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MINIREVIEW ARTICLE

# Polyamine catabolism: target for antiproliferative therapies in animals and stress tolerance strategies in plants

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**Abstract** Metabolism of polyamines spermidine and spermine, and their diamine precursor, putrescine, has been a target for antineoplastic therapy since these naturally occurring alkyl amines were found essential for normal mammalian cell growth. Intracellular polyamine concentrations are maintained at a cell type-specific set point through the coordinated and highly regulated interplay between biosynthesis, transport, and catabolism. A correlation between regulation of cell proliferation and polyamine metabolism is described. In particular, polyamine catabolism involves copper-containing amine oxidases and FAD-dependent polyamine oxidases. Several studies showed an important role of these enzymes in several developmental and disease-related processes in both animals and plants through a control on polyamine homeostasis in response to normal cellular signals, drug treatment, environmental and/or cellular stressors. The production of toxic aldehydes and reactive oxygen species, $H_2O_2$ in particular, by these oxidases using extracellular and intracellular polyamines as substrates, suggests a mechanism by which the oxidases can be exploited as antineoplastic drug targets. This minireview summarizes recent advances on the physiological roles of polyamine catabolism in animals and plants in an attempt to highlight differences and similarities that may contribute to determine in detail the underlined mechanisms involved. This information could be useful in evaluating the possibility of this metabolic pathway as a target for new antiproliferative therapies in animals and stress tolerance strategies in plants.

**Keywords** Polyamines · Polyamine oxidase · Amine oxidase · Tumor cells · Reactive oxygen species · Plants

**Abbreviations**

| | |
|---|---|
| ABA | Abscisic acid |
| ADC | Arginine decarboxylase |
| ALDH | Aldehyde dehydrogenase |
| AMADH | Aminoaldehyde dehydrogenase |
| APAO | $N^1$-acetylpolyamine oxidase |
| ATAO | *Arabidopsis thaliana* CuAO |
| AtPAO | *Arabidopsis thaliana* PAO |
| BENSpm | Bis(ethyl)norspermine |
| BSAO | Bovine serum amine oxidase |
| CHO | Chinese hamster ovary |
| CPENSpm | $N^1$-ethyl-$N^{11}$-[(cyclopropyl)methyl]-4,8-diazaundecane |
| CuAO | Copper amine oxidase |
| Dap | 1,3-diaminopropane |
| DFMO | Difluoromethylornithine |
| DX | Doxorubicin-resistant |
| eIF5A | Eukaryotic translation initiation factor 5A |
| FBS | Fetal bovine serum |
| GABA | $\gamma$-aminobutyric acid |
| HO· | Hydroxyl radical |
| HR | Hypersensitive response |

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| | |
|---|---|
| JA | Jasmonic acid |
| MDR | Multidrug-resistant |
| NSAIDS | Non-steroidal anti-inflammatory drugs |
| ODC | Ornithine decarboxylase |
| PAO | Polyamine oxidase |
| PBS-BSA | Phosphate buffered saline-bovin serum albumin |
| PC-Acro | Protein conjugated acrolein |
| PCD | Programmed cell death |
| P-gp | P-glycoprotein |
| Put | Putrescine |
| ROS | Reactive oxygen species |
| SA | Salicylic acid |
| SAMDC | *S*-adenosylmethionine decarboxylase |
| SMO | Spermine oxidase |
| Spd | Spermidine |
| SPDS | Spd synthase |
| Spm | Spermine |
| SPMS | Spm synthase |
| SSAT | Spd/Spm $N^1$-acetyltransferase |
| Ther-Spm | Thermospermine |
| TMV | Tobacco mosaic virus |
| TNFα | Tumor-necrosis factor α |
| TPQ | 2,4,5-trihydroxyphenylalaninequinone |
| ZmPAO | Maize PAO |

## Introduction

Polyamines are polycationic biogenic amines required for both eukaryotic and prokaryotic cell growth and differentiation (Pegg 1986). They have attracted interest because of their multiple functions in cell biology (Cohen 1998; Mattoo et al. 2010). During the last two decades, polyamines have been found in practically every microbe, plant, or animal studied. Furthermore, considerable progress has been made in understanding their metabolic pathways and numerous enzymes involved in polyamine synthesis and degradation have been isolated and characterized. In particular, several developments in polyamine catabolism have provided avenues to test this metabolic pathway as a target for therapeutic applications in animals and stress tolerance strategies in plants. This minireview deals with the recent developments on the physiological roles of polyamine catabolism in animals and plants, with the aim to describe similarities and differences between the two kingdoms.

## Polyamines in animals and plants

The most common polyamines in eukaryotes are putrescine (Put), spermidine (Spd) and spermine (Spm). In addition to these commonly found polyamines, other polyamines, such as 1,3-diaminopropane (Dap), cadaverine, agmatine, norspermidine, sym-homospermidine and norspermine are found in many organisms as minor components of cellular polyamine pools. Plants also have a small amount of thermospermine (Ther-Spm), an isomer of Spm which has not as yet been detected in mammalian cells (Minguet et al. 2008; Vera-Sirera et al. 2010).

Being positively charged at physiological pH, polyamines are mostly ionically bound to negatively charged macromolecules, such as DNA and RNA, proteins and phospholipids. In this way, the concentration of the freely available polyamines is considered much lower than that of the total intracellular pool (Igarashi and Kashiwagi 2010). Polyamines also occur as soluble and insoluble conjugated forms. Among these, the acetylated forms of Spd and Spm are found in very small amounts both in animals and plants. In particular, in animals $N^1$-acetyl-Spd and $N^1$-acetyl-Spm are the major polyamines exported from the cells and found rarely in normal cells. However, they are present at high levels in cancer cells, providing a link between alterations in polyamine metabolism and carcinogenesis (see next paragraph) and contributing to the development of new approaches to the therapy of cancer and of other diseases. In plants, acetylated polyamines have been detected in some species (Bagni and Tassoni 2001) although a gene homologous to the animal Spd/Spm $N^1$-acetyltransferase (SSAT), which catalyzes the transfer of an acetyl group from acetyl-coenzyme A to the $N^1$-position of either Spd or Spm, has still to be characterized. In plants, but not in animals, polyamines also occur as hydroxycinnamic acid conjugates which have been suggested to be implicated in protecting against pathogens, detoxifying phenolic compounds, and/or serving as a reserve of polyamines that are available to actively proliferating tissues (Fellenberg et al. 2009; Fuell et al. 2010; Kaur et al. 2010). In most organisms, polyamines can also be covalently bound to glutamine residues of certain proteins by the action of transglutaminases forming insoluble conjugates (Serafini-Fracassini et al. 2009).

