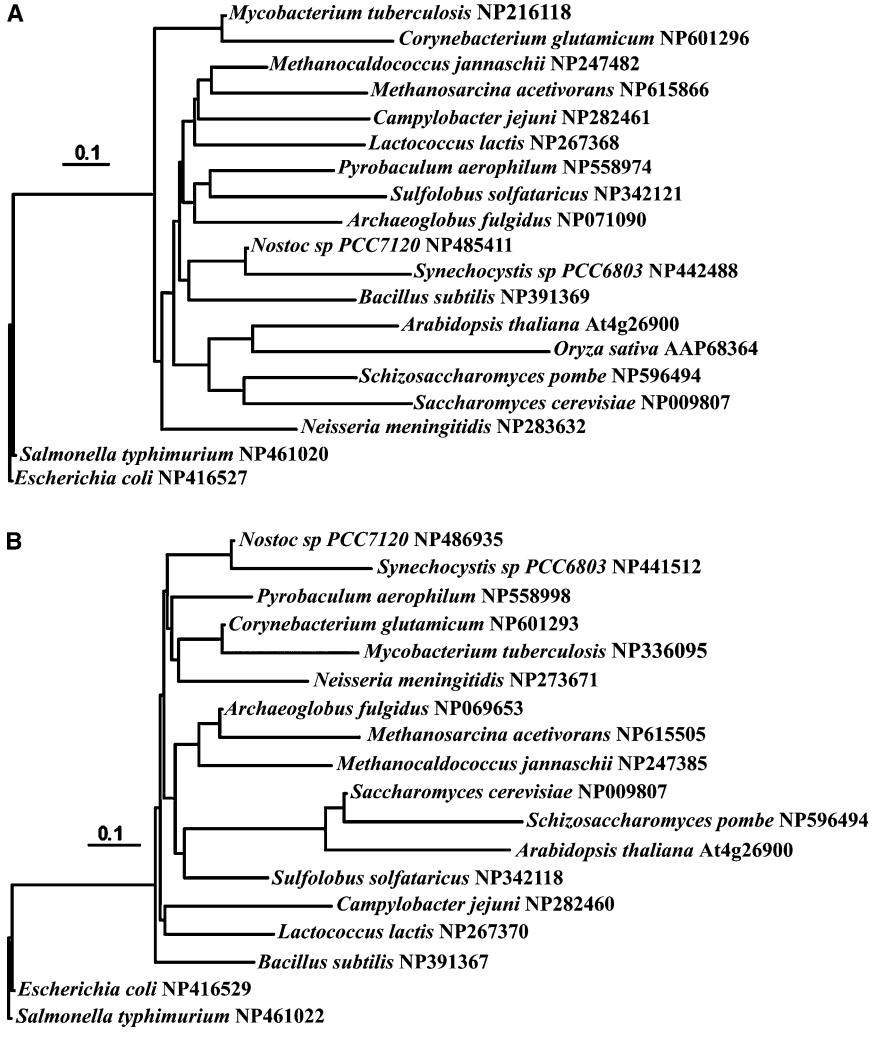

Fig. 8. Phylogenetic tree of HisHF orthologs. Phylogenetic analysis of protein sequences of the corresponding archaeal, eubacterial HisH (**A**) and HisF (**B**) protein sequences as well as plant HISN4 and yeast HIS7 orthologs were analyzed as explained for Fig. 5. Taxa are represented as follows: actinobacteria (*M. tuberculosis* and *C. glutamicum*), archaea (*M. jannaschii*, *M. acetivorans*, *P. aerophilum*, *S. solfataricus*, *A. fulgidus*), cyanobacteria (*Synchocystis* sp. and *Nostoc* sp.), bacteria and proteobacteria (*C. jejuni*, *L. lactis*, *B. subtilis*, *N. meningitidis*, *S. typhimurium*, *E. coli*), Viridiplantae (*A. thaliana* and *O. sativa*), Eukaryota (*S. pombe* and *S. cerevisiae*)

counterparts indicated that the HIS7/HISN4 sequences have a conserved region absent in either the prokaryotic *hisH* or *hisF* genes. This region encodes a linker peptide that separates the amidotransferase and cyclase domains (Brilli and Fani, 2004a).

