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Update on Regulation

Polyamines: Small Molecules Triggering Pathways in Plant Growth and Development[1,2]

Richard Walden*, Alexandra Cordeiro, and Antonio F. Tiburcio

Max Planck Institut für Züchtungsforschung, Carl von Linné Weg 10, 50829 Cologne, Germany (R.W., A.C.); and Unitat de Fisiologia Vegetal, Facultat de Farmàcia, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain (A.F.T.)

Plant biologists fall into two categories: those who believe polyamines play an important role in plant growth and development, and those who are skeptical. The latter group by far exceeds the former. Why is this? Over the years polyamines have been implicated in being involved in a wide array of processes in plants, ranging from triggering organogenesis to protecting against stress. However, the problem has been that a particular response or a developmental event has generally been correlated only with changes in polyamine levels and spectra. The question of how direct or indirect the effect of polyamines is has remained open to debate. Although parallels are often drawn with animal systems in which polyamines have been linked with cell proliferation, their exact role has yet to be firmly established. It is no surprise that a formative review with the provocative title "Do Polyamines Have Roles in Plant Development?" concluded that although this was indeed likely, there were no definitive supporting conclusions (Evans and Malmberg, 1989). Since 1989, however, several important advances have been made in plant polyamine research. Most of the genes encoding polyamine biosynthetic enzymes have been isolated, and antibodies to some are available. Moreover, transgenic plants, or mutants changed in polyamine metabolism, have been created. Thus, it would seem an opportune time to reconsider the issue of what role polyamines play in plant development.

BIOSYNTHESIS

The polyamine biosynthetic pathway is relatively well established, and inhibitors are available for many of the biosynthetic enzymes (Fig. 1). Putrescine can be produced directly from Orn by the action of ODC, or indirectly from Arg by ADC. SAMDC probably is the rate-limiting activity that provides the aminopropyl moiety that is used by spermidine and spermine synthases, respectively, to convert putrescine to spermidine and spermine. S-Adenosylmethionine is also a precursor in ethylene biosynthesis; thus, increases in polyamine biosynthesis, particularly via SAMDC activity, are likely to affect the rates of ethylene synthesis. Because polyamine concentrations are much higher than those of both ACC and ethylene, changes in polyamines are more likely to affect ACC and ethylene biosynthesis than vice versa. Currently, the control of turnover of polyamines by polyamine and diamine oxidase is little understood. Apparently, polyamines are present in all plant cells, and putrescine and other polyamines can form conjugates that can represent up to 90% of the total polyamine content. Cells undergoing division contain high levels of free polyamines, whereas low levels are found in cells undergoing expansion (Galston and Kaur-Sawhney, 1995).

Increases in polyamine biosynthetic activities have been observed in a variety of developmental or stress situations (Evans and Malmberg, 1989). One of the best documented effects of polyamine accumulation relates to organogenesis in the tobacco thin cell layer system. These are strips of stem epidermal tissue removed from plants that have flowered and formed fruit. The stem strips, when introduced into culture, can be triggered into differing organogenic programs, depending on the media supplied (Fig. 2). During vegetative growth and floral bud formation there are increases in ADC activity but not in ODC activity. Subsequent development, however, appears to be dependent on ODC activity, with spermidine appearing to be the most effective polyamine. Conversely, putrescine, formed by ADC, appears to be important in triggering root formation (Tiburcio et al., 1989).

PLANT POLYAMINE BIOSYNTHETIC GENES

Cloning the genes encoding polyamine biosynthetic enzymes from plants using heterologous probes initially proved difficult because of the low degree of sequence

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* Corresponding author; e-mail walden@mpiz-koeln.mpg.de; fax 49-221-5062-213.

Abbreviations: ADC, Arg decarboxylase; ODC, Orn decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; Tet, tetracycline.

