Chapter 2

Exploring Polyamine Biosynthetic Diversity Through Comparative and Functional Genomics

Anthony J. Michael

Abstract

The existence of multiple, alternative pathways for polyamine biosynthesis, and the presence of alternative polyamine structural analogs, is an indication of the physiological importance of polyamines and their long evolutionary history. Polyamine biosynthesis is modular: diamines are synthesized directly or indirectly from amino acids, and triamines are synthesized from diamines by transfer of aminopropyl, carboxyaminopropyl, or aminobutyl groups to the diamine. Diversification of polyamine biosynthesis has depended on gene duplication and functional divergence, on gene fusion, and on horizontal gene transfer. Four examples of polyamine biosynthetic diversification are presented here with a discussion of methodological and conceptual approaches for identification of new pathways.

Key words: Polyamine, Evolution, Biosynthetic diversity, Metabolic pathway, Operon, Comparative genomics, Functional genomics

1. Introduction

Most studies of polyamine metabolism have been motivated by biomedical considerations focused on preventing growth of cancer cells through disruption of polyamine homoeostasis (1). Although the cancer intervention strategy has turned out to be a far more frustrating endeavor than initially conceived, polyamine metabolism appears to be a promising target for intervention in parasite diseases, especially in kinetoplastidia such as trypanosomes and leishmania (2). Study of the regulation of polyamine metabolism in mammalian cells and in the budding yeast *Saccharomyces cerevisiae* has revealed an extraordinarily complex hierarchy of polyamine-responsive feedback systems based on posttranscriptional mechanisms (3). There is a wide evolutionary conservation of the

Anthony E. Pegg and Robert A. Casero, Jr. (eds.), *Polyamines: Methods and Protocols*, Methods in Molecular Biology, vol. 720,
DOI 10.1007/978-1-61779-034-8_2,

polyamine-responsive feedback systems, exemplified by the ornithine decarboxylase antizyme programmed frameshifting mechanism that is found in both fungi and animal cells. The antizyme system does not appear to be present in plants, or green and red algae, but plants and animals do share a polyamine-responsive feedback mechanism convergently centered on the translational regulation of *S*-adenosylmethionine decarboxylase (AdoMetDC; EC 4.1.1.50) mRNA by a ribosome-stalling peptide.

Polyamine biosynthesis in animal cells and yeasts is very similar. The committing step in polyamine biosynthesis in both systems is decarboxylation of ornithine by ornithine decarboxylase (ODC; EC 4.1.1.17) to produce putrescine (1,4-diaminobutane). Spermidine is synthesized from putrescine by the transfer of an aminopropyl group donated from decarboxylated *S*-adenosylmethionine, accomplished by the aminopropyltransferase spermidine synthase (SpdSyn; EC 2.5.1.16). In most animal cells, spermine can be formed from spermidine by transfer of an aminopropyl group to the aminobutyl end of spermidine, carried out by spermine synthase (EC 2.5.1.22). Some animal cells such as nematode worms have lost spermine synthase (4). Most fungi except the closely related true yeasts (Saccharomycotina), such as *S. cerevisiae* and *Candida albicans*, do not possess a spermine synthase gene (4) and, in the Basidiomycota, spermidine synthase is fused to the lysine biosynthetic enzyme saccharopine dehydrogenase. In plants, and green and red algae, spermine synthase is found only in the flowering plants (angiosperms). Spermine synthase has evolved independently on at least three occasions, in the ancestor of animal and choanoflagellates, in the ancestor of flowering plants, and in the ancestor of Saccharomycotina yeasts. Thus, the spermine synthase of plants has evolved within the last 200 million years, whereas the animal spermine synthase predates the origin of the metazoa and may have arisen in the pre-Cambrian (4). In contrast, plants and algae can synthesize a spermine structural analog, thermospermine, which is synthesized from spermidine by transfer of an aminopropyl group to the N^1- aminopropyl end of spermidine. The aminopropyltransferase responsible for the synthesis of thermospermine (EC 2.5.1.B4), thermospermine synthase, is evolutionarily much more ancient than the plant spermine synthase.

