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Expression and sequence analysis of cDNAs induced during the early stages of tuberisation in different organs of the potato plant (*Solanum tuberosum* L.)

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Abstract

cDNA clones of two genes (*TUB8* and *TUB13*) which show a 25–30-fold increase in transcript in the stolon tip during the early stages of tuberisation, have been isolated by differential screening. These genes are also expressed in leaves, stems and roots and the expression pattern in these organs changes on tuberisation. Southern analysis shows homologous sequences in the non-tuberising wild type potato species *Solanum brevidens* and in *Lycopersicon esculentum* (tomato). Sequence analysis reveals a high degree of similarity between the *TUB13* cDNA, and a human *S*-adenosylmethionine decarboxylase gene. The predicted *TUB8* peptide sequence shows several repeats of alanine, glutamate and proline which suggests a structural role for the encoded protein.

Introduction

Tuberisation is a complex process that results in the differentiation of a specialised shoot, the stolon, into a storage organ, the tuber. Histological studies have described in detail the changes in cell type and growth pattern that occur during tuberisation in potato (reviewed in [25]). Potato tubers develop initially from enlargement of existing pith cells in the sub-apical region of the stolon, followed rapidly by cell divisions in most parenchyma cells, particularly those associated with the perimedulla and inner cortex. These divisions are thought to cease early in tuber development (once the tuber has reached 30–40 g fresh weight). The final tuber size is determined by a further increase in cell volume of perimedullary and cortical parenchyma tissues [25].

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers Z11679 (*S. tuberosum TUB8* cDNA) and Z11680 (*S. tuberosum TUB13* cDNA).

A wide variety of environmental and hormonal stimuli are known to be involved in the induction of tuberisation (reviewed in [19, 20, 31]). These include photoperiod, temperature, nitrogen supply, and a range of plant growth hormones, including abscisic acid, cytokinins, ethylene and gibberellins. Recently, a tuber-inducing substance termed tuberonic acid (a jasmonic acid derivative [15]) has been suggested to function by disrupting cortical microtubules in stolon cells. This allows lateral expansion in the stolon tip and hence the radial growth associated with tuberisation [1]. We have observed changes in the expression pattern of isoforms of the major microtubule polypeptides, α and β-tubulin, which may suggest a reorganisation of the cytoskeleton occurs during tuberisation [30].

Other changes in the pattern of gene expression in the early stages of tuberisation have been identified from analysis of proteins extracted from stolon tips and tubers during tuberisation [8, 9, 30]. *In vitro* translation of RNA extracted from the same stages of development supports the idea that changes in gene expression occur early in tuberisation [9, 30]. With the exception of these studies, little is known of the molecular mechanisms that underpin the early stages of tuberisation.

Previous molecular studies have concentrated on the expression of tuber storage proteins such as patatin [reviewed in 24] and various classes of serine protease inhibitor genes [9, 14]. Under some circumstances, starch and protein deposition can occur without the morphogenesis and cell proliferation associated with tuberisation [24]. For example, when the axillary bud is removed from a single leaf stem cutting, large amounts of patatin and starch accumulate in the petiole [22]. Unlike the tuberisation response which in some varieties (for example *Solanum andigena*) is totally dependent on short photoperiods, this accumulation of storage polymers is independent of daylength [22]. Furthermore, substantial suppression of patatin synthesis by 'antisense' inhibition does not result in tubers with significantly changed morphology [10]. Hence, although the regulation of storage polymer biosynthesis shares some common aspects with the regulation of tuber morphology, the two processes can be separated.

Differential screening of a potato tuber cDNA library with probes against leaf and tuber poly(A)$^+$ RNAs resulted in the isolation of patatin and serine protease inhibitor clones [29]. In this study we have used a similar approach to isolate two genes (*TUB8* and *TUB13*) that are induced in the early stages of tuberisation. We show that these two genes are expressed in tissues in addition to the tuberising stolon and their levels in these other tissues alter at the onset of tuberisation, in an independent manner to their expression levels in the stolon. The deduced amino acid sequence of *TUB13* shows it to be a homologue of the human *S*-adenosylmethionine decarboxylase (SAMDC) gene, which is involved in polyamine biosynthesis. *TUB8* contains some unusual repeated motifs and may encode a polypeptide with a structural role.

