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Polyamines in plants: An overview

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Abstract

This article presents an overview of the role of polyamines (PAs) in plant growth and developmental processes. The PAs, putrescine, spermidine and spermine are low molecular weight cations present in all living organisms. PAs and their biosynthetic enzymes have been implicated in a wide range of metabolic processes in plants, ranging from cell division and organogenesis to protection against stress. Because the PA pathway has now been molecularly and biochemically elucidated, it is amenable to modulation by genetic approaches. Genes for several key biosynthetic enzymes namely, arginine decarboxylase, ornithine decarboxylase and S-adenosyl methionine decarboxylase have been cloned from different plant species, and antibodies to some genes are now available. Both over-expressed and antisense transgenic approaches to PA biosynthetic genes have provided further evidence that PAs are required for plant growth and development. However, molecular mechanisms underlying PA effects on these processes remain unclear. Analysis of gene expression by using DNA microarray genomic techniques should help determine the precise role of these compounds. The potential of proteomics to unravel the role of PAs in particular cellular processes has also been examined. The extensive use of the two-hybrid system and other proteomic approaches will provide new insights into the role of PAs in signal transduction. Furthermore, there is evidence that proteomics provides an excellent tool for determining supramolecular organizations of PA metabolic enzymes which may help in understanding homeostatic control of this metabolic pathway.

Key words: Polyamines, mutants, transgenic plants, genomics, proteomics

Bitkilerde poliaminler: Genel bir bakış

Özet

Bu makalede poliaminlerin (PA) bitki büyüme ve gelişme olaylarındaki rolüne genel bir bakış yapılmaktadır. PA ler putresin, spermidin ve spermin, düşük molekül ağırlıklı ve tüm canlı organizmalarda mevcut olan maddelerdir. PA lerin ve bunların biyosentetik enzimlerinin bitkileri strese karşı korumaya yönelik olarak hücre bölünmesinden organogeneze kadar değişen geniş bir metabolik olaylar zincirinde yer aldığı ortaya konmuştur. Günümüzde PA yolu moleküler ve biyokimyasal yönden açıklığa kavuştuğu için genetik yaklaşımlarla düzenlenmeye uygundur. Çeşitli anahtar biyosentez enzimleri, arginin dekarboksilaz, ornitin dekarboksilaz ve S-adenozil metiyonin dekarboksilazın genleri farklı bitki türlerinde klonlanmıştır ve günümüzde bazı genlerin antikorlarını elde etmek mümkündür. PA biyosentezi genlerine hem over-ekspres ve hem de antisens transgenik yaklaşımlar PA lerin bitki büyüme gelişmesi için gerekliliğini daha da ortaya koymuştur. Bununla birlikte bu olaylardaki PA etkilerinin moleküler mekanizması hala açıklığa kavuşmamıştır. DNA mikroarray genom teknikleri kullanılarak yapılan gen ekspresyon analizleri bu bileşiklerin rollerini kesin olarak belirlemeye yardımcı olacaktır. PA lerin özellikle hücresel olaylardaki rolünü ortaya koymaya yönelik olarak proteomiğin potansiyeli de araştırılmıştır. İki-hibrit sistemi ve diğer proteomik yaklaşımların yoğun kullanımı, PA lerin sinyal iletimindeki rolüne yeni bir bakış açısı getirecektir. Bundan başka proteomiğin, PA metabolik yolunun homeostatik kontrolünü anlamaya yardımcı olabilecek, PA metabolizma enzimlerinin supramoleküler organizasyonunun belirlenmesinde çok önemli bir araç olduğu konusunda veriler mevcuttur.

Anahtar sözcükler: Poliaminler, mutantlar, transgenik bitkiler, genomik, proteomik

1. Introduction

Polyamines (PAs) are low molecular weight polycations found in all living organisms (Cohen, 1998). They are known to be essential for growth and development in prokaryotes and eukaryotes (Tabor and Tabor, 1984; Tiburcio et al., 1990). In plant cells, the diamine putrescine (Put), triamine spermidine (Spd) and tetramine spermine (Spm) constitute the major PAs. They occur in the free form or as conjugates bound to phenolic acids and other low molecular weight compounds or to macromolecules such as proteins and nucleic acids. As such, they stimulate DNA replication, transcription and translation. They have been implicated in a wide range of biological processes in plant growth and development, including senescence, environmental stress and infection by fungi and viruses. Their biological activity is attributed to their cationic nature. These findings have been discussed in several recent review articles (Tiburcio et al., 1993; Galston et al.,1997; Bais and Ravishankar, 2002).