Plant polyamine metabolism differs from that in animals and fungi, not just because of thermospermine biosynthesis but because plants can also synthesize putrescine from arginine via a pathway consisting of arginine decarboxylase (ADC; EC 4.1.1.19), agmatineiminohydrolase (EC 3.5.3.12), and *N*-carbamoylputrescine amidohydrolase (EC 3.5.1.53) (5). Both thermospermine synthase and the ADC pathway were likely derived from the cyanobacterial ancestor of the chloroplast, and through endosymbiotic gene transfer, the two pathways were transferred from the chloroplast ancestral genome to the host nuclear genome. Subsequently,

ADC was retargetted back to the chloroplast. Plants also possess a wide variety of enzymes that *N*-acylate polyamines for the synthesis of alkaloids and hydroxycinnamic amides, e.g., putrescine *N*-methyltransferase and spermidine disinapoyltransferase.

The polyamine biosynthetic strategies used by animals, fungi, and plants may seem quite different, but phylogenetically, animals and fungi are sister groups within the same eukaryotic supergroup known as the Opisthokonta. Plants and red and green algae, along with glaucocystophyte algae, are in another supergroup, the Archaeplastidia. There are four other eukaryotic supergroups: the Amoebozoa, including true amoebae and slime molds, which are the group closest to the Opisthokonta; the Chromalveolata, which includes the Apicomplexan parasites such as *Plasmodium* and *Toxoplasma*; the Excavata, containing other well-known parasites such as the kinetoplastid trypanosomes and the diplomonad *Giardia* and the parabasaliad *Trichomonas*; and finally the Rhizaria supergroup including foraminiferans and radiolarians (6). Polyamine metabolism is poorly sampled and characterized in the Amoebozoa, Chromalveolata, Rhizaria, and Excavata because these supergroups are relatively poorly understood at a biological level and sparsely sampled at a genomic level. It can be seen therefore that there probably remains significant uncharted polyamine biosynthetic diversity within eukaryotes. Many bacteriovorus single-celled eukaryotes have acquired genes from bacteria by horizontal gene transfer and so part of the uncharted polyamine biosynthetic diversity may be based on bacterial-derived acquisitions.

Although there is considerable polyamine biosynthetic diversity within the eukaryotes, there is far greater diversity among the prokaryotes, which consist of the other two domains of life, the bacteria and archaea (7, 8). There are now over a thousand published prokaryotic genomes, with several thousand other genomes in preparation. As a generalization, it is thought that eukaryotes share more in common with bacterial operational genes, i.e., metabolism, and with archaeal informational genes, i.e., translation and transcription. Much of the analysis of bacterial polyamine metabolism has been performed with the γ-proteobacterium *Escherichia coli* (9). Like eukaryotes, *E. coli* synthesizes putrescine and employs AdoMetDC and SpdSyn to make spermidine from putrescine. However, *E. coli* synthesizes putrescine from both ornithine and arginine. The *E. coli* biosynthetic ODC is an entirely different fold of enzyme to the eukaryotic ODC and is similar to the *Lactobacillus* ODC (10). Eukaryotic and *E. coli* ODC are an example of convergent biosynthetic evolution. The biosynthetic ADC of *E. coli* is similar to the plant ADC (11) and is related to the eukaryotic ODC. However, *E. coli* also contains a biodegradative ADC, a large decameric complex activated by acid stress (12), is and involved in acid resistance. It also possesses a biodegradative ODC, very similar to the biosynthetic ODC, and is

involved in acid stress resistance (13). Adding complexity to this picture is the fact that in *E. coli*, a structural analog of spermidine, aminopropylcadaverine, can be synthesized when putrescine is limiting (14). Cadaverine can be synthesized by an acid-inducible lysine decarboxylase and a constitutively expressed paralog (15). The AdoMetDC/SpdSyn pathway for spermidine biosynthesis is found in many bacteria and archaea. However, there are many bacteria that contain spermidine but do not contain AdoMetDC/SpdSyn orthologs, and there are many bacteria that contain *sym*-homospermidine rather than spermidine (16). Some bacteria also contain *sym*-norspermidine. The exploration of polyamine biosynthetic diversity through comparative and functional genomics has been made easier because of prior published detailed biochemical analyses of what was at the time regarded as unusual or unique polyamine biosynthetic enzymes and pathways. With the benefit of complete genome sequences, the development of molecular biological approaches in diverse organisms, and the availability of commercial gene synthesis, comparative genomic approaches for exploring polyamine biosynthetic diversity can now be biochemically validated through functional genomics and recombinant enzymology.

