Evolutionary Diversification in Polyamine Biosynthesis

Eugenio G. Minguet,* Francisco Vera-Sirera,* Alberto Marina,† Juan Carbonell,* and Miguel A. Blázquez*

*Instituto de Biología Molecular y Celular de Plantas (UPV-Consejo Superior de Investigaciones Científicas), Universidad Politécnica de Valencia, Valencia, Spain; †Departamento de Genómica y Proteómica, Instituto de Biomedicina de Valencia (Consejo Superior de Investigaciones Científicas) and CIBERER, Valencia, Spain

Polyamine biosynthesis is an ancient metabolic pathway present in all organisms. Aminopropyltransferases are key enzymes that mediate the synthesis of spermidine, spermine, and thermospermine. The relatively high sequence similarity between aminopropyltransferases and their similarity with putrescine N-methyltransferases (PMT) raises the question of whether they share a common ancestor or have evolved by convergence. Here we show that aminopropyltransferases and PMT are phylogenetically interconnected, and the different activities have been generated by unusually frequent events of diversification of existing functions. Although all spermidine synthases (SPDSs) derive from a common ancestor preceding the separation between prokaryotes and eukaryotes, they have been the origin of a variety of new activities. Among those, spermine synthases (SPMSs) represent a novelty independently arisen at least 3 times, in animals, fungi, and plants. The most parsimonious mechanism would involve the duplication and change of function of preexisting SPDS genes in each phylum. Although spermine is not essential for life, the repeated invention of SPMS and its conservation strongly argues for an evolutionary advantage derived from its presence. Moreover, the appearance of thermospermine synthase (tSPMS) in several genera of Archaea and Bacteria was accompanied by a loss of SPDS, suggesting that the new activity originated as a change of function of this enzyme. Surprisingly, tSPMS was later acquired by plants at an early stage of evolution by horizontal gene transfer and has proven to be essential for vascular development in tracheophytes. Finally, the synthesis of nicotine and tropane alkaloids in Solanales was favored by the origination of a new activity, PMT, as a duplication and change of function from SPDS.

Introduction

Polyamines are small positively charged aliphatic molecules with primary and secondary amines, widely present in all organisms (Tabor CW and Tabor T 1984; Galston and Sawhney 1990; Igarashi and Kashiwagi 2000; Wallace et al. 2003; Kuehn and Phillips 2005). Depending on the species, the relative proportions of the different compounds vary, and they can reach high concentrations, in the millimolar range. The most common polyamines in eukaryotes are the diamine putrescine, the triamine spermidine, and the tetraamine spermine. Although prokaryotes do not seem to have spermine synthase (SPMS) activity, several Archaea and Bacteria can synthesize a different tetraamine named thermospermine, whose presence has also been detected in lower eukaryotes and plants (Oshima 1979; Hamana et al. 1991, 1994; Kroger et al. 2000; Knott et al. 2007).

The importance of polyamines in physiology is illustrated by the changes in polyamine concentration that accompany certain developmental transitions or exposure to stress conditions, the alterations in development and the protection against stress that are triggered by exogenous application of polyamines, and the phenotype caused by loss-of-function mutations in polyamine metabolism genes. In fact, putrescine and spermidine synthesis has been shown to be essential for life in all organisms evaluated (Hamasaki-Katagiri et al. 1997; Chattopadhyay et al. 2002; Imai et al. 2004; Urano et al. 2005).