Materials and methods

Growth of plant material

Seed tubers of potato (*Solanum tuberosum* L.) cv. Record were grown as described [30]. Plants were harvested between 21 and 60 days after planting to obtain material at various developmental stages. For stage A (Fig. 1), stolons were harvested from plants shortly after the emergence of leafy shoots (ca. 21 days after planting). There was a complete absence of swelling stolons on any of the plants from this stage. Non-swelling stolons (stage B, Fig. 1) were taken from plants on which some stolons had started to tuberise. The stolons selected from this stage did not show any signs of swelling (harvested 28–35 days after planting). Swelling stolons (stage C, Fig. 1), were selected from plants in which the diameter of the swelling stolon was less than twice the diameter of the stem. Small tubers (up to 1 g fresh weight) were harvested after approximately 42 days (stage D, Fig. 1) and 25 g tubers at 60 days after planting (stage E). Roots, internodal green stem

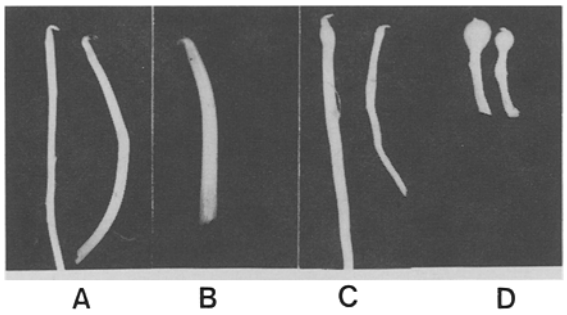

Fig. 1. Stages in stolon development. Stage A, non-swelling stolons harvested 21 days after planting, stage B, non-swelling stolons harvested 35 days after planting, stage C, swelling stolons also harvested 35 days after planting, stage D small tubers up to 1 g fresh weight.

segments and leaves were harvested from plants in which there were no visible signs of tuberisation (21 days after planting) and from plants which had started to tuberise (42 days after planting).

RNA extraction

Plant material was harvested, washed in distilled water and rapidly frozen in liquid nitrogen. Total RNA was extracted from ca. 2 g fresh weight stolon tips (apical 5 mm), whole leaves, roots and green stems using Qiagen-pack 100 cartridges (Qiagen Inc., Studio City, USA) following the manufacturer's protocol. Poly(A)$^+$ RNA was prepared by affinity chromatograhy using an oligo(dT) cellulose column or by using oligo(dT)-coated magnetic beads (Dynal Ltd., Liverpool, UK).

Library construction

A cDNA library was constructed from poly(A)$^+$ RNA extracted from swelling stolon tips (stage C in Fig. 1). cDNA was prepared from 5 μg of poly(A)$^+$ RNA using a Pharmacia cDNA synthesis kit. The cDNA was methylated and *Eco* RI links were ligated to the termini. The cDNA was ligated into the *Eco* RI site of the lambda Zap II vector (Stratagene), packaged using an *in vitro* packaging kit (Amersham) and plated on the XL1-Blue strain of *Escherichia coli* (Stratagene). The library contained ca. 1.5×10^6 pfu with a mean insert size of 1.2 kb.

Differential screening

Differential screening was carried out as described [18]. Single-stranded cDNA probes, labelled with [α-^{32}P]dCTP, were prepared from 1 μg poly(A)$^+$ RNA from non-swelling stolon tips (stage A) and from swelling stolon tips (stage C) using a reverse transcriptase kit (Gibco BRL). The probe specific activities were adjusted to equality and equivalent amounts of radioactivity per ml hybridisation solution were used [7]. After hybridisation (16 h) filters were washed sequentially in 2 × SSC, 0.1% SDS, and then 0.1 × SSC, 0.1% SDS for 2 × 20 min changes in each solution at 65 °C and exposed to X-ray film (Fuji) at −70 °C, with intensifying screens for 48–72 h. Plaques that hybridised to the probe from swelling stolons (stage C) but not the non-swelling stolon probe (stage A) were selected and purified to homogeneity by two further rounds of plaque screening. Seven recombinants with these characteristics were isolated from screening of approximately 40 000 initial plaques. The *in vivo* excision protocol of Stratagene with the R408 helper phage was used to rescue putative tuberisation induced cDNAs in pBluescript SK (−) plasmids. The plasmids were purified using Qiagen-100 cartridges.