2. Methods and Notes: Four Vignettes

To illustrate the methods and conceptual approaches used for exploring alternative polyamine biosynthetic pathways, four biosynthetic vignettes are presented below. The first is the characterization of the alanine racemase-fold family of amino acid decarboxylases that consists of arginine, ornithine, carboxynorspermidine, and diaminopimelate decarboxylases (17). Secondly, a consideration of the aminopropyltransferase family, which includes spermidine, norspermidine, spermine, thermospermine, norspermine, and aminpropylagmatine synthases, is shown. Thirdly, an alternative pathway for norspermidine and spermidine biosynthesis based on carboxypolyamine intermediates is discussed, and lastly, the identification of spermidine *N*-acyltransferases in plants is described.

2.1. The Alanine Racemase-Fold Family of Amino Acid Decarboxylases

1. One of the driving forces in evolution is the expansion of gene families through gene duplication and subsequent divergence. Horizontal gene transfer allows the dissemination of individual members of gene families. The alanine racemase-fold family of enzymes, which includes the biosynthetic ADC, ODC (EC 4.1.1.17), carboxynorspermidine decarboxylase (CANSDC; EC 4.1.1.), and the lysine biosynthetic enzyme diaminopimelate decarboxylase (DAPDC; EC 4.1.1.20), is a typical example of the evolution of enzyme catalytic diversity through gene duplication. Each member of the family uses pyridoxal

5' phosphate as a cofactor and binds similar substrates. Key residues in the active site of each enzyme are conserved and the enzymes are of roughly the same size except for arginine decarboxylase, which is approximately 50% bigger than the others. The AR-fold ODC was thought to be a eukaryotic-specific ODC. Another class of ODC belonging to the aspartate aminotransferase fold had been described in *Lactobacillus sp.* and *E. coli*. However, a eukaryotic-type ODC was identified and characterized in the firmicute bacterium *Selenomonas ruminantium* (ODC) and was found to be active as both an ODC and lysine decarboxylase (LDC) (18).

2. To explore the prevalence of the AR-fold ODC in bacteria, a phylogenetic analysis was performed on this family of enzymes (17). The main tool for the phylogenetic analysis was BLASTP (19). Several validated ODC proteins were used to screen the protein database, including mammalian, plant, fungal, trypanosome, and *S. ruminantium* enzymes. In addition, the functionally validated ADC proteins of Arabidoposis and *E. coli*, multiple, characterized DAPDC proteins, and the *Vibrio alginolyticus* CANSDC were also used to screen the protein database by BLASTP. A quick phylogenetic tree of the related sequences can be obtained using the fast minimum evolution tree of the BLASTP results (Fig. 1). A subset of proteins exhibiting similarity to the probe sequences was collected and all sequences were aligned using the alignment program CLUSTALW (20) in the CLUSTALX package. After automated alignment, the N- and C-termini of the amino acid sequences were trimmed to optimize the alignment and then, after another automated round of sequence alignment, some manual adjustment was performed to optimize the alignment. The alignment was then used to build a Neighbor Joining tree using the program PAUP* (21). However, this approach has been superseded by the phylogenetic package MEGA Version 4.0 (22), which is free and can be downloaded from http://www.megasoftware.net. For an excellent explanation of the use of MEGA and of phylogenetic methods for nonspecialists, an inexpensive, how-to manual, "Phylogenetic Trees Made Easy," third edition, is available and I highly recommend this concise and readable paperback book (23). The phylogenetic tree figure configuration was modified using the program TREEVIEW (24) and the cladogram image imported into a graphics program for further annotation. Confidence levels for clades, i.e., the bootstrap supports, were calculated separately using PAUP* and the bootstrap values were added manually to the phylogenetic tree using the graphics program.