The use of PA biosynthesis inhibitors has shown a causal relationship between changes in endogenous PA levels and growth responses in plants. These observations led to further studies into undestanding the mode of PA action. Some of the important observations suggest that PAs can act by stabilizing membranes, scavenging free radicals, affecting nucleic acids and protein synthesis, RNAse, protease and other enzyme activities, and interacting with hormones, phytochrome, and ethylene biosynthesis (reviewed in Slocum et al., 1984; Galston and Tiburcio, 1991). Because of these numerous biological interactions of PAs in plant systems, it has been difficult to determine their precise role in plant growth and development.

In recent years, however, investigations into molecular genetics of plant PAs have led to isolation of a number of genes encoding PA biosynthetic enzymes and development of antibodies to some of the genes. Furthermore mutants and transgenic plants with altered PA metabolism have also been developed. Genomic and proteomic approaches are being used to further gain an understanding into the role of PAs in plant developmental processes. These findings will hopefully lead to a better understanding of their specific functions in plants. Several useful reviews on these aspects have been published (Galston et al., 1997; Walden et al., 1997; Malmberg et al., 1998; Martin-Tanguy, 2001; Bais and Ravishankar, 2002).

This article presents an overview of the role of PAs in plants with particular emphasis on recent investigations using molecular and genetic approaches.

2. Polyamine biosynthesis

The PA biosynthetic pathway in plants has been thoroughly investigated and reviewed in detail (Evans and Malmberg, 1989; Tiburcio et al., 1990; Slocum, 1991a; Martin-Tanguy, 2001). Briefly, PAs are synthesized from arginine and ornithine by arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) as illustrated in Figure 1. The intermediate agmatine, synthesized from arginine, is converted to Put, which is further transformed to Spd and Spm by successive transfers of aminopropyl groups from decarboxylated S-adenosylmethionine (dSAM) catalysed by specific Spd and Spm synthases. The aminopropyl groups are derived from methionine, which is first converted to S-adenosylmethionine (SAM), and then decarboxylated in a reaction catalyzed by SAM decarboxylase (SAMDC). The resulting decarboxylated SAM is utilized as an aminopropyl donor. SAM is a common precursor for both PAs and ethylene, and SAMDC regulates both biosynthetic pathways as illustrated in Figure 1.

A number of investigators have used PA inhibitors to modulate the cellular PA titer in order to determine their role in various plant processes. Four commonly used inhibitors of PA synthesis are: 1. Difluoromethylornithine (DFMO), an irreversible inhibitor of ODC (reviewed in Bey et al., 1987); 2. Difluoromethylarginine (DFMA), an irreversible inhibitor of ADC (Bitonti et al., 1987); 3. Methyl-glyoxyl-bis guanylhydrazone (MGBG), a competitive inhibitor of S-adenosyl-methionine decarboxylase (SAMDC) (Williams-Ashman and Schenone,1972); and 4. Cyclohexylamine (CHA), a competitive inhibitor of spermidine synthase (Hibasami et al., 1980). Common oxidases are diamine oxidase and polyamine oxidase (PAO), as reviewed by Smith and Marshall (1988). Each PA has been found to be catabolized by a specific oxidase.

Several investigations have dealt with localization of PAs and their biosynthetic enzymes in plants (reviewed by Slocum, 1991b). However, paucity of information regarding the exact cellular and subcellular localization of these entities remains one of

