
The two classes of genes for the major potato tuber protein, patatin, are differentially expressed in tubers and roots

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ABSTRACT

The major potato tuber protein, patatin, is a family of 40kd glycoproteins that constitutes forty per cent of the soluble protein in tubers but is generally undetectable in other tissues. Fused rocket immunoelectrophoresis was used to detect in roots patatin that is immunologically different from tuber patatin. Western blots of SDS-polyacrylamide gels show root patatin to have a different molecular weight distribution than tuber patatin isoforms, but immunoprecipitation of in vitro translation products shows the patatin precursors to be of similar molecular weight in both tissues. This suggests that post-translational processing may differ in tubers and roots. Northern blots show that tuber and root patatin mRNAs are of similar size, but tuber transcripts are about 100-fold more abundant. 5' S1 nuclease and primer extension mapping suggests the class of patatin transcripts expressed in roots (class II transcripts) to be a subset of patatin transcripts expressed in tubers (classes I and II). Class II patatin mRNAs differ from class I transcripts by the presence of a 22 nucleotide insertion just upstream of the initiation codon. These data demonstrate that expression of the patatin multigene family is differentially regulated in tubers and roots.

INTRODUCTION

Patatin is a family of glycoproteins that constitutes approximately 40% of the soluble protein in potato tubers. This protein family can be purified by DEAE and Con A chromatography (1) and has a molecular weight of about 40,000. Patatin has been detected in tubers of all cultivated varieties that have been examined. Though patatin isoforms are heterogeneous within and between varieties as judged by SDS-polyacrylamide gel electrophoresis and isoelectric focusing, all tuber isoforms appear immunologically identical (2). Patatin has generally only been detected in tubers, but can be induced to accumulate elsewhere. In stem cuttings from which the axillary bud has been removed, patatin accumulates in petioles to high levels, accompanied by the accumulation of other tuber proteins and massive amounts of starch (3). The patatin that accumulates in petioles resembles tuber patatin by SDS-PAGE and is immunologically identical to tuber patatin.

The physiological role of patatin remains unknown, but the evidence suggests that as a dimer patatin is a potent lipid acyl hydrolase (4). Lipid acyl hydrolase activity could potentially result in the production of cytotoxic compounds and free radicals and thereby play a role in defense against microbial infection. Another possibility is that as a putative lipid acyl hydrolase, patatin may be involved in the metabolic shift from carbohydrate to lipid-based respiration following wounding (5). However, the correlation between starch accumulation and patatin accumulation in tubers and in petioles of the stem cutting system (3) remains intriguing, though possibly coincidental.

Three essentially full-length patatin cDNA clones have been sequenced (6,7) from two different varieties of potato. The cDNAs are highly homologous (greater than 95%) and encode proteins of 363 amino acids plus an additional 23 amino acid signal sequence (6). Using cDNA probes a number of genomic clones have been isolated and sequenced (7,8,9), none of which have thus far matched the sequence of any cDNAs that have been characterized. The four patatin genes that have been described are 4-5kb in length and are interrupted by six introns. Based on sequence comparison of the 5' untranslated regions of the clones, the known patatin genes can be separated into two types: one type resembles the full-length cDNA pGM01 (6), the other type is characterized by the presence of a 22 nucleotide insertion relative to the cDNA just upstream of the initiation codon (7,8). Recent work in this laboratory to characterize more than twenty patatin genomic clones shows that approximately one-half the clones are of each type (Mignery *et al.*, in preparation).

In this paper we report the detection in roots of patatin that is immunologically different from tuber patatin as demonstrated by fused rocket immunoelectrophoresis and western blotting. We characterize the root patatin mRNA by northern blot, S1, and primer extension analysis and demonstrate the existence of tuber-specific (class I) patatin transcripts and a class of patatin transcripts common to both tubers and root (class II). Finally, we show that class II transcripts contain the 22 nucleotide insertion in the 5' untranslated leader sequence that is characteristic of several completely sequenced genomic clones. This is the first evidence of differential organ-specific gene expression among members of the patatin multigene family.