Polyamines are synthesized in most organisms by a conserved pathway that is initiated by the decarboxylation of ornithine, or arginine in the case of Bacteria and plants, to eventually synthesize putrescine. Transfer of an aminopropyl group by spermidine synthase (SPDS) from decarboxylated S-adenosylmethionine (dcAdoMet) to putrescine renders spermidine. The activity of SPMS or thermospermine synthase (tSPMS) incorporates a new aminopropyl group to synthesize the spermine or thermospermine, respectively (Ikeguchi et al. 2006) (fig. 1). In addition, putrescine N-methyltransferases (PMTs) catalyze the methylation of putrescine with the donation of the methyl group from AdoMet, in a reaction that initiates the synthesis of several groups of alkaloids in plants (Ghosh 2000). All aminopropyltransferases (SPDS, SPMS, and tSPMS) and PMT share a high degree of sequence similarity including the putative active centers, although they display different specificity of substrate (Hashimoto et al. 1998; Teuber et al. 2007). This observation, together with the assumption that polyamine biosynthesis is an ancient metabolic pathway because it is present in all cellular organisms, raises the question of whether the different steps in polyamine biosynthesis are phylogenetically interconnected or can the similarity in sequence be explained by convergence due to functional constrains. Moreover, several hypotheses have been proposed to explain the evolution of metabolic pathways (Schmidt et al. 2003). In particular, the backward evolution model proposes that the selective pressure targets the production of the end product of a pathway, whereas the forward evolution model suggests that changes in a pathway (usually in secondary metabolism) result from gradual addition of steps. The aim of this work was to reconstruct the evolutionary pathways of genes involved in polyamine biosynthesis and provide plausible interpretations for the structural features that distinguish the different enzyme activities.

Key words: aminopropyltransferases, polyamine, putrescine, spermidine, spermine, evolution.

E-mail: mblazquez@ibmcp.upv.es.

doi:10.1093/molbev/msn161
Advance Access publication July 24, 2008

© The Author 2008. Published by Oxford University Press on behalf of the Society for Molecular Biology and Evolution. All rights reserved.
For permissions, please e-mail: journals.permissions@oxfordjournals.org

Materials and Methods

Sequence Data

Extensive Blast search was carried out in different databases (National Center for Biotechnology Information [http://www.ncbi.nlm.nih.gov]; TIGR [http://www.tigr.org];
MGI [http://www.noble.org/medicago/index.html]; and JGI [http://www.jgi.doe.gov]) using ACL5 (GI:18419941), SPMS (GI:23397200), and SPDS (GI:30688573 and GI:30698029) sequences from Arabidopsis thaliana; SPE4 (GI:6323175) from Saccharomyces cerevisiae; SPD (GI:1786312) from Escherichia coli; and SPM (GI:21264340) from Homo sapiens. When no unigene or complete coding sequences were found, expressed sequence tag (EST) information was used to reconstruct a contig containing the complete sequence.

Sequence Analysis

Amino acid sequences in Fasta format were analyzed with the CLANS program (Frickey and Lupas 2004) with default parameters (cutoff P value $1.0 \times 10^{-50}$, 110 rounds). For genomic exon–intron structure comparison, genomic sequences were searched in the corresponding databases. For exon–intron identification, manual alignment was performed between comparing genomic and cDNA sequences.

Amino acid sequence alignment was performed with ClustalX using default parameters (Thompson et al. 1997). The “ProtTest” program (Abascal et al. 2005) was used for amino acid model substitution selection for phylogenetic analysis: the Whelan and Goldman (WAG) model was the best choice, followed by the Jones, Taylor, and Thornton (JTT) model, and both of them were used in our studies. Alignments were also analyzed with the Gblock software to eliminate the less informative sites, and the block obtained was subject to bootstrap and phylogenetic analysis. For bootstrap generation, distance methods, and consensus tree, the PHYLIP package (version 3.66) was used (Felsenstein 2005). Maximum likelihood analyses were performed using “PHYML” program (version 2.4.4) (Guindon and Gascuel 2003). Tree visualization and manipulation was achieved with “TreeView” (Page 1996).