With the aim of determining the evolutionary origin of plant HISN4, two neighbor-joining trees were constructed in which the eukaryotic HIS7/HISN4 orthologs were separately compared with the prokaryotic HisH and HisF enzymes (Fig. 8A, B). The analysis was not particularly helpful in determining the origin of the eukaryotic HisH domain of the HISN4 because it appears to be equally distant to a range of representative prokaryotic HisH orthologs. In contrast, the HisF domain of eukaryotic HIS7/HISN4 formed a subgroup clustered with the archaeal HisF enzymes, suggesting closer lineage between these two groups (Fig. 8B).

A gene that is distantly related to the HisH-type amidotransferases exists on chromosome 5 (locus At5g60540). The enzyme derived from this gene shows 26% identity with *E. coli* HisH and 20% identity with the amino-terminal domain of AtHISN4. Whether that gene encodes a separate glutamine amidotransferase involved in histidine biosynthesis is possible but uncertain. One reason is the discrepancy in the predicted subcellular localization of the protein sequence of At5g60540 predicted with PSORT and TargetP suggesting that it is localized within mitochondria, whereas the plant histidine biosynthesis pathway is most likely localized within chloroplasts. In addition, At5g60540 shows slightly better homology with a class of glutamine amidotransferases found in bacteria and fungi that are required for an as yet uncharacterized step in pyridoxine (vitamin B6) biosynthesis (Galperin and Koonin, 1997; Ehrenshaft and Daub, 2001). For example, the amino acid sequence of At5g60540 shows 33% identity with PDX2 of the fungus *Cercospora nicotianae*, the first member of this class to have been characterized (Ehrenshaft and Daub, 2001). Therefore, pyridoxine biosynthesis must also be considered as a candidate function for At5g60540.

IGP dehydratase

IGPD is a monofunctional enzyme in yeasts, encoded by the *HIS3* gene. In most, but not all prokaryotes it is part of a bifunctional enzyme along with histidinol phosphatase encoded by the *hisB* gene (Chariotti et al., 1986; Staples and Houston, 1979; Brilli and Fanni, 2004b). In plants, IGPD activity was first detected in crude extracts from shoots of barley, oat, and pea (Wiater et al., 1971). Later, IGPD was purified to apparent homogeneity from wheat germ as a protein having a molecular mass of 600 to 670 kDa and composed of 24 identical subunits (Mano et al., 1993). Detailed biochemical analysis showed that the wheat IGPD protein, like the yeast enzyme, is a monofunctional polypeptide lacking histidinol phosphatase activity. In addition, the wheat enzyme shares many biochemical properties with related IGP dehydratases, including similar K_m towards IGP, sensitivity to the inhibitor aminotriazole, and a catalytic requirement for Mn^{2+} and a disulfide-reducing agent (2-mercaptoethanol [Mano et al., 1993]). Mn^{2+} was found to be necessary for assembly of subunits into a catalytically active holoenzyme (Tada et al., 1995). However, the role of the reducing agent is enigmatic since wheat IGPD lacks a Cys residue.

cDNAs encoding IGPD were isolated from *Arabidopsis* (AtHISN5) and *Triticum aestivum* (wheat HISN5) by complementing an *E. coli* mutant carrying a *hisB* allele lacking only IGP dehydratase activity, but retaining histidinol phosphatase activity (Tada et al., 1994, 1995). Analysis of the *Arabidopsis* genome revealed the presence of two *HISN5* genes located on chromosome 3 (locus At3g22425) and chromosome 4 (locus At4g14910) encoding HISN5A and HISN5B proteins, respectively. Comparison of the two members of this family from *Arabidopsis* revealed that they show 82% amino acid identity, whereas the protein sequence derived from the wheat HISN5 cDNA (accession nr. U02690) was 87% and 86% identical to AtHISN5A and AtHISN5B, respectively. Antibodies against wheat HISN5, derived from expression of the cloned cDNA in a baculovirus–insect cell system, was found to cross-react with two bands of different molecular masses in wheat germ extracts, raising the possibility that this species might also contain 2 isoforms of HISN5 (Tada et al., 1995). Another plant HISN5 cDNA was isolated from *T. goesingense* (accession nr. AF023140) by its ability to functionally complement an *E. coli hisB* mutant (Persans et al., 1999). A single rice HISN5 ortholog has been located on chromosome 4 (accession nr. XP_473903) corresponding to locus Os04g52710. With the aim of tracing the evolutionary relationships of plant HISN5, a phylogenetic analysis was carried out. The resulting neighbor-joining tree (Fig. 9) shows the evolutionary divergence of the eukaryotic IGPD enzymes, since yeast HIS3 proteins clustered together with prokaryotic counterparts from archaeal origin, four plant HISN5 sequences formed a coherent cluster clearly separated from archaeal and bacterial HisB sequences, and the partial wheat HISN5 protein shared an evolutionary lineage with hisB orthologs from members of the cyanobacteria.