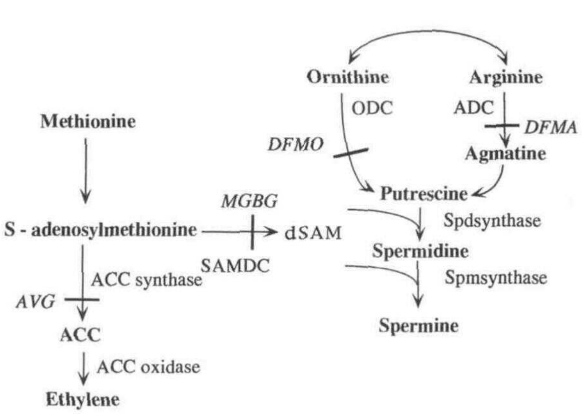

Figure 1. The polyamine biosynthetic pathway and its linkage to ethylene biosynthesis. The substrates and biosynthetic enzymes are ACC, ADC, decarboxylated *S*-adenosylmethionine (dSAM), ODC, and SAMDC. The inhibitors are aminoethoxyvinylglycine (AVG), DL-α-difluoromethylarginine (DFMA), DL-α-difluoromethylornithine (DFMO), and methylglyoxal-bis(guanylhydrazone) (MGBG).

similarity. Recently, however, clones representing ODC from *Datura* (Michael et al., 1996); ADC from oat (Bell and Malmberg, 1990), tomato (Rastogi et al., 1993), pea (Pérez-Amador et al., 1995), Arabidopsis (Malmberg and Watson, 1996), and soybean (Nam et al., 1996); and SAMDC from potato (Mad Arif et al., 1994), spinach (Bolle et al., 1995), periwinkle (Schröder and Schröder, 1995), carnation (Chang et al., 1996), and *Tritordeum* (Dresselhaus et al., 1996) have been described.

The *Datura* ODC gene, although similar to other eukaryotic ODCs, lacks the long 3′ and 5′ untranslated regions present in mammalian ODC mRNAs, which are thought to affect translational efficiency. Its mRNA accumulates to higher levels in rapidly growing, transformed root cultures than in tissues with more limited cell division, such as leaves or stems (Michael et al., 1996).

Each of the cloned ADC genes is similar to the biosynthetic *Escherichia coli* ADC and encodes proteins containing the conserved, putative substrate-binding site that has been found in all eukaryotic ADCs and ODCs reported to date. It is interesting that they also have residues that are similar to those described as functioning as binding sites for pyridoxal phosphate and DL-α-difluoromethylornithine (Poulin et al., 1992), suggesting that the catalytic mechanisms of ADCs and ODCs from eukaryotes are similar. The expression patterns for ADC support the idea that it is the primary source of putrescine during cell elongation. ADC mRNA levels increase from the immature green to the breaker stage in tomato (Rastogi et al., 1993), and pea ADC expression is higher in young developing tissues and early stages of fruit growth than in mature organs (Pérez-Amador et al., 1995). However, ADC activity does not appear to correlate with mRNA levels, suggesting that expression may be subject to translational and/or posttranslational regulation. Indeed, pea and soybean mRNAs have long 5′ untranslated regions, which may be involved in this (Pérez-Amador et al., 1995).

Despite a low overall sequence similarity, plant SAMDC genes share regions of similarity with all SAMDC genes analyzed to date, e.g. a putative PEST sequence, which is characteristic of peptides with high turnover rates and a pro-enzyme cleavage site. Indeed, SAMDC in potato and periwinkle is made as a precursor that is posttranslationally cleaved to form two subunits forming the functional enzyme (Mad Arif et al., 1994; Schröder and Schröder, 1995). Potato SAMDC is highly expressed in actively dividing and differentiating tissues of both vegetative and reproductive organs (Mad Arif et al., 1994). In *Tritordeum*, SAMDC mRNA accumulates in wounded tissues and its levels follow a circadian rhythm, suggesting expression in the absence of cell division (Dresselhaus et al., 1996). Plant SAMDC mRNAs have a conserved region in the long 5′ leader containing a short open reading frame. Thus, control of expression is probably also exerted posttranscriptionally.