Experimental Determination of Aminopropyltransferase Activity

The full-length cDNA clone of the tomato SPMS gene was amplified from a tomato fruit cDNA library using the following oligos, both introducing a SalI restriction site (underlined) for cloning purposes. Oligo1: 5’-GG-GTCGACGGTTTGGACAGCCGGAGGAC-3’; Oligo2: 5’-GGGGTCGACTTGAGCTTACTCCAGCAGCA-3’). The full-length cDNA clone-#2 from Picea glauca was obtained through the Arborea Project databank (http://www.arborea.ulaval.ca). This clone was amplified by polymerase chain reaction with Oligo3 (5’-GCGTAATACGACTCACT-3’) and Oligo4 (5’-CACTAAAGGGAAACAAAG-3’). The amplified products were introduced into pCR2.1 (Invitrogen, San Diego, CA) and shuffled to the yeast expression vector pCM190 (Gari et al. 1997) after sequencing. The activity of the encoded enzyme was evaluated by complementation of the S. cerevisiae spe4 mutant (Hamasaki-Katagiri et al. 1998) grown in minimal medium. In all, 100-mg (wet weight) aliquots from yeast cultures were centrifuged, and the pellet was resuspended in 100 µl of 0.2 M perchloric acid containing 1,6-diaminohexane as an internal standard (0.5 µmol/g). Detection and quantification of polyamines was performed by high-performance liquid chromatography (HPLC) (Carbonell and Navarro 1989).

Modeling and Structural Analysis

The initial 3D structural model of the Nicotiana tabacum PMT (GI:6093754) was produced using the
MODWEB server (http://salilab.org/modweb) (Pieper et al. 2004) and the protein full-length sequence as template. Program default values were used in the calculation with the exception of the speed that was changed to “very low” value. The best model (e value = 2 × 10−31) was based in the Bacillus subtilis SPDS structure (PDB accession code 1Y9) and enclosed the residues 86–373 of N. tabacum PMT sequence, which is the stretch that showed a high sequence similarity with the A. thaliana SPDS sequence (Supplementary fig. S4, Supplementary Material online). The PMT model and the SPDS structure from A. thaliana (PDB accession code 2Q41) were superimposed using the LSQKAB program (CCP4 1994), and the AdoMet molecule in the active center of PMT was generated using as template the dcAdoMet molecule coming from the SPDS structure. To improve the initial PMT–AdoMet complex model, simulated annealing using torsion angle dynamics and energy minimization was applied as implemented in the program Crystallography and NMR System (Brunger et al. 1998). The quality of the final model was assessed using the PROCHECK suite of programs (Morris et al. 1992), revealing that 98.9% of the residues fall within the allowed (92.3%) or generously allowed (6.6%) regions of the Ramachandran plot. Only the residues F205, E277, and D329, which are located outside of the AdoMet binding site, fall in the disallowed region of the plot. In agreement with the correct geometry of the model, the internal bad contacts per 100 residues are reduced to 5.2, a usual value for structures that have been solved at atomic resolution (under 2 Å).

To evaluate the polyamine-binding sites of SPDS, SPMS, and tSPMS proteins, the structures of the tSPMS from Thermus thermophilus (PDB accession code: 1UIR) and the human SPDS and SPMS crystallized in presence of putrescine and spermine (PDB accession codes: 2006 and 2QFM), respectively, were superposed using the LSQKAB program as implemented in the CCP4 (1994) suite. Polyamine-binding analysis in the different active sites was carried out with the CCP4 suite and visualized with the graphic program COOT (Emsley and Cowtan 2004). The structural representations were generated with the PYMOL program (DeLano 2002).

Results and Discussion
High Sequence Similarity between All Polyamine Biosynthesis Enzymes

Two observations suggest that there may be a phylogenetic relationship between polyamine biosynthesis enzymes that use putrescine (SPDS and PMT) and spermidine (SPMS and tSPMS) as substrates. First, the reactions are enzymatically very similar and share an equivalent substrate (dcAdoMet for aminopropyltransferases and AdoMet for methyl transferases) (fig. 1), and second, the reported degree of similarity between the sequences of plant SPDS, SPMS, tSPMS, and PMT is high (Hashimoto et al. 1998; Paniot et al. 2002; Teuber et al. 2007). To challenge the hypothesis that these reactions are phylogenetically connected, we performed extensive iterative searches of public databases queried with representative sequences from each enzyme class and constructed a set of 127 sequences from Archaea, Bacteria, Metazoans, Fungi, and Plants. Several of these sequences were assembled from partial cDNA and EST sequences, and the final full-length sequences used in this study are shown in Supplementary file 1 (Supplementary Material online). As shown in the inset of figure 2, pairwise comparisons between all sequences produced a number of clusters based on Blast similarity score, when interpreted by CLANS (Frickey and Lupas 2004). This analysis confirmed the statistically significant similarity (P value < 1.0 × 10−50) between protein sequences corresponding to enzymes of the 4 different groups, with metazoan SPMS clustering in a more distant group. This result suggests that the functional similarity between all members is accompanied by sequence similarity across all kingdoms.