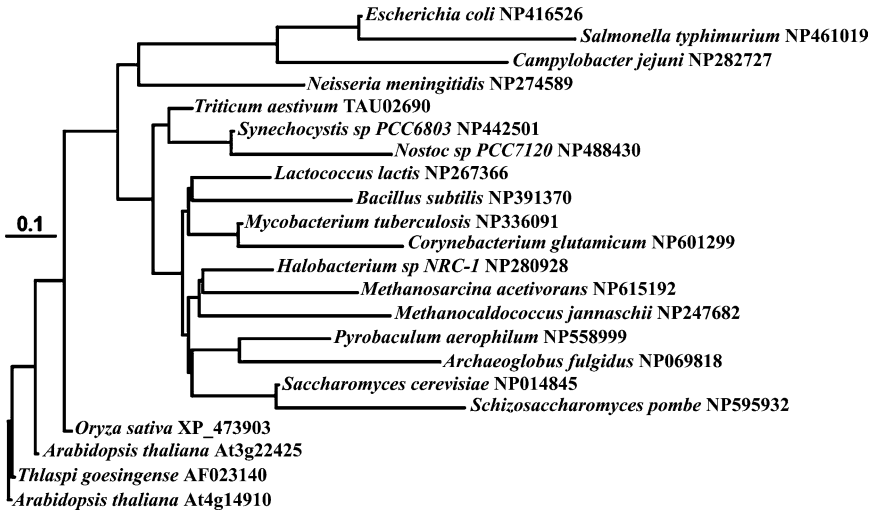

Fig. 9. Phylogenetic tree of IGPD orthologs. Phylogenetic analysis of the IGPD moiety from divergent organisms was carried out as described for Fig. 5. Taxa are represented as follows: bacteria and proteobacteria (*E. coli* to *N. meningitidis*, *L. lactis*, *B. sabtilis*), Viridiplantae (*T. aestivum* and *O. sativa* to *A. thaliana*), cyanobacteria (*Synchocystis* sp. and *Nostoc* sp.); actinobacteria (*M. tuberculosis* and *C. glutamicum*), archaea (*Halobacterium* sp. to *A. fulgidus*), Eukaryota (*S. pombe* and *S. cerevisiae*)

Histidinol phosphate aminotransferase

HPA is a monfunctional enzyme encoded in *E. coli* by *hisC* and in yeasts by *HIS5*. The first plant cDNAs encoding HISN6 were isolated from *Nicotiana tabacum* (Y09204 [El Malki et al., 1998]) and *Nicotiana plumbaginifolia* (AJ278767 [El Malki and Jacobs, 2001]). Southern blot analysis of these *Nicotiana* species revealed the existence of at least two homologous genes in each. The calculated molecular mass of the HPA from *Nicotiana tabacum* was around 45 kDa representing the precursor form of the protein containing an N-terminal transit peptide for

