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Characterisation of the *S*-adenosylmethionine decarboxylase (SAMDC) gene of potato

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Abstract

S-adenosylmethionine decarboxylase (SAMDC) is involved in the biosynthesis of the polyamines, spermidine and spermine. Recently, we reported the isolation of a putative cDNA clone of the SAMDC clone of potato (*Plant Mol Biol* 20; 641–651). In order to confirm that the potato genes does encode SAMDC, a complementation experiment with a yeast strain that possesses a null mutation in the SAMDC gene was performed. The yeast strain contains a deletion-insertion mutation in the SAMDC gene and has an absolute requirement for the addition of exogenous spermidine for growth. When the full-length potato cDNA was expressed in the mutant yeast strain there was no longer a requirement for exogenous spermidine. Immunoblotting experiments suggest that the potato SAMDC gene product has an apparent molecular mass of 39 kDa. Expression of the SAMDC gene was high in the young and actively dividing tissues and low in the mature and non-dividing tissues of both vegetative and reproductive organs. Additionally, isolation and characterisation of the corresponding genomic clone is reported. The gene has one intron in its 5′-untranslated sequence but otherwise the transcribed portion is identical to the cDNA clone.

Introduction

S-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50) is a key enzyme in the biosynthesis of the polyamines spermidine and spermine. The substrate for this enzyme is *S*-adenosylmethionine (SAM) and the product is decarboxylated SAM which provides the aminopropyl moiety required for spermidine and spermine biosynthesis from putrescine (Fig. 1) [1]. SAMDC activity has been shown to be rate limiting for the biosynthesis of these polyamines [2] and there are no alternative pathways reported in the literature.

Genes encoding SAMDC have been characterised from a number of mammalian sources [3, 4], from yeast (*Saccharomyces cerevisiae* [5]) and from *Escherichia coli* [6]. Although no plant sequences have been reported, SAMDC activity has been characterised in a variety of plant tissues including developing roots, fruits, floral organs and tuberising stolons from potato [7, 8, 9].

Although SAMDC has been purified from several plant species, no amino acid sequence data

are available for these proteins [10]. There is little nucleotide sequence similarity between the *E. coli* SAMDC gene and those from eukaryotic sources [4]. Although yeast and mammalian genes share only ca. 35% sequence identity, all the mammalian sequences are very similar [3]. Two common features are shared by all SAMDC enzymes studied to date. They all contain a covalently linked pyruvoyl group essential for activity and all are synthesised as precursors [1]. The pre-protein cleavage site is well conserved between the eukaryotes and is at a glutamyl-serine bond. In *E. coli* it is at a lysyl-serine site [11].

Despite their ubiquitous presence in all living organisms the physiological functions of polyamines remain unclear [1, 12, 38]. At cellular pH values, polyamines are cations, and can interact with anionic macromolecules such as DNA, RNA, phospholipids and certain proteins [13]. The association with nucleic acids can influence transcription and translation processes [10]. Binding of polyamines to membrane phospholipids results in altered patterns of solute permeation [1]. Polyamines can also bind covalently to the glutamic acid residues of polypeptides in a reaction catalysed by transglutaminases and perhaps other enzymes [14]. An absolute requirement for polyamines has been demonstrated for the growth and normal development of *E. coli* and *S. cerevisiae* [1]. For example, a mutation in the SAMDC gene of yeast results in severe inhibition of its growth and development [15]. However, in plants similar types of well-defined mutants have not yet been produced, although a large body of correlative data has implicated polyamines in a wide range of developmental processes. These include embryogenesis, tuberisation, and growth and development of roots, floral organs and fruits [7, 9]. Polyamines, especially spermidine, are generally abundant in actively dividing young tissues and non-senescent organs, and decline to lower levels as organs age and senesce [16]. Of additional interest in plants is the relationship between polyamine biosynthesis and the biosynthesis of the plant growth regulator, ethylene. SAM is a common precursor in both pathways; it may be converted to 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene, or it may be decarboxylated by SAMDC and used in spermidine and spermine synthesis (Fig. 1) [17]. Indeed, in some cases the synthesis of ethylene and these polyamines have been shown to be inter-dependent [18].

