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BOTANICAL BRIEFING

Polyamines: ubiquitous polycations with unique roles in growth and stress responses

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• *Background* Polyamines are small polycationic molecules found ubiquitously in all organisms and function in a wide variety of biological processes. In the past decade, molecular and genetic studies using mutants and transgenic plants with an altered activity of enzymes involved in polyamine biosynthesis have contributed much to a better understanding of the biological functions of polyamines in plants.
• *Possible roles* Spermidine is essential for survival of *Arabidopsis* embryos. One of the reasons may lie in the fact that spermidine serves as a substrate for the lysine → hypusine post-translational modification of the eukaryotic translation initiation factor 5A, which is essential in all eukaryotic cells. Spermine is not essential but plays a role in stress responses, probably through the modulation of cation channel activities, and as a source of hydrogen peroxide during pathogen infection. Thermospermine, an isomer of spermine, is involved in stem elongation, possibly by acting on the regulation of upstream open reading frame-mediated translation.
• *Conclusions* The mechanisms of action of polyamines differ greatly from those of plant hormones. There remain numerous unanswered questions regarding polyamines in plants, such as transport systems and polyamine-responsive genes. Further studies on the action of polyamines will undoubtedly provide a new understanding of plant growth regulation and stress responses.

Key words: Polyamines, putrescine, spermidine, spermine, thermospermine, ACL5, translation, uORF.

INTRODUCTION

Polyamines are organic polycations having variable hydrocarbon chains and two or more primary amino groups. The diamine putrescine, triamine spermidine and tetraamine spermine are widespread in living organisms, accumulate to a high concentration in actively proliferating cells, and are involved in a variety of fundamental cellular processes, including transcription, RNA modification, protein synthesis and the modulation of enzyme activities (Tabor and Tabor, 1999). Because polyamines in mammals and bacteria occur mainly as a polyamine–RNA complex, their effects on RNA secondary structure are thought to be one of the primary actions of polyamines (Igarashi and Kashiwagi, 2000). Thermophilic bacteria contain a number of unusual polyamines, including long-chain and branched polyamines, which are implicated in stabilizing DNA and RNAs at high temperatures (Oshima, 2007).

The simplest polyamine, putrescine, is derived either directly from ornithine by ornithine decarboxylase (ODC) or from arginine through several steps catalysed by arginine decarboxylase (ADC), agmatine iminohydrolase and *N*-carbamoylputrescine amidohydrolase (Fig. 1). In contrast to animals and fungi, in which ODC is the first and rate-limiting enzyme in the synthesis of polyamines, plants typically use ADC. The *Arabidopsis thaliana* genome lacks a gene encoding ODC (Hanfrey *et al.*, 2001). Putrescine is converted to spermidine and spermine by successive activities of spermidine synthase and spermine synthase with the use of decarboxylated *S*-adenosyl methionine (dcSAM) as an aminopropyl donor. The dcSAM is produced by *S*-adenosylmethionine decarboxylase (SAMDC) from SAM. SAMDC is thought to be a major regulatory enzyme in the synthesis of spermidine and spermine and is also known to influence the rate of ethylene production in plants given that the precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid, is derived from SAM. Polyamines are further metabolized by oxidation and conjugation with other molecules (Bagni and Tassoni, 2001; Cona *et al.*, 2006; Moschou *et al.*, 2008*b*).

Plant polyamines are preferentially detected in actively growing tissues and under stress conditions and have been implicated in the control of cell division, embryogenesis, root formation, fruit development and ripening, and responses to biotic and abiotic stresses (Kumar *et al.*, 1997). The molecular mechanism of how polyamines act in these processes had remained unclear. In the past decade, however, molecular and genetic studies with mutants and transgenic plants having no or altered activity of enzymes involved in the biosynthesis of polyamines have contributed much to a better understanding of the biological functions of polyamines in plants. In this Briefing, we summarize the recent findings on the individual functions of each polyamine in plant cells and discuss the mode of action at the molecular level. A more detailed review covering a wide range of topics is also given by Kusano *et al.* (2008).