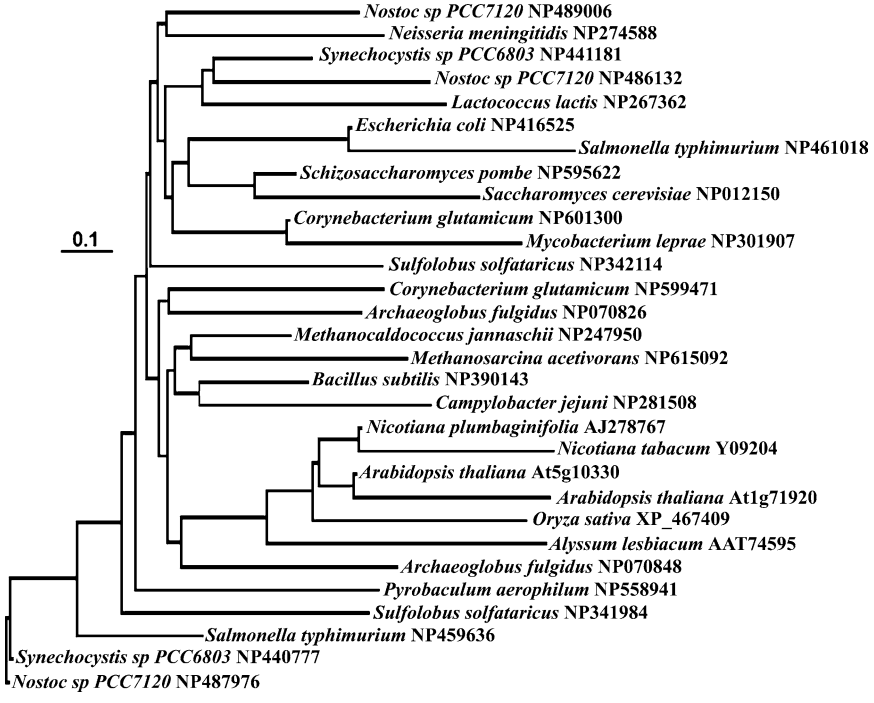

Fig. 10. Phylogenetic analysis of HisC orthologs. Queries chosen from the Clusters of Orthologous Groups (COG) database (http://www.ncbi.nlm.nih.gov/COG/). Some eubacterial and archaeal genomes include a number of HisC orthologs, such as *Nostoc* sp. PCC 7120, *Synechocystis* sp. PCC 6803, *Salmonella tiphmurium*, *Corynebacterium glutamicum*, *Sulfolobus solfataricus* and *Archaeoglobus fulgidus.* Taxa are represented as follows: cyanobacteria (*Synchocystis* sp. and *Nostoc* sp.), bacteria and proteobacteria (*N. meningitidis*, *L. lactis*, *E. coli*, *S. typhimurium*, *B. subtilis*, *C. jejuni*), Eukaryota (*S. pombe* and *S. cerevisiae*), actinobacteria (*M. tuberculosis* and *C. glutamicum*), archaea (*S. solfataricus*, *A. fulgidus*, *M. jannaschii* and *M. acetivorans*), Viridiplantae (*N. plumbaginifolia* to *A. lesbiacum*)

chloroplast localization. It is interesting to note that the yeast HIS5 protein lacking a transit peptide was able to complement an HPA-deficient, histidine auxotrophic mutant haploid cell culture of *N. plumbaginifolia* (El Malki et al., 1998). This finding is remarkable because it suggests that HPA need not be localized within the plastids of plants in order to function in histidine biosynthesis.

Two genes (*HISN6A* and *HISN6B*) encoding HPA can be readily identified in the *Arabidopsis* genome on chromosomes 1 and 5 on the basis of sequence similarity with homologs from microorganisms (loci At1g71920 and At5g10330). Putative HISN6 homologs also exist in rice encoded by locus Os02g47940 on chromosome 2 (accession nr. XP_467409) and in *A. lesbiacum* (accession nr. AAT74595). Phylogenetic analysis of eukaryotic HPA orthologs revealed the divergence of the yeast HIS5 enzymes clustered with bacterial HisC orthologs and the plant counterparts that seemed to share more close relationship with *Archaeoglobus fulgidus* HisC and other representatives of the Archaea (Fig. 10).

Histidinol phosphate phosphatase

Hol-P is encoded by the *HIS2* gene in yeasts and by the amino-terminal domain of *hisB* in *E. coli*. Hol-P phosphatases fall into two superfamilies that do not share any discernable sequence homology. The first is represented by the orthologous protein group COG0241, which includes the amino-terminal domain of HisB. The second lineage is represented by COG1387 and includes the yeast HIS2 and related monofunctional prokaryotic enzymes such as *Bacillus subtilis* HisJ (Le Coq et al., 1999; Brilli and Fani, 2004b). The *Arabidopsis* genome does not contain genes encoding proteins that are closely related to either of these two COGs. The closest relative is At3g14890, annotated as a phosphoesterase, which shows weak homology (E Value of 8^{-24}) with the most distant representative of COG1387, corresponding to a polynucleotide phosphatase from *Schizosaccharomyces pombe* (locus tag SPAC23C11.04c). The protein localization prediction programs TargetP and LOCtree predict that the At3g14890 protein may be chloroplast localized, suggesting that At3g14890 should be explored experimentally for involvement in histidine biosynthesis.