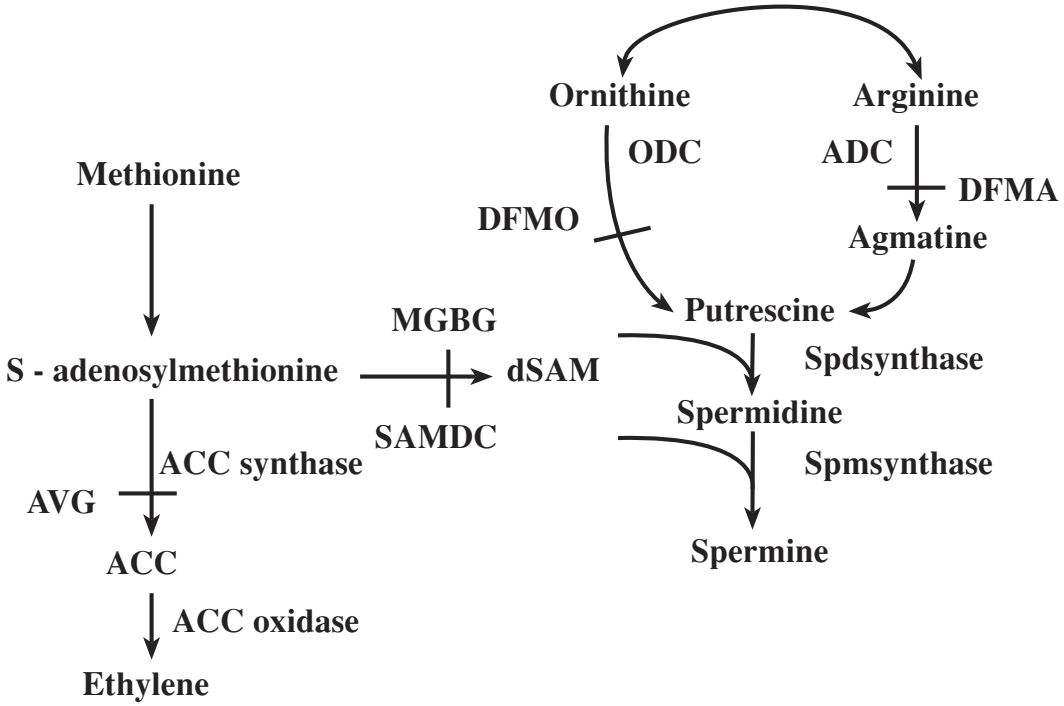

Figure 1: Polyamine biosynthetic pathway and its linkage to ethylene biosynthetis. Biosynthetic enzymes are ADC, ODC and SAMDC and the inhibitor DFMA, DFMO and MGBG.

the obstacles in understanding their biological role. Recent studies have shown that PAs are present in the cell wall fractions, vacuole, mitochondria and chloroplasts (Torrigiani et al., 1986; Slocum, 1991b; Tiburcio et al., 1997). The biosynthetic enzymes, ODC, SAMDC, and Spd synthase have been reported to be localized in the cytoplasm, whereas ADC is localized in the thylakoid membrane of chloroplast (Borrell et al., 1996; Tiburcio et al., 1997) and PAO in the cell wall (Kaur-Sawhney et al., 1981). ODC activity has also been observed in the nucleus (Slocum, 1991b). However, these findings have to be interpreted with caution because various procedural problems can mask the results. Despite these advances in understanding the metabolic processes involving PAs and their localization in plant cells, the precise role of PAs in plant morphogenesis remains elusive.

3. Polyamines in plant growth and development

The availability of specific inhibitors of PA biosynthesis has helped in investigating the mechanisms involved in PA interactions to some extent, providing a partial understanding of their physiological role in plant growth and development. Clearly, PAs are involved in many plant developmental processes, including cell division, embryogenesis, reproductive organ development, root growth, tuberization, floral initiation and development, fruit development and ripening as well as leaf senescence and abiotic stresses (reviewed by Evans and Malmberg, 1989; Galston et al., 1997; Bais and Ravishankar, 2002; Tiburcio et al., 2002). Changes in free and conjugated PAs and their biosynthetic enzymes, namely ADC, ODC, and SAMDC have been found to occur during these developmental processes. Earlier experiments had shown that increases in PAs and their biosynthetic enzymes are associated with rapid cell division in many plant systems e.g., carrot embryogenesis (Montague

and Koppenbrink, 1978; Feirer et al., 1984), tomato ovaries (Heimer and Mizrahi, 1982), tobacco ovaries (Slocum and Galston, 1985), and fruit development (reviewed in Kakkar and Rai, 1993). Similar results have been reported for many other plant species (reviewed in Bais and Ravishankar, 2002). In contrast, several other studies have suggested that correlations between PAs and their biosynthetic enzymes and plant growth processes, especially somatic embryogenesis, are not universal and may be species specific (reviewed in Evans and Malmberg, 1989; Galston et al., 1997; Bais and Ravishankar, 2002).