POSTTRANSLATIONAL CONTROL AND LOCALIZATION OF POLYAMINE BIOSYNTHETIC ENZYMES

Cofactor requirements and regulation of activity by spermidine and spermine suggest that the tomato and the tobacco ODCs are similar to the mammalian enzymes (Heimer and Mizrahi, 1982). But unlike their mammalian counterpart ODCs, the *Datura* enzyme lacks the carboxy-terminal extension, which is thought to be involved in rapid turnover. This may contribute to a higher stability of the plant enzyme (Michael et al., 1996).

Oat ADC is synthesized as a 66-kD precursor, which is cleaved to produce an amino-proximal 44-kD peptide and a carboxy-proximal 22-kD peptide linked by disulfide bonds to form the active enzyme (Watson and Malmberg, 1996). In osmotically stressed oat leaves spermine inhibits posttranslational processing of the ADC precursor, with subsequent decreases in mature ADC (Borrell et al., 1996). Antibodies to ADC have shown that the enzyme is

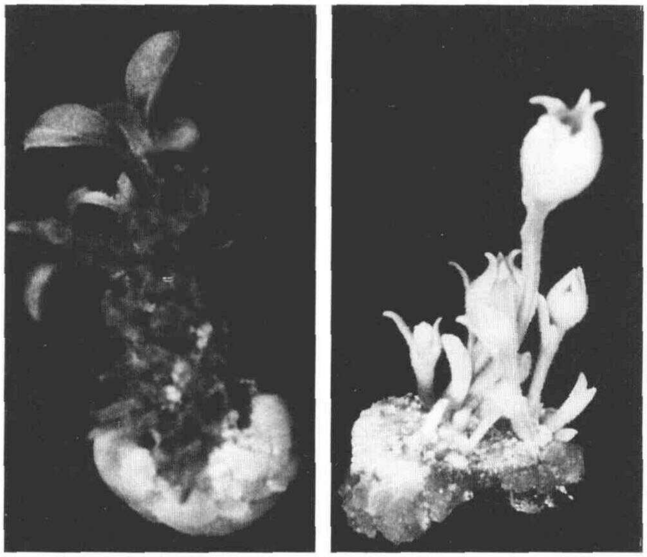

Figure 2. The effect of spermidine in thin cell layer explants of tobacco. Explants were cultured in the presence (right) or absence (left) of 5 mM spermidine. The photograph was taken when the cultures were 1 month old and buds were fully developed. (Reproduced from Kaur-Sawhney et al. [1988] with permission.)

chloroplast-localized (Borrell et al., 1995). ODC is generally considered to be cytoplasmic; thus, the two biosynthetic pathways leading to putrescine might be physically separated within the plant cell.

EFFECT OF EXPRESSION OF HETEROLOGOUS POLYAMINE BIOSYNTHETIC GENES IN TRANSGENIC PLANTS

In the absence of homologous genes, the study of the effect of the expression of polyamine biosynthetic genes in transgenic tissue initially relied on creating transgenic plants, generally tobacco, engineered to express genes from nonplant sources. Generally, the genes were linked to the cauliflower mosaic virus 35S RNA promoter, thus producing high levels of constitutive expression.

The yeast ODC gene expressed in tobacco hairy root cultures produced up to a 3-fold increase in ODC activity and doubled the nicotine content (Hamill et al., 1990). Considering that the promoter used contained a doubled enhancer sequence, one might have expected higher activities. The authors suggest that there might be a tight control of flux through the nicotine biosynthetic pathway. The mouse ODC gene introduced into tobacco produced 2- to 3-fold increases in putrescine in leaf tissue and a 4- to 12-fold increase in callus (DeScenzo and Minocha, 1993). Most of the transformants appeared morphologically normal, although those with the highest ODC activities and putrescine levels displayed stunted growth, wrinkled leaves, and flowers with reduced stamens. In both of these studies only an increase in putrescine levels was observed, raising the issue of whether SAMDC, spermidine synthase, or spermine synthase activities are limiting in plants.

The notion that SAMDC may be limiting in the polyamine biosynthetic pathway received support from studying tobacco transgenic for the human SAMDC (Woon Noh and Minocha, 1994). In this study transgenic plants displayed a 2- to 4-fold increase in SAMDC activity, putrescine levels were significantly reduced, and amounts of spermidine were increased 2- to 3-fold.