Attempts to use eukaryotic SAMDC genes as hybridisation probes for cloning the corresponding plant genes have proved unsuccessful, suggesting a low degree of sequence similarity (S.C. Minocha, personal communication). Recently, however, a cDNA clone of a putative SAMDC gene from potato was isolated by differential screening of a swelling stolon tip library [19]. The degree of similarity at the level of deduced amino acid sequence between the potato cDNA and the other eukaryotic genes was ca. 35%. In this paper we confirm that the potato cDNA encodes SAMDC by demonstrating its ability to complement a yeast null mutant. Furthermore, we describe the isolation and characterisation of a potato genomic clone corresponding to the putative cDNA clone of the SAMDC gene. Finally, we discuss the potential use of this gene in improving our understanding of the role of polyamines in plant developmental processes.

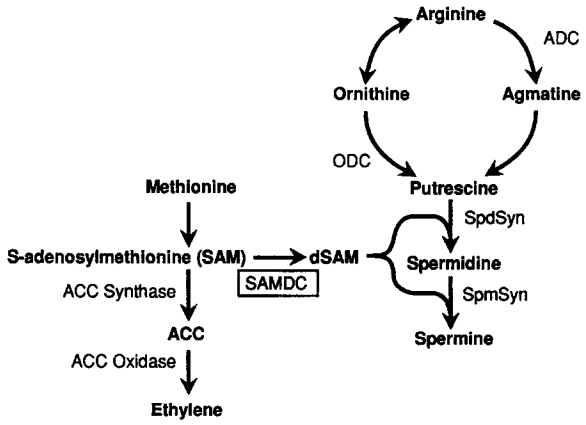

Fig. 1. Polyamine and ethylene biosynthetic pathways and their inter-relationships (adapted from 7). ODC, Ornithine decarboxylase; ADC, arginine decarboxylase; ACC, 1-aminocyclopropane-1-carboxylic acid. SAMDC, S-adenosylmethionine decarboxylase; dSAM, decarboxylated S-adenosylmethionine.

Materials and methods

Yeast strains, media and growth conditions

A yeast mutant strain, Y342, *MAT6α ura3-52 leu2 spe2Δ::LEU2*, was kindly provided by Drs Celia Tabor and Herbert Tabor, National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Health, Bethesda, MD 20892, USA.

Standard yeast culture and manipulation methods used were as described [20]. YPAD medium (a rich medium) and SD medium (a minimal medium, containing glucose but no amino acids and polyamines) were used for maintenance of strains and growth studies, respectively. SD medium required supplementation of uracil (100 μm) to support growth of Y342 and therefore *ura3* was used as a selectable marker for transformation. All liquid media were filter-sterilised using 0.25 μm Millipore membranes and previously autoclaved ultra-pure agarose (FMC, Flowgen) was used to make solid media. All precautions were taken to avoid traces of spermidine and spermine contamination in the culture media as recommended [15]. In all cases new sterile plastic culture vessels were used. Cultures were grown at 30 °C and when required in rotary shaking conditions.

Veçtor construction and yeast transformation

To express the full-length cDNA clone of the potato SAMDC gene in yeast, a plasmid YCpGAL containing the bi-directional GAL1-GAL10 promoter [21] was used initially (Fig. 2) to clone the entire coding region of SAMDC gene plus the untranscribed 3′ and 5′ ends of the gene by excising with *Sal* I and *Pst* I from pBluescript and ligating into the *Sal* I and *Pst* I cloning sites of the plasmid YCpGAL (A. Sneddon and M.J.R. Stark, unpublished). The chimaeric SAMDC gene with the GAL promoter (*GAL10::SAMDC*) was then excised from the plasmid YCpGAL with *Pst* I and *Sst* I and was ligated into the *Pst* I and *Sst* I cloning sites of the plasmid YCplac33 to make a vector, *YCpGAL10::SAMDC* (Fig. 2). The

