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Research article

Role of polyamines in plant vascular development

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ABSTRACT

Several pieces of evidence suggest a role for polyamines in the regulation of plant vascular development. For instance, polyamine oxidase gene expression has been shown to be associated with lignification, and downregulation of S-adenosylmethionine decarboxylase causes dwarfism and enlargement of the vasculature. Recent evidence from *Arabidopsis thaliana* also suggests that the active polyamine in the regulation of vascular development is the tetraamine thermospermine. Thermospermine biosynthesis is catalyzed by the aminopropyl transferase encoded by *ACAULIS5*, which is specifically expressed in xylem vessel elements. Both genetic and molecular evidence support a fundamental role for thermospermine in preventing premature maturation and death of the xylem vessel elements. This safeguard action of thermospermine has significant impact on xylem cell morphology, cell wall patterning and cell death as well as on plant growth in general. This manuscript reviews recent reports on polyamine function and places polyamines in the context of the known regulatory mechanisms that govern vascular development.

1. Introduction

Putrescine, spermidine, and spermine have been described as the most common polyamines in eukaryotes. However, in plants, recent evidence has shown that spermine is found only in angiosperms while thermospermine is likely present throughout the whole plant kingdom [33].

The importance of polyamines has become clear from the changes in polyamine levels that accompany certain developmental transitions or exposure to stress conditions. In addition, exogenous applications of polyamines have frequently been shown to affect plant growth and as well as the response against various stress factors [14,30]. Loss of function mutations in polyamine metabolism genes have shown that the diamine putrescine and the triamine spermidine are essential for life in all organisms evaluated [6,16,24,25,46]. With respect to tetraamines, although they have been proven to be dispensable for strict survival in plants, they have been shown to be involved in stress tolerance [2,48,49]. Moreover, recent results have highlighted the regulation of vascular differentiation by the tetraamine thermospermine, as reviewed in the next sections [7,20,27,34].

2. Brief overview of vascular development

All vascular tissues are derived from undifferentiated, meristematic cells (e.g. the procambium and cambium) [50]. The body plan for the vasculature in the adult plant is already established in the embryo. Certain cells along the embryo axis acquire procambial identity, and they will sustain primary growth of the vasculature. In a later developmental stage, the vascular cambium is formed giving rise to the secondary, radial growth of the vasculature in the stem. Procambial and cambial cells are polar in the sense that depending on their position, they give rise either to the phloem or the xylem. The initiation of xylem differentiation depends on the concerted action of several players [45]. Auxin plays an important role as a trigger of the developmental program, while the activity of a family of homeodomain transcription factors (HD-ZIP III) provides the necessary spatial information for the acquisition of xylem identity. Among these genes is *ATHB8*, which is an early marker for procambial activity [3,4]. Overexpression of *ATHB8* provokes overproliferation of xylem cells [4]. Downregulation of another member of the HD-ZIP III family, *ATHB15*, results in plants with increased

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vascular tissue, while its overexpression leads to smaller vascular bundles [28,41]. This suggests that not all HD-ZIP III genes act as positive regulators of cambial cell specification and proliferation.

Xylem is composed of the water-transporting tracheary elements, such as tracheids and the vessel elements, and the xylem fibers that provide the necessary support to the growing tissue [35]. The xylem elements typically undergo a differentiation program that involves, in a sequential manner, cell expansion, deposition of secondary cell wall material including lignin, and cell death.

Cell death of tracheary elements is a well recognized example of programmed cell death during plant development, in which specific genes are upregulated to trigger destruction. This process involves the collapse of the vacuole followed by hydrolysis of the nuclear DNA and the cytoplasmic contents as a result of the activation or the release of the hydrolytic enzymes released from the vacuole [12,26]. Even though the process of xylem cell death is well described, little is known about the molecular mechanism that regulates it.

3. Broad range effects of polyamines on vascular development

An early link between polyamines and plant vasculature was the identification of relatively high concentrations of putrescine, spermidine and spermine in xylem exudates of sunflower, grapevine, mung bean, and orange trees [10]. However, this finding was logically interpreted in the key of long-distance translocation of polyamines and their role in the regulation of plant development along the whole organism. This idea was pursued later by careful examination of the levels of the different polyamines along the axis of the plant, establishing correlations between the concentrations of putrescine, spermidine and spermine, and the size of the cells and the age of the tissues [38].

More relevant to vascular development, polyamine metabolism has been related to the formation of the reactive oxygen species (ROS) that play a key role in cell wall expansion and growth, vascular differentiation, and lignin polymerization [40]. During vascular differentiation, lignin is formed with the concurrence of H_2O_2 and cell wall bound peroxidases [9]. Enzymes that may be responsible for H_2O_2 generation include diamine oxidase (DAO) [42,47], and polyamine oxidase (PAO) [8]. The strong upregulation of DAO and PAO in vascular tissues and their direct correlation with peroxidase gene expression in tobacco [39] could make amine oxidases an attractive candidate for a role in the generation of H_2O_2 for protein cross-linking and lignification.