CONTROLLED EXPRESSION OF POLYAMINE BIOSYNTHETIC GENES AS TRANSGENES

Controlled expression of a transgene allows a more detailed study of the effects of its expression on enzyme activity and plant morphology compared with constitutive overexpression. It also overcomes the concern that high levels of transgene expression might be lethal to plant cells undergoing regeneration as part of the transformation process. For example, potential overexpression of a homologous SAMDC gene in potato has been reported to be lethal. Thus, the Tet repressor system has been used to regulate expression of the gene in a sense and an antisense orientation (Kumar et al., 1996). In leaf explants of plants containing the sense orientation SAMDC/Tet construct, a 2- to 6-fold increase in the SAMDC transcript level was observed following Tet induction. These explants also displayed increases in levels of SAMDC activity, spermidine, spermine, and, intriguingly, putrescine. Although these data confirm the utility of the Tet system in manipulating a biochemical pathway, since the experiments were carried out in leaf discs it does not shed light on the function of polyamines in the intact plant.

The authors cite difficulties in the uptake of Tet in the hydroponic system that was used to feed the transgenic plants. The work involving the antisense SAMDC plants was more informative. These display a variety of phenotypes, including stunted growth, short internodes, stem branching, and small leaves. The phenotypes are accompanied by decreases in stable SAMDC transcripts, a reduction in SAMDC activity, and reductions in levels of putrescine, spermidine, and spermine. It is interesting that the plant displaying the most dramatic phenotype, as well as the maximal decreases in mRNA levels, enzyme activities, and polyamines, also had a 46-fold increase in ethylene evolution compared with the wild type. This supports the idea of a competitive interaction between polyamine and ethylene biosynthesis. Whereas the regenerated plants display some features that are suggestive of ethylene overproduction, there are differences that lead the authors to suspect that the observed phenotypes result from the combined effects of depleted polyamine levels and elevated levels of ethylene, rather than being an ethylene effect alone.

Recently, further success with the Tet repressor system has been achieved in tobacco in which the oat ADC gene has been induced in intact plants (Masgrau et al., 1996). Following Tet induction, ADC activities as well as putrescine levels were increased, and these increases correlated with morphological effects, such as leaf wrinkling, chlorosis, necrosis, and reduced stem and root growth.

MUTATIONS IN POLYAMINE METABOLISM

Relatively little work has been carried out regarding mutants in polyamine metabolism/action. In petunia and tomato, floral morphology mutants have been shown to have increases in putrescine content and ADC activity, although the basis of the mutation has not been characterized (Gerats et al., 1988; Rastogi and Kaur-Sawhney, 1990). Formative work from Evans and Malmberg (1989) involved tobacco suspension cell cultures that were tolerant to inhibitors of SAMDC and ODC. The mutants obtained, which were able to regenerate, showed an array of phenotypic characteristics, including dwarfing and dramatic floral aberrations. Unfortunately, the severity of the phenotypes obtained precluded detailed genetic analysis, but the results support the idea that polyamines are important in floral morphogenesis.

More recently, activation T-DNA tagging has been used to create methylglyoxal-bis(guanylhydrazone)-resistant tobacco cell lines (Fritze et al., 1995). A variety of methylglyoxal-bis(guanylhydrazone)-resistant plant lines were recovered, the majority containing single T-DNA inserts representing independent insertion events. Regenerated plants display characteristic leaf and floral malformation as well as parthenocarpy. In two lines studied in detail, SAMDC activity was increased in callus, and in one line this was coupled with elevated levels of putrescine and spermidine. The tagged plant genomic DNA was isolated and used to isolate a functional cDNA that is currently being characterized (Cordeiro et al., 1996).

CONCLUSIONS

These are exciting times in polyamine research. The appearance of molecular probes to the genes encoding the polyamine biosynthetic enzymes, along with the known biochemistry of the enzymes, should clarify when and where polyamine biosynthesis occurs in the intact plant. This, coupled with a genetic analysis, should finally establish the role of polyamines in plants.