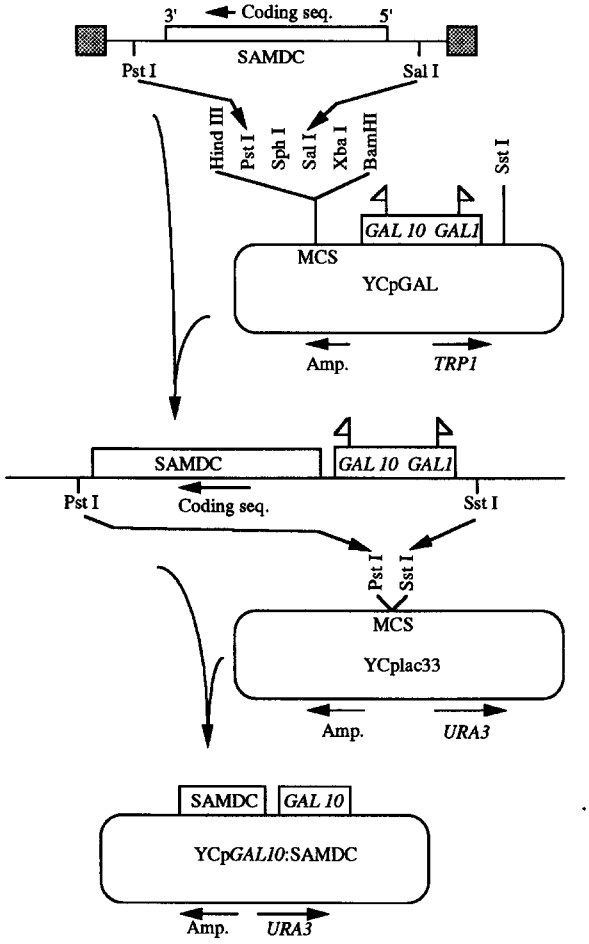

Fig. 2. Construction of a chimaeric SAMDC gene under the control of a yeast *GAL10* promoter (*YCpGAL10::SAMDC*) in yeast transformation vector YCplac33 [22]. For details, see the text.

plasmid YCplac33 contains the *URA3* gene as the selectable marker for transformants [22] and this plasmid was used as a control.

The lithium acetate transformation method [23] was used to introduce plasmids *YCpGAL10::SAMDC* and YCplac33 (as a control) independently into the yeast strain Y342. The mutant cells were grown in SD medium with 100 μM uracil and 1 μM spermidine and were used for the transformation. Transformants were initially selected in SD medium with 1 μM spermidine, but without uracil. In Fig. 3 single yeast transformed colonies containing the plasmids *YCpGAL10::SAMDC* or YCplac33 were grown

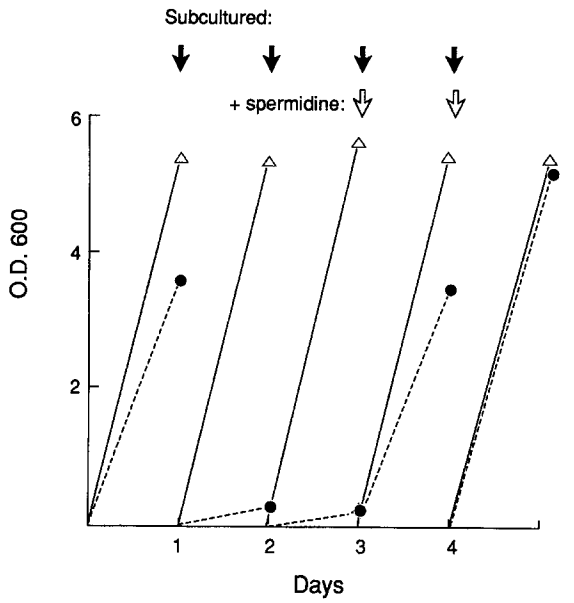

Fig. 3. Yeast cell growth measurements of the transformed Y342 mutant line with YCplac 33 vector (indicated as ----●) or with *YCpGAL10::SAMDC* vector (—△). Growth was measured by determining the optical density at 600 nm in a Beckman spectrophotometer. Transformed yeast lines were grown in SD medium with or without 1 μM spermidine for 24 h and then subcultured into fresh medium (for details, see the text). Closed arrows (—▸) represent each subcultured stage and open arrows (⇒) represent the addition of spermidine at the time of subculture.

independently (five tubes for each transformant) for 24 h in SD medium, and 10 μl from each tube were subcultured again in 5 ml of fresh SD medium. This subculture step was again repeated. In the third and fourth subcultured stages spermidine (1 μM) was added to the SD medium and both transformants were grown for 24 h. Growth was measured by determining the optical density at 600 nm in a Beckman spectrophotometer at each subcultured stage and shown in Fig. 3. The standard errors were less than 1% of the mean values and so not shown in the Fig. 3. The transformed line carrying *YCpGAL10::SAMDC* was also grown in the synthetic medium supplemented with raffinose or galactose.