Northern blotting

Northern blotting of RNA was carried out using Hybond-N (Amersham) following the manufacturers' protocols. Total RNA (15 μg per track) was resolved by electrophoresis on 1.2% agarose gels containing formaldehyde. Probes were labelled with [α-^{32}P]dCTP to high specific activity (typically 1×10^9 cpm/μg) using random primers [6]. Following hybridisation (16 h at 42 °C in 50% formamide) filters were washed sequentially

in 2× SSC, 0.5% SDS followed by 2× SSC, 0.1% SDS and then 0.1× SSC, 0.1% SDS for 20 min per wash at 52 °C. The filters were then exposed to X-ray film at −70 °C for between 24 and 96 h. The hybridisation signal on northern blots was quantified by densitometry using a Quantimet 900 image analyser, Leica, Cambridge, UK. Equal loading of gels was verified by re-probing stripped filters with a 25S potato ribosomal RNA gene probe.

DNA extraction and Southern blots

Plant genomic DNA was extracted from leaves using cetyl-triethylammonium bromide (CTAB) as described [4]. Five μg of each DNA was digested with *Eco* RI and/or *Hin*d III and resolved by electrophoresis on 0.8% agarose gels. DNA was transferred under vacuum to nylon membranes (Hybond-N). Filters were hybridised with the labelled inserts of the *pTUB8* and *pTUB13* clones, prepared as above. Filters were washed at high stringency (0.1× SSC, 0.1% SDS at 65 °C) and exposed to X-ray film for 24–72 h at −70 °C with intensifying screens.

DNA sequence analysis

DNA sequence was obtained from both strands of alkaline denatured plasmid by dideoxysequencing [28] using Sequenase version 2.0 (United States Biochemical Corporation). DNA sequence data were compiled and compared using the Genetics Computer Group programs for the VAX [3]. Alignments of complete sequences were carried out using the GAP program.

Results

Analysis of patatin expression in tuberised and untuberised plants

To ensure that the tissues in these experiments were expressing genes associated with tuberisation, northern blots of RNAs from different stages in tuberisation were probed for patatin gene expression. As has previously been observed [21], patatin expression increased dramatically during tuberisation (Fig. 2). No patatin transcript was detectable in non-swelling stolon tips from plants harvested at stage A (Fig. 2, lane 1). In contrast, a significant level of patatin RNA (20% of the maximum) was detected in RNA extracted from stolon tips taken from plants on which some swelling stolons were also observed (stage B; Fig. 2, lane 2). Expression of patatin mRNA was maximal in small tubers (1 g fresh weight) but declined to ca. 35% of this level in larger tubers (25 g) (Fig. 2, lanes 3 and 4). Compared to the level of expression in swelling stolon tips and tubers, patatin transcript levels were low (1–2% of the small tuber level) in other parts of the plant (leaves, roots and green internodal stem sections).

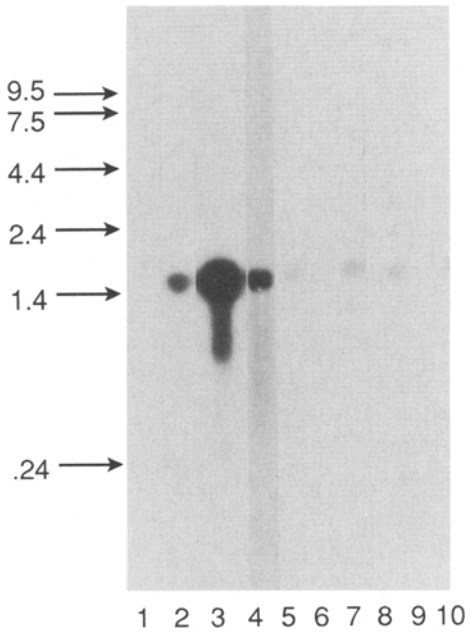

Fig. 2. Northern analysis of patatin expression. Hybridisation of patatin probe to 15 μg total RNA extracted from stolon tips from stage A (lane 1); stolon tips from stage B (lane 2); small tubers from stage D (lane 3), 25 g fresh weight tuber (lane 4), leaf, root, stem from plants harvested 21 days after planting (lanes 5, 6 and 7, respectively), leaf, root and stem from plants harvested 42 days after planting (lanes 8, 9 and 10, respectively).

Similar levels in leaf, root and stem were observed in material obtained from tuberising (stage C) or non-tuberising plants (stage A) (Fig. 2, lanes 5–10).