To date, molecular evidence essentially supports the biochemical data, suggesting that free polyamines accumulate via ODC in actively dividing cells, whereas ADC is active in elongating cells. Although mRNA accumulation is important in the control of the polyamine biosynthetic pathway, it is clear that other controls exist, most probably at translation and enzyme processing and assembly. In addition, subcellular compartmentation of the enzymes is likely to be important.

Accumulating evidence from transgenic plants expressing the genes encoding the polyamine biosynthetic enzymes shows that flux through the polyamine pathway can be manipulated. On the one hand, this is significant because polyamines are precursors of many important secondary metabolites. On the other hand, changes in polyamine biosynthesis appear to have a reciprocal effect on ethylene biosynthesis. With the latter point in mind, manipulating the polyamine biosynthetic pathway may deserve closer attention from a biotechnological viewpoint.

The analysis of transgenic plants, together with results from mutant analysis, indicates that changes in levels of polyamines can affect leaf morphology, root growth, and flower formation. However, caution is required before using these observations to infer a role for polyamines in plant growth. First, ectopic expression of the genes most probably does not reflect the normal pattern of their expression in plants. Second, over- or underproduction of polyamines may affect other processes, ethylene biosynthesis being but one example.

Probably one of the greatest gaps at the moment in plant polyamine research is the need to create mutants in which the action of single polyamine biosynthetic genes is knocked out. With this in mind, the utility of the antisense technology has already been demonstrated with SAMDC by Kumar et al. (1996). Nevertheless, probably the definitive method of obtaining down-regulation of gene expression is the creation of gene knockouts. Malmberg and Watson (1996) recently reported the isolation of ODC and ADC mutants from ethyl methanesulfonate-mutagenized populations of Arabidopsis. We predict that the genetic analysis of these mutants, along with other molecular and biochemical approaches, should resolve whether the majority of plant biologists were correct in thinking that polyamines do not play an essential role in plant growth and development, or whether that small band of polyamine believers has been right all along.

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LITERATURE CITED

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

Bolle C, Herrmann RG, Oelmüller R (1995) A spinach cDNA with homology to *S*-adenosylmethionine decarboxylase. Plant Physiol **107:** 1461–1462

Borrell A, Besford RT, Altabella T, Masgrau C, Tiburcio AF (1996) Regulation of arginine decarboxylase by spermine in osmotically stressed oat leaves. Physiol Plant **98:** 105–110

Borrell A, Culiañez-Macià A, Altabella T, Besford RT, Flores D, Tiburcio AF (1995) Arginine decarboxylase is localized in chloroplasts. Plant Physiol **109:** 771–776

Chang KS, Nam KH, Lee MM, Lee SH, Park KY (1996) Nucleotide sequence of cDNA (accession no. U63832) encoding *S*-adenosylmethionine decarboxylase from carnation flowers (PGR 96–092). Plant Physiol **112:** 863

Cordeiro A, Fritze K, Schell J, Walden R (1996) T-DNA tagging genes influencing polyamine metabolism (abstract no. 41002). Plant Physiol **111:** S-21

DeScenzo RA, Minocha SC (1993) Modulation of cellular polyamines in tobacco by transfer and expression of mouse ornithine decarboxylase cDNA. Plant Mol Biol **22:** 113–127

Dresselhaus T, Barceló P, Hagel C, Lörz H, Humbeck K (1996) Isolation and characterization of a *Tritordeum* cDNA encoding S-adenosylmethionine decarboxylase that is circadian-clock-regulated. Plant Mol Biol **30:** 1021–1033

Evans PT, Malmberg RL (1989) Do polyamines have roles in plant development? Annu Rev Plant Physiol Plant Mol Biol **40:** 235–269

Fritze K, Czaja I, Walden R (1995) T-DNA tagging of genes influencing polyamine metabolism: isolation of mutant plant lines and rescue of DNA promoting growth in the presence of a polyamine biosynthetic inhibitor. Plant J **7:** 261–271

Galston AW, Kaur-Sawhney R (1995) Polyamines as endogenous growth regulators. *In* PJ Davies, ed, Plant Hormones: Physiology, Biochemistry and Molecular Biology, Ed 2. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 158–178