SAMDC enzyme assays

The mutant yeast strain Y342 and the two transformants containing plasmids *YCpGAL10::SAMDC* or YCplac33 were grown to log phase and harvested by centrifugation and 400 mg wet weight of each were used for SAMDC determination. Cells of *YCpGAL10::SAMDC* and YCplac33 were resuspended in 1 ml of lysis buffer as described [24] from which 50 μl each of *YCpGAL10::SAMDC* and of YCplac33 samples were dispensed in culture tubes. 50 μg of lyticase (Sigma) was added to each suspension. The incubation with lyticase and addition of *S*-adenosyl-*L*-(carboxy-^{14}C)methionine was performed as described [24]. The method described [25] was used in the subsequent assay of *S*-adenosylmethionine decarboxylase, except that the incubation time was increased to 2–3 h and putrescine was added to the reaction mixture. The YCplac33 transformant was used as a negative control.

Plant material

Flowers, buds, pollen and stolons were obtained from glasshouse-grown, tuberising potato plant (*Solanum tuberosum* L. cv. Désirée).

Production of polyclonal antibodies

A synthetic peptide corresponding to residues 11 to 25 (GFEKRLEISFVEPGL) of the deduced amino acid sequence of the putative SAMDC cDNA clone was synthesised using an Applied Biosystems Peptide Synthesiser, Model 431A. Polyclonal antibodies were raised against this peptide in rabbit as described [26].

Immunoblotting

Total protein extracted from swelling stolons of tuberising potato plants, was separated on SDS-polyacrylamide gels and immunoblotted as described [27].

Nucleic acid manipulation

Standard techniques for DNA manipulations were as described [28] and modifying enzymes

were used according to the manufacturer's instructions.

RNA analysis

RNA extractions and blots were performed as described [19]. Even loading of tracks was confirmed by probing with a 25S rRNA gene probe from potato (data not shown).

Isolation of genomic clone

The library used for the isolation of the SAMDC genomic clones was constructed in λEMBL-3 SP6/T7 (purchased from Clontech, Palo Alto, CA). Potato (cv. Désirée) genomic DNA was partially digested with the restriction endonuclease *Sau*3A and cloned in the *Bam* HI site of the vector. The average insert size was 15 kb. Screening was performed by using Hybond-N (Amersham) and ^{32}P-labelled probes were synthesised using standard techniques [29]. The template for the probe was the full-length cDNA clone of the putative SAMDC gene of potato [19].

DNA sequence analysis

DNA sequencing was performed by the dideoxy chain termination method [30] using a Sequenase Sequencing Kit (United States Biochemical, Cleveland, OH). A number of primers and subclones enabled both strands of the genomic clone to be sequenced. By comparison with the putative cDNA of the SAMDC gene the entire coding region and upstream of the initiation codon (ATG) were sequenced.

Results

Complementation of a yeast SPE2 mutant

To confirm that the putative cDNA clone of the potato SAMDC gene does encode SAMDC, its ability to complement a yeast *spe2* gene (i.e. SAMDC gene) was investigated. This *spe2* null mutant was generated by a deletion-insertion mutation in the SAMDC gene using a one-step disruption technique in which the coding sequence was replaced by the *LEU2* gene [15]. The *spe2* null mutant has no detectable SAMDC activity, endogenous spermidine and spermine and has an absolute requirement for exogenous spermidine or spermine for growth [15, 31]. To express a full-length cDNA clone of the SAMDC gene of potato in yeast, a plasmid YCpGAL containing the bi-directional *GAL1-GAL10* promoter was used to construct a vector *YCpGAL10::SAMDC* as described in the Materials and methods and shown in Fig. 2. Complementation of the *ura3* mutation in the mutant Y342 by the functional gene on the vector *YCpGAL10::SAMDC* allowed selection of transformants on medium without uracil. The presence of the SAMDC cDNA sequence in the transformant was confirmed by PCR analysis using a pair of primers specific to the potato SAMDC gene (data not shown).