In general, cells undergoing division contain high levels of free PAs synthesized via ODC, and cells undergoing expansion and elongation contain low levels of free PAs synthesized via ADC (see review by Galston and Kaur-Sawhney, 1995). High levels of endogenous PAs and their conjugates have also been found in apical shoots and meristems prior to flowering (Cabbane et al., 1981) and flower parts of many plants (Martin-Tanguy, 1985). Our experiments using callus cultures derived from thin layer explants of pedicels from tobacco inflorescence show that endogenous Spd increased more rapidly than other PAs in floral buds than in vegetative buds. Addition of CHA, an inhibitor of Spd synthesis, to the culture medium reduced flower formation in a dose dependent manner and such inhibition was correlated with a switch to initiation of vegetative instead of flower buds. This inhibition was reversed by the addition of exogenous Spd (Kaur-Sawhney et al., 1988). More recently, we have found that higher levels of endogenous PAs occur in flowers and siliques when compared with their levels in leaves and bolts of certain strains of *Arabidopsis*. Addition of the PA biosynthetic inhibitors, DFMA and CHA to the culture medium, at time of seed germination, inhibited bolting and flower formation and this was partially reversed by addition of exogenous Spd (Applewhite et al., 2000). These results clearly show that Spd is involved in flower initiation and development. Similar results have been reported in other plants also (reviewed by Galston et al.,1997; Bais and Ravishankar, 2002).

Many plant growth and development processes known to be regulated by plant hormones, such as auxins, 2,4-D, GA and ethylene, have also been correlated with changes in PA metabolism. These changes occur in both endogenous levels of PAs and their biosynthetic enzymes and appear to be tissue specific (reviewed by Galston and Kaur-Sawhney,1995). Thus, PAs which may or may not be mobile in plants (Young and Galston, 1983; Bagni and Pistocchi, 1991) can serve as intracellular mediators of hormone actions (Galston and Kaur-Sawhney, 1995). Supporting evidence for this hypothesis has been obtained in experiments using specific inhibitors of PA biosynthesis (Bagni et al., 1981; Egea-Cortines and Mizrahi, 1991; reviewed in Galston et al., 1997; Bais and Ravishankar, 2002).

Of the major plant hormones, ethylene has been most intensively investigated with respect to PA metabolism. The two metabolites, PAs and ethylene, play antagonistic roles in plant processes. While PAs inhibit senescence of leaves (Kaur-Sawhney et al., 1982), cell cultures of many monocot and dicot species (Muhitch et al., 1983) and fruit ripening (Kakkar and Rai, 1993), ethylene promotes these processes. The most commonly held view is that PAs and ethylene regulate each other's synthesis, either directly or through metabolic competition for SAM, a common precursor for their biosynthesis (Figure 1). PAs inhibit ethylene biosynthesis, perhaps by blocking the conversion of SAM to ACC and of ACC to ethylene (Apelbaum et al., 1981; Suttle, 1981; Even-Chen et al., 1982; Furer et al., 1982). Ethylene, on the other hand, is an effective inhibitor of ADC and SAMDC, key enzymes in PA biosynthetic pathway (Apelbaum et al., 1985; Icekson et al., 1985). Thus, PAs may affect senescence and fruit ripening by modulating PA and ethylene biosynthesis.

Apparently, PAs are essential members of an array of internal metabolites required in many plant developmental processes, but their precise role in these processes has yet to be established. Whereas, specific PAs at specific concentrations may be required at critical stages of growth and morphogenetic events, no definitive data are available to establish their role as plant hormones.

4. Manipulation of the polyamine pathway

The PA pathway is ubiquitous in living organisms and is relatively short (see Section 2) in terms of the number of enzymes involved. Most of the genes coding for enzymes involved in the pathway have been cloned from different sources (Kumar et al., 1997; Walden et al., 1997; Galston et al., 1997; Tiburcio et al., 1997; Malmberg et al., 1998; Kumar and Minocha, 1998; Panicot et al., 2002b). Thus, the PA pathway

represents an excellent model to test various hypotheses and to answer fundamental biological questions derived from pathway manipulation (Thu-Hang et al., 2002; Bhatnagar et al., 2002).