The availability of decarboxylated S-adenosylmethionine (dcSAM) for the synthesis of longer-chain polyamines like spermidine and spermine also seems to be an important limiting step in the correct development of the vasculature. Compared with wild-type, the morphology of the vascular bundles of the *bud2* mutant, defective in *SAMDC4*, one of the four SAM decarboxylase genes in *Arabidopsis*, is greatly affected, due to a significant increase in bundle size [13]. Moreover, the lignin content in *bud2* mutants was at least 30% higher than in wild-type inflorescences, although cellulose content did not vary. On the other hand, the bushy phenotype of *bud2* suggests a possible involvement of polyamines in signal transduction of auxin and cytokinin.

4. Thermospermine and xylem formation

4.1. ACL5 and vascular development

Beyond the influence of polyamine synthesis and degradation on the formation of cell walls and lignin biosynthesis through the interference with redox metabolism, the most solid evidence available for the involvement of a polyamine in the formation of the vasculature was derived from molecular genetic analyses of *Arabidopsis* mutants impaired in stem elongation. In an attempt to identify genes controlling the architecture of the aerial part of the plant, five *acaulis* mutants were isolated with a defect in stem elongation which couldn't be rescued by the application of hormones known to control organ growth, such as gibberellins and brassinosteroids [19,44]. Particular attention was devoted to *ACAULIS5* (*ACL5*), whose loss of function caused severe shortening of internodes, smaller mature leaves, and, more importantly, over-proliferation of xylem vessel elements in the vascular bundles of the inflorescence stems. Given that the general cell size in elongating organs was smaller in *acl5* mutants compared to the wild-type, and that the orientation of cortical microtubules in epidermal cells of the *acl5* mutant was mostly longitudinal instead of transversal, as it is the case in wild-type cells, ACL5 was initially attributed a role in signaling during cell elongation in inflorescence stems.

ACL5 was cloned and proposed to encode a spermine synthase based on the similarity with aminopropyl transferases and the ability of the ACL5 protein expressed in *Escherichia coli* to catalyze the conversion of spermidine into a tetraamine identified as spermine by HPLC analysis [20]. Interestingly, an apparent paradox was raised when another gene encoding a spermine synthase (SPM1) was identified in *Arabidopsis* [37], the corresponding knockout mutant did not display any defect in stem elongation [24]. But in fact, this contradiction is explained because the spermidine aminopropyl transferase encoded by *ACL5* in fact catalyzes the in vitro formation of thermospermine, and not spermine [29]. These two tetraamines cannot be distinguished with the HPLC method used in previous studies, although they are easily separated by alternative procedures, such as thin-layer chromatography. Thermospermine had been detected at low concentrations mainly in archaea, bacteria and certain aquatic plants [15,36], and its presence in *Arabidopsis* wild-type (but not *acl5*) extracts has been recently confirmed in a qualitative manner [27]. Therefore, the most likely cause of the stem growth defect displayed by *acl5* mutants is the lack of thermospermine, although exogenous supply of this polyamine to mutant plants allowed only very partial rescue of the wild-type phenotype [27].

Although it has been suggested that the xylem defects associated with *acl5* were the result of deficient auxin transport [7], two other pieces of evidence make this a less likely possibility: a slight increase in the concentration of the natural auxin, indole-3-acetic acid (IAA), and increased expression of the IAA marker line *DR5::GUS* in the hypocotyls of young *acl5* seedlings (Fig. 1), which must be the result of IAA transport from the apical meristem and the youngest leaves where it is synthesized. Therefore, it seems that IAA can be transported in young *acl5* seedlings. It is possible that the reduced auxin transport capacity demonstrated earlier in excised pieces of the inflorescence stem is a secondary effect due to altered xylem specification and impaired growth of the *acl5* mutant.

Apart from the overproliferation of vascular cells observed in the stems of *acl5* mutants, additional pieces of evidence suggest that thermospermine has an important role in the correct development of the vasculature. First, expression of the *ACL5* gene is upregulated upon auxin treatments [20]. This regulation might be highly significant because, as indicated above, auxin serves as a signal for the initiation of cell differentiation at the vascular cambium. Second, *ACL5* transcripts are detected only associated to the vascular cambium, in cells that initiate differentiation [7,34]. And third, thickvein (*tkv*) mutants are allelic to *acl5*, and they present an overall increase in leaf vascularization and in vein thickness [7]. Besides, it is noteworthy that the morphological defects of the knockout mutants in *Arabidopsis BUD2/SAMDC4*, with lower capacity to synthesize one of the substrates for thermospermine synthesis, closely resemble those displayed by *acl5* mutants [13].