Transformants of the mutant strains containing either the *YCpGAL10::SAMDC* or YCplac33 vectors were initially grown in SD medium with 1 μM spermidine. To test, if the expression of the chimaeric SAMDC gene in the transformed mutant strains was able to complement for the deficiency of SAMDC (thereby restoring the biosynthesis of spermidine and spermine), the transformants were grown in media without any exogenous spermidine. Since the SAMDC cDNA was fused to the *GAL* promoter we expected that complementation of the *spe2* deletion would be galactose dependent, since the *GAL10* promoter is largely inactive in the absence of galactose, induced some 1000-fold in its presence and subject to glucose repression [36]. However, transformants of Y342 carrying *YCpGAL10::SAMDC* were able to grow in the absence of polyamines using galactose (inducing), raffinose (non-inducing) or glucose (repressing) as the carbon source, both on plates and on repeated subculturing in liquid media (Fig. 3). On the contrary, the mutant line transformed with the same vector (i.e. YCplac33) but lacking only the chimaeric

GAL10::SAMDC gene was unable to grow in the absence of any exogenous spermidine. However, the growth rate of the mutant transformed with YCplac33 decreased with several cycles of subculturing in media lacking spermidine (Fig. 3). Presumably this reflects the dilution of spermidine initially present in the starter culture. Furthermore, activity of SAMDC in the transformed yeast line with *YCpGAL10::SAMDC* was detectable (Table 1). No SAMDC activity was observed in the original mutant strain Y342 and in Y342 transformed with YCplac33.

Immunoblot analysis

Rabbit polyclonal antibodies were raised against a synthetic peptide as described in Materials and methods. Immunoblotting of a total protein extract from swelling potato stolons which are high in SAMDC activity [9] was performed. The antibodies cross-reacted monospecifically with a polypeptide of ca. 39 kDa (Fig. 4) as judged by comparison with the molecular weight markers.

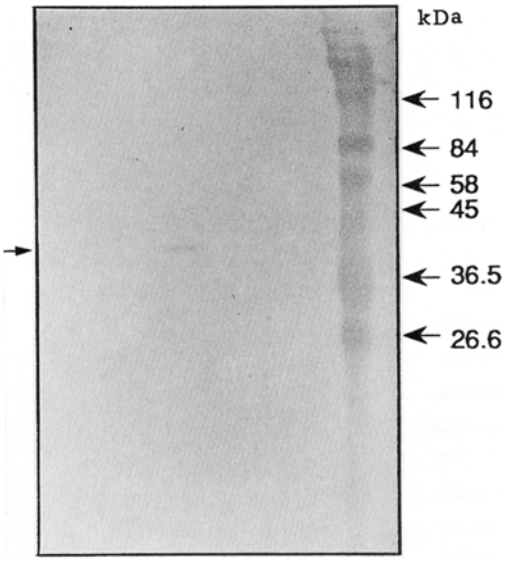

Fig. 4. Immunoblot showing the product of the SAMDC gene as a single band of ca. 39 kDa. Total protein from swelling stolons was separated on SDS polyacrylamide gels and immunoblotted with rabbit polyclonal antibodies raised against a synthetic peptide corresponding to the N-terminal of the SAMDC pre-protein (for details, see the text).

Expression of SAMDC gene in the reproductive organs

Expression of the SAMDC gene in reproductive tissues such as young flower buds (15–20 mm), whole mature flowers and mature pollen from tuberising potato plants were investigated. RNA was isolated from these tissues and RNA blot analysis was performed using the labelled SAMDC cDNA as a probe, as described in the Materials and methods. An RNA transcript of ca. 1.8 kb was observed in all these tissues (Fig. 5). The highest level of SAMDC transcript

Table 1. Measurements of SAMDC activity in the tranformed Y342 lines with YCplac33 vector or with *YCpGAL10::SAMDC* vector (for details, see the text).

Sample	Incubation time (h)	SAMDC activity*
YCpGAL10::SAMDC		
50 µl	2	40
	3	146
YCplac33		
50 µl	2	0
	3	0

* pmol $^{14}CO_2$ released per hour per gramme fresh weight.

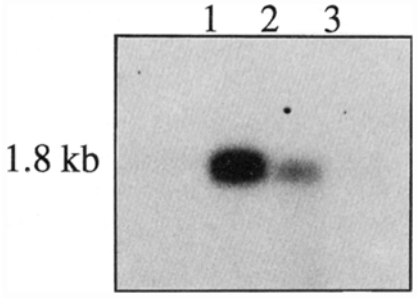

Fig. 5. RNA blot showing the expression levels of the SAMDC gene in (1) buds, (2) flowers, and (3) mature pollen of potato. 15 µg of total RNA from each organ was loaded in each lane and was separated on a denaturing agarose gel, transferred to nylon membrane and hybridised with the ^{32}P-labelled cDNA clone of the SAMDC gene of potato (for details, see the text).

was observed in the young flower buds, only a lower level could be detected in the mature flowers and in the mature pollen a very low level of the transcript could be seen.