MATERIALS AND METHODS

Plant Material

Tubers were harvested from field-grown *Solanum tuberosum* L. cv. Superior. Roots were harvested from field-grown plants or plantlets propagated in vitro.

Protein and RNA Extraction

Protein was extracted as described (10) in cold 25mM sodium phosphate (pH 7.0), 0.1% (w/v) sodium bisulfite, 0.1% (w/v) diethyldithiocarbamate, 0.1% (w/v) polyvinylpyrrolidone. Protein concentration was determined (11) after clearing homogenates of cell debris by centrifugation. Root RNA was extracted by homogenization in 4M guanidinium thiocyanate, 0.1M 2-mercaptoethanol (12). Tuber RNA preparation, and poly A+ RNA isolation by chromatography on oligo d(T) cellulose (Collaborative Research) was as described previously (6).

Protein Analysis

Fused rocket immunoelectrophoresis (13) was performed in 1mm thick 1% agarose horizontal gels containing patatin antiserum diluted 1:150 (3). SDS-PAGE in 12.5% slab gels was according to Laemmli (14). For western blotting (15), horseradish peroxidase conjugated goat anti-rabbit (Bio-Rad) was used as the second antibody.

Affinity Purification of Patatin Antibody

Approximately 7mg of SDS-PAGE purified tuber patatin was coupled to CNBr-activated Sepharose 4B using conditions recommended by the supplier (Pharmacia). Patatin antiserum (3) was diluted 1:10 in PBS (136mM NaCl, 1.5mM KH_2PO_4 , 8.1mM Na_2HPO_4 , 2.7mM KCl, pH 7.4) and passed over the column twice. The column was washed with ten column-volumes of PBS, and bound patatin antibody was eluted with 3M ammonium thiocyanate. Fractions (0.5ml) were collected and tested for the NH_4SCN by red color reaction with 1% $\text{Fe}(\text{NH}_4)(\text{SO}_4)$. Two column volumes were collected after first color reaction, and the antibody was separated from NH_4SCN by chromatography on Sephadex G-25 (Pharmacia). Fractions showing high absorbance at 280nm were pooled and used for western blot detection of patatin.

In Vitro Translation

Root and tuber poly A+ RNA were translated in a wheat-germ extract system (Amersham) using ^{35}S -Methionine (800 Ci/mmol, Amersham). Immunoprecipitation of patatin was as described (6).

Preparation of Probes

Isolation of the pGM01 222bp Taq 1 restriction fragment, 5' end-labelling with T4 Kinase (BRL), and nick-translation of cDNA insert was accomplished using standard techniques (16). The specific activity of end-labelled probes was about 5×10^6 c.p.m./microgram, nick-translated probes were about 10^8 c.p.m./microgram.

Analysis of RNA

RNA was size fractionated on 1.4% agarose gels containing methylmercuric

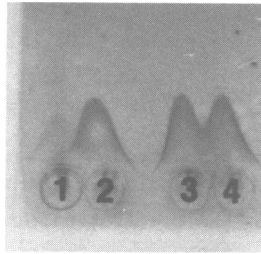


Figure 1. Fused rocket immunoelectrophoresis of root and tuber protein from the potato cultivar Superior. Two micrograms of root protein was loaded in the first well. 0.6 micrograms of tuber protein was loaded in wells 2, 3, and 4. The volume loaded in each well was 15 microliters. Samples were allowed to diffuse 30 minutes then were subjected to electrophoresis into 1% agarose containing polyclonal antibody raised against tuber patatin.

hydroxide as the denaturant (17) and transferred to nitrocellulose (Schleicher and Schuell). Northern blot hybridization was by standard methods (16). S1 mapping was essentially according to Berk and Sharp (18). Primer extension (19) was with 5' end-labelled synthetic oligodeoxynucleotides and was done as follows: RNA and labelled primer were mixed and the volume adjusted to 8 microliters with H₂O. Two microliters of 0.5M NaCl, 0.1M Tris pH 8.0, 0.5mM EDTA was added, and the mixture incubated at 55C for 10 minutes, then placed on ice. Ten microliters of chilled reaction buffer (80mM Tris pH 7.0, 10mM MgCl₂, 4mM DTT, 0.4mM each dNTP) containing 50 units Murine Moloney Leukemia Virus (M-MLV) reverse transcriptase was then added, and the reaction incubated at 37C, 30 minutes. Nucleic acid was precipitated with ethanol, and resuspended in sequencing sample buffer containing 20mM NaOH prior to electrophoresis on 8% sequencing gels.