Polyamines interplay with a variety of fundamental cellular processes, including DNA replication, transcription, RNA modification, protein synthesis, ion-channel regulation, free radical scavenging, cell cycle regulation, gene expression and signal transduction (Bachrach et al. 2001; Childs et al. 2003; Srivastava et al. 2007). Spd is also a substrate for a two-step post-translational modification of the ε-amino group of a specific lysine residue of the eukaryotic translation initiation factor 5A (eIF5A) which is essential for eukaryotic cell growth and proliferation (Park et al. 2006) and is implicated in apoptosis in several organisms (Wang et al. 2003; Taylor et al. 2007). In animals, polyamines also have an important role in cell

differentiation and proliferation. Indeed, mice with specific inactivation of the Spm synthase gene (*SPMS*) has severe developmental defects (Pegg and Michael 2010). Furthermore, polyamine synthesis is down-regulated as cells become senescent in many tissues of adults. Administration of Spd markedly extends the lifespan of yeast, flies and worms and human immune cells. This treatment triggers epigenetic deacetylation of histone H3 through inhibition of histone acetyltransferase, suppressing oxidative stress and necrosis. The altered acetylation status of chromatin trigger autophagy, which is crucial for enhancing longevity (Eisenberg et al. 2009). On the other hand, dysregulated polyamine metabolism has been associated with neoplastic transformation and cancer cell growth (Pegg 1988; Pegg and Feith 2007). Polyamines affect numerous processes in carcinogenesis (see "Animal polyamine catabolism during carcinogenesis"). In fact, polyamines are often present at increased concentration in both tumor cell cultures and solid tumors, as determined in breast and colon cancer (Heby and Persson 1990), while polyamine depletion leads to inhibition of tumor growth (Averill-Bates et al. 2005). It has been demonstrated that polyamines can also induce programmed cell death (PCD) in various animal cell types (Wallace et al. 2003; Igarashi and Kashiwagi 2010), thus indicating a bivalent function for these molecules, promoting both cell growth and cell death, likely depending on their concentration and other developmental and environmental signals (Wallace et al. 2003; Toninello et al. 2006).

In plants, polyamines are implicated in growth and developmental processes, as well as in defense responses to biotic and abiotic stresses (Wargo et al. 2002; Groppa and Benavides 2008; Alcázar et al. 2010; Minocha et al. 2010). Indeed, *Arabidopsis thaliana* double mutant for two arginine decarboxylase genes (*ADC1* and *ADC2*), which cannot produce polyamines, died at the embryo stage (Urano et al. 2005). Furthermore, in a double *Arabidopsis* mutant for the two Spd synthase genes (*SPDS1* and *SPDS2*), embryo development is arrested at the heart stage indicating a requirement for Spd during the course of embryogenesis (Imai et al. 2004a). On the other hand, Spm, though not essential for viability of *Arabidopsis* (Imai et al. 2004b), has been shown to be involved in stress tolerance (Groppa and Benavides 2008; Alcázar et al. 2010). A role in defense responses to biotic and abiotic stresses has been also shown for Spd and Put (Alcázar et al. 2010). Furthermore, in plants Ther-Spm has been shown to be involved in the regulation of vascular differentiation (Vera-Sirera et al. 2010).

In general, both in animals and plants, changes in polyamine metabolism occur in response to a variety of physiological and pathological conditions, and although the precise physiological function and mechanism of action of polyamines still remain unclear, polyamine metabolism is being used as a target for antiproliferative therapies in animals and stress tolerance strategies in plants.

## Polyamine catabolism in animals and plants

Since polyamines are implicated in such a divergent array of processes, their intracellular concentrations are strictly regulated through biosynthesis, conjugation, transport and catabolism. Polyamines are oxidatively deaminated by amine oxidases in a reaction consuming $O_2$ and $H_2O$ and producing an aldehyde, the removed amine moiety, and $H_2O_2$ in stoichiometric amounts. The various amine oxidases involved in polyamine catabolism show differences among one other in substrate and inhibitor specificity, mechanism of substrate oxidation and subcellular localization. On the basis of the cofactor involved they can be classified into two subclasses, the copper-containing amine oxidases (CuAO) and the flavin-containing polyamine oxidases (PAO) (Agostinelli et al. 2010; Angelini et al. 2010).

Copper-containing amine oxidase are homodimers in which each subunit of about 70–90 kDa with 33 fully conserved residues near the catalytic site contains a tightly bound $Cu^{2+}$ ion coordinated to three His residues and a 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor. The TPQ cofactor is generated by post-translational autocatalytic modification of an active site Tyr residue, which is part of the consensus sequence Asn-TPQ-Asp (or Glu) (Mu et al. 1992; Mitchell Guss et al. 2009), in a reaction shown to be dependent on both molecular oxygen and a mononuclear copper center. CuAOs catalyze the oxidation of a wide variety of biogenic amines including mono-, di- and polyamines. Furthermore, they are either membrane-bound or soluble, the latter with an intracellular or extracellular localization. We consider here only the soluble CuAOs that oxidize di- and polyamines. CuAOs from animals and plants are involved in the terminal catabolism of the polyamines. They oxidize Put at the primary amino groups to produce $H_2O_2$, ammonia and 4-aminobutanal which spontaneously cyclizes to $\Delta^1$-pyrroline. The cyclized product, $\Delta^1$-pyrroline, can be further converted to the nonprotein aminoacid $\gamma$-aminobutyric acid (GABA) by a NAD-dependent aminoaldehyde dehydrogenase (AMADH; Petřivalský et al. 2007). CuAOs are also able to oxidize Spd and Spm producing $H_2O_2$ and ammonia together with 4-aza-8-amino-octan-1-al and 4,9-diaza-12-amino-dodecan-1-al, respectively. The latter is further oxidized to 4,9-diaza-dodecan-1,12 dialdehyde by CuAO. The two aminoaldehydes, produced by Spd and Spm oxidation, when not previously further oxidized by aldehyde dehydrogenases (ALDH), may undergo spontaneous degradation forming Put and Spd, respectively, and the highly toxic

aldehyde acrolein (Fig. 1) (Agostinelli et al. 2006a, 2010). Thus, these studies suggested that CuAOs may be involved in a polyamine back-conversion pathway. However, no evidence exists for its physiological significance. Importantly, while the intracellular animal CuAOs and all plant CuAOs thus far characterized have a lower catalytic efficiency for Spm and Spd than Put, animal serum CuAOs, such as bovine serum amine oxidase (BSAO), preferentially oxidizes Spd and Spm. Other substrates of CuAOs are Dap, cadaverine, agmatine, histamine and several other naturally occurring amines (e.g., benzylamine, heptylamine) (Agostinelli et al. 2010) as well as synthetic polyamine analogues. Overall, CuAOs have relatively broad substrate specificity although with greatly varying $K_M$ and $k_{cat}$ values (Agostinelli et al. manuscript in preparation).

In animals, CuAOs are present at considerably different levels in various tissues and organs, as well as in some biological fluids. CuAOs in mammals have been well characterized from pig kidney, intestine and human placenta. Interestingly, in humans, serum CuAO activity rises during pregnancy or under some pathological conditions, such as cancer (Agostinelli et al. 2010). Plant CuAOs occur at high levels in dicots (particularly pea, chickpea, lentil and soybean seedlings) loosely associated to cell walls of tissues often characterized by rapid extension growth (Cona et al. 2006). In *Arabidopsis,* about ten putative CuAO genes have been identified by database search, some of which with a predicted apoplastic localization while others with an intracellular localization. Some of the putative *Arabidopsis* CuAOs have a predicted peroxisomal localization and thus they may represent the first CuAOs involved in a peroxisomal catabolic pathway of polyamines. The expression of some plant CuAOs has been shown to be modulated during development, pathogen attack, wound healing and salt stress. Plant hormones, as for example jasmonic acid (JA) and abscisic acid (ABA), were also shown to regulate expression of plant CuAOs (Møller et al. 1998; Cona et al. 2006; An et al. 2008; Toumi et al. 2010; Quinet et al. 2010). A class of CuAOs, involved in the oxidative deamination of *N*-methyl-Put to 4-methylaminobutanal, is also present in plants; 4-methylaminobutanal represents a precursor of alkaloids, well-known molecules that improve plant fitness in the natural environment while also used by humans for medicinal purposes (Heim et al. 2006; Katoh et al. 2007).