PUTRESCINE IS REQUIRED FOR STRESS TOLERANCE

ADC is a key enzyme in the synthesis of putrescine in plants (Fig. 1). The *Arabidopsis* genome has two genes encoding

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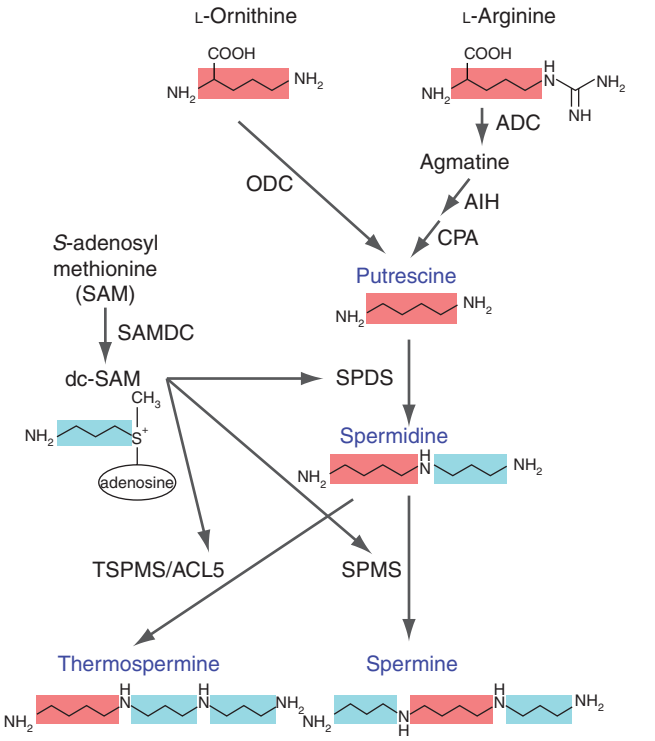

FIG. 1. Biosynthetic pathways of polyamines in plants. Abbreviations: ACL5, ACAULIS5; ADC, arginine decarboxylase; AIH, agmatine iminohydrolase; CPA, *N*-carbamoylputrescine amidohydrolase; ODC, ornithine decarboxylase; SAMDC, *S*-adenosylmethionine decarboxylase; SPDS, spermidine synthase; SPMS, spermine synthase; TSPMS, thermospermine synthase.

ADC: *ADC1* and *ADC2*. In contrast to *ADC1*, which is constitutively expressed in all tissues, *ADC2* is responsive to abiotic stresses such as drought and wounding (Soyka and Heyer, 1999; Pérez-Amador *et al.*, 2002). A loss-of-function mutant of *ADC2* fails to show the osmotic-stress-induced increase in ADC activity observed in the wild-type, but exhibits no obvious phenotype under normal growth conditions (Soyka and Heyer, 1999). This was the first report on a genetically mapped mutant allele of a polyamine biosynthetic gene in plants. Another mutant allele of *ADC2* was later shown to be more sensitive to salt stress than wild-type plants (Urano *et al.*, 2004). This mutant has reduced levels of putrescine, but not of spermidine or spermine, and its stress tolerance is restored by exogenously supplied putrescine. These findings suggest a direct protective role of putrescine in abiotic stress tolerance. It is also clear that putrescine is important as a precursor for the biosynthesis of higher polyamines. According to a threshold model based on studies using transgenic plants with altered putrescine levels (Capell *et al.*, 2004), the putrescine level must exceed a certain threshold to enhance the synthesis of spermidine and spermine under stress, such synthesis being necessary for recovery from the stress. The double mutant of *ADC1* and *ADC2*, which could not produce polyamines, dies at the embryo stage (Urano *et al.*, 2005).

Putrescine also serves as a precursor for the biosynthesis of pyridine and tropane alkaloids such as nicotine in tobacco and hyoscyamine in *Datura stramonium*. The first committed step is catalysed by putrescine *N*-methyltransferase, which converts putrescine into *N*-methylputrescine and is structurally related to spermidine synthase (Fig. 2; Hashimoto *et al.*, 1998).