RESULTS

Fused rocket immunoelectrophoresis of tuber and root patatin into agarose containing polyclonal antibody raised against purified tuber patatin shows that patatin in roots is immunologically different from tuber patatin (Figure 1). Unlike the tuber-tuber fused rocket, the root-tuber fusion does not form a continuous precipitin line when complexed with the antibody. Instead, the more intense precipitin line formed by the reaction of tuber patatin with the antibody crosses the fainter root-antibody precipitin line, indicating that the antibody population reacts with antigenic determinants present in tuber protein that are lacking in roots. We have observed the same phenomenon in

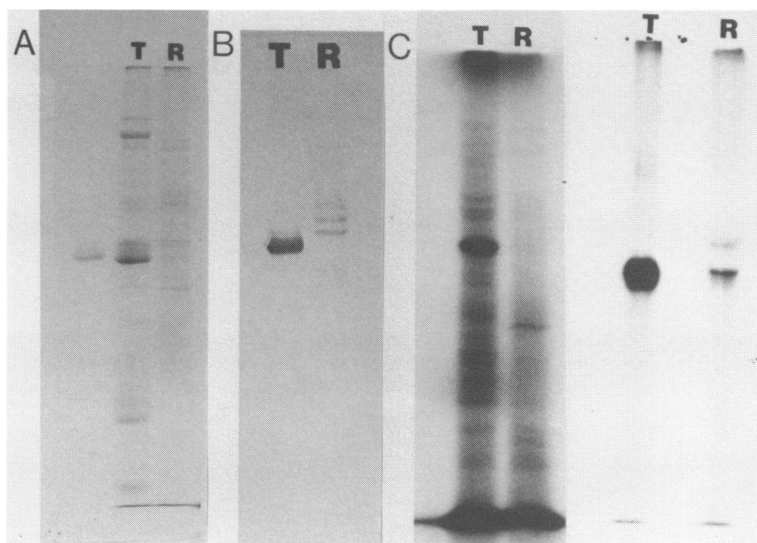


Figure 2. Electrophoretic analysis of protein from roots and tubers. (A) Coomassie stained gel of 10 micrograms of root and tuber protein resolved by SDS-PAGE compared to purified patatin. (B) Western blot of 6 micrograms of tuber protein and 25 micrograms of root protein resolved by SDS-PAGE. (C) Total *in vitro* translation products of tuber and root poly A+ RNA (left pair of lanes) and *in vitro* translation products immunoprecipitated with patatin antibody (right pair of lanes).

fused rockets of root and tuber protein extracts from the potato variety, Kennebec (not shown).

Patatin isoforms are the predominant tuber proteins seen in coomassie blue stained SDS-polyacrylamide gels, but are not evident in root extracts (Figure 2A). However western blotting reveals patatin in roots whose apparent molecular weight distribution is different from tuber patatin (Figure 2B). It is noteworthy that the antibody used for the western blot was raised against patatin purified by the method of Racusen and Foote (1) and was further purified by affinity chromatography over a column of SDS-PAGE purified tuber patatin linked to CNBr-activated Sepharose.