Polyamine oxidases are monomeric proteins of about 55 kDa which bear a non-covalently bound molecule of FAD as a cofactor. They catalyze the oxidation of Spm, Spd and/or their acetylated derivatives at the secondary amino groups. In animals, the first discovered PAO is involved in a two-step pathway. The first step catalysis involves the cytosolic enzyme SSAT, which has a short half-life and is highly inducible at the mRNA and protein synthesis/stabilization level by polyamine themselves and polyamine analogues, as well as by a variety of physiopathological stimuli as toxins, hormones, cytokines, anti-inflammatory drugs, stress pathways, and ischemia-reperfusion injury. It catalyzes the transfer of an acetyl group from acetyl-coenzyme A to the $N^1$ position of either Spd or Spm, respectively, forming $N^1$-acetyl-Spd or $N^1$-acetyl-Spm (Wallace et al. 2003). In the second step of this catabolic pathway, $N^1$-acetyl-Spd and $N^1$-acetyl-Spm are oxidized by a peroxisomal PAO, recently named $N^1$-acetylpolyamine oxidase (APAO), to produce stoichiometric amounts of Put or Spd, respectively, together with 3-acetamidopropanal and $H_2O_2$ (Fig. 1). Among these, 3-acetamidopropanal can be metabolized by an ALDH to form *N*-acetyl-*β*-alanine (Fig. 1). After its deacetylation by a selective hydrolase, *β*-alanine is formed. This catabolic pathway, the rate limiting factor of which is SSAT, is considered a polyamine back-conversion pathway recycling Spd and Put from Spm and Spd, respectively. Interestingly, although APAO is constitutively expressed in most normal tissues and its activity is normally regulated by the availability of the substrates provided by SSAT, rather than by changes in APAO levels, its levels considerably change in human breast cancer tissues (Wallace et al. 2000). Furthermore, APAO has the ability to oxidize a subset of antitumor polyamine analogues, suggesting that this oxidase activity could have a significant effect on determining tumor sensitivity to these or similar agents (Wang et al. 2005; Casero and Pegg 2009).

In animals, a cytosolic/nuclear PAO is also present which was named Spm oxidase (SMO) since it specifically oxidizes Spm to produce Spd, 3-aminopropanal and $H_2O_2$ (Fig. 1) (Wang et al. 2001; Vujcic et al. 2002; Cervelli et al. 2003). An AMADH may further metabolize 3-aminopropanal to form *β*-alanine or 3-aminopropanal may be converted to the toxic acrolein by *β*-elimination. Interestingly, the single copy mammalian SMO gene encodes for many splice variants, both in human and mouse (Cervelli et al. 2004; Murray-Stewart et al. 2008; Amendola et al. 2009). SMO is highly inducible in response to a variety of stimuli, including the tumor necrosis factor-α (TNF-α), antitumor polyamine analogues, *Helicobacter pylori* infection, prostatic neoplasia and during cell differentiation (Goodwin et al. 2008; Cervelli et al. 2009; Pegg 2009; Casero and Pegg 2009). Importantly, all of the analogues that induce SSAT also induce SMO expression (Casero and Pegg 2009).

In plants, the first PAO to be characterized was maize PAO (ZmPAO). ZmPAO is an apoplastic enzyme which oxidizes both Spd and Spm producing 4-aminobutanal and *N*-(3-aminopropyl)-4-aminobutanal, respectively, in addition to Dap and $H_2O_2$ (Fig. 1). Thus, this enzyme is not involved in the recycling of polyamines, but it is involved