Initially, approaches to manipulate the PA pathway made use of suicide inhibitors, but the effects of DFMO and DFMA on ODC and ADC respectively, are variable in different plant systems, ranging from inhibition to stimulation or no effect and depending on the concentration, plant system tested and the existence of compensatory mechanisms (Slocum and Galston, 1987). Therefore, alternative approaches to manipulate polyamine metabolism have been developed during the recent years.

4.1. Mutants

Mutants deficient in PA biosynthesis have been isolated from several biological systems. Hafner et al. (1979) isolated PA mutants in *Escherichia coli* showing decreased growth and increased sensitivity to paraquat (Milton et al., 1990). Yeast mutants presenting ODC as the sole pathway, show reduced growth and altered sporulation on PA deficient medium (Cohn et al., 1980; Whitney and Morris, 1978). Chinese hamster ovary cells lacking ODC activity do not grow in medium lacking PA (Steglich and Scheffler, 1983) and a moderately reduced brood size was observed in a *Caenorhabditis elegans* ODC deletion mutant (Macrae et al., 1995). Mutations in genes affecting Spd and Spm biosynthesis have also been isolated in yeast. The *spe3 Spd* synthase mutation causes a growth arrest, which can be complemented with externally added Spd (Hamasaki-Katagiri et al., 1997), while the yeast *spe4* mutant is defective in Spm biosynthesis (Hamasaki-Katagiri et al., 1998).

Less is known about mutants affecting PA metabolism in plants. Mutants with high levels of ADC activity have been identified in petunia because of their abnormal morphology (Geerats et al., 1988), but the basis of the mutation is still not known. Screening for resistance to the SAMDC inhibitor MGBG (Malmberg and Rose, 1987) or to inhibitory concentrations of Spm (Mirza et al., 1997), yielded mutants that showed reduced sensitivity to the respective agent, but these mutants have not been further exploited for the analysis of PA function. Watson et al. (1998) isolated EMS mutants of *A. thaliana* that are reduced in ADC activity. The mutants fall into two complementation groups, *spe1* and *spe2*, which may correspond to the two gene copies encoding ADC, *ADC1* and *ADC2* (Watson et al., 1998). The mutations have not been mapped and therefore it cannot be excluded that other functions, i.e. regulatory elements, are affected (Soyka and Heyer, 1999). More recently, Hanzawa et al. (2000) reported that the inactivation of the *Arabidopsis ACAULIS5* (ACL5) gene causes a defect in the elongation of stem internodes by reducing cell expansion. It was suggested that ACL5 encodes a Spm synthase, but the possibility that ACL5 may exhibit broad amine substrate specificities and be involved in the synthesis of other polyamines could not be excluded (Hanzawa et al., 2000).

Thus far the only well characterized plant polyamine biosynthetic mutant has been generated by using reverse genetics. The availability of mutant collections generated either by transposon or T-DNA tagging now facilitates the identification of knockouts in any gene of interest using PCR-based mutant screening techniques (Ferrando et al., 2002). By using these techniques, Soyka and Heyer (2000) isolated an *Arabidopsis thaliana* mutant line carrying an insertion of the *En-1* transposable element at the *ADC2* locus which should be regarded as a complete loss-of-function or knockout mutation. The *ADC2* knockout mutant shows no obvious phenotype change under normal growth conditions, but is completely devoid of ADC induction by osmotic stress. As *ADC1* gene expression was not affected in the mutant, it was concluded that *ADC2* is the gene responsible for induction of ADC and PA biosynthesis under osmotic stress (Soyka and Heyer, 2000). More recently, Pérez-Amador et al. (2002) have shown that *ADC2* gene expression is induced in response to mechanical wounding and methyl jasmonate treatment in *Arabidopsis thaliana*. All these observations appear to indicate that *ADC2* is a key gene involved in the PA response to abiotic stress in *Arabidopsis*. We envisage that the extensive use of functional genomics and reverse genetic studies will facilitate the isolation of novel knock-out mutants affected in other PA biosynthetic genes.