SPERMIDINE IS ESSENTIAL FOR PLANT GROWTH

Spermidine-deficient mutants in *Escherichia coli* are viable with no growth defects, while the yeast *spe3* mutant, which has no spermidine synthase activity, requires spermidine for growth (Tabor and Tabor, 1999). There are two genes encoding spermidine synthase, *SPDS1* and *SPDS2*, in the *Arabidopsis* genome. Each single mutant of these genes shows no growth defects but embryo development of the double mutant is arrested at the heart stage, indicating a requirement for spermidine during the course of embryogenesis (Imai *et al.*, 2004*a*). On the other hand, as described below, spermine is not essential for viability of *Arabidopsis* (Imai *et al.*, 2004*b*). Although it remains to be examined whether spermine can functionally substitute for spermidine or not, spermidine may be specifically required for some aspects of development. For instance, spermidine is a substrate for deoxyhypusine synthesis. The unusual amino acid deoxyhypusine is produced by deoxyhypusine synthase (DHS), which transfers the butylamine moiety of spermidine to the ε-amino group of highly conserved lysine-50 of inactive eukaryotic translation initiation factor 5A (eIF5A) precursor. The deoxyhypusine-eIF5A is further converted to the active hypusine-eIF5A, which functions in the transport of newly transcribed mRNAs from the nucleus to cytoplasm. The fact that activated eIF5A is essential for eukaryotic cell growth and proliferation (Park, 2006) is consistent with the absolute requirement for spermidine in plant embryo development. A mutation in the *DHS* gene results in the arrest of embryo-sac development in *Arabidopsis* (Pagnussat *et al.*, 2005) probably because deoxyhypusine-eIF5A cannot be supplied by maternal tissues.

Plant polyamines are present not only as free molecules but also as conjugates to cinnamic acids such as *p*-coumaric, ferulic and caffeic acids (Bagni and Tassoni, 2001). The resulting conjugates are known as hydroxycinnamic acid amides. Recently, two genes encoding spermidine disinapoyltransferase (SDT) and spermidine dicoumaroyltransferase (SCT), which mediate the production of spermidine conjugates, were identified in *Arabidopsis* (Luo *et al.*, 2009). *SDT* and *SCT* are highly expressed in developing embryos and root tips, respectively. In addition, an anther tapetum-specific gene encoding spermidine hydroxycinnamoyltransferase was cloned from *Arabidopsis* (Grienenberger *et al.*, 2009). The spermidine conjugates produced are implicated in protecting against pathogens, detoxifying phenolic compounds, and/or serving as a reserve of polyamines that are available to actively proliferating tissues, although not always essential for survival. In most organisms, polyamines can also be covalently bound to glutamine residues of certain proteins by the action of transglutaminase (TGase). The widespread occurrence of TGase activity in all plant tissues suggests the significance of inter- or intra-molecular cross-link formation of the proteins by polyamines (Serafini-Fracassini and Del Duca, 2008).