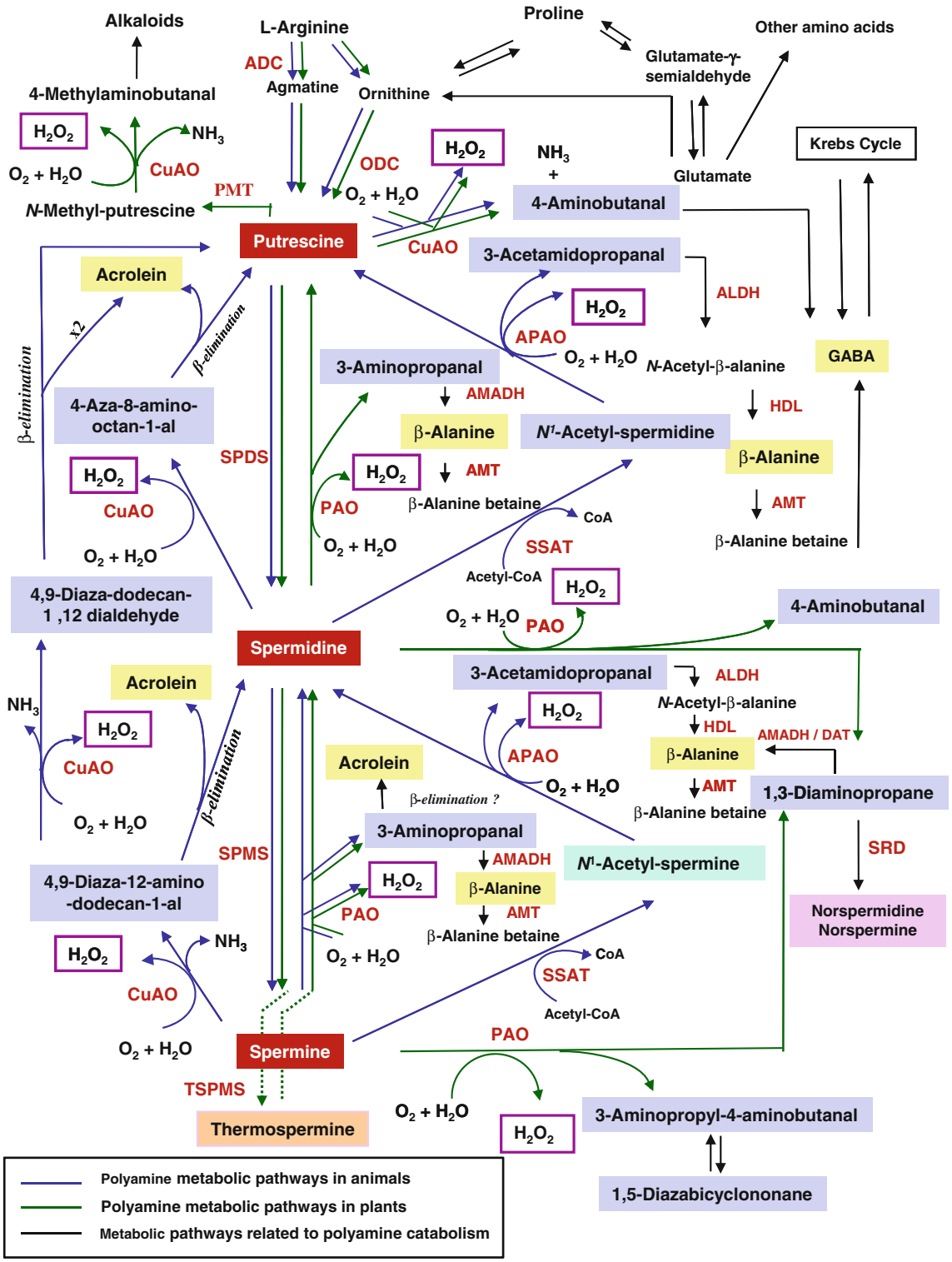


**Fig. 1** Schematic representation of the polyamine catabolic pathways in animals and plants and related matabolites. *ADH* aldehyde dehydrogenase, *ADC* arginine decarboxylase, *AMADH* aminoaldehyde dehydrogenase, *AMT* β-alanine *N*-methyltransferase, *DAT* 1,3-diaminopropane-aminotransferase, *HDL* *N*-acetyl-β-alanine amidohydrolase, *ODC* ornithine decarboxylase, *PMT* putrescine *N*-methyltransferase, *SPDS* spermidine synthase, *SPMS* spermine synthase, *SRD* Schiff-base reductase/decarboxylase, *TSPMS* thermospermine synthase

in a terminal catabolic pathway of polyamines. The difference in the reaction products between animal PAOs and ZmPAO is due to differences in the mode of substrate oxidation, which in turn is due to a different mode of substrate binding inside the catalytic site resulting in the oxidation of a different carbon atom. Recently, biochemical characterization of four of the five *A. thaliana* PAOs (*AtPAOs*) evidenced that *AtPAO1–AtPAO4* resemble the animal PAOs in that they are involved in a polyamine back-conversion pathway (Fig. 1) (Tavladoraki et al. 2006; Kamada-Nobusada et al. 2008; Moschou et al. 2008a; Fincato et al. 2011; Takahashi et al. 2010). Furthermore, *AtPAO1* has a predicted cytosolic localization and oxidizes only Spm and not Spd and thus can be considered the plant counterpart of the animal SMOs. In addition, *AtPAO2*, *AtPAO3* and *AtPAO4* have a peroxisomal localization similar to the animal APAOs, though they preferentially oxidize the non-acetylated form of Spd and Spm. Expression of plant PAOs is highly induced during de-etiolation, pathogen interaction and wound-healing, under salt stress conditions and following treatment with the plant–pathogen elicitor flagellin 22 and plant hormones, such as ABA, salicylic acid (SA) and JA (Yoda et al. 2003; Angelini et al. 2008; Moschou et al. 2008a, b, c; Xue et al. 2009; Toumi et al. 2010; Hewezi et al. 2010; Quinet et al. 2010).

It should be added here that Dap produced from the terminal catabolism of Spd and Spm can also be converted to $\beta$-alanine by a Dap-aminotransferase, reported in bacteria but not yet in plants, and an AMADH (Fig. 1) (Cona et al. 2006). $\beta$-Alanine in turn might be metabolized to the osmoprotectant $\beta$-alanine betaine by $\beta$-alanine *N*-methyltransferase. Dap is also a precursor of the uncommon polyamines norspermidine and norspermine present in plants and associated with stress tolerance (Cona et al. 2006).

## Physiological roles of polyamine catabolism in animals and plants

In animals, polyamine catabolism contributes to important physiopathological processes such as cell proliferation and differentiation, apoptosis, amine detoxification and cell signaling through both regulation of polyamine levels and their oxidatively deaminated reaction products, mainly $H_2O_2$, aminoaldehydes or dialdehyde and acrolein (Sharmin et al. 2001; Averill-Bates et al. 2008; Agostinelli et al. 2009).

As evidenced by the complex role of polyamines in cell growth and proliferation, optimal polyamine levels are necessary for mammalian health. In fact, an important difference between normal and tumor cells is polyamine content. To ensure optimal polyamine levels, polyamine homeostasis is tightly regulated at various steps of polyamine metabolism and transport (Wallace et al. 2003), thus becoming difficult to be perturbated through inhibition of a single biosynthetic or catabolic reaction. Despite this tight control, it has been shown that an altered polyamine catabolism can cause changes in polyamine homeostasis. Indeed, constitutive or inducible over-expression of SSAT in animal cells brought a substantial reduction in Spd and Spm pools as well as a large increase in Put and $N^1$-acetylSpd intracellular levels and export of acetylated polyamines (Jänne et al. 2005; Zahedi et al. 2007). Furthermore, over-expression of SMO in mouse neuroblastoma cells and HEK293 cells caused a statistically significant decrease in Spm levels and an increase in Put levels (Vujcic et al. 2002; Amendola et al. 2005; Zahedi et al. 2007). Notably, the changes in polyamine levels through polyamine catabolism were often accompanied by increased DNA damage and changes in cell proliferation (Zahedi et al. 2007). These data suggest that polyamine catabolism has an important role in controlling polyamine content and thus can be used as a therapeutic target for several diseases. The other catabolic product, $H_2O_2$, which can get converted into the highly reactive hydroxyl radical (HO·) through Fenton-like-catalysis (Fig. 2), is able either to impair cell growth and proliferation or to regulate signal transduction and gene expression, depending on its concentration. Indeed, it has been demonstrated that, in human breast cancer cells, the SMO-derived $H_2O_2$ in response to treatment with the polyamine analogue bis(ethyl)norspermine (BENSpm) is cytotoxic (Fig. 2) (Pledgie et al. 2005; Casero and Pegg 2009). Furthermore, the $H_2O_2$ produced by purified BSAO and Spm exogenously supplied to human colon adenocarcinoma (LoVo) and melanoma (M14) cells has been also shown to cause cytotoxicity (Calcabrini et al. 2002; Agostinelli et al. 2006a). However, it is still an open question whether or not $H_2O_2$, formed by polyamine catabolism, is always pathologic, or has a role in cell signaling (Wang and Casero 2006).