tSPMS Was Derived from SPDS and Horizontally Transferred from Prokaryotes to Plants

To determine if the sequence similarity between SPDS, PMT, SPMS, and tSPMS reflects convergence or indicates common ancestry, we aligned the full-length amino acid sequences using ClustalX (Supplementary fig. S1, Supplementary Material online) and produced a phylogenetic tree using the maximum likelihood method (for details, see Materials and Methods). Interestingly, trees obtained with different amino acid substitution models displayed equivalent topology as the distance tree shown in figure 2. The clear separation of defined clades that correspond to the different enzyme classes in the different phyla is supported by high bootstrap values in all cases. The only exceptions are a few Archaea and bacterial sequences, which are not resolved in a single clade. Remarkably, 2 of the 3 prokaryotic classes are paraphyletic with the rest of SPDS sequences in all organisms (fig. 2) and might therefore represent SPDS enzymes. However, a second group of sequences cluster with the plant tSPMS sequences and have probably been wrongly annotated as SPDS (fig. 2). This is the case of T. thermophilus, in which thermospermine is a very abundant polyamine, and, more specifically, the only tetraamine found in this one and related prokaryotes (Oshima 1979; Hamana et al. 2001). It is thus very likely that the prokaryotic sequences conforming this clade are the enzymes responsible for the synthesis of thermospermine, and they are not SPDS. The absence of SPDS activity in these prokaryotes is not surprising, given that, at least in T. thermophilus, spermidine is synthesized from N1-aminopropylagmatine and not from putrescine (Ohnuma et al. 2005). A subgroup of prokaryotic SPDS-related sequences, that contains a sequence of a cadaverine aminopropyltransferase from Pyrococcus furiosus (Cacciapuoti et al. 2007), is clearly separated from its paraphyletic SPDS, indicating that it can also be a group of sequences loosely annotated as SPDS (fig. 2).

More importantly, the close phylogenetic relationship between plant tSPMS sequences and prokaryotic “tSPMS-like” sequences and the absence of such sequences in animals and fungi suggests that either these organisms lost their respective ancestral sequences early in their lineage or plants acquired the ability to synthesize thermospermine by horizontal gene transfer from Archaea or Bacteria.
Although not very common, horizontal gene transfer from prokaryotes to plants has also been documented in other cases, such as for the origin of plant enolase (Keeling and Palmer 2001), glycerol transporters (Zardoya et al. 2002), and WHy protein domains (Ciccarelli and Bork 2005). Moreover, another set of reactions integral to polyamine biosynthesis, the route that synthesizes putrescine from arginine, has also been presumably acquired by plants from the cyanobacterial precursor of the chloroplast (Illingworth et al. 2003), but it is difficult to assess if both acquisitions
would have occurred in a single event or in successive periods during evolution. It is noteworthy that the function of tSPMS in plants must be very relevant for plant life because the ability to synthesize thermospermine it has remained unaltered in plants since the appearance of tSPMS. The A. thaliana acl5 mutant, devoid of tSPMS activity, shows severe defects in stem elongation and in the formation of the vasculature (Hanzawa et al. 2000; Knott et al. 2007), which suggests a role for thermospermine in the correct differentiation of vascular bundles in tracheophytes.