4.2. Transgenic plants

With the availability of most of the genes involved in PA metabolism, it has become possible to manipulate this metabolic pathway using sense and antisense transgenic approaches. Thus, cellular PA content has

been modulated by overexpression or down regulation of the key genes *ODC*, *ADC* or *SAMDC* (Kumar et al., 1997; Walden et al., 1997; Malmberg et al., 1998; Kumar and Minocha, 1998; Capell et al., 1998; Rajam et al.,1998; Roy and Wu, 2001; Bhatnagar et al., 2002). Most of the studies have used the constitutive 35S promoter, but only few of them were successful in using either inducible (Masgrau et al., 1997; Panicot et al., 2002a; Mehta et al., 2002) or tissue-specific promoters (Rafart-Pedros et al., 1999). Overexpression of heterologous *ODC* or *ADC* cDNAs generally causes the production of high levels of Put (DeScenzo and Minocha, 1993; Bastola and Minocha, 1995; Masgrau et al., 1997; Capell et al., 1998; Bhatnagar et al., 2002; Panicot et al., 2002a), but in most cases only a small increase or even no change in Spd and Spm has been observed. This indicates that elevated levels of Put resulting from genetic manipulation of a single step located upstream of the PA biosynthetic pathway (i.e. ODC or ADC) are not accompanied by an increase in subsequent biosynthetic reactions (i.e. Spd and Spm biosynthesis) (Bhatnagar et al., 2002). In contrast, overexpression of genes located downstream of the pathway (i.e. SAMDC or SPDS) generally lead to increased levels of Spd or Spm or both (Thu-Hang et al., 2002; Mehta et al., 2002). Taken together these results suggest that the levels of Spd and Spm in the cells are under a tight homeostatic regulation (Bhatnagar et al., 2002), which possibly could be related to a supramolecular organization of some of these enzymes (see Section 5).

Discrepancies observed among different studies may have several causes. These include: transgene source, positional effects, recipient plant system, plant material analyzed and type of promoter used. A hierarchical accumulation of polyamines in different transgenic tissues/organs has been observed (Lepri et al., 2001). In general, less metabolically active tissues accumulate higher levels of polyamines (Lepri et al., 2001). These results are in line with experiments in which metabolites such as vitamin A and pharmaceutical antibodies accumulate at high levels in seeds of different species. It is reasonable to assume that dormant or less metabolically active tissues provide a conducive environment for the accumulation of transgenic products (Thu-Hang et al., 2002). In this regard, it should be stressed that the most remarkable results have been obtained by controlled expression of transgenes using inducible or tissue-specific promoters. For example, tissue-specific expression of SAMDC gives rise to smaller potato tubers without affecting tuber yield (Rafart-Pedros et al., 1999). The distribution of tuber weights is of agronomic importance, and generally a reduction of tuber-size variation is economically advantageous, so that more tubers fall into a given size grade either for seed or ware (Rafart-Pedros et al., 1999). Similarly, fruit-specific expression of heterologous SAMDC in tomato resulted in ripening-specific accumulation of Spd and Spm which led to an increase in lycopene, prolonged vine life, and enhanced fruit juice quality (Mehta et al., 2002). Besides the agronomic interest of this finding, this latter study constitutes one of the most striking evidence regarding the *in vivo* involvement of polyamines in a particular developmental process, i.e. fruit ripening (Mehta et al., 2002).

5. Understanding the role of polyamines

Phenotypic analyses of mutants and transgenic plants with altered PA levels gives further support to the previous physiological studies (see Section 3) with regard to the involvement of these compounds in several plant processes (reviewed by Tiburcio et al., 2002). These include somatic embryogenesis (Bastola and Minocha, 1995), stem elongation and flowering (Gerats et al., 1988; Masgrau et al., 1997; Hanzawa et al., 2000; Panicot et al., 2002a), root growth (Watson et al., 1998; Cordeiro et al., unpublished), tuber development (Kumar et al., 1996; Rafart-Pedrós et al., 1999), fruit ripening (Mehta et al., 1997; 2002), abiotic stresses (Minocha and Sun, 1997; Soyka and Heyer, 1999; Roy and Nu, 2001). However, most of these mutants and transgenic plants have not been further exploited for the analysis of PA function. Application of advanced genomic and proteomic approaches will help to elucidate the role of PA in particular plant processes.