The aminoaldehydes produced through polyamine catabolism have been shown to be cytotoxic on animal cells (Fig. 2a), probably due to the inhibition of nucleic acid and protein synthesis (Nocera et al. 2003; Wallace et al. 2003). Indeed, it has been reported that 3-aminopropanal and acrolein produced from polyamine catabolism (Fig. 1) are intimately involved in cell damage during ischemia in rats (Igarashi and Kashiwagi 2010). It was also observed that renal failure patients had increased levels of SMO activity and both free and protein conjugated acrolein (PC-Acro). Furthermore, PC-Acro increased at the locus of infarction after induction of stroke in mice (Igarashi and Kashiwagi 2010). Moreover, acrolein has been shown to have an inhibitory effect on cell growth. In particular, it has been determined that the toxicity of acrolein on cells in

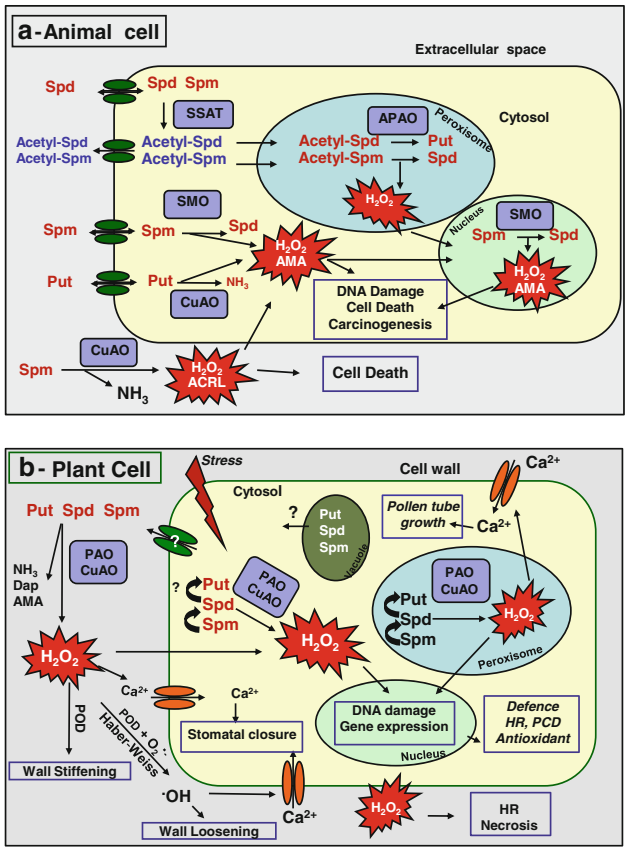


**Fig. 2** Physiological roles of polyamine catabolism in animals (**a**) and plants (**b**). *AMA* aminoaldehyde, *ACRL* acrolein, *Dap, 1,3* diaminopropane, *HR* hypersensitive response, *PCD* programmed cell death,*POD* peroxidase

culture medium containing fetal bovine serum (FBS), with amine oxidase activity, is greater than that caused by $H_2O_2$ (Sharmin et al. 2001). However, it was observed that acrolein is not formed under normal conditions, likely due that polyamines mainly exist as a RNA-polyamine complex, rather than free molecules. It was shown that infarction led to RNA damage. As a consequence, polyamines were released from RNA and acrolein was produced from polyamines, especially from spermine by SMO (Fig. 1). It was therefore hypothesized that acrolein is formed when cells are damaged, so that the aldehyde might be used as a biochemical marker for pathologies involving cell damage (Igarashi and Kashiwagi 2010; Saiki et al. 2011).

There is an ongoing debate about whether $H_2O_2$ or the Spm-derived aldehydes is the major effector. Although a contribution of the aldehydes derived from polyamine catabolism is without a doubt, it is still not known which Spm-derived aldehyde is most important. According to Sharmin et al. (2001), acrolein is among the Spm metabolites nearly exclusively responsible for the induction of cell death. However, a role of acrolein in the early, most active phase of cell damage is unlikely, because its spontaneous formation from the aldehydes, derived from Spm, is a time-dependent process of unknown velocity, while, in contrast, $H_2O_2$ is produced immediately by the enzymatic reaction (Calcabrini et al. 2002; Agostinelli et al. 2006a). $H_2O_2$ seems to be earliest cytotoxic catabolite of Spm, while acrolein induces cell death only after accumulation, at the later stage of the Spm oxidation reaction. In fact, the prevention of cytotoxicity of Spm metabolites by catalase and ALDH, separately added to the incubation mixture, confirmed these results (Calcabrini et al. 2002; Agostinelli et al. 2007, 2010; Averill-Bates et al. 2008). It is worth mentioning here that early studies had pointed out that the products of the oxidative catabolism of acetylated polyamines were considerably less cytotoxic than those of non-derivatized polyamines, likely because 3-acetamidopropanal, in contrast to 3-aminopropanal, is not a precursor of acrolein (Seiler 2004). The cytotoxic effect of APAO-reaction products may be less severe than that of the SMO and CuAO reaction products, due to the fact that APAO is localized in peroxisomes, a subcellular compartment harboring elevated amounts of catalase and antioxidant molecules. It would seem that a main role of APAO could be in $H_2O_2$-mediated signaling or control of polyamine homeostasis, while that of SMO in the control of cell proliferation.

Various data argue against a regulatory role for CuAOs in polyamine homeostasis. Instead, mainly for the secretory CuAOs, a protective role against pathologic accumulation of polyamines has been suggested. Detoxification of diamines of gastrointestinal origin within the intestinal mucosa, and the protection of the embryo against these amines by high placental CuAO activities are established examples of the protective function of these enzymes (Seiler 2004). Moreover, BSAO and other CuAOs may play a role in the post-translational modification of some proteins (Mondovì et al. 2003). At the cellular level, BSAO has been shown to increase significantly the outward rectifier $K^+$ channel current in NIE-115 neuroblastoma cells, thus modulating the electronic properties of the neuronal $K^+$ ionic channels (Wu et al. 1996). At the organ level, BSAO shows an antioxidant and cardio-protective effect in vivo against oxidative injury to heart produced by reactive oxygen species (ROS) (Mateescu et al. 1997). Also, BSAO has an anti-arrhythmic effect on ischemic isolated hearts at reperfusion (Mondovì et al. 2003). The cardioprotective effect of BSAO is probably related, among other factors, to its biogenic amine oxidizing activity (Mondovì et al. 2003). A purified CuAO from pea seedlings exerted a significant cardioprotection against post-ischemic reperfusion damage in vivo, probably through histamine oxidation and as a ROS scavenger, thus presenting a good perspective for a novel therapeutic

approach in the treatment of ischemic heart pathology (Masini et al. 2003).

In plants, polyamine catabolism has been shown to have important roles in plant development and stress responses through both regulation of polyamine levels and their reaction products. Although a key role of the polyamine biosynthetic pathway in polyamine homeostasis has been highlighted, recent evidence would suggest that the polyamine catabolic pathways equally play an important role in the regulation of the polyamine levels. Indeed, it has been shown that increased polyamine levels are accompanied by a concomitant increase in their catabolism (Bhatnagar et al. 2002). Recently, it has been also shown that it is possible to induce changes in the levels of the specific polyamines through manipulation of the catabolic pathways (Kamada-Nobusada et al. 2008; Moschou et al. 2008b, c; Fincato et al. 2011). A tight regulation of polyamine levels is very important not only because polyamines have a direct role in several physiopathological processes as discussed above, but also because polyamine metabolism has a central role in cellular metabolism (Mattoo et al. 2010; Mohapatra et al. 2010).