3. Based on the prominent clades of enzymes in the NJ tree, and cross-validation with known substrate preferences of some of the proteins in the tree, a number of bacterial putative ODCs

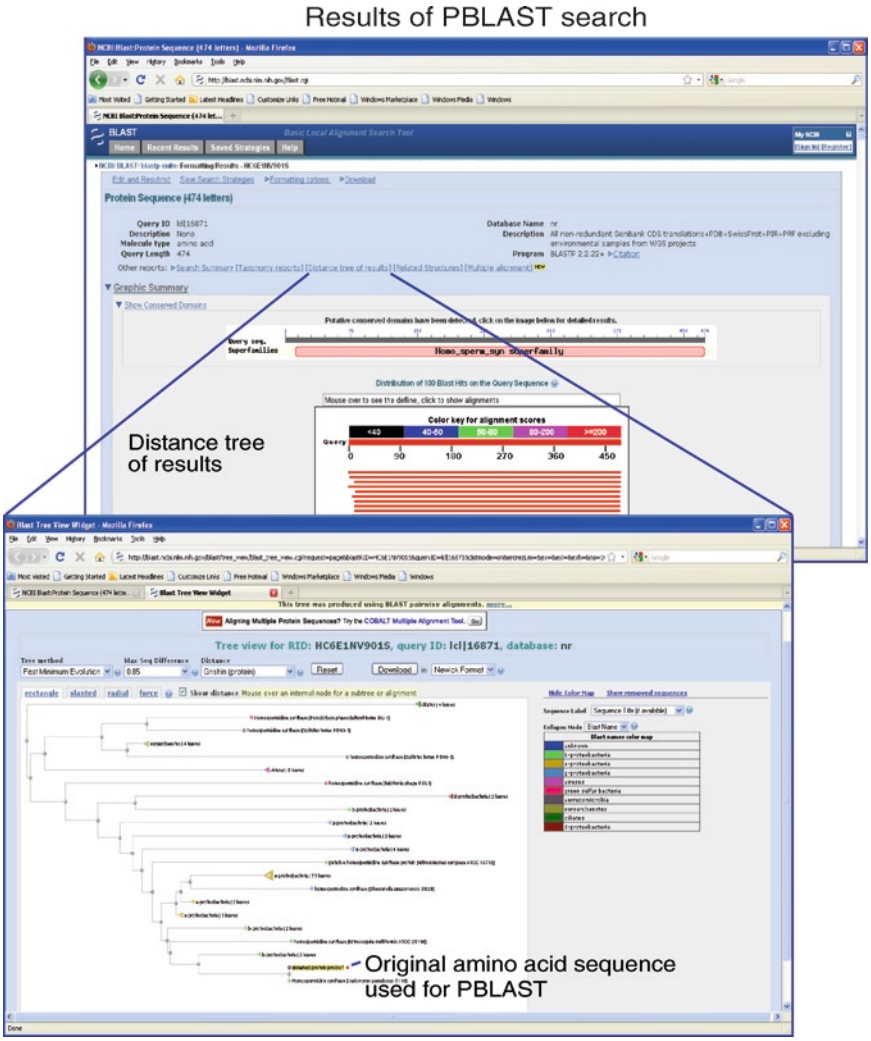

Fig. 1. Fast Minimal Evolution tree from a BLASTP search. A quick phylogenetic tree analysis can be obtained from a BLASTP search by clicking on the "distance tree of results" button at the top of the BLASTP results page. The fast minimum evolution tree individual clades and species can be clicked on to reveal subclades and species, and sequence alignments of the subtrees.

were chosen for biochemical characterization. Within the putative ODC clade, there were three subclades: one clade was comprised of the eukaryotic ODCs containing enzymes with confirmed biochemical function, another small subclade contained the bifunctional *S. ruminatium* ODC/LDC, and a third subclade contained bacterial enzymes of unknown function. Genomic DNA samples of bacterial and eukaryotic species were obtained and putative ODC and ODC/LDC genes were cloned and expressed in *E. coli* (from the eukaryotic protist parasite *Giardia lamblia*, the euryarchaeote *Methanosarcina*

mazei, and the bacterial species *Aquifex aeolicus*, *Thermotoga maritima*, *Nitrosomonas europea*, *Bartonella henselae*, *Pseudomonas aeruginosa,* and *Vibrio vulnificus*). Each enzyme was biochemically determined to be a specific ODC except for the *V. vulnificus* enzyme, which was a bifunctional ODC/LDC. A crystal structure was determined for the bifunctional *V. vulnificus* ODC/LDC protein and the mechanistic basis of the bifunctional nature of the enzyme was shown to be dependent on the presence or absence of a well-ordered water molecule in the active site. The ADC and DAPDC clades were further validated by cloning and expression in *E. coli* of the *V. vulnificus* orthologs from those clades. An additional validation of the CANSDC clade was provided later, by the characterization of the *Vibrio cholerae* ortholog (25).