Independent Origin of SPMSs in Animals, Fungi, and Plants

The topology of the phylogenetic tree (fig. 2) indicates that animal SPDS and animal SPMS possess a common ancestor, which is different from the ancestors of plant and fungal SPMS. Indeed, the phylogenetic analysis indicates that fungal SPMS sequences are paraphyletic to fungal SPDS (fig. 2). The annotation of this group as fungal SPMS has been thoroughly confirmed by the availability of yeast mutants harboring knockouts for these genes (Hamasaki-Katagiri et al. 1997, 1998; Chattopadhyay et al. 2002), which leads to a scenario where fungal SPMS and SPDS derive from a common ancestor in fungi. A similar topology is observed in the plant SPDS clade, which also includes sequences annotated as SPMS. In this case, the only experimental indication that the proteins included in the SPMS group class of plant enzymes are SPMS and not SPDS had been originally reported only for the A. thaliana SPM1 protein (Panicot et al. 2002). However, 2 more members of this clade also display unequivocal SPMS activity: the SPM gene from Malus domestica (cultivated apple) has been reported to complement the S. cerevisiae spe4 mutant devoid of SPMS activity (Kitashiba et al. 2005); and we have also found that the Solanum lycopersicum (tomato) SPM gene encodes an SPMS, which is functional in yeast (Supplementary fig. S2, Supplementary Material online).

The hypothesis for a common ancestor to plant SPDS and plant SPMS enzymes can be extended to PMT, according to the position of the PMT clade in the phylogram (fig. 2), and the fact that these enzymes have so far been found only in Solanales. Furthermore, the exon–intron structure of genes encoding enzymes of these 3 groups has been conserved not only in closely related plant species but also between monocots and dicots (fig. 3A), which dates the
common ancestor to at least 130 MYA (Crane et al. 2002). With respect to the origin of these duplications, we have found evidence only for the most recent event in *A. thaliana*, which involves a large translocation within chromosome 1, as indicated by the presence of a paralogon including the 2 SPDS genes in this species (fig. 3B). An explanation for the common origin of SPDS, SPMS, and PMT is a series of recent events of duplication and change of function of an ancestral SPDS. This scenario would then represent an example of a metabolic pathway evolving in a forward direction (Granick 1957), as opposed to the more common cases of retroevolution (Horowitz 1945) or patchwork evolution (Jensen 1976). This latter hypothesis would be based in the existence of an ancestral enzyme endowed with broad catalytic specificity able to perform the transfer of both aminopropyl and methyl groups using dcAdoMet and AdoMet, respectively, a requirement which is rather difficult to meet given the structural requirements at the catalytic center for each reaction (see below, fig. 6).

A caveat in our phylogenetic analysis of plant SPMS is the lack of other than angiosperms full-length sequences that fall into this class. To know if SPMS had originated before the separation of angiosperms from the other plant groups, in addition to all the full-length sequences, we extended the search also for partial cDNA clones, with sequence similarity to the *A. thaliana* SPMS. Not surprisingly, we retrieved sequences annotated as putative SPMS, SPDS, and PMT, but the alignment and the phylograms constructed with these sequences (and a few sequences from other taxa, for reference) rendered a clearer view of the different functions of the retrieved sequences (fig. 4A). All plants (including Gymnosperms and the moss *Physcomitrella patens*) possess sequences clearly identifiable as SPDS or tSPMS, but SPMS sequences were found only in angiosperms. Only the ancient tracheophyte *Selaginella* and the gymnosperm *P. glauca* had 3 sequences in the databases, one of which (*Selaginella* #2 and *P. glauca* #2) could not be unambiguously assigned to any of the 3aminopropyltransferase classes (fig. 4A). However, expression of the *P. glauca* full-length cDNA clone #2 in yeast allowed complementation of the SPDS mutant spe3 but not of the SPMS mutant spe4, indicating that *P. glauca* possesses one extra SPDS but no SPMS enzyme.

Considering that most primitive organisms (Archaea and Bacteria) and plants other than angiosperms lack SPMS activity, it is unlikely that spermine synthesis could have arisen only once in evolution. This scenario would require the loss of SPMS genes in several phyla, and the topology of the phylogenetic tree is not compatible with this idea unless multiple losses of genes were invoked. Alternatively, the most parsimonious explanation would favor independent origins for animal, fungal, and plant SPMS enzymes (fig. 5).