Similar to animals, polyamine catabolism in plants plays an important role through the production of $H_2O_2$, which is necessary for several plant developmental processes, such as cell expansion, polar growth, gravitropism, stomatal aperture, flower development and stress responses. In plants, $H_2O_2$ produced via apoplastic degradation of polyamines drives peroxidase-mediated oxidative cross-linking of structural cell wall components contributing to cell wall strengthening during development and under stress conditions, such as wound-healing and pathogen attack (Cona et al. 2006; Angelini et al. 2008, 2010; Moschou et al. 2009). $H_2O_2$ has also been identified as an important second messenger in signal transduction networks in which downstream activation of ion channels and modulation of gene expression are involved. Indeed, in *A. thaliana* $H_2O_2$ produced by PAO-mediated Spd oxidation triggers the opening of hyperpolarization-activated $Ca^{2+}$-permeable channels in pollen, thereby regulating pollen tube growth (Wu et al. 2010), a process important for sexual plant reproduction. Plant polyamine catabolism is also involved in the regulation of gene expression as shown in *AtPAO4*-deficient *Arabidopsis* mutants altered in the expression of genes related to abiotic stress responses and flavonoid and/or lignin metabolism (Kamada-Nobusada et al. 2008). It has been reported that polyamine catabolism is involved in the regulation of gene expression also under stress conditions (see below). $H_2O_2$ produced by polyamine catabolism has been also proposed to activate PCD associated with developmental differentiation. Indeed, the presence of an *A. thaliana* CuAO (ATAO1; Møller and Mc Pherson 1998; Møller et al. 1998) and ZmPAO in developing tracheary elements and root cap cells suggests their involvement in PCD which both cell types eventually undergo (Cona et al. 2006). $H_2O_2$ produced by polyamine catabolism has been shown to induce PCD also as a defense response to abiotic and biotic stresses (see below).

Polyamine catabolism contributes to the formation of GABA, an important cellular metabolite which is also synthesized by cytosolic glutamate decarboxylase (Yu and Sun 2007). GABA is rapidly produced in plants in response to biotic and abiotic stresses (Petřivalský et al. 2007; Xing et al. 2007; Dittami et al. 2011). Furthermore, polyamine catabolism contributes to the formation of $\beta$-alanine, which in turn can be further converted in plants to the osmoprotectant, $\beta$-alanine betaine. Dap is also a precursor of the uncommon polyamines norspermidine and norspermine which in plants are associated with stress tolerance (Cona et al. 2006). However, the exact contribution of polyamine catabolism to plant development and defense responses through production of these metabolites has still to be evaluated. Further studies are necessary to verify whether, similar to what is reported in animals, the aminoaldehydes derived from polyamine catabolism in plants have cytotoxic activity and whether acrolein is also formed.

## Polyamine catabolism during carcinogenesis in animals and under abiotic and biotic stress conditions in plants

### Animal polyamine catabolism during carcinogenesis

Several tumors contain higher levels of polyamines as compared to normal tissues (Heby and Persson 1990). Indeed, clinical studies evidenced a significant increase in ODC and *S*-adenosylmethionine decarboxylase (SAMDC) transcripts in human prostatic cancer relative to benign hyperplasia (Kee et al. 2004). Increased polyamine levels have also been observed in chemically and virus-induced tumors (Bachrach 2004). Also patients with various types of cancer excrete acetylated polyamines in their urine and it has been suggested that measuring acetylated polyamines in urine or cell extracts may be useful in the diagnosis of cancer (Seiler 2000). The presence of increased levels of acetylated polyamines is usually a reliable marker of increased SSAT activity, but their accumulation also depends on their catabolic rate, as was shown in breast tumors where both decreased APAO levels and increased SSAT levels contributed to an increase in acetylated polyamines (Wallace et al. 2000). In breast cancer tissues, also decreased SMO expression levels have been found comparing to non neoplastic tissues (Cervelli et al. 2010). The observed decrease in APAO and SMO levels in these tissues may contribute to tumor growth either through an increase in polyamine levels or through a locally reduced

production of $H_2O_2$ and thus a decreased rate of endogenous apoptosis. However, increased SMO expression levels were found in patients diagnosed with prostate cancer, prostatic intraepithelial neoplasia, or proliferative inflammatory atrophy compared to unaffected individuals (Goodwin et al. 2008). It has been suggested that the prostatitis-induced SMO expression and its concomitant $H_2O_2$ production results in oxidative DNA damage and carcinogenesis, and that SMO activity is associated with inflammation, probably linking chronic inflammation and carcinogensis (Goodwin et al. 2008). On the other hand, it has been also shown that in non-small cell lung cancer cells SSAT expression is induced by TNFα. This factor is a potent pleiotropic proinflammatory cytokine produced by numerous different cells in response to infection and inflammatory stress with actions directed towards both tissue destruction and recovery from damage. The SSAT induction leads to lower intracellular polyamine content, decreased cell growth and increased apoptosis through APAO-mediated $H_2O_2$ production, and thus a beneficial effect (Babbar et al. 2006). These data suggest a general association between polyamine catabolism and inflammation, with both desirable and undesirable effects depending on the polyamine catabolic pathway involved, the extent of the inflammatory stimuli and the cell type (Babbar et al. 2007).

CuAOs are also particularly elevated in rapidly proliferating tissues, such as placenta, fetal tissues and organs undergoing hyperplastic, hypertrophic or neoplastic processes (Keskinege et al. 2001). These enzymes have been suggested to eliminate excessive amounts of polyamines (Toninello et al. 2006; Agostinelli et al. 2010).

In general, it is important to note that the information available thus far is not sufficient to draw any conclusion about the physiological role of polyamine catabolism in carcinogenesis, and further studies are necessary to throw light on the seemingly opposite findings. However, the promising results obtained from a broad number of investigations suggest that the polyamine catabolic pathways are good targets for antiproliferative therapies.

## Plant polyamine catabolism under abiotic and biotic stress conditions

Numerous studies in different plant species have shown that polyamine levels are modulated in response to several biotic and abiotic stresses (Bouchereau et al. 1999; Walters 2003; Groppa and Benavides 2008; Kusano et al. 2008; Alcázar et al. 2010). In particular, the expression levels of genes involved in polyamine biosynthesis change under stress conditions (Alcázar et al. 2010). Furthermore, several studies on plant responses to various biotic and abiotic stresses have shown an association with stimulation of polyamine catabolism. Indeed, inducible expression of plant PAOs and CuAOs was observed under various stress conditions and following treatment with stress-related hormones (Møller et al. 1998; An et al. 2008; Xue et al. 2009; Hewezi et al. 2010; Quinet et al. 2010; Toumi et al. 2010).

The contribution of polyamine catabolism in plant defense responses has been mainly shown for extracellular polyamine catabolic enzymes and interestingly it is linked to polyamine transport in the apoplast where only limiting amounts of polyamines are present under normal growth conditions (Kusano et al. 2008; Moschou et al. 2008c; Takahashi et al. 2010). Stress-related processes that have been shown so far to induce polyamine transport in the apoplast are: incompatible and compatible plant–pathogen interactions, salt stress and treatment with the stress hormone ABA (Yoda et al. 2003, 2006, 2009; Marina et al. 2008; Moschou et al. 2009; Toumi et al. 2010). These data suggest that polyamine catabolism in the apoplast may be a general defense response against several stresses. The difference among the various studies reporting polyamine transport in the apoplast is the identity of the transported polyamine. In fact, the transported polyamine in some cases is Spd (Yoda et al. 2003, 2006; Moschou et al. 2008c, 2009), while in others it is either Spm (Yamakawa et al. 1998) or Put (Marina et al. 2008; Toumi et al. 2010). These differences may be due to either the different experimental systems used, the distinct biochemical properties of the catabolic enzymes involved, and/or the fact that under some conditions the catabolic product were probably identified but not the transported polyamine.