2.2. Aminopropyltransferases

1. Aminopropyltransferases are typified by spermidine and spermine synthases. However, there are also recently characterized thermospermine synthases, norspermidine and norspermine synthases, as well as agmatine aminopropyl transferases and long-chain polyamine synthases. The recently solved crystal structure of the human spermine synthase revealed that there is an N-terminal domain that has structural homology to the bacterial *S*-adenosylmethionine decarboxylase; no corresponding catalytic activity but the domain is required for dimerization of spermine synthase and so is essential for spermine synthase activity (26). The rest of the human spermine synthase structure is very similar to spermidine synthase.
2. To investigate the evolution of aminopropyltransferases, the spermidine synthase-like domains of various functionally validated aminopropyltransferase amino acid sequences were used to screen the protein database and expressed sequence tag databases. A file of diverse aminopropyltransferase sequences was aligned as described in Figure 1. The NJ tree produced based on the alignment revealed a number of important evolutionary developments of the aminopropyltransferase gene family. A key finding was that spermine synthase has evolved at least three times independently in eukaryotes, in the ancestor of metazoa and choanoflagellates, in the true yeasts (Saccharomycotina) but not in the rest of the fungi, and in flowering plants but not the rest of land plants and algae. In contrast, thermospermine synthase is present in all plants and algae and is also found in the Chromalveolata supergroup, most likely due to endosymbiotic gene transfer from the cyanobacterial ancestor of the chloroplast to the host cell that took up the chloroplast ancestor to form a red alga, and then from a red algal nucleus to the heterotrophic eukaryotic host nucleus that was the ancestral

host cell of the Chromalveolata (27). Thus, thermospermine synthase is found in nonplant, photosynthetic eukaryotes, such as diatoms and coccolithophores, and in cells that have lost the ability to photosynthesize, such as *Perkinsus* and *Phytophthora*.

2.3. The Carboxy(nor) Spermidine Pathway for Norspermidine and Spermidine Biosynthesis

1. Biosynthesis of norspermidine in *Vibrio alginolyticus* is dependent on the synthesis of diaminopropane from glutamate and aspartate β-semialdehyde, via a diaminobutyric acid intermediate (25). The diaminopropane is converted to norspermidine via the intermediate carboxynorspermidine. Although a diaminobutyrate decarboxylase activity had been reported in *V. alginolyticus*, the gene encoding the activity had not been identified. However, diaminobutyrate aminotransferase (DABA AT), which produces diaminobutyrate from glutamate and aspartate β-semialdehyde, and diaminobutyrate decarboxylase (DABA DC), which produces diaminopropane from diaminobutyrate, had been characterized and cloned from another γ-proteobacterium *Acinetobacter baumanii*. In the genome of *V. alginolyticus*, the DABA AT and DABA DC orthologs are present as a gene fusion (25). Conversion of carboxynorspermidine to norspermidine is achieved by another PLP-dependent enzyme, carboxynorspermidine decarboxylase (CANSDC), which had been identified originally in *V. alginolyticus*. The missing, unidentified gene in the norspermidine biosynthetic pathway was carboxynorspermidine synthase, which transfers an aspartate β-semialdehyde-derived carboxyaminopropyl group to diaminopropane to form the carboxynorspermidine intermediate. However, in *Vibrio* species, the DABA AT/DC fusion protein ORF is clustered together with the CANSDC ORF, with an ORF of unknown function juxtaposed between them. The genomic configuration of these three ORFs suggested that the ORF of unknown function might encode the missing carboxynorspermidine synthase enzyme. Using a combination of heterologous expression of the *V. cholerae* genomic cluster in *E. coli* and gene deletions strains of the corresponding genes of *V. cholerae*, it was functionally demonstrated that the ORF of unknown function was in fact the carboxyspermidine synthase, which was subsequently renamed carboxyspermidine dehydrogenase (CANSDH). This is an example of the "guilt by association" approach to identifying genes of unknown function.
2. Gene clusters around genes of interest can be visualized in genome sequences by using the gene ID/genome map facility provided with the BLASTP results output (Fig. 2). Outside of the Vibrionaceae, no other bacterial species have the entire