Histidinol dehydrogenase

HDH is encoded by *hisD* in many prokaryotes and by a domain of a trifuctional enzyme, along with PRA-PH and PRA-CH in *S. cerevisiae* known as HIS4. The occurrence of a monofunctional plant HDH was demonstrated first by Nagai and Scheidegger (1991), who purified the enzyme to homogeneity from cabbage (*Brassica oleracea*). Later, Nagai et al. (1991) reported the isolation of the corresponding cDNA (HISN8, accession nr. M60466). A single gene encoding HDH exists in *Arabidopsis* on chromosome 5 (*AtHISN8*, At5g63890) and an orthologous gene exists in rice on chromosome 1 corresponding to locus Os01g13190. Comparison of the *Arabidopsis* and rice HISN8 (accession nr. BAD81372) protein sequences revealed that they are 79% identical. Two partial mRNAs with homology to HISN8 were cloned from *A. lesbiacum* (AY570535) and *T. goesingense* (AF023141 [Persans et al., 1998]). Phylogenetic analysis of HDH orthologs from archaeal, bacterial, and eukaryotic origins is shown in Fig. 11. Plant HISN8 orthologs share an evolutionary relationship with the HDH domain of HIS4 protein of yeast. There was no clear evolutionary relationship of plant HISN8 with cyanobacterial HisD. Thus, despite the organizational similarity between plant and bacterial HDH (both are monofunctional), the plant enzyme is more closely aligned with eukaryotic bifunctional enzymes.

Detailed kinetic studies of the recombinant cabbage HDH revealed that the enzyme oxidizes L-histidinol to L-histidine via two sequential reactions following a Bi

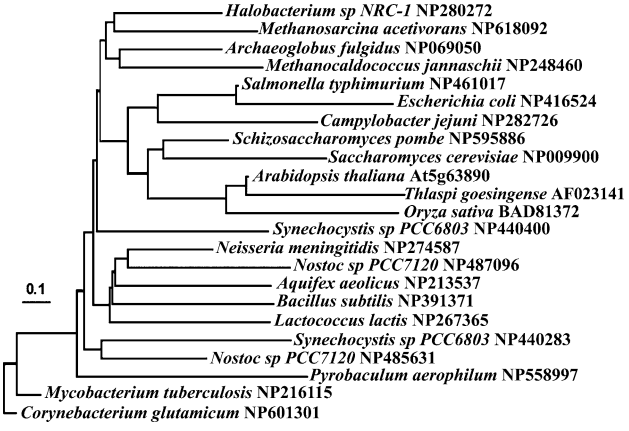

Fig. 11. Phylogenetic analysis of HisD orthologs. Phylogenetic analysis of HisD protein sequences from the divergent organisms, including Eubacteria, Archaea and Eukaryota was carried out as described for Fig. 5. Genomes of *Nostoc* sp. PCC 7120 and *Synechocystis* sp. PCC 6803 are represented by two HisD protein sequences. Taxa are represented as follows: archaea (*Halobacterium* sp. to *M. jannaschii*, *P. aerophilum*), bacteria and proteobacteria (*S. typhimurium* to *C. jejuni*, *N. meningitidis*, *A. aeolicus* to *L. lactis*), Eukaryota (*S. pombe* and *S. cerevisiae*), Viridiplantae (*A. thaliana* to *O. sativa*), cyanobacteria (*Synchocystis* sp. and *Nostoc* sp.), actinobacteria (*M. tuberculosis* and *C. glutamicum*)

Uni Uni Bi Ping Pong mechanism as was established for the corresponding *S. typhimurium* enzyme (Nagai et al., 1992). According to this scheme, binding of histidinol to HDH leads to subsequent NAD^+ binding and release of NADH from the enzyme before the second NAD^+ is bound. Moreover, the inhibition constant for NADH on the oxidation of histidinol to histidinaldehyde was higher than that of the oxidation of histidinaldehyde to histidine and the rate constant value of the histidinaldehyde oxidation to histidine was higher than the oxidation of histidinol to histidinaldehyde. These observations suggest that histidinaldehyde oxidation could be the rate-determining step for the cabbage HDH enzyme. This was not the case for HDH of *Salmonella typhimurium*. Since the calculated dissociation constant for the HDH–histidinaldehyde complex is much smaller than that of HDH–histidinol, it can explain the failure to detect free histidinaldehyde during the reaction (Kheirolomoon et al., 1994). Despite its plastid compartmentalization, the cabbage HDH enzyme shows an unusual specificity toward NAD^+ rather than $NADP^+$ (Nagai et al., 1992).