Gerats AGM, Kaye C, Collins C, Malmberg RL (1988) Polyamine levels in *Petunia* genotypes with normal and abnormal floral morphologies. Plant Physiol **86:** 390–393

Hamill JD, Robins RJ, Parr AJ, Evans DM, Furze JM, Rhodes MJC (1990) Over-expression of a yeast ornithine decarboxylase gene in roots of transgenic *Nicotiana rustica* can lead to enhanced nicotine accumulation. Plant Mol Biol **15:** 27–38

Heimer YM, Mizrahi Y (1982) Characterization of ornithine decarboxylase of tobacco cells and tomato ovaries. Biochem J **201:** 373–376

Kaur-Sawhney R, Tiburcio AF, Galston AW (1988) Spermidine and floral bud differentiation in thin-layer explants of tobacco. Planta **173:** 282–284

Kumar A, Taylor M, Mad Arif SA, Davies H (1996) Potato plants expressing antisense and sense S adenosylmethionine decarboxylase (SAMDC) transgenes show altered levels of polyamines and ethylene:antisense plants display abnormal phenotypes. Plant J **9:** 147–158

Mad Arif SA, Taylor MA, George LA, Butler AR, Burch LR, Davies HV, Stark MJR, Kumar A (1994) Characterization of the S-adenosylmethionine decarboxylase (SAMDC) gene of potato. Plant Mol Biol **26:** 327–338

Malmberg RL, Watson MB (1996) Genetic analysis of polyamine biosynthesis in Arabidopsis (abstract no. 41001). Plant Physiol **111:** S-21

Masgrau C, Altabella T, Farrás R, Flores D, Thompson AJ, Besford R, Tiburcio AF (1996) Inducible overexpression of oat arginine decarboxylase in transgenic tobacco plants. Plant J (in press)

Michael AJ, Furze JM, Rhodes MJC, Burtin D (1996) Molecular cloning and functional identification of a plant ornithine decarboxylase cDNA. Biochem J **314:** 241–248

Nam KH, Lee SH, Lee JH (1996) A cDNA encoding arginine decarboxylase (accession no. U35367) from soybean hypocotyls (PGR 95-142). Plant Physiol **110:** 714

Pérez-Amador MA, Carbonell J, Granell A (1995) Expression of arginine decarboxylase is induced during early fruit development and in young tissues of *Pisum sativum* (L.). Plant Mol Biol **28:** 997–1009

Poulin R, Lu L, Ackermann B, Bey P, Pegg AE (1992) Mechanism of the irreversible inactivation of mouse ornithine decarboxylase by α-difluoromethylornithine. J Biol Chem **267:** 150–158

Rastogi R, Dulson J, Rothstein SJ (1993) Cloning of tomato (*Lycopersicon esculentum* Mill.) arginine decarboxylase gene and its expression during fruit ripening. Plant Physiol **103:** 829–834

Rastogi R, Kaur-Sawhney V (1990) Polyamines and flower development in the male sterile stamenless-2 mutant of tomato. I. Levels of polyamines and their biosynthesis in normal and mutant flowers. Plant Physiol **93:** 439–445

Schröder G, Schröder J (1995) cDNAs for S-adenosyl-L-methionine decarboxylase from *Catharanthus roseus*, heterologous expression, identification of the proenzyme-processing site, evidence for the presence of both subunits in the active enzyme, and a conserved region in the 5′ mRNA leader. Eur J Biochem. **228:** 74–78

Tiburcio AT, Gendy CA, Tran Thanh Van K (1989) Morphogenesis in tobacco subepidermal cells: putrescine as marker for root differentiation. Plant Cell Tissue Org Cult **19:** 43–54

Watson MB, Malmberg RL (1996) Regulation of *Arabidopsis thaliana* (L.) Heynh arginine decarboxylase by potassium deficiency stress. Plant Physiol **111:** 1077–1083

Woon Noh E, Minocha SC (1994) Expression of a human *S*-adenosylmethionine decarboxylase in transgenic tobacco and its effects on polyamine biosynthesis. Transgenic Res **3:** 113–127