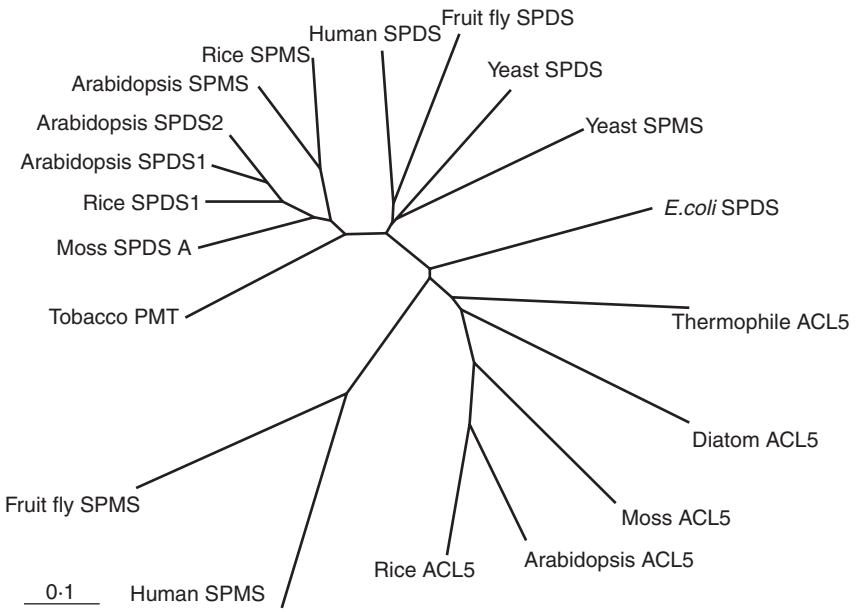

FIG. 2. Molecular phylogenetic tree of the amino acid sequences of spermidine/spermine synthase-related proteins. The full-length amino acid sequences were aligned by use of Clustal X and the tree was drawn by the neighbour-joining method with the software TreeView. ACL5 represents thermospermine synthase or its putative orthologues, which may have been acquired by an ancestor of the plant lineage through horizontal gene transfer from archaea or bacteria, as suggested by the absence of ACL5-like sequences in animals and fungi (Minguet *et al.*, 2008). Abbreviations: PMT, putrescine *N*-methyltransferase; diatom, *Thalassiosira pseudonana*; moss, *Physcomitrella patens*; thermophile, *Thermus thermophilus*.

In some plant species, an aminobutyl group of spermidine is transferred to putrescine by the action of homospermidine synthase (HSS), which catalyses the formation of an uncommon polyamine, homospermidine. Homospermidine is the first intermediate of the biosynthesis of the pyrrolizidine alkaloids that serve as defence compounds in such families as Asteraceae, Boraginaceae and Orchidaceae. The *HSS* gene from *Senecio vernalis* was cloned and shown to be derived from the *DHS* gene (Ober and Hartmann, 1999).

SPERMINE PLAYS VERSATILE ROLES IN STRESS RESPONSES

The *E. coli* genome does not contain a gene for spermine synthase. The yeast *spe4* mutant, which lacks spermine synthase activity, shows normal growth in the absence of spermine (Tabor and Tabor, 1999). In mammals, the mutant male mouse strain that has a deletion of the *SpmS* gene on the X chromosome displays a greatly reduced size, is sterile, and has neurological abnormalities and a short life span. These phenotypes are reversed by the transgenic expression of *SpmS*. Detailed studies of the deafness in this mutant mouse show that the absence of spermine synthase leads to loss of endocochlear potential, which is regulated by inward-rectifier potassium channels (Wang *et al.*, 2009). The inward-rectifier potassium channels are blocked directly by spermine. Furthermore, there are many reports that polyamines, in particular spermine, interact with functionally diverse ion channels and receptors (Williams, 1997). In general, the efficacy of polyamines in modulating or blocking these types of proteins decreases in the order spermine > spermidine > putrescine. In plants, no requirement for spermine under normal growth conditions has been demonstrated in a loss-of-function mutant of *SPMS* in *Arabidopsis* (Imai *et al.*, 2004*b*) but the existence of a polyamine metabolon, a large protein complex containing both spermidine synthase and spermine synthase in *Arabidopsis*, is probably responsible for the efficient production of spermine in plant cells (Panicot *et al.*, 2002). The *spms* mutant appears to be more sensitive to drought and salt stresses than the wild-type (Yamaguchi *et al.*, 2007). This phenotype might be related to the fact that inward potassium currents across the plasma membrane of guard-cells are blocked by intracellular polyamines (Liu *et al.* 2000). Blocking of ion channels by polyamines in plants has also been reported for vacuolar cation channels in barley and red beet (Dobrovinskaya *et al.*, 1999), and for non-selective cation channels in pea mesophyll cells (Shabala *et al.*, 2007).

Although increasing evidence supports a modulating role for spermine in the control of ion channel and receptor activities, one of the most important roles of spermine, which occurs in millimolar concentrations in the nucleus, has been thought to be in protecting DNA from free radical attack and subsequent mutation (Ha *et al.*, 1998). On the other hand, spermine plays a role as a mediator in defence signalling against plant pathogens (Yamakawa *et al.*, 1998; Takahashi *et al.*, 2003). This 'spermine signalling pathway' involves accumulation of spermine in the apoplast, upregulation of a subset of defence-related genes such as those encoding pathogenesis-related proteins and mitogen-activated protein kinases, and a type of programmed cell death known as the hypersensitive response. This response is triggered by spermine-derived H_2O_2, produced through the action of polyamine oxidase (PAO) localized in the apoplast (Cona *et al.*, 2006; Kusano *et al.*, 2008; Moschou *et al.*, 2008*b*). Taken together, these data indicate double-edged roles of spermine in cell survival:

as a free radical scavenger in the nucleus and as a source of free radicals in the apoplast, although the possibility cannot be ruled out that the interaction of spermine with other molecules is involved in the cell death. These paradoxical roles of polyamines are reminiscent of those in mammals, in which excessively accumulated polyamines induce the initiation of apoptosis of several cell types, either through direct action or via H_2O_2 produced by the intracellular spermine/spermidine acetyltransferase/PAO pathway (Seiler and Raul, 2005). It remains unclear how plant pathogens lead to the accumulation of spermine in the apoplast.

STEM ELONGATION REQUIRES THERMOSPERMINE

Thermospermine is a structural isomer of spermine first discovered in thermophilic bacteria (Oshima, 2007). Thermospermine is synthesized from spermidine in a similar reaction to spermine synthesis (Fig. 1). The *Arabidopsis* gene encoding thermospermine synthase, *ACL5*, was previously identified as encoding spermine synthase from the *acaulis5* (*acl5*) mutant, which shows a severely dwarfed phenotype with over-proliferation of xylem tissues (Hanzawa *et al.*, 2000), but a detailed biochemical study of *ACL5* and its orthologue cloned from the diatom *Thalassiosira pseudonana* reveals that they encode thermospermine synthase (Knott *et al.*, 2007). A study of the evolutionary pathways of genes involved in polyamine biosynthesis suggests that plants acquired the ability to synthesize thermospermine at an early stage of evolution by horizontal gene transfer from a prokaryote (Fig. 2; Minguet *et al.*, 2008). Exogenous application of thermospermine but not spermine to the *acl5* mutant partially rescues plant height and reduces the *acl5* transcript level that is increased in the mutant (Kakehi *et al.*, 2008). Reduction in *ACL5* expression by thermospermine is also observed in wild-type plants and indicates a negative feedback control of thermospermine synthesis. *ACL5* shows a preferential expression in developing xylem vessel elements, suggesting a role in preventing premature cell death in xylem differentiation (Clay and Nelson, 2005; Muñiz *et al.*, 2008). We have confirmed that the dwarf phenotype of *acl5* was partially overcome by transgenic expression of a moss *ACL5* orthologue in *acl5* plants (Fig. 3; our unpubl. data). An interesting question is how the role of thermospermine was integrated into the regulation of stem elongation during the evolution of higher plants. In experiments designed to elucidate the mode of action of thermospermine in stem elongation, suppressor mutants of *acl5*, named *sac*, that show a recovery from the dwarf phenotype in *acl5* have been isolated. One of the responsible genes, *SAC51*, encodes a bHLH-type (basic-helix-loop-helix) transcription factor and the *sac51-d* allele has a point mutation in the 4th upstream open reading frame (uORF) of the five uORFs present in the *SAC51* 5′ leader sequence (Imai *et al.*, 2006). The dominant nature of the *sac51-d* phenotype in *acl5* can be attributed to an increased translational efficiency of the *SAC51* main ORF because the 4th uORF of *SAC51* has an inhibitory effect on the translation of the main ORF. The gene for another mutant, *sac52-d*, encodes ribosomal protein L10 (RPL10A) and the *sac52-d* allele also increases the translational efficiency of the *SAC51* main ORF (Imai *et al.*, 2008). In addition, we have found that a newly identified mutant, *sac56-d*, has a defect in a gene encoding RPL4 and shows a similar effect to that of *sac52-d* on *SAC51* translation (our unpubl. data). These results suggest that SAC51 is one of the key transcription factors controlling stem elongation and that thermospermine plays a crucial role in its uORF-mediated translational regulation.

FIG. 3. Phenotype of the *Arabidopsis acl5-1* mutants, which are defective in the synthesis of thermospermine. Forty-day-old plants of *acl5-1* (left) and transgenic *acl5-1* expressing a full-length cDNA of the moss *ACL5B* (GenBank accession number EDQ54752) (right) are shown.