Regulation of the histidine pathway

From studies with microorganisms it is known that histidine biosynthesis is integrated with multiple metabolic pathways. The metabolic interconnections suggest that the pathway is very likely exquisitely controlled. In support of this idea, multiple regulatory mechanisms operating at the levels of both gene expression and enzyme regulation have been reported to control the histidine pathway in bacteria and lower eukaryotes (Winkler, 1987; Alifano et al., 1996; Hinnebusch, 1992; Tice-Baldwin et al., 1989). In plants, our knowledge of the regulatory mechanisms that control the rate of histidine biosynthesis is still very limited. However, a few isolated studies indicate that plants may be similar to microorganisms in exerting tight control over histidine biosynthesis and that some of the regulation mechanisms are shared with those operating in microorganisms and lower eukaryotes.

Regulation of histidine biosynthesis at the enzyme level in plants

In bacteria and yeasts, ATP-PRT is feedback inhibited by L-histidine. The same regulation mechanism appears to operate in plants. Thus, the ATP-PRT from pea was found to be inhibited by L-histidine, but it was much less sensitive than the bacterial enzyme (Wiater et al., 1971). Ohta et al. (2000) found that the *Arabidopsis* ATP-PRT isoenzymes were both inhibited by L-histidine. The IC_{50} of L-histidine for AtHISN1A was 40 μM, whereas that for AtHISN1B was 320 μM, suggesting that these isoenzymes have diverged regulatory functions. ATP-PRT1 is much more likely to be regulated under physiological conditions than is ATP-PRT2. The IC_{50}s of L-histidine observed with AtATP-PRT1 were in the same range as those reported with the microbial enzymes (Ohta et al., 2000).

In addition to histidine, two molecules, PRPP and ATP, whose levels are likely linked to the cellular energetic and metabolic state, stimulate the activity of the bacterial ATP-PRT (Alifano et al., 1996). The apparent K_m values of the two purified *Arabidopsis* ATP-PRTs for PRPP and ATP were comparable to the kinetic constants for ATP-PRT from other species (Ohta et al., 2000). However, further investigations are required to determine whether these two compounds play a role in regulating histidine biosynthesis in plants.

Regulation of histidine biosynthesis at the gene expression level in plants

A survey of the steady-state mRNA levels of the *Arabidopsis HISN1*, *HISN2*, and *HISN3* genes revealed no evidence for developmental or organ-specific expression. The tobacco *HISN6* gene also appeared to be constitutively expressed in green tissues, especially in the seedling, and in leaves and flowers (El Malki et al., 1998). These results suggest that histidine is synthesized and supplied throughout the plant (Fujimori and Ohta, 1998a, b; Tada et al., 1994). Future studies must focus on more detailed expression studies, especially for histidine biosynthesis enzymes accounted for by more than one gene, to determine whether spatial and temporal regulation occurs.

The steady-state pool of free histidine appeared to correlate with the level of *HISN1* gene expression. Thus, a comparison of total HISN1 transcript levels in tissues of the *Arabidopsis* and three *Alyssum* species using an internal fragment of the AtHISN1B cDNA revealed transcript abundance in roots to be in the order *A. lesbiacum* $\gg$ *A. serpyllifolium* > *A. montanum* > *A. thaliana*, which correlated closely with the size of the steady-state pool of root free histidine (Ingle et al., 2005). Moreover, overexpression of an *A. lesbiacum* HISN1B cDNA in transgenic *Arabidopsis* plants boosted the pool of endogenous free histidine up to 15-fold, providing experimental support for the tight correlation between the high level of *HISN1* gene expression and histidine pool size (Ingle et al., 2005).

It was recently shown that inhibition of the histidine pathway leads to changes in expression of genes involved

in histidine biosynthesis. In *N. plumbaginifolia*, a histidine auxotrophic mutant cell line transferred to histidine-free medium resulted in the induction of *HISN6* expression (El Malki and Jacobs, 2001). Similarly, inhibition of IGPD enzyme in *Arabidopsis* using the experimental herbicide IRL 1803 also resulted in significant induction of HISN8 and AIRS mRNA expression (Guyer et al., 1995). The latter is involved in the imidazole ring-closing step of de novo purine biosynthesis, the pathway that shares a known metabolic link with histidine biosynthesis.

In addition to the effect on genes involved in histidine and purine biosynthesis pathways, histidine starvation was found to increase the expression of eight genes from metabolically unrelated amino acid pathways leading to synthesis of lysine, tyrosine, and phenylalanine (Guyer et al., 1995). In addition, the free pools of some but not all amino acids, such as alanine, aspartate, glutamate, phenylalanine, proline, threonine, tryptophan, tyrosine, and valine also increased between 1.5- and 2-fold in response to inhibition of histidine biosynthesis (Guyer et al., 1995). These observations imply that plants may have a gene regulation mechanism resembling the general control response in *S. cerevisiae*.