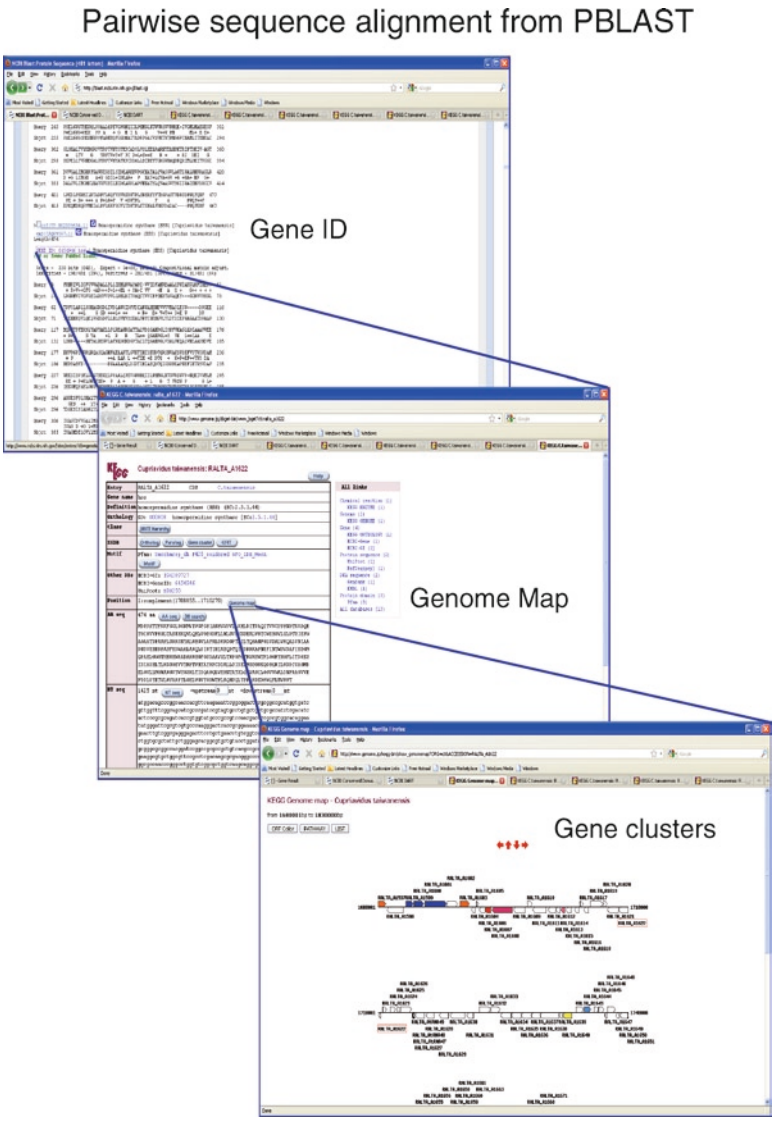

Fig. 2. Gene cluster maps from a BLASTP search. The genomic organization of local gene clusters can be graphically visualized by clicking on "Gene ID" hyperlink associated with each pairwise sequence alignment from the results of a BLASTP search. From the new page, clicking on "Genome Map" will provide the gene cluster graphical map.

norspermidine biosynthetic pathway clustered as an operon. The *V. cholerae* CANSDH/CANSDC pathway was also able to use putrescine, as well as diaminopropane, to synthesize spermidine. It is important to note that most of the bacterial species that possess orthologs of CANSDH and CANSDC do not possess orthologs of DABA AT and DABA DC, strongly suggesting that the CANSDH/CANSDC pathway is used primarily to synthesize spermidine rather than *sym*-norspermidine.