POLYAMINES ACT IN UPSTREAM ORF-MEDIATED TRANSLATION

It is well known that spermidine and spermine regulate the translation of SAMDC with a small uORF of its mRNA that encodes a conserved hexapeptide (MAGDIS) in mammals (Ruan *et al.*, 1996). The MAGDIS peptide causes ribosome stalling at its own termination codon in the presence of elevated levels of polyamines through polyamine-dependent interaction of the peptide with a component of the translational machinery; consequently, MAGDIS blocks translation of the SAMDC main ORF and the synthesis of polyamines. A similar autoregulatory circuit is found in the SAMDC translation in *Arabidopsis* but its regulation involves two uORFs, the first and shorter of which is responsible, at reduced levels of polyamines, for repressing translation of the second and longer uORF, which in turn blocks SAMDC translation constitutively irrespective of polyamine levels (Hanfrey *et al.*, 2005). The predicted role of spermidine and spermine in bypassing the inhibitory effect of the first uORF on the translation of the second uORF seems to be opposite to that in mediating the effect of the MAGDIS uORF in mammals

and, rather, to be related to that of thermospermine in enhancing the *SAC51* main ORF translation. For polyamines to bypass the inhibitory effect of uORFs, several possible mechanisms are conceivable, including translational frame-shifting and ribosome shunting. Translational frame-shifting is known to occur in response to high polyamine levels to induce the synthesis of the ODC antizyme, which destabilizes ODC in mammals and yeasts (Ivanov *et al.*, 2000). Ribosome shunting, a mechanism by which ribosomes physically shunt over the 5′ leader of mRNAs to reach their initiation codon, is also suggested to be stimulated by polyamines in certain mRNAs in mammals (Nishimura *et al.*, 2009). Molecular targets and actions of polyamines in uORF-mediated translational regulation in plants are expected to be identified in the near future.

POLYAMINE OXIDATION

While putrescine is catabolized by the copper-containing diamine oxidase to 4-aminobutanal with concomitant production of NH_3 and H_2O_2, spermidine, spermine and probably thermospermine are catabolized by PAOs (Bagni and Tassoni, 2001). PAOs are classified as those de-aminating spermidine and spermine to 4-aminobutanal and *N*-(3-aminopropyl)-4-aminobutanal, respectively, along with 1,3-diaminopropane and H_2O_2, and those back-converting spermine to spermidine and spermidine to putrescine with concomitant production of 3-aminopropanal and H_2O_2 (Moschou *et al.*, 2008*b*). These de-amination reactions occur mainly in the apoplast and the resulting H_2O_2 plays a role in mediating a complex array of defence responses to microbial pathogens as described above. Furthermore, polyamine-derived H_2O_2 has been implicated in cell-wall maturation and lignification during development as well as in wound-healing and cell-wall reinforcement during pathogen invasion (Cona *et al.*, 2006). During abiotic stress responses, spermidine is secreted into the apoplast and oxidized to produce H_2O_2 (Moschou *et al.*, 2008*a*). PAOs possessing back-conversion activity are sorted into peroxisomes in *Arabidopsis* as in mammals but their physiological roles remain unclear (Moschou *et al.*, 2008*b*).

PROSPECTS

The physiological roles of polyamines in plants are gradually being elucidated at the molecular level. Among them, the role of extracellular polyamines as a source of H_2O_2 and that of thermospermine in stem elongation seem to be unique to higher plants. Further knowledge of polyamine catabolism, including degradation and conjugation, will shed light on the maintenance of polyamine homeostasis in plant cells. Although the export system of polyamines in plants needs to be investigated, their import mechanism will also need to be addressed, in view of the presumed occurrence in the soil of sufficient polyamines for the root to utilize. On the other hand, thermospermine synthesis is maintained through a feedback regulation of *ACL5* gene expression in *Arabidopsis*, the mechanism of which remains unknown. Detailed characterization of a series of *sac* mutants and their responsible genes should aid in deciphering the precise mode of action of thermospermine in stem elongation. Further identification of polyamine-responsive genes might also help us to understand the role of each polyamine. The molecular mechanisms of the action of polyamines are obviously different from those of plant hormones, in which ubiquitin–proteasome pathways play a central part (Santner and Estelle, 2009), and undoubtedly provide a new understanding of plant growth regulation and stress responses.

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