General control is mediated by GCN4, a B-ZIP transcription factor that up-regulates at least 539 genes in every amino acid biosynthesis pathway except cysteine and genes encoding amino acid precursors, vitamin biosynthetic enzymes, peroxosomal components, mitochondrial carrier proteins, and autophagy proteins (Natarajan et al., 2001). Analysis of the *Arabidopsis* genome sequence revealed that although B-ZIP-type transcription factors exist, there are no genes with close sequence homology to yeast GCN4. It has been reported that the maize Opaque2 protein was able to bind to the promoters of GCN4-activated yeast genes and to complement a yeast *GCN4* mutant (Mauri et al., 1993). In plants, GCN4-like binding sites have been identified in a series of gene promoters that encode not only enzymes of amino acid metabolism but also storage proteins and enzymes from amino acid metabolism pathways that are unrelated to histidine (Muler and Knudsen, 1993; Ghislain et al., 1994; Albani et al., 1997). Promoter analysis of the *Arabidopsis HISN1*, *HISN2*, *HISN3*, *HISN4*, *HISN5*, and *HISN6A* genes using the PlantCARE database (Lescot et al., 2002) revealed the presence of multiple putative GCN4-responsive elements in their 5′ untranslated regions. (Approximately 2 kb of 5′-untranslated genomic regions upstream of ATG of the corresponding histidine genes were screened for putative GCN4 regulatory elements using PlantCARE Web program [Lescot et al., 2002; http://intra.psb.ugent.be:8080/PlantCARE/].) Moreover, most of these promoter regions contain putative AACA-core sequences, known to be closely associated with the GCN4 motif. The presence of the GCN4 promoter element in all *Arabidopsis* histidine biosynthesis genes could suggest a possible coordinated regulation of the pathway by GCN4 as well as together with other unrelated amino acid metabolism pathways.

In contrast to the effect of histidine starvation, excess of histidine as a result of constitutive expression of the *A. lesbiacum HISN1B* gene in *Arabidopsis* had little or no effect on the concentrations of any other amino acids in this species (Ingle et al., 2005). A correlation between histidine and the concentrations of eight other minor amino acids has been shown in potato shoot (*Solanum tuberosum*), while in wheat (*Triticum aestivum*) no such correlation was apparent (Noctor et al., 2002). Taking into consideration all the accumulated evidences to date, the question of the extent to which histidine biosynthesis is generally coordinated with other amino acid metabolism in plants remains to be resolved.

Role of histidine in metal ion homeostasis in plants

Several lines of investigation have recently highlighted the role of histidine in chelating nickel ions (Ni^{2+}) in several nickel-hyperaccumulating plant species (Krämer et al., 1996; Liao et al., 2000; Kerkeb and Krämer, 2003). Thus, high cellular histidine levels in roots of several nickel hyperaccumulators in the genus *Alyssum* have been reported to correlate with nickel tolerance (Krämer et al., 1996). Since, histidine is an effective chelator of nickel at cytoplasmic pH, Dawson et al. (1986) proposed that histidine may function as a chelator of Ni^{2+} during transport and/or storage in the *Alyssum* Ni hyperaccumulators. In support of this idea, a large increase in the concentration of free histidine in the xylem as well as the presence of putative [Ni^{2+}–His] complexes in the xylem sap, root, and shoot tissue of *A. lesbiacum* were observed in response to nickel exposure (Krämer et al., 1996).

The first direct evidence for involvement of histidine in nickel chelation and transport was reported by Kerkeb and Krämer (2003), who found that treatment of nonaccumulator species *Brassica juncea* L. cv. Vitasso and *Alyssum montanum* L. with histidine increased nickel accumulation. The results obtained in that study made it clear that Ni tolerance, Ni loading into xylem as well as Ni accumulation in plant shoot could be induced by increasing the pool

of exogenous free histidine (Kerkeb and Krämer, 2003). The significance of the positive correlation between histidine level and nickel tolerance have been recently confirmed by Wycisk et al. (2004), who generated transgenic *Arabidopsis* plants engineered to express a feedback-insensitive form of the *S. typhimurium* HisG. The overexpression of *S. typhimurium* HisG increased the endogenous pool of free histidine and resulted in enhanced tolerance of the transgenic *Arabidopsis* plants to Ni^{2+} ions (Wycisk et al., 2004). Overexpression of *HISN1B* from the nickel hyperaccumulator species *A. lesbiacun* in *Arabidopsis* increased the endogenous concentration of free histidine and also elevated the tolerance to nickel, strongly suggesting that a high capacity for histidine biosynthesis is a major determinant of nickel tolerance (Ingle et al., 2005).