Examination of total *in vitro* translation products of poly A+ RNA shows patatin mRNA to be abundant in tubers but not in roots. However, patatin can be immunoprecipitated from the *in vitro* translation products of both tuber and root mRNA (Figure 2C). The major immunoprecipitated *in vitro* translation products have the same apparent molecular weight in both tubers and roots,

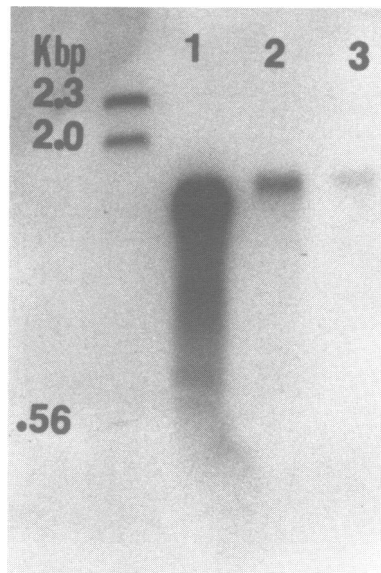


Figure 3. Northern blot of tuber and root RNA probed with nick-translated patatin cDNA insert. Lane 1, 0.1 microgram of tuber poly A+ RNA. Lane 2, 1.0 microgram of root poly A+ RNA. Lane 3, 6.7 micrograms of root total RNA. Sizes of DNA molecular weight markers are shown.

which is in contrast to the western blot results in which root patatin isoforms were of higher apparent molecular weight. One possible explanation for this discrepancy is that post-translational modifications such as signal peptide cleavage and glycosylation may differ for patatin synthesized in tubers and in roots. However, there is a minor immunoprecipitable *in vitro* translation product of higher molecular weight in roots. This suggests that some patatin mRNAs in roots may have longer coding sequences than others. The appearance of this higher molecular weight band was variable among experiments, however, and may be artifactual. Western blots and immunoprecipitation results as shown in Figure 2 have also been obtained using protein and RNA from the cultivar Kennebec (not shown).

Northern blots of poly A+ RNA probed with nick-translated insert DNA of the full-length cDNA pGM01 show that patatin mRNA is about 1500 nucleotides long in both tubers and roots (Figure 3). An approximately ten-fold less intense hybridization signal is obtained using ten-fold more root poly A+ RNA than tuber RNA. Therefore we estimate that per microgram of RNA, patatin is about 100-fold less abundant in roots than in tubers.