Isolation of a potato SAMDC genomic clone

SAMDC cDNA was used as a probe to isolate the corresponding genomic clone. The DNA sequence of this genomic clone is shown in Fig. 6. The transcribed sequences of the cDNA and genomic clones are identical. By comparison with the cDNA sequence, the SAMDC gene contains only one intron located in the 5′-untranslated part of the gene. The intron boundaries are in good agreement with plant splice junction consensus sequences [32]. The SAMDC gene has an untranslated 5′ sequence of over 500 nucleotides.

Located in the 5′-flanking region is a putative TATA box-like sequence (nucleotides −586 to −575 underlined in Fig. 2) which shows excellent agreement with the plant TATA box consensus (TCACTATATATAG) [33]. The size of the SAMDC transcript as determined by RNA blot analysis (Fig. 5) is 1.7–1.8 kb nucleotides consistent with the transcription start site being within 25–40 bp downstream of the presumed TATA box. There are no other candidate TATA box-like sequences in this region of the gene. Upstream of the TATA box there is no CAAT box present although there is an AGGA element at nucleotides −683 and −686 [33]. A polyadenylation signal (AATAAA) is located at nucleotides 1317 to 1322.

A comparison of the deduced amino acid sequence of potato SAMDC gene with sequences from other eukaryotic sources is shown in Fig. 7. The percentage sequence identities with the yeast [11], golden hamster [34], bovine [35] and human [4] sequences are 30.7, 35.6, 35.6 and 34.9 respectively. Although the similarity of the potato sequence to other eukaryotic sequences is limited, a number of regions are highly conserved. These include a region of eight amino acids from residues 66 to 73 (LSESSMFV) in the potato sequence which is identical to the mammalian and yeast pro-enzyme cleavage site [11]. Another highly conserved region is at residues 247 to 262 (TIHITPEDGFSYASFE) which is a putative PEST sequence [3], a characteristic of polypeptides that are rapidly turned over.

Discussion

In the absence of any cloned SAMDC genes from plants and in view of the low degree of sequence similarity between the potato and the other eukaryotic SAMDC genes it was necessary to confirm that the potato sequence did encode SAMDC. Therefore, a complementation experiment with a yeast strain that possesses a null mutation in the SAMDC gene was performed. The strain contains a deletion-insertion mutation in the SAMDC gene and has an absolute requirement for spermidine or spermine for growth [15, 31]. When the full-length potato cDNA was expressed in the mutant yeast strain there was no longer a requirement for exogenous spermidine. This result confirms that the cDNA clone is from a *bona fide* SAMDC gene of potato. Moreover, this result also suggests that despite the low level of nucleotide sequence similarity between the SAMDC gene of potato and yeast there is sufficient functional similarity at the enzyme level. This is consistent with some functionally important domains being conserved between the potato and yeast (Fig. 7). For example, one important conserved part of SAMDC are residues surrounding the cleavage site of the pro-enzyme. The conservation of the cleavage site suggests that a processing mechanism similar to that shown for other SAMDC pro-enzymes may be operating for the potato enzyme.

Transformants carrying *YCpGAL10::SAMDC* were able to grow in the absence of polyamines using galactose (inducing), raffinose (non-inducing) or glucose (repressing) as the carbon source [36]. This implies that either the requirement of yeast cells for SAMDC is very low and can be supplied by basal expression from *GAL10* or that expression from the construct was 'leaky'. Similarly, yeast expressing *PPH21* or *PPH22* from

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        E  F  S  I  A  L  H  A  D  V  A  T  K  L  L  E  R  T  C  S  V  D  V  K  G  Y  S  L  A  E
  949  gtggagtccagaagagtttggcgaaggcggttccattgtctaccagaagttcactagaactccttactgtgaatctcccaagtccgttct  1038
        W  S  P  E  E  F  G  E  G  G  S  I  V  Y  Q  K  F  T  R  T  P  Y  C  E  S  P  K  S  V  L
 1039  gaagggctgctggaaggaggaagagaaagaaggaaaggagtagtgttgtcttgagggtcgttttgttgtttttgtttctgtttcagtgtc  1128
        K  G  C  W  K  E  E  E  K  E  G  K  E  *
 1129  tgtcgtttgctcatgttttccctgtttctcagaataatggacttatacgtccaaacttgtgtctgtcgaatttgcaacgtctgtgtgcaa  1218
 1219  attctgaactagtcttgccttttggtgttccaccagaagcctttatgtgtctgcattttgaattgtgtcatgttgttggtctctgttccg  1308
 1309  ttgcgtctaataaaattgtgtttg  1332
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Fig. 6. The nucleotide sequence of the genomic clone of the potato SAMDC gene. Shown in bold is the 5′ intron sequence. The putative TATA box and the polyadenylation signal are underlined. The deduced stop codon is indicated by an asterisk.