Compared to other known low-molecular-weight metal chelators such as phytochelatins and nicotianamine, histidine is of relatively low metabolic cost. Histidine biosynthesis does not involve the assimilation of sulfate as is required for the biosynthesis of phytochelatins, and it contains six C and three N atoms compared with nicotinamine (12 C and 3 N) or phytochelatins (approximately 18 or 36 C, 5 or 10 N, and 2 or 4 S). However, not all nickel-hyperaccumulating plant species use histidine as a major chelator of Ni^{2+} ions. In a *Thlaspi* nickel hyperaccumulator species, exposure to Ni^{2+} failed to induce histidine accumulation in root, xylem sap, and shoot tissue and did not affect the expression of genes of the histidine biosynthesis pathway, suggesting that nickel exposure did not regulate histidine biosynthesis either at transcriptional or posttranscriptional levels (Persans et al., 1999). However, the study found that histidine could be involved in general nickel transport in *Thlaspi* species since treatment with D–His reduced the accumulation of Ni in shoots of the nonaccumulator *T. arvense*, suggesting that the [D–His–Ni] complex may compete with endogenous [L–His–Ni] complex for transport to the shoot (Persans et al., 1999).

Histidine was also found to act as chelator of zinc (Zn^{2+}) ions. In such a complex, zinc is coordinated by the neutral ring and amino nitrogen of histidine and by the weakly interacting negative carboxyl oxygen to form [Zn–$(His)_2$]. By the noninvasive technique of X-ray absorption spectroscopy, putative [Zn–$(His)_2$] complexes have been observed in the roots of the Zn hyperaccumulator *Thlaspi caerulescens* (Salt et al., 1999). The accumulation of Zn in *T. caerulescens* requires its transport across the cytoplasm of root cells and translocation in the xylem to the shoots. Conceptually, the histidine is an ideal chelator of zinc under the pH conditions that exist in the cytoplasm because of the high stability constant of [Zn–$(His)_2$] complex and the protonation constants of histidine. However, at the low pH that exists in the xylem sap, the imidazole nitrogen of histidine would become protonated, which would result in dissociation of the [Zn–$(His)_2$] complexes, favoring the transportation of Zn by organic acids including citrate.

Recently, histidine has been reported to act as a copper (Cu) carrier in the xylem sap of chicory (*Cichorium intybus* L. cv. Grasslands Puna) and tomato (*Lycopersicon esculentum* Mill cv. Rondy) plants (Liao et al., 2000). Moreover, exposure to Cu resulted in a significant proportional increase in histidine concentration in xylem sap of chicory and tomato plants, indicating that increased external Cu concentration may induce synthesis of histidine (Liao et al., 2000).

The sum of these findings point out the intriguing possibility that besides being a proteogenic amino acid, histidine also plays an important role in metal ion homeostasis in plants. A similar function of histidine has been reported in microorganisms and mammals. Thus, yeast histidine auxotrophs are far more sensitive to a range of metal ions as compared to wild type (Pearse and Sherman, 1999) and a [Ni–His] complex also seems to be involved in nickel detoxification in *S. cerevisiae* (Joho et al., 1990). Furthermore, in animals, histidine is known to stimulate the absorption of zinc (Wapnir et al., 1983).

Role of histidine in plant growth and development

It has been reported that histidine may be essential for embryo development. DeFraia and Leustek (2004) recently demonstrated that a TDNA insertion mutant of *AtHISN3* resulted in embryo lethality. The aborted mutant embryos could be rescued by histidine feeding, suggesting that the phenotype resulted from the inability of the embryo to synthesize histidine. The knockout mutation was demonstrated to be recessive and heterozygous individuals were completely normal. A little bit later, the essential role of BBMII isomerase in embryo development in *Arabidopsis* was confirmed by Noutoshi et al. (2005). The significance of this result is that the *AtHISN3* mutant embryos appear to be unable to obtain histidine from the heterozygous parent plant that is phenotypically normal and fully capable of histidine biosynthesis. Thus, the result indicates that embryos must be prototrophic for histidine, in contrast to other amino acids, which are known to be transported to embryos from the parent.

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