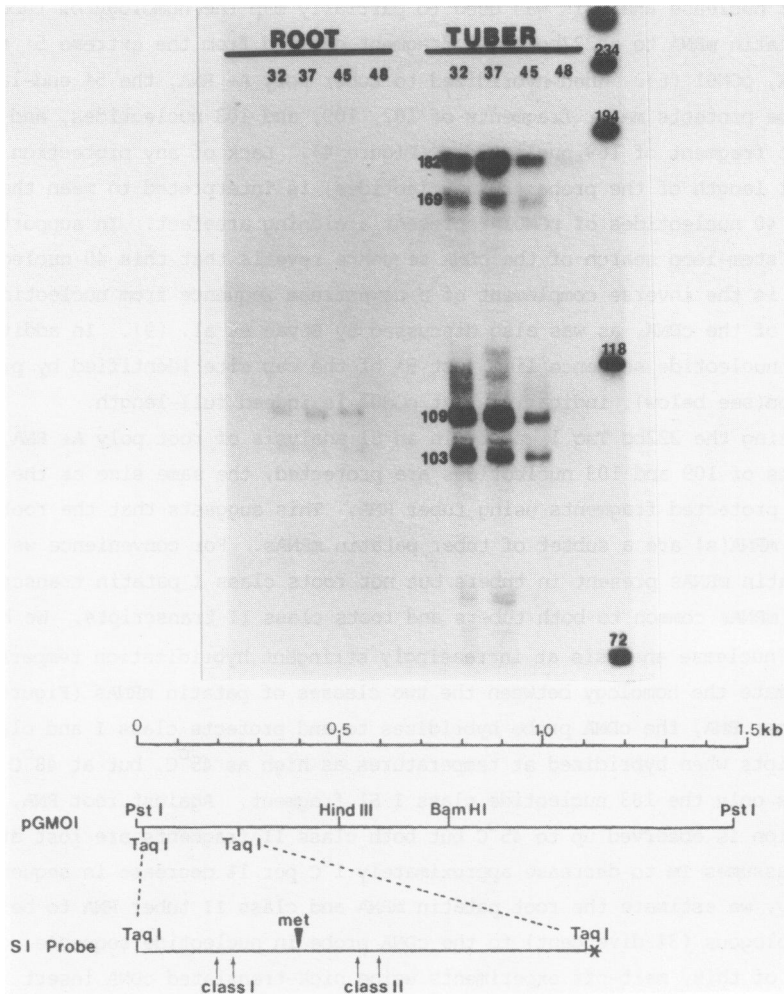


Figure 4. S1 nuclease protection patterns of tuber and root poly A+ RNA. Two micrograms of root poly A+ RNA or 0.2 micrograms of tuber poly A+ RNA were hybridized for two hours at 32, 37, 45, or 48°C to a 5' end-labelled 222bp Taq I fragment representing the extreme 5' end of the full-length cDNA pGM01. Hybrids were then treated with S1 Nuclease (200 Units/ml.) for 30 minutes, precipitated with ethanol and subjected to electrophoresis on an 8% sequencing gel. Sizes of molecular weight markers and protected fragments are shown. The diagram summarizes the data and shows a partial restriction map of the cDNA and the origin of the S1 probe. Arrows mark the positions corresponding to the endpoints of protected fragments. The position of the initiating methionine codon (met) is also shown.

S1 nuclease analysis was used to partially map the homology of tuber and root patatin mRNA to a 222bp Taq 1 fragment derived from the extreme 5' end of the cDNA, pGM01 (6). When hybridized to tuber poly A+ RNA, the 5' end-labelled Taq probe protects major fragments of 182, 109, and 103 nucleotides, and a less abundant fragment of 169 nucleotides (Figure 4). Lack of any protection over the full length of the probe (222 nucleotides) is interpreted to mean that the 5'-most 40 nucleotides of pGM01 represent a cloning artefact. In support of this, a stem-loop search of the cDNA sequence reveals that this 40 nucleotide stretch is the inverse complement of a downstream sequence from nucleotides 186-226 of the cDNA, as was also discussed by Bevan *et al.* (9). In addition, this 40 nucleotide sequence lies just 5' of the cap site identified by primer extension(see below), indicating that pGM01 is indeed full-length.

Using the 222bp Taq 1 probe in an S1 analysis of root poly A+ RNA, fragments of 109 and 103 nucleotides are protected, the same size as the smaller protected fragments using tuber RNA. This suggests that the root patatin mRNA(s) are a subset of tuber patatin mRNAs. For convenience we term the patatin mRNAs present in tubers but not roots class I patatin transcripts, and the mRNAs common to both tubers and roots class II transcripts. We have used S1 nuclease analysis at increasingly stringent hybridization temperatures to estimate the homology between the two classes of patatin mRNAs (Figure 4). With tuber RNA, the cDNA probe hybridizes to and protects class I and class II transcripts when hybridized at temperatures as high as 45°C, but at 48°C protects only the 183 nucleotide class I S1 fragment. Against root RNA, protection is observed up to 45°C but both class II fragments are lost at 48°C. If one assumes T_m to decrease approximately 1°C per 1% decrease in sequence homology, we estimate the root patatin mRNA and class II tuber RNA to be about 97% homologous (3% divergent) to the cDNA probe in nucleotide sequence. In support of this, melt-off experiments using nick-translated cDNA insert hybridized to tuber and root RNA dot-blot show a 2-3°C difference in T_m . This also suggests approximately 97% homology between the two classes (Hannapel and Park, unpublished observations).