Several data based on the use of PAO-specific inhibitors and transgenic plants evidenced that polyamine catabolism in the apoplast contributes to stress defense responses through $H_2O_2$ production. Indeed, it has been shown that the $H_2O_2$ produced by polyamine catabolism in the apoplast contributes to the second phase of ROS production during tobacco mosaic virus (TMV)-induced hypersensitive response (HR), a plant response which is developed during an incompatible plant–pathogen interaction and consists of rapid ROS production, PCD and induction of defense responses aiming to restrict pathogen expansion (Yoda et al. 2003). Similarly, $H_2O_2$ produced by polyamine catabolism in the apoplast contributed to the synthesis of the ROS that accumulated under abiotic stress conditions (Moschou et al. 2008c) or following treatment with ABA, inducing adaptive responses and acting as a key regulator of stomatal apertures to restrict transpiration and reduce water loss (An et al. 2008; Xue et al. 2009; Toumi et al. 2010). $H_2O_2$ produced by polyamine catabolism in the apoplast under stress conditions and/or the apoplastic polyamines themselves trigger a downstream signal cascade pathway leading to increased

expression of specific genes for proteins, such as superoxide dismutase, ascorbate peroxidase, pathogenesis-related proteins, protein kinases, transcriptional factors and other stress responsive proteins (Yamakawa et al. 1998; Mitsuya et al. 2007; Moschou et al. 2008c, 2009; Xue et al. 2009). Interestingly, exogenous application of Spm to tobacco leaves, which mimics the apoplastic accumulation of polyamines upon an incompatible plant–pathogen interaction, increased expression of HR marker genes, an effect which was suppressed by CuAO and PAO inhibitors (Kusano et al. 2008). Furthermore, ABA-inducible generation of $H_2O_2$ by Put catabolism in the apoplast of guard cells signals stomatal closure through a mechanism involving $Ca^{2+}$ as a second messenger (An et al. 2008).

It has been proposed that $H_2O_2$ produced during polyamine catabolism in the apoplast upon stress may also lead to PCD. In particular, it has been shown that accumulation and further oxidation of free polyamines in the apoplast induce PCD during tobacco defense against infection by microorganisms with diverse pathogenesis strategies, i.e. microorganisms establishing host and non-host incompatible interactions, such as TMV and *Pseudomonas cichorii* in tobacco, *P. syringae* in *Arabidopsis* and *Magnaporthe grisea* in rice (Yoda et al. 2003, 2006, 2009). Under salt stress conditions, the levels of $H_2O_2$ and PCD were found higher in transgenic plants over-expressing the apoplastic ZmPAO than in the wild-type plants (Moschou et al. 2008c). Accumulation and further oxidation of free polyamines in the apoplast-enhanced necrotic cell death, and thus disease severity, following infection of *N. tabacum* plants with the necrotrophic pathogen *Sclerotinia sclerotiorum*, an effect blocked by PAO- and CuAO-specific inhibitors (Marina et al. 2008). However, when the biotrophic bacterial pathogens *P. viridiflava*, *P. syringae pv tabaci* or hemibiotrophic pathogen oomycete *Phytophthora parasitica* var *nicotianae* were tested in *N. tabacum* host plants, polyamine oxidation in the apoplast strongly decreased bacterial growth in planta and caused a reduction in the oocyte-induced necrosis (Marina et al. 2008; Moschou et al. 2009). These data suggest that increased polyamine catabolism in the apoplast may have opposing effects against pathogens with different pathogenic strategies: a beneficial effect in plant defense responses against biotrophic pathogens that depend on living tissue for successful host colonization and a detrimental effect in plant defense responses against necrotrophic pathogens that feed on necrotic tissue (Marina et al. 2008). The opposing functions of polyamine metabolism in general and of polyamine catabolism in particular can be also observed in *Arabidopsis* plants infected with the cyst nematode *Heterodera schachtii* (Hewezi et al. 2010). During early plant parasitism, the interaction of the cyst nematode secretory protein 10A06 with the plant SPDS2-induced changes in polyamine metabolism in the syncytium (specialized feeding structures formed in the root vasculature of the host plant). This resulted in both the induction of cellular antioxidant machinery and disruption of SA-dependent defense signaling through a mechanism involving also the polyamine catabolic pathway. In this way, a protective antioxidant environment with inhibited plant defense responses is formed which contributes to the successful parasitism by the cyst nematode (Hewezi et al. 2010).

$H_2O_2$, together with the other ROS ($O_2^{\cdot-}$ and ·OH), has an important role in cell wall architecture. Indeed, $H_2O_2$ promotes cell wall stiffening through peroxidase-mediated formation of covalent cross-links between structural cell wall components, while ·OH promotes polysaccharide degradation leading to wall relaxation and thus pressure-driven extension growth (Fig. 2b). Thus, relative levels of ROS in the apoplast seem to play an important role in cell wall modulation. $H_2O_2$ produced via apoplastic degradation of polyamines has been shown to contribute to cell-wall strengthening under stress conditions, such as wound-healing and pathogen attack (Cona et al. 2006; Angelini et al. 2008, 2010; Moschou et al. 2009). Recently, it has been also reported that increased polyamine oxidation in the apoplast changes the equilibrium among the various ROS in such a way as to sustain plant growth under salt stress conditions (Rodríguez et al. 2009; Campestre et al. 2011).

Polyamine catabolism in plants has also been correlated to wound healing and salt stress resistance through accumulation of GABA. Mechanical injury of pea stems elicited a parallel increase in CuAO, peroxidase and AMADH activity at the wound site in spatial correlation with intensive lignification (Petřivalský et al. 2007), while salt stress in soybean-induced GABA accumulation in roots through stimulation of CuAO activity (Xing et al. 2007). Increased GABA levels, probably derived from Put oxidation, have been observed in the brown alga *Ectocarpus siliculosus* under hyper-saline stress (Dittami et al. 2011).

Although the data described above strongly support the contribution of the apoplastic amine oxidases involved in the terminal catabolism of polyamine to the plant defense responses, more definitive studies are necessary to determine in detail the involved mechanism(s), to comprehend in depth the pleitrophic effects of the polyamine catabolic pathways and to unravel co-interacting metabolic and signaling pathways. More studies are also necessary to understand the contribution of the newly identified polyamine back-conversion pathways (Tavladoraki et al. 2006; Kamada-Nobusada et al. 2008; Moschou et al. 2008a; Fincato et al. 2011; Takahashi et al. 2010) to plant defense responses.