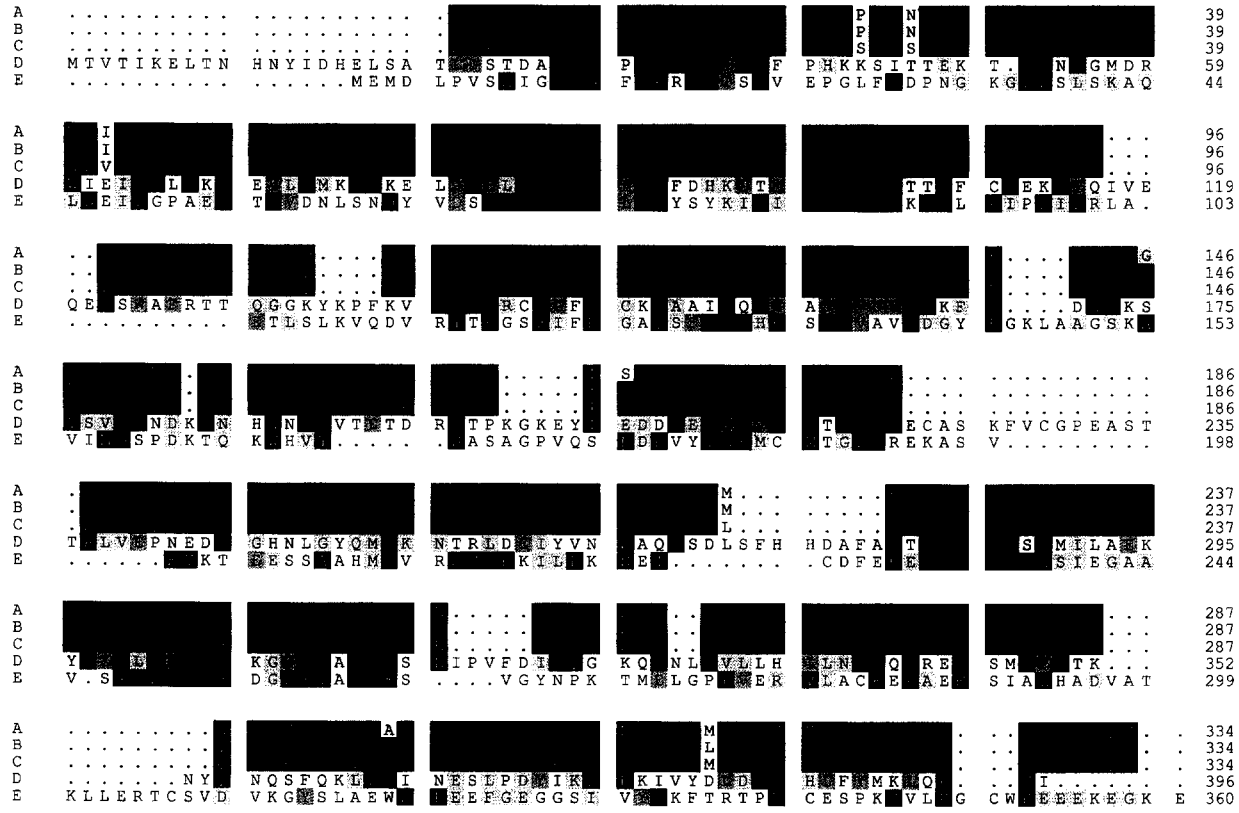

Fig. 7. Comparison of the deduced amino acid sequence of SAMDC from (a) human, (b) bovine, (c) golden hamster, (d) yeast, and (e) potato. Conserved amino acid residues are outlined in black and conservative changes are light-shaded.

GAL1 as their sole source of protein phosphatase 2A, an essential enzyme in yeast, can grow at the wild type rate on glucose as the carbon source (A. Sneddon and M.J.R. Stark, unpublished). The ability to express a plant SAMDC gene in yeast should prove a quick and reliable means of characterising other putative cDNA clones of SAMDC genes. Additionally, site-directed mutagenesis of the nucleotide sequence of the gene in conjunction with this heterologous expression system should allow detailed analysis of the enzyme.