Primer extension of tuber poly A+ RNA using a 21 nucleotide synthetic oligodeoxynucleotide complementary to a sequence in the middle of exon 1 yields a major product of about 158 nucleotides and minor products of 154 and 180 nucleotides (Figure 5). Extension of the same primer hybridized to root poly A+ RNA gives only the 180 nucleotide product. As with the S1 nuclease experiments, primer extension demonstrates the existence of class I transcripts that are present in tubers but not roots (the 154 and 158 nucleotides extension products) and class II transcripts present in both tubers and roots (the 180

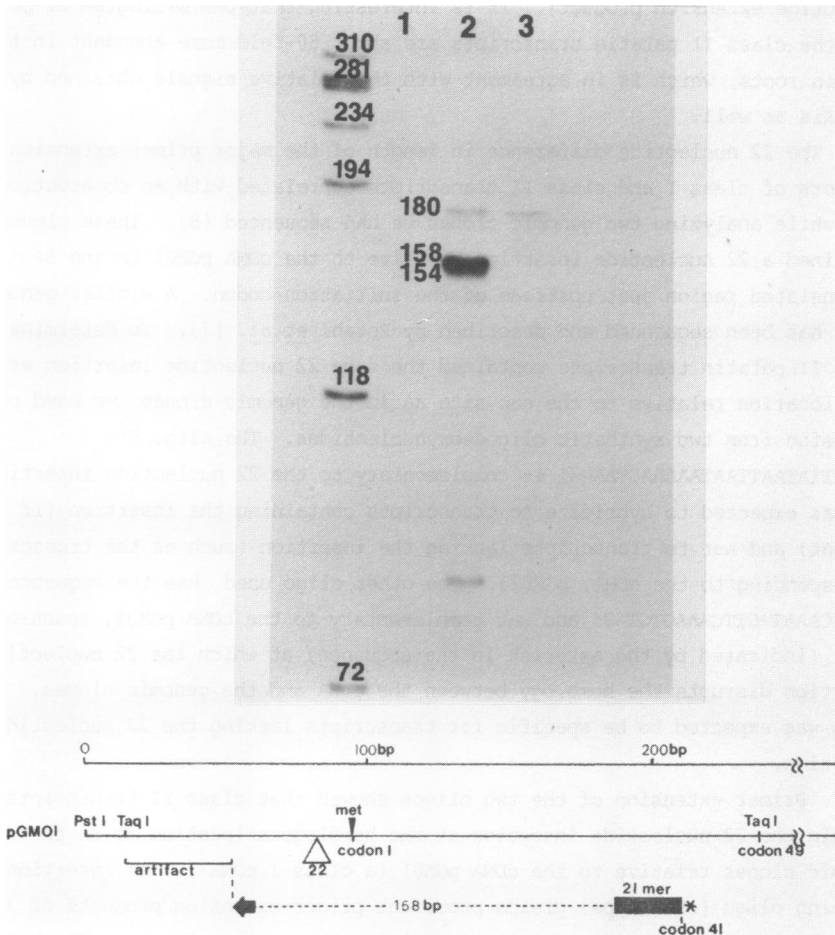


Figure 5. Mapping tuber and root patatin mRNA 5' ends by primer extension. Approximately 50,000 cpm of primer was hybridized to 0.2 micrograms of tuber poly A+ RNA (lane 2) or 2.0 micrograms of root poly A+ RNA (lane 3) and extended with reverse transcriptase. Lane 1 is a control of probe only (no RNA) treated like the other samples. The sizes of molecular weight markers and extension products are shown. The unlabelled, faster migrating bands in lanes 2 and 3 are apparently due to non-specific stops as they were variable between experiments. The diagram shows an expanded map of the 5' end of pGM01 and the relative positions of the 5' end-labelled oligonucleotide primer, initiation codon, and 22 nucleotide insertion (open triangle) present in a number of genomic clones. From the nucleotide complementary to the labelled end of the primer to the 3' boundary of the artifactual sequence in the cDNA is 158 nucleotides. For clones containing the insertion, this distance is 180 nucleotides.

nucleotide extension product). It is interesting that per microgram of poly A+ RNA, the class II patatin transcripts are about 50-fold more abundant in tubers than in roots, which is in agreement with the relative signals obtained by S1 analysis as well.