Differential gene expression during tuberisation

A cDNA library was constructed from poly(A)$^+$ RNA from swelling stolon tips (stage C). This library was screened with radioactive cDNA from non-swelling (stage A) stolon tips and swelling stolon tips (stage C). From a total of 40000 plaques screened, 7 were found to be differentially expressed between these stages. Two of the seven (*TUB8* and *TUB13*) were selected for further analysis.

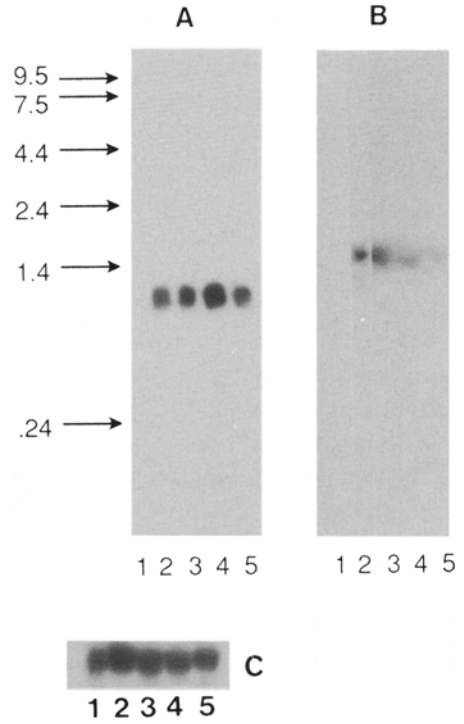

Fig. 3. Northern analysis of *TUB8* and *TUB13* expression. A. Hybridisation of *TUB8* to 15 μg total RNA extracted from stolon tips from stage A (lane 1), stolon tips from stage B (lane 2), stolon tips from stage C (lane 3), small tubers from stage D (lane 4), tubers (25 g fresh weight) (lane 5). B. Hybridisation of *TUB13* to 15 μg total RNA (lanes as in A). C. Hybridisation of 25S rRNA probe to stripped filter used in Fig. 3A (lanes as in A).

Expression levels of *TUB8* and *TUB13* were determined by northern analysis (Figs. 3A and 3B respectively). In common with patatin, there was virtually no expression of either of these genes in stolon tips from non-swelling plants from stage A (lanes 1). In non-swelling stolon tips from stage B, the expression level increased dramatically (29 times for *TUB8* (Fig. 3A) and 25 times for *TUB13* (Fig. 3B). *TUB13* expression was maximal in non-swelling stolon tips from this stage (Fig. 3B, lane 2) that is in plants that contained a mixture of swelling and non-swelling stolon tips. The transcript level of *TUB13* then declined as the tuber increased in size, the level falling to 8% of the maximal level in tubers of 25 g fresh weight (Fig. 3B, lane 5). In contrast, *TUB8* transcript level remained high as the tuber developed to 25 g fresh weight (Fig. 3A, lanes 3–5).

Filters were stripped and hybridised with a potato 25S ribosomal RNA probe. An equivalent hybridisation signal was detected for RNA extracted from tissue at all stages. Fig. 3C shows the data for the filter used in Fig. 3A (the *TUB8* blot). Similar data were obtained for the *TUB13* and patatin blots (not shown).

Expression of TUB8 *and* TUB13 *in other parts of the potato plant*

Northern analysis of RNA extracted from leaves, roots and stems of stage A and stage C plants was also carried out using the labelled inserts of *pTUB8* and *pTUB13* as probes (Fig. 4). *TUB8* was highly expressed in green internodal stems of plants harvested at stage A (Fig. 4A, lane 3) but was expressed at very low relative levels in leaves and roots (Fig. 4A, lanes 1 and 2). In the tuberising plant (stage C), however, a large induction (ca. 50-fold) was observed in the transcript level in roots and leaves (Fig. 4A, lanes 4 and 5). The high expression level observed in green stems was maintained in the tuberised plant (Fig. 4A, lane 6). In contrast, in plants harvested at stage A, significant transcript levels of *TUB13* were observed in leaves, stems and roots (ca. 40%, 32% and 36% respectively of the maximal stolon