To study the properties of SAMDC in plants, polyclonal antibodies were raised against a putative N-terminal sequence. These polyclonal antibodies should recognise the pro-enzyme, but would not be expected to cross-react with the large subunit following processing when the N-terminal segment is cleaved. The antibodies reported here recognise a polypeptide of apparent Mr of 39 kDa consistent with the calculated molecular weight of the deduced pro-enzyme (39.7 kDa). No smaller fragments corresponding to the N-terminal fragment following cleavage could be detected. Mammalian SAMDC protein has a rapid turnover rate and represents a very minor component of the total cellular protein [37]. It is synthesised as a 38 kDa pro-enzyme that is autocatalytically cleaved in a putrescine reaction. The products of 31 kDa and 7 kDa are both required for activity whereas the pro-enzyme is inactive [1]. It is possible that in plants, once cleavage has taken place, the enzyme is rapidly turned over and, as in mammalian systems, there is a very low steady-state level of the enzyme that cannot be detected easily in total protein extracts.

Expression of the SAMDC gene in some of the reproductive tissues of potato was determined by RNA blot analysis. There was a high level of transcript in the young flower buds, a low level of

transcript in the mature flowers and a very low level of transcript in the mature pollen. Similarly, detailed analysis of the flower buds, anthers and pollen of tobacco showed high levels of SAMDC transcript in the young and actively dividing tissues and low levels of transcript in mature tissues (S. Mad Arif, M. Roberts, M. Taylor and A. Kumar, unpublished). Previously, we have shown that the SAMDC transcript in stolon tips is high at the initial stages of tuberisation but is greatly reduced in mature tubers [19]. Other vegetative tissues such as leaves and roots also show a high level of SAMDC transcript when cells are actively dividing and differentiating, but much lower levels in mature tissues (data not shown). These results suggest that the SAMDC gene is highly expressed in actively dividing and differentiating tissues of both vegetative and reproductive organs. This is consistent with the many reports of maximum SAMDC activity and polyamine levels in rapidly dividing and differentiating tissues [7 and references therein].

Using a previously isolated cDNA clone of a putative SAMDC gene of potato, the corresponding genomic clone has been isolated. The transcribed parts of the cDNA and genomic clones are identical. Interestingly, the SAMDC gene contains only one intron located in the 5′-untranslated part. Furthermore, the gene is unusual in that it has an untranslated 5′ sequence of over 500 nucleotides. The mammalian SAMDC gene also has a long (300 nucleotide) transcript leader that is highly conserved between different species and contains a 21 nucleotide upstream open reading frame [4]. The human SAMDC gene 5′ translation leader sequence can confer cell specificity in the translational efficiency of RNA transcripts by having an influence on ribosomal loading [35]. There is little sequence similarity between the long leader sequences of potato and mammalian genes and the functional significance of the long leader sequence in plants remains to be tested. The large changes in the levels of the steady state SAMDC transcript levels imply that there is a strong transcriptional control in operation. Promoter studies using the 5′-upstream sequence reported here should enable identification of the sequences that have an important regulatory function.

The physiological function of polyamines remain unclear despite their ubiquitous presence in all living organisms. In plants, very little is known about either the mechanisms that regulate polyamine biosynthesis or their subcellular localisation. These two aspects are critical to understanding their functions in plant cell proliferation and morphogenesis. Previous studies based on various inhibitors have provided some useful insight into the role played by polyamines in plant cell proliferation and morphogenesis [7, 16, 38]. However, because the inhibitors are non-specific and not well characterised for their permeability and stability in cells, the interpretations of the results are not precise. With the SAMDC gene now available, it will be possible to manipulate polyamine biosynthesis more precisely using cellular and molecular techniques. We are transforming potato with engineered SAMDC genes in both antisense and sense orientations to both down-regulate and over-express the SAMDC transcript in transgenic plants. This will allow precise manipulation of the biosynthesis of polyamines and consequently, should provide valuable insight into the functions of polyamines in plant developmental processes. Additionally, any modulations in the expression of the SAMDC gene are likely to not only affect the biosynthesis of polyamines but should also affect the biosynthesis of ethylene since SAM is a common precursor (Fig. 1) in both biosynthetic pathways.

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