## Polyamine catabolism as a target for antiproliferative therapies in animals and stress tolerance strategies in plants

During the past decades, considerable research has been devoted to the discovery of new and more effective strategies for clinical antitumor therapy, involving the polyamine catabolic pathway. Inhibitors of ODC (the enzyme required for the first stage in polyamines synthesis), such as difluoromethylornithine (DFMO), and agents that stimulate polyamine acetylation and export, such as non-steroidal anti-inflammatory drugs (NSAIDS), act at least additively to arrest growth in human cell models and suppress intestinal carcinogenesis in mice (Babbar and Casero 2006; Gerner et al. 2007). In this context, toxic polyamine metabolites are currently explored as probable candidates for a new strategy in tumor therapy (Agostinelli et al. 2004; Agostinelli and Seiler 2006; Agostinelli et al. 2007), since it has been observed that the growth of mouse melanoma cells (B16-F0) was inhibited by exposure to subcytotoxic concentrations of BSAO and exogenous Spm (Averill-Bates et al. 2005, 2008). The same enzymatic system caused inhibition of cell proliferation and cell death in Chinese hamster ovary (CHO) cells and in several types of human tumor cell cultures such as colon adenocarcinoma cells (LoVo), melanoma cells (M14), and osteosarcoma cells (U2OS). However, in C57BL mice, prior injection of B16 mouse melanoma cells, cytotoxicity occurred in presence of endogenous polyamines, after directly injection of BSAO into the solid tumors (Calcabrini et al. 2002; Arancia et al. 2004; Averill-Bates et al. 2005, 2008; Agostinelli et al. 2006a, b, c, 2009, 2010). The cytotoxic effect appeared to be mediated by the enzymatic oxidation products of Spm, inducing both necrotic and apoptotic processes. Importantly, the products of BSAO-catalyzed oxidation of Spm were also able to overcome multidrug resistance (MDR) in cancer cells. In fact, previous findings on P-glycoprotein (P-gp) overexpressing MDR in CHO cells (Lord-Fontaine et al. 2001) demonstrate that doxorubicin-resistant human cancer cells (M14 DX and LoVo DX) are significantly more susceptible than their parental sensitive counterparts (M14 WT and LoVo WT) to hydrogen peroxide and aldehyde(s), the products of BSAO catalyzed oxidation of Spm, suggesting a possible new strategy against MDR tumors (Agostinelli et al. 2009, 2010).

The high expression of SMO seen in response to various inflammatory stimuli, its ability to produce mutagenic ROS, and its demonstrated capacity to damage DNA suggest that SMO may also represent a legitimate target for chemoprevention. Indeed, over-expression of murine SMO in mouse neuroblastoma cells caused a direct oxidative DNA damage which was more pronounced along with radiation exposure. Furthermore, following irradiation SMO over-expression drastically enhanced cell death, thus indicating an acquired hypersensitivity (Amendola et al. 2005). On the other hand, due to the importance of oxidative damage in inflammation-associated epithelial carcinogenesis, inhibition of SMO and/or SSAT activity would appear to be attractive targets for chemopreventive therapy.

The suggested involvement of polyamine metabolism in cancer and other hyper-proliferative disorders led to the synthesis of new pharmacological agents including polyamine analogues. These molecules have to be slightly different from the natural polyamines to be able to act as metabolic modulators, but not so different as to be excessively toxic to normal tissues. Several polyamine analogues have demonstrated significant activity against a great number of different cancer cell lines (Wallace and Niiranen 2007; Casero and Woster 2009; Häkkinen et al. 2010). The cytotoxic effects of many of these substances, including BENSpm and $N^{1}$-ethyl-$N^{11}$-[(cyclopropyl)methyl]-4,8-diazaundecane (CPENSpm), are believed to act, in part, through induction of SSAT and/or SMO. Polyamine analogues that can be substrates of SMO and/or APAO or their inhibitors are known. Among the polyamine analogues, BENSpm and CPENSpm decrease intracellular polyamine levels by down-regulating polyamine biosynthesis and, in specific instances, significantly up-regulating polyamine catabolism (Casero et al. 2003). BENSpm has significant activity against several human cancer cells in vitro and in vivo and has been examined in phase I and II clinical studies (Wallace and Niiranen 2007). In phase I trials, the drug was shown to be safe to administer on once-a-day schedule for 5 days (Hahm et al. 2002) with minimal toxicity but, unfortunately, in phase II trials there was little evidence of clinical activity (Wolff et al. 2003). Further optimization of polyamine analogues and schedules is needed to test if clinical results can be improved. Furthermore, combining standard chemotherapeutic agents with polyamine analogues that induce SSAT and SMO may be a rational approach to treating various types of cancers (Pledgie-Tracy et al. 2010).

Studies with the use of transgenic plants in which apoplastic polyamine catabolic enzymes were either over-expressed or down-regulated revealed the importance of polyamine catabolism in the induction of tolerance to a series of different biotic and abiotic stresses (Yoda et al. 2006; Moschou et al. 2008a, b, c, 2009; Marina et al. 2008). These studies opened the possibility of applying biotechnological strategies to improve resistance against a broad spectrum of pathogens and environmental stresses in agronomically important crops. An alternative approach to the biotechnological ones for crop improvement is the exogenous application of polyamines or polyamine

analogues (Cona et al. 2004; Maiale et al. 2008) which may have a direct protective role and/or may modulate the polyamine catabolic and/or biosynthetic pathways. The use of specific inhibitors for the polyamine catabolic pathways may have a beneficial effect in some cases, as for example in the cases of plant interaction with a necrotrophic pathogen in which an increased rate of polyamine catabolism has a detrimental effect on plant defense responses (Marina et al. 2008). In this context, the development and characterization of polyamine analogues that specifically inhibit the distinct polyamine catabolic enzymes may be very useful in plant research for determining the specific physiological roles of these enzymes and to efficiently design stress tolerance strategies.

## Conclusions and perspectives

Despite some important differences between animal and plant polyamine catabolism, several similarities also exist. In particular, the recent characterization of AtPAOs has bridged the gap between the two polyamine catabolic pathways, showing that the polyamine back-conversion pathway is also present in plants. Furthermore, the importance of extracellular polyamine catabolism in the control of cell proliferation and defense responses in both animals and plants is now documented. Notably, in both animals and plants, polyamine catabolism may have opposite effects depending on the catabolic pathway involved, the cell type, the external stimuli, and cellular metabolism.

Enough evidence suggests the potential of using amine oxidase(s) in the presence of polyamines in cancer therapy (Agostinelli et al. 2004, 2010). Since endogenous polyamines are present at high concentrations in cancer cells and growing tissues, it is expected that by delivering BSAO into cancer cells, toxic enzymatic oxidation products could be produced intracellularly for selective in situ killing of proliferating cells. Therefore, strategies could be developed to find out how the enzyme could be delivered in vivo, for possible clinical application. In fact, in cultured normal chick fibroblasts or in fibroblasts transformed by Rous sarcoma viruses, Bachrach et al. (1987) had observed an inhibition of the synthesis of proteins and nucleic acids when the cells were enriched with amine oxidase via microinjection. Transformed cells were more sensitive than normal controls, presumably due to higher polyamine content. Moreover, attempts were made to incorporate the enzyme into liposomal vesicles, and to prepare amine oxidase-gold complexes that are bound and incorporated by hepatocytes (Agostinelli et al. 2010). Thus, providing a scenario whereby endogenous polyamines could be targeted and oxidized by the enzyme. An extension of these studies with the aim to further improve the cytotoxic effect of Spm metabolites appears promising.

In conclusion, further studies are necessary to express in detail the physiological role(s) of the polyamine catabolic pathways, and extend further the knowledge on polyamine catabolism as targets for antiproliferative therapies in animals and stress tolerance strategies in plants.

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