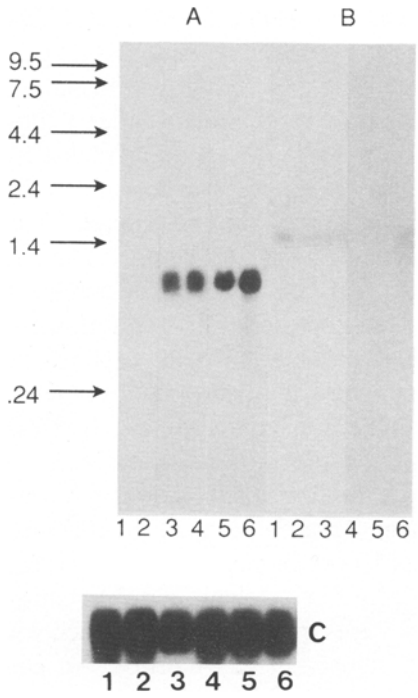

Fig. 4. Northern analysis of *TUB8* and *TUB13* in leaves, stems and roots. A. Hybridisation of *TUB8* to 15 μg total RNA extracted from leaf, root and stem (lanes 1–3 respectively) from plants harvested 21 days after planting (non-tuberising), lanes 4, 5, 6 RNA extracted from leaf, root and stem respectively of plants harvested 42 days after planting (tuberising). B. Hybridisation of *TUB13* to 15 μg total RNA extracted from leaf, stem, root (lanes 1–3 respectively) from plants harvested 21 days after planting and 42 days after planting (lanes 4–6 respectively). C. Hybridisation of 25S RNA probe to stripped filter used for the *TUB8* blot shown in Fig. 4A.

tip level (Fig. 4B, lanes 1–3). In the tuberising plants (stage C) these levels fell to 25% of the maximal stolon tip level in roots (lane 6) and 5% in leaves and stems (lanes 4 and 5). Again the filters were stripped and hybridised with a potato ribosomal probe in order to confirm equal loading of lanes (Fig. 4C).

Sequence analysis of TUB8 *and* TUB13

The sequences of the cDNA clones of *TUB8* and *TUB13* are shown in Fig. 5A and C respectively. The size of the insert in *pTUB8* is 823 bp. A potential long open reading frame (ORF) from the nucleotide position 2 to the TAA stop codon at nucleotide 634 was identified (Fig. 5A). There is a methionine at position 23 of the deduced amino acid sequence for this ORF but it is not known if this corresponds to the translational initiation point because the ORF extends to the 5′ end of the insert. The size of the transcript was estimated to be 1000 to 1100 bases from Fig. 3A, by comparison with the RNA size markers. Allowing for a poly(A)$^+$ tail, there cannot be more than 100–200 nt missing from the 5′ end of the insert in *pTUB8* and the entire coding region may well be present. There is a typical eukaryotic polyadenylation signal AATAAA in the 3′ untranslated sequence (underlined in Fig. 5A)

The deduced amino acid sequence of the *TUB8* ORF was compared with known sequences using the Genetic Computer Group programs for the VAX [3]. *TUB8* did not show significant homology with any known sequences in the databases (EMBL, GenBank); however the deduced amino acid sequence does have a number of interesting features. There are several repeating motifs in the central region of the sequence (between residues 57 and 173). There are three repeats of the hexapeptide EEPAAA and altogether, within this part of the polypeptide, a three alanine residue motif is repeated 7 times (underlined in Fig. 5A). These repeats are interspersed with 6 pairs of glutamate residues and the sequence contains 11 pairs of glutamate residues in all (underlined in Fig. 5A). The position of the alanine and glutamate motifs are shown in Fig. 5C. Five of the three alanine repeat motifs are either followed or preceded directly by a proline residue, the central region being high in proline content.

The size of the insert in *pTUB13* is 1594 bp. The largest ORF runs from the methionine initiation codon at position 261 to the TAG termination codon at nucleotide 1341. This ORF encodes a polypeptide of 360 amino acid residues (Fig. 5C). A polyadenylation signal, AATAAA, is present in the 3′ untranslated sequence (underlined in Fig. 5C). The sequence of the *TUB13* cDNA clone is homologous to the SAMDC genes in man, rat and yeast with the highest homology to the human amino acid sequence. There is 53%