Furthermore, 2 more recent duplications and changes of function from a plant SPDS gene are the cause for the appearance of a second SPMS isoenzyme and PMT activity in several plant families.

The repeated diversification of enzyme activities involved in polyamine metabolism, with its origin in ancestral SPDS proteins, prompts at least 2 questions: 1) does the function of spermine in the cells justify the creation and maintenance of spermine synthesis in multiple occasions during evolution? and 2) is there a mechanism that favors the modification of SPDS activity to change the specificity for its substrates?

There are multiple examples for an essential role of spermidine in growth and development in eukaryotes because the elimination of SPDS activity causes lethality in yeasts (Hamasaki-Katagiri et al. 1997; Chattopadhyay et al. 2002), protists (Roberts et al. 2001), and plants (Imai
et al. 2004). However, it is possible to obtain viable eukaryotes deficient in spermine biosynthesis, although they show different degrees of dysfunction. For instance, yeast mutants do not require exogenous spermine to live in minimal media (Hamasaki-Katagiri et al. 1998), mice lacking spermine show fertility and growth problems (Wang et al. 2004), mouse embryonic stem cells without SPMS are more sensitive to DNA damage–inducing agents (Korhonen et al. 2001), and spermine-deficient mutants in *A. thaliana* are viable although they are hypersensitive to drought and high salt stress (Yamaguchi et al. 2006, 2007). Therefore, spermine must play a very important role, albeit not essential, in eukaryotes, that justifies its conservation during evolution.

### Structural Features of Polyamine Biosynthesis Enzymes with Evolutionary Implications

Regarding the functional diversification that SPDS has suffered during evolution to give rise to SPMS and PMT activities in several occasions, a likely explanation might reside within the active site of these enzymes. To identify the relevant amino acids that could account for the diverse substrate specificity, we have compared the protein structures of the different enzymes. Among the solved structures present in public databases, we centered our analysis in SPDS and SPMS from *H. sapiens* (PDB codes: 2Q06, 2Q0L, and 2QFM), SPDS from *A. thaliana* (PDB code: 2Q41), and tSPMS from *T. thermophilus* (PDB code: 1UIR). Despite their evolutionary distance, they show a high degree of conformational similarity in the active center, which led us to assume that modeling would be a reasonable strategy to obtain 3-dimensional information of the more recently diverged PMT using SPDS structures as the template.

A comparison between the active centers of SPDS and PMT model revealed important changes affecting the active center and its interaction with AdoMet (fig. 6). Particularly, important seem the changes of D131 into I172 and Q107 into T148 in PMT, given that these residues introduce a negative charge in aminopropyltransferases, that would prevent AdoMet from fitting in the active center because of the carboxyl group that is missing in dcAdoMet (Korolev et al. 2002). On the contrary, the substitutions of V134 into T175 and L98 into H129 in PMT, changing hydrophobic by polar residues, seem to be needed for the binding and stabilization of the carboxyl group of AdoMet (fig. 6A). In fact, substitution of only the residue equivalent to I172 by D in a *Datura stramonium* PMT sequence did not change its activity into SPDS but eliminated any activity (Teuber et al. 2007).
The observation that the different catalytic properties of PMT and SPDS may reside in fairly discrete amino acid changes rendering mutually exclusive active centers, supports the view that the mechanism for the appearance of PMT activity is neofunctionalization from an ancestral SPDS rather than subfunctionalization of an ancestor with both catalytic activities (Ohno 1970; Force et al. 1999; He and Zhang 2005), and is in agreement with our phylogenetic analysis and the conservation of exon–intron structures (figs. 2 and 3).