5.1. Genomic approaches

The availability of complete genome sequences permits the use of approaches to explore gene expression variations on a large genome scale. Either cDNAs or large oligonucleotide collections are attached on surfaces to create a *microarray*. The hybridisation of the microarray with fluorescent labelled RNA or cDNA yields an overall image of gene expression or ‘transcriptome’ (Lockhart and Winzeler,

2000). The global examination of gene expression should reveal the coincidence of spatial and temporal transcript expression profiles that may reflect a requirement of co-ordinated gene product expression in response to different type of signals. The technology developed for the *Arabidopsis* genome has been accelerated in the recent years both by public funding through the Arabidopsis Functional Genomics Consortium in the USA and the GARNet in the UK, and also by private initiatives like Monsanto, Affymetrix or Synteny/InCyte (Wisman and Ohlrogge, 2000).

Although there are already many examples in the literature showing the utility of this approach for unraveling complex plant responses and signal transduction processes (Schena et al., 1995; Schaffer et al., 2000), the use of this technology in our field is unfortunately in its infancy. So far, DNA microarray analysis has been used to reveal the induction of ADC genes during drought stress (Ozturk et al., 2002) or in response to wounding and methyl jasmonate treatment (Sasaki et al., 2001; Pérez-Amador et al., 2002).

We envisage that global analysis of gene expression in well characterized mutant and transgenic plants with altered polyamine metabolism will provide novel clues in the near future for understanding the molecular mechanisms underlying polyamine effects on plant growth and development.

5.2. Proteomic approaches

Proteomics' uses biochemical approaches aimed at systematically characterizing the 'proteome' or the 'protein complement of the genome' (Wasinger et al., 1995) in a given organism, tissue, cell or subcellular compartment. The means of proteome characterization include protein localization, expression and most importantly protein interaction maps. A plethora of innovative procedures has been employed in recent years for the large-scale analysis of protein signalling pathways, including the yeast two-hybrid system (Fields and Song, 1989), protein purification methods linked to detection by mass spectrometry (Neubauer et al., 1997; Verma et al., 2000); protein localization (Ferrando et al., 2000; 2001; Farràs et al., 2001), and protein microarray techniques (Zhu et al., 2001).

The yeast two-hybrid system is a genetic tool to describe *in vivo* protein interactions using the yeast cell as a test tube. Each separated module of the GAL4 transcription factor, either the DNA binding domain (DBD) or the transcriptional activation domain (AD), is translationally fused to proteins of interest X or Y, generating respectively the hybrid proteins X-DBD (bait) and Y-AD (prey). A powerful aspect of the yeast molecular genetics involves the facility to isolate the corresponding cDNAs coding for proteins X or Y, introduced in the form of plasmid DNA. This latter feature immediately favored the use of this system to identify interacting partners for a given bait protein X using cDNA libraries as a prey (reviewed by Walhout et al., 2000). The number of studies that have used proteomics in our field is still scanty. Here we will provide two examples that demonstrate the potential of these techniques to (i) unravel the role of PA in transcription; and (ii) to identify PA metabolons (see below).

Although the potential role of PAs in affecting gene expression had already been reported, the molecular mechanisms underlying their effects were unknown (Wang et al., 2002). The identification of a polyamine responsive element and corresponding transacting protein factors that respond to polyamines has opened up an exciting new area to study the function of these compounds in transcription (Wang et al., 1999). By using the two-hybrid system, it was recently found that the human homologue of the *Arabidopsis* subunit COP9 signalosome complex binds to such transacting protein factors with the potential to directly affect gene expression (Wang et al., 2002). Remarkably, the COP9 signalosome proteins were first identified in *Arabidopsis* and have been demonstrated to form a regulatory complex involved in light-activated development and playing a role in intracellular signalling (Deng et al., 2000). We envisage that similar type of experiments will be performed in the plant PA field that hopefully will provide new insights into the role of PAs in plant signal transduction.