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   1  TTTTGCCCCTTCCATTTCTTCATTTATTTTCCTCTTTCCCATTTTCTTATTTTTCAACTT   60
      ---------+---------+---------+---------+---------+---------+
   1   F  A  P  S  I  S  S  F  I  F  L  F  P  I  F  L  F  F  N  F    20
  61  TATTCCAATGGCCAGTGTTGAGGTTGAATCTGCACCAGTAGCAGCAGTAGAGACTACTAC   120
      ---------+---------+---------+---------+---------+---------+
  21   I  P  M  A  S  V  E  V  E  S  A  P  V  A  A  V  E  T  T  T    40
 121  TCCAGCTGAGGTTGAGGCTACCCCAGCTCCAGAGGTAACCAAGGTGGAGGAACCAGCCCC   180
      ---------+---------+---------+---------+---------+---------+
  41   P  A  E  V  E  A  T  P  A  P  E  V  T  K  V  E  E  P  A  P    60
 181  AGTTGTAGAAAAGGAAGTCGAGGTCGAATCAGCACCAGCACCAGTAGAAGAAGAAGCTGC   240
      ---------+---------+---------+---------+---------+---------+
  61   V  V  E  K  E  V  E  V  E  S  A  P  A  P  V  E  E  E  A  A    80
 241  TCCTGTTGCGGAGGAAGCAGCAGCCCTTGTAGCTGAAGAGCCTGCAGCTGCAGAACCTAC   300
      ---------+---------+---------+---------+---------+---------+
  81   P  V  A  E  E  A  A  A  L  V  A  E  E  P  A  A  A  E  P  T    100
 301  AGCCGCAGTGGCAGCTGCAGTAGAACCTGTTGCAGCCCCAGTTGAGGAACCTGCGGCTGC   360
      ---------+---------+---------+---------+---------+---------+
 101   A  A  V  A  A  A  V  E  P  V  A  A  P  V  E  E  P  A  A  A    120
 361  AGAAGAGCCTGCAGCCGCAGAGGAACCGGTGGCTGCAGCACCCGTTGAGGAGGCTGCAGC   420