The 22 nucleotide difference in length of the major primer extension products of class I and class II transcripts correlated with an observation we made while analyzing two genomic clones we had sequenced (8). These clones contained a 22 nucleotide insertion relative to the cDNA pGM01 in the 5' untranslated region just upstream of the initiation codon. A similar genomic clone has been sequenced and described by Rosahl *et al.* (7). To determine if class II patatin transcripts contained the same 22 nucleotide insertion at the same location relative to the cap site as in the genomic clones, we used primer extension from two synthetic oligodeoxynucleotides. The oligo 5'-GCAGATTATAATTAATAAAAACTAA-3' is complementary to the 22 nucleotide insertion and was expected to hybridize to transcripts containing the insertion (if present) and not to transcripts lacking the insertion (such as the transcript corresponding to the cDNA, pGM01). The other oligo used has the sequence 5'-TTTTGCAAAT*GTTCAAAGTGT-3' and was complementary to the cDNA pGM01, spanning the point (indicated by the asterisk in the sequence) at which the 22 nucleotide insertion disrupts the homology between the cDNA and the genomic clones. This oligo was expected to be specific for transcripts lacking the 22 nucleotide insertion.

Primer extension of the two oligos showed that class II transcripts contain the 22 nucleotide insertion at the homologous location as in the genomic clones relative to the cDNA pGM01 (a class I cDNA). The insertion spanning oligo (cDNA type) yields prominent primer extension products of 34 and 38 nucleotides if hybridized to tuber poly A+ RNA (Figure 6A, lane 2), but no extension products if hybridized to root poly A+ RNA (lane 3). The endpoints of these fragments correspond to the positions of the 154 and 158 nucleotide (class I) products extended from the mid exon 1 primer used for the data in Figure 5. Had the insertion spanning oligo also hybridized to class II transcripts, an extension product was expected with both tuber and root RNA. The reciprocal experiment using the oligo complementary to the 22 nucleotide insertion present in the genomic clones yields a primer extension product of 57 nucleotides with both tuber and root poly A+ RNA (Figure 6B) which maps to the same position as the 180 nucleotide (class II) primer extension product previously obtained with the mid exon 1 primer. Thus the insertion spanning oligo and the oligo corresponding to the 22 nucleotide insertion are specific for class I and class II patatin transcripts, respectively. Furthermore, the

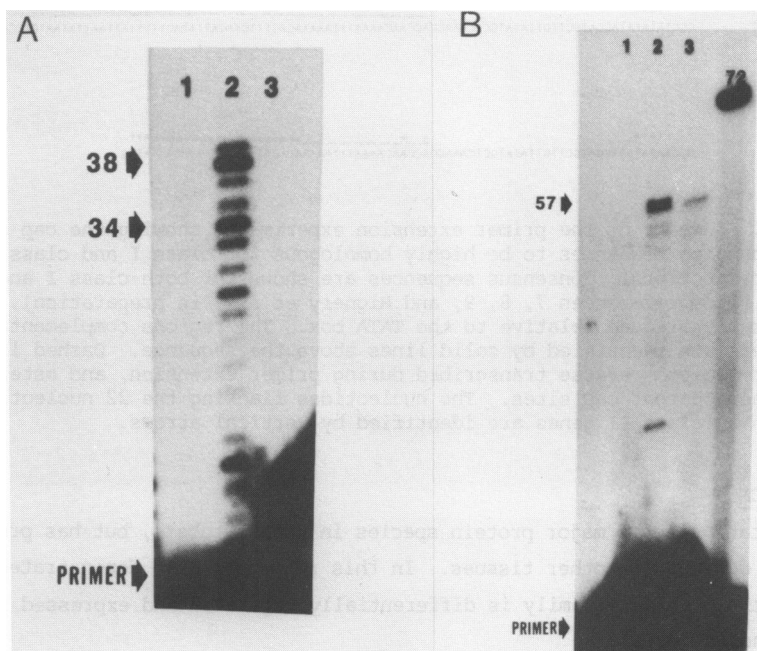


Figure 6. Discrimination between tuber-specific (