2.4. Spermidine Hydroxycinnamoyl Transferases

1. Flowering plants often conjugate polyamines with hydroxycinnamic acids, secondary metabolites derived from phenylalanine. The tyramine *N*-hydroxycinnamoyl transferase has been identified in potato and tobacco and is a small acyltransferase belonging to the huge GNAT superfamily of acyltransferases. However, polyamine *N*-acyltransferases have been biochemically characterized from diverse plant species and the subunit size has been found to be about 55 kDa, roughly twice the size of the tyramine *N*-hydroxycinnamoyl transferase. This larger size corresponds to the monomer of another class of acyl transferases in plants, the BAHD family of transferases, which includes an agmatine *N*-hydroxycinnamoyl transferase. There are over 60 members of the BAHD family of genes in the model plant Arabidopsis. The main class of polyamine hydroxycinnamoyl conjugates in Arabidopsis are spermidine forms and particularly 5-hydroxyferuloyl and sinapoyl spermidine compounds.
2. To identify the genes encoding the enzymes responsible for spermidine hydroxycinnamoyl conjugate formation, a combination of bioinformatic analysis, recombinant enzyme characterization, and functional genomics was used (28). The main localization of the hydrophobic N^1,N^8-disinapoyl spermidine conjugates was in seeds. Expression patterns of all BAHD family members were analyzed using the GENEVESTIGATOR microarray result database (29) and a gene expressed strongly in seeds was identified. The coding sequence of the gene (At2g23510) was cloned by PCR and expressed in *E. coli*. CoA-activated hydroxycinnamic substrates were synthesized and coumaroyl-CoA, caffeoyl-CoA, feruloyl-CoA, and sinapoyl-CoA were tested with putrescine, spermidine, *sym*-homospermidine, *sym*-norspermidine, and spermine as acyl acceptors. The enzyme encoded by At2g23510 was found to be specific for the acylation of spermidine by sinapoyl-CoA to form N^1,N^{10}-disinapoyl spermidine. Plants containing a homozygous transposon insertion mutant of the gene were found to completely lack disinapoyl spermidine. Other authors using a similar approach found another BAHD acyltransferase that was responsible for the formation of N^1,N^5,N^{10}-*tris*(feruloyl)spermidine in Arabidopsis anthers (30).

References

1. Wallace HM, Fraser AV, Hughes A (2003) A perspective of polyamine metabolism. Biochem J 376:1–14
2. Bacchi CJ, Yarlett N (2002) Polyamine metabolism as chemotherapeutic target in protozoan parasites. Mini Rev Med Chem 2:553–563
3. Ivanov IP, Atkins JF, Michael AJ (2010) A profusion of upstream open reading frame mechanisms in polyamine-responsive translational regulation. Nucleic Acids Res 38:353–359
4. Pegg AE, Michael AJ (2010) Spermine synthase. Cell Mol Life Sci 67:113–121