      ---------+---------+---------+---------+---------+---------+
 121   E  E  P  A  A  A  E  E  P  V  A  A  A  P  V  E  E  A  A  A    140
 421  ACCCAAAGCTGAACCAGAAGAAGCCCCAGTTTCTGAACCAGAAGCAGAGAAAGCAGAGGA   480
      ---------+---------+---------+---------+---------+---------+
 141   P  K  A  E  P  E  E  A  P  V  S  E  P  E  A  E  K  A  E  E    160
 481  AGCTTCCCCTGTATCTGAGGAACCAGAGAAAGTTGAAGAGATAATTCAATGGTTGATGAA   540
      ---------+---------+---------+---------+---------+---------+
 161   A  S  P  V  S  E  E  P  E  K  V  E  E  I  I  Q  W  L  M  N    180
 541  TGATGGTTTTTTTTTTATTATTGAAGTTTGGTTGTGTTTATTTTCCTTCTTAGTTGAGTA   600
      ---------+---------+---------+---------+---------+---------+
 181   D  G  F  F  F  I  I  E  V  W  L  C  L  F  S  F  L  V  E  Y    200
 601  TTTTATTTACTACTATTATTACTATATTTTAATATAATATGGTTACATAGAGGGCATATG   660
      ---------+---------+---------+---------+---------+---------+
 201   F  I  Y  Y  Y  Y  Y  Y  I  L  I  *                           220
 661  TTAGCATTAACATATGTCTAGGTCTAGGTGGTCGGGTGGGATATTTTACACAGTTTTTTT   720
      ---------+---------+---------+---------+---------+---------+
 721  AAAGCTATGTTTGTTATCTTGTCCAGGGTTCAGTTTGTCATGGTTTGATTAAAGATGATA   780
      ---------+---------+---------+---------+---------+---------+
 781  TGAGATTTGATCTCTAGTTATGATAATAATAATAAAGGGGTAA  823
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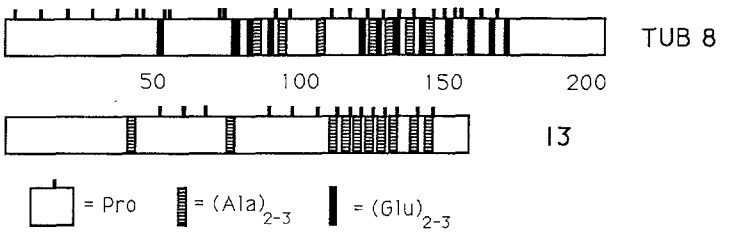
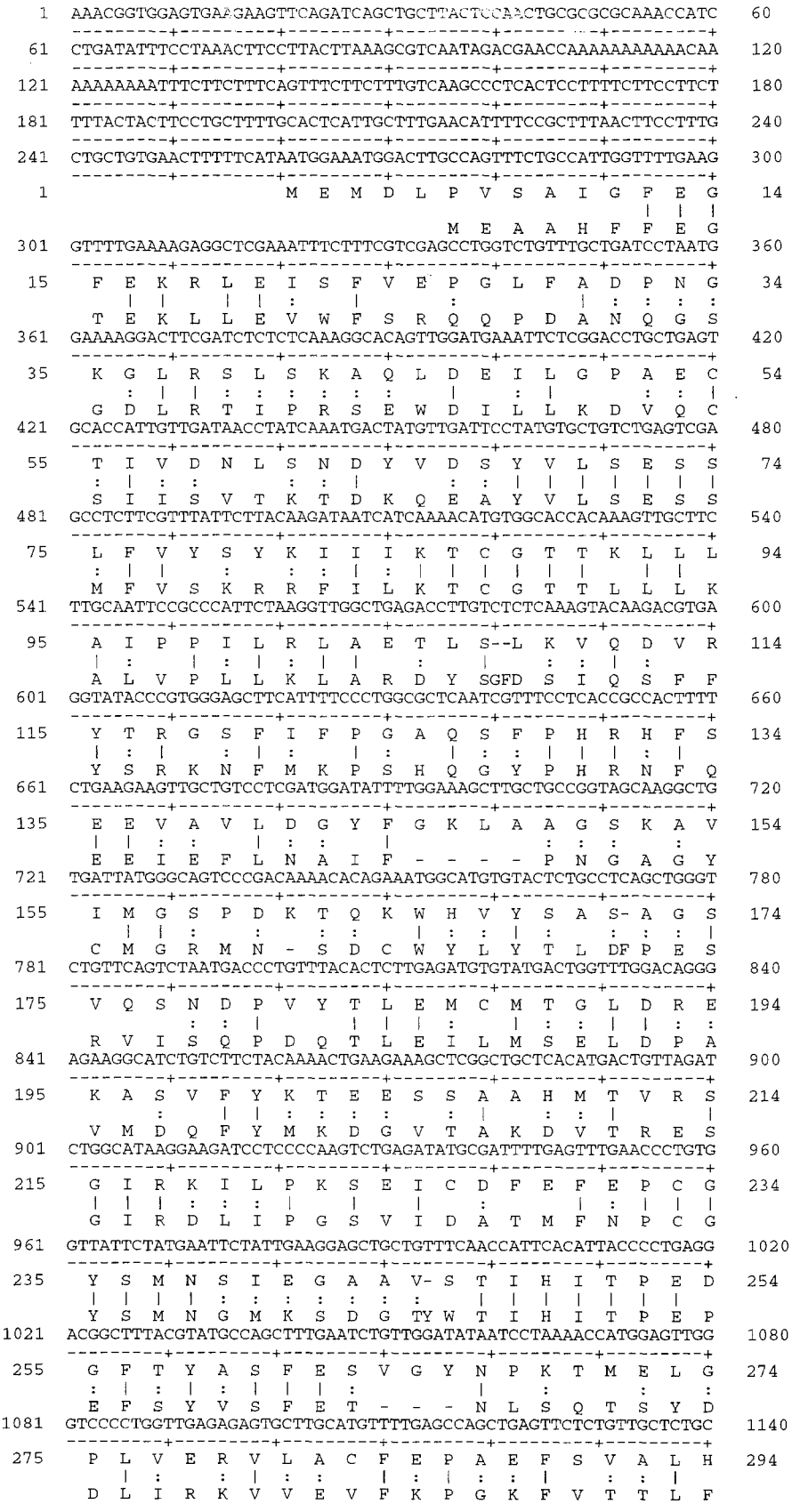