Increasing number of reports document that many metabolic reactions are catalysed by complexes of sequentially acting enzymes that show highly ordered structural organization (reviewed in Srere, 1987). In such multienzyme complexes the metabolites pass from one active enzyme site to the next through a process termed 'substrate channeling'. The supramolecular arrangement of enzymes involved in such metabolic reactions is referred to as 'metabolon'. Metabolons are multienzyme complexes in both prokaryotes and eukaryotes that represent highly organized assemblies of sequential enzymes in a metabolic pathway and are thought to provide

increased metabolic efficiency and higher substrate selectivity. Metabolons may also help to coordinate the activities of enzymes by sharing intermediates in a given pathway, as well as to ensure protection of labile substrates and sequestration of toxic intermediates (Sugumaran et al., 2000). In addition, the formation of multienzyme metabolon complexes may enhance enzyme stability, improve enzymatic performance and provide a means for adaptation to alterations of input of metabolic reactions, especially during demanding physiological conditions (Abadjieva et al., 2001).

The relevant information about intrinsic properties of 'metabolon' formation can be acquired by studies of protein-protein interactions using modern proteomic approaches (Ferrando et al., 2002). In this regard, our laboratory has recently analyzed possible interactions between the SPDS and SPMS enzymes of polyamine biosynthetic pathway in the yeast two-hybrid system (Panicot et al., 2002b). Using the *Arabidopsis* spermidine synthase as bait, two similar proteins were identified to interact with SPDS2 that were named SPDS1 and SPMS. Yeast and bacterial mutant complementation tests revealed that SPDS1 encodes a novel spermidine synthase, whereas SPMS displays spermine synthase activity. The heterodimerization capabilities of enzymes catalyzing the two last steps of polyamine biosynthesis were also demonstrated *in vivo* by co-immunoprecipitation using epitope tagged SPDS1, SPDS2 and SPMS proteins (Ferrando et al., 2000; Ferrando et al., 2001). Immunoaffinity purification and size fractionation of SPDS and SPMS enzymes labeled with different HA and c-Myc epitopes revealed that the SPDS and SPMS proteins co-purify with large multiprotein complexes of 650 to 750 kDa. Further analysis of subunits of isolated SPDS-SPMS metabolon(s) by mass spectrometry is expected to yield important information about yet unknown regulatory subunits of SPDS-SPMS metabolon in the PA biosynthesis pathway. The available data support the conclusion that Spd synthesized by SPDS is effectively channeled to SPMS to control the formation of the end-product Spm thereby regulating the synthesis of high molecular weight polyamines (Panicot et al., 2002b).

6. Conclusions

Considerable evidence indicates that polyamines are involved in a wide array of plant processes, including DNA replication, transcription of genes, cell division, organ development, fruit development and ripening, leaf senescence and abiotic stresses. Despite ample evidence of their involvement in these processes, their precise role in these specific processes remains to be established. Recent developments of PA-deficient mutants and transgenic plants as well as of molecular genetic investigations should further our understanding of their role in plant growth and development.

The polyamine pathway is now amenable to modulation by genetic approaches because it has been elucidated molecularly and biochemically in plants. Reverse genetics has identified an *Arabidopsis* knockout mutation of ADC2 gene which reveals inducibility by osmotic stress. Extensive use of functional genomics and reverse genetics studies will facilitate the isolation of novel knockout mutants affected in other polyamine metabolic genes. Sense and antisense transgenic approaches have revealed the feasibility of modulating cellular PA contents. Generally, genetic manipulation of single steps located upstream of the PA pathway (i.e. ODC or ADC) lead to elevated levels of Put, but no changes occur in the higher PAs, Spd and Spm. By contrast, overexpression of genes located downstream of the pathway (i.e. SAMDC or Spd synthase) generally leads to increased levels of Spd and Spm, indicating that the levels of Spd and Spm are under a tight homeostic cellular control. Phenotypic analyses of mutants and transgenic plants affected in polyamine metabolism further support previous physiological evidence, but the molecular mechanisms underlying PA effects on plant growth and development remain to be elucidated. Global analysis of gene expression by using the available DNA microarray genomic techniques will help to understand the role of these compounds. The potential of proteomics to unravel the role of polyamines in particular cellular processes is also examined. We envisage that the extensive use of the two-hybrid system and other proteomic approaches will provide new insights into the role of PAs on plant signal transduction. Furthermore, we provide evidence that proteomics is an excellent tool to unravel supramolecular organizations of PA metabolic enzymes which may help to understand homeostatic control of this metabolic pathway.

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