5. Illingworth C, Mayer MJ, Elliott K, Hanfrey C, Walton NJ, Michael AJ (2003) The diverse bacterial origins of the Arabidopsis polyamine biosynthetic pathway. FEBS Lett 549:26–30
6. Adl SM, Simpson AG, Farmer MA, Andersen RA, Anderson OR, Barta JR, Bowser SS, Brugerolle G, Fensome RA, Fredericq S, James TY, Karpov S, Kugrens P, Krug J, Lane CE, Lewis LA, Lodge J, Lynn DH, Mann DG, McCourt RM, Mendoza L, Moestrup O, Mozley-Standridge SE, Nerad TA, Shearer CA, Smirnov AV, Spiegel FW, Taylor MF (2005) The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. J Eukaryot Microbiol 52:399–451
7. Koonin EV, Wolf YI (2008) Genomics of bacteria and archaea: the emerging dynamic view of the prokaryotic world. Nucleic Acids Res 36:6688–6719
8. Fraser C, Alm EJ, Polz MF, Spratt BG, Hanage WP (2009) The bacterial species challenge: making sense of genetic and ecological diversity. Science 323:741–746
9. Chattopadhyay MK, Tabor CW, Tabor H (2009) Polyamines are not required for aerobic growth of Escherichia coli: preparation of a strain with deletions in all of the genes for polyamine biosynthesis. J Bacteriol 191:5549–5552
10. Momany C, Ernst S, Ghosh R, Chang NL, Hackert ML (1995) Crystallographic structure of a PLP-dependent ornithine decarboxylase from Lactobacillus 30a to 3.0 A resolution. J Mol Biol 252:643–655
11. Bell E, Malmberg RL (1990) Analysis of a cDNA encoding arginine decarboxylase from oat reveals similarity to the Escherichia coli arginine decarboxylase and evidence of protein processing. Mol Gen Genet 224:431–436
12. Andrell J, Hicks MG, Palmer T, Carpenter EP, Iwata S, Maher MJ (2009) Crystal structure of the acid-induced arginine decarboxylase from Escherichia coli: reversible decamer assembly controls enzyme activity. Biochemistry 48:3915–3927
13. Kashiwagi K, Watanabe R, Igarashi K (1994) Involvement of ribonuclease III in the enhancement of expression of the speF-potE operon encoding inducible ornithine decarboxylase and polyamine transport protein. Biochem Biophys Res Commun 200:591–597
14. Igarashi K, Kashiwagi K, Hamasaki H, Miura A, Kakegawa T, Hirose S, Matsuzaki S (1986) Formation of a compensatory polyamine by Escherichia coli polyamine-requiring mutants during growth in the absence of polyamines. J Bacteriol 166:128–134
15. Nagano T, Kikuchi Y, Kamio Y (2000) High expression of the second lysine decarboxylase gene, ldc, in Escherichia coli WC196 due to the recognition of the stop codon (TAG), at a position which corresponds to the 33th amino acid residue of sigma38, as a serine residue by the amber suppressor, supD. Biosci Biotechnol Biochem 64:2012–2017
16. Hamana K, Matsuzaki S (1992) Polyamines as a chemotaxonomic marker in bacterial systematics. Crit Rev Microbiol 18:261–283
17. Lee J, Michael AJ, Martynowski D, Goldsmith EJ, Phillips MA (2007) Phylogenetic diversity and the structural basis of substrate specificity in the beta/alpha-barrel fold basic amino acid decarboxylases. J Biol Chem 282:27115–27125
18. Takatsuka Y, Yamaguchi Y, Ono M, Kamio Y (2000) Gene cloning and molecular characterization of lysine decarboxylase from Selenomonas ruminantium delineate its evolutionary relationship to ornithine decarboxylases from eukaryotes. J Bacteriol 182:6732–6741
19. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410
20. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680
21. Wilgenbusch JC, Swofford D (2003) Inferring evolutionary trees with PAUP*. Curr Protoc Bioinformatics, Chapter 6, Unit 6 4
22. Kumar S, Tamura K, Nei M (1994) MEGA: molecular evolutionary genetics analysis software for microcomputers. Comput Appl Biosci 10:189–191
23. Hall BG (2008) Phylogenetic trees made easy, 3rd edn. Sinauer Associates, Sunderland, MA
24. Page RD (1996) TreeView: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358
25. Lee J, Sperandio V, Frantz DE, Longgood J, Camilli A, Phillips MA, Michael AJ (2009) An Alternative Polyamine Biosynthetic Pathway is Widespread in Bacteria and Essential for Biofilm Formation in *Vibrio Cholerae*. J Biol Chem 284:9899–9907
26. Wu H, Min J, Zeng H, McCloskey DE, Ikeguchi Y, Loppnau P, Michael AJ, Pegg AE, Plotnikov AN (2008) Crystal structure of human spermine synthase. J Biol Chem 283:16135–16146
27. Pegg AE, Michael AJ (2009) Spermine synthase. Cell Mol Life Sci 67:113–121
28. Luo J, Fuell C, Parr A, Hill L, Bailey P, Elliott K, Fairhurst SA, Martin C, Michael AJ (2009) A novel polyamine acyltransferase responsible

for the accumulation of spermidine conjugates in Arabidopsis seed. Plant Cell 21:318–333
29. Zimmermann P, Hirsch-Hofmann M, Hennig L, Gruissem W (2004) GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. Plant Physiol 136:2621–2632
30. Grienenberger E, Besseau S, Geoffroy P, Debayle D, Heintz D, Lapierre C, Polllet B, Heitz T, Legrand M (2009) A BAHD acyltransferase is expressed in the tapetum of Arabidopsis anthers and is involved in the synthesis of hydroxycinnamoyl spermidines. Plant J 58:246–259

http://www.springer.com/978-1-61779-033-1

Polyamines
Methods and Protocols
Pegg, A.E.; Casero, Jr., R.A. (Eds.)
2011, XIV, 524 p., Hardcover
ISBN: 978-1-61779-033-1
A product of Humana Press