In the case of SPMS and tSPMS, it is not easy to identify specific amino acid changes that explain the new activities putatively derived from SPDS ancestors. Nevertheless, the overall conformation of the channel that accommodates the polyamine substrate presents subtle structural differences in SPDS compared with SPMS and tSPMS that could significantly influence substrate specificity. For instance, direct comparison of the geometry of the active sites by overlapping the 3 corresponding structures (fig. 7A) shows the close proximity between the opposite walls that build the polyamine channel in SPDS (boxes 1 and 2), unlike in SPMS and tSPMS. Besides, the presence of box3 in SPDS would prevent the accommodation of a spermidine molecule in the polyamine channel. On the other hand, there are no obvious amino acid differences to explain why putrescine should not fit the active center in SPMS and tSPMS, compared with that of SPDS. The lack of crystal structures obtained from SPMS and tSPMS with their substrates precludes a definitive answer, but when putrescine and spermidine are modeled into the available structures, a few amino acid differences seem relevant. For instance, the D176 residue that stabilizes one of the amino groups of putrescine in SPDS (fig. 7B) is missing in SPMS (fig. 7C) and present but more distant from putrescine in tSPMS (fig. 7D). Given that putrescine has a smaller size and only 2 amine groups, it would not reach to establish the necessary interactions with the walls of the substrate channel in SPMS and tSPMS.

The conclusion that the change in activity needs to be based on the additive effect of subtle amino acid substitutions that nevertheless maintain the structure of the polyamine channel is compatible with the idea that, at some point in evolution, the ancestral and the novel activities might coexist in a single protein, which would be a bifunctional enzyme. Support for this hypothesis comes from the ability of some aminopropyltransferases to use a broader range of polyamine substrates, such as the cadaverine aminopropyltransferase sequence reported from *P. furiosus* that our phylogenetic analysis relates with prokaryotic SPDS (Cacciapuoti et al. 2007) (fig. 2).
Concluding Remarks

The polyamine biosynthetic pathway seems to have been subject to unusually frequent diversification events from a single enzyme already existing in early prokaryotes, which has allowed the independent appearance of novel enzyme activities several times in the course of evolution. Indeed, besides the evidence presented here, the origin of the synthesis of another polyamine used as precursor of alkaloids, homospermidine, has also been traced back to deoxyhypusine synthases (Ober et al. 2003), indicating that part of the alkaloid biosynthetic pathway has arisen thanks to the generation of novel activities based on diversification of polyamine-related enzymes. Besides, there are traces of a bacterial origin of the plant tSPMS acquired by horizontal gene transfer, which might have occurred in parallel to the acquisition of the enzymes required to synthesize putrescine from arginine (Illingworth et al. 2003). In the case of tSPMS, it is noteworthy that this enzyme has become an essential contributor to the proper differentiation of vascular tissues in plants (Hanzawa et al. 2000; Clay and Nelson 2005), and it illustrates the important impact of the new activities in the fitness of the organisms. The large variety of polyamine biosynthesis enzymes that have been generated from SPDS through only a few changes in the coding sequence makes this pathway a very suitable model for structure–function studies, the interest of which partly lies in the impact that this knowledge may have in targeted metabolic engineering.

Supplementary Material

Supplementary files 1 and 2, and figures S1–S4 are available at Molecular Biology Evolution online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

We thank Dr H. Dopazo (Centro de Investigaciones Príncipe Felipe, Valencia, Spain) for help with the use of bioinformatic tools and useful discussions; Dr F. Navarro-García (Universidad Complutense de Madrid, Madrid, Spain) for material and discussions; and Drs C. Ferrándiz, F. Madueño, and M. A. Pérez-Amador (IBMCP, Valencia, Spain) for critical comments on the manuscript. Work in the authors’ laboratory is supported by grants BIO2004-02355, BIO2007-60923, BIO2005-00153, and BIO2005-07156-C02-01 from the Spanish Ministry of Education and Science (MEC) and by the Generalitat Valenciana. M.A.B. is an EMBO Young Investigator. E.G.M. has been funded by Fellowships from the Spanish MEC and the Spanish National Research Council I3P Program.

Literature Cited


Claudia Schmidt-Dannert, Associate Editor

Accepted July 15, 2008