Fig. 5. Nucleotide and deduced amino acid sequences of the cDNA clones of *TUB8* (Fig. 5A) and *TUB13* (Fig. 5C). In Fig. 5A (top) repetitive elements in the deduced amino acid sequence are underlined. Fig. 5B (bottom) highlights the repetitive elements in the deduced amino acid sequence of *TUB8* and for comparison shows the location of an AAA motif in the I3 gene from *Brassica napus*. A global alignment of the deduced amino acid sequence of *TUB13* with the human *S*-adenosylmethionine decarboxylase sequence is shown (Fig. 5C, next pages). Putative polyadenylation signals in *TUB8* and *TUB13* are underlined.

similarity and 34% identity between the two sequences, with homology extending throughout a 330 amino acid overlap (Fig. 5B). We conclude that the *TUB13*-specific mRNA encodes a potato SAMDC.



Southern analysis

Southern blots of genomic DNA from *Solanum tuberosum* cv. Record (a cultivated tetraploid), *Solanum brevidens* (a wild-type, non-tuberising diploid) and from the related diploid solanaceous species, *Lycospercicum esculentum* (tomato), were probed with *TUB8* and *TUB13* (data not shown). With both these probes the greatest number of hybridising bands was observed with the tetraploid cultivar Record. Strongly hybridising bands were also detected with *S. brevidens* and tomato DNA. Therefore, *TUB8* and *TUB13* or homologous sequences are not confined to tuberising plants. Both *S. brevidens* and *L. esculentum* are diploid and so the reduced number of hybridising bands is not surprising.

Discussion

We have used differential screening to isolate cDNA clones which show greatly enhanced expression in stolon tips in the very early stages of tuberisation. Both of the differentially expressed genes described here were also expressed in plant organs other than the stolon (see Results). The expression patterns in these other tissues also changed on tuberisation although the patterns were not the same for *TUB8* and *TUB13*. This shows that tuber induction is coincident with (and may perhaps cause) changes throughout the whole plant. It also suggests that the function(s) of these genes are not necessarily exclusive to tuberisation. It seems likely that during tuberisation genes with a wide variety of functions are recruited. This may be necessary in order to bring about the complex processes of development associated with tuberisation.

To understand more fully the factors which influence the expression of *TUB8* and *TUB13* it will be necessary to analyse their promoters. *Cis*-regulatory elements in the patatin [17] and protease inhibitor II [13, 14] promoters have been identified. It will be interesting to see if there are similar elements in the promoters of the *TUB8* and *TUB13* genes.

To assign possible functions to *TUB8* and *TUB13* the DNA and deduced amino acid sequences of these cDNA clones have been compared to sequences of known function in databases. *TUB13* is probably the SAMDC gene, on the basis of its homology with the human [23], rat [26] and yeast [12] genes. This is the first plant SAMDC gene to be sequenced. SAMDC is an enzyme involved in polyamine biosynthesis. Decarboxylated *S*-adenosylmethionine provides the propylamino moiety for the conversion of putrescine to spermidine [23]. Polyamines are known to stimulate plant growth and enhanced polyamine biosynthesis often occurs concurrently

with growth [5]. Although polyamine levels in stolon tips of potato during tuberisation have not been measured, rapid increases in polyamine levels and in the associated biosynthetic enzymes have been observed on tuberisation of *Helianthus* [2]. Similarly, there is an increase in polyamine levels and in related biosynthetic enzymes during dormancy break in sprouting potato tubers [12]. Therefore it is reasonable to propose that the SAMDC gene is transcriptionally induced in the early stages of tuberisation to provide an impetus to the rapid growth required at this time.

Although *TUB8* did not show significant homology with any sequences in the databases (EMBL, GenBank) its deduced amino acid sequence does contain several repetitive units rich in alanine, glutamate and proline. A micro-spore specific gene (I3) isolated from anthers of *Brassica napus* shares some similar features with *TUB8*. In particular I3 has eight repeats of three alanine residues followed directly by a proline [27]. This is similar to the seven, three alanine repeats present in *TUB8*, five of which are closely associated with proline residues (see Fig. 5B). It has been suggested that the I3 gene may have a role in the cell wall architecture [27] because cell wall structural genes often contain repeated elements in their sequence and are proline-rich. On this basis, we suggest tentatively that *TUB8* may also encode a structural element in the cell wall. Altered expression of such genes would certainly be expected with the changes in cell size and shape that occur during tuberisation.

It will be interesting to determine the role(s) played by these two genes, and the others we have isolated, in the process of plant development in general and tuberisation in particular. This will require a concerted approach to determine both their exact expression profile in the different cell types of developing organs and the effects of inhibiting their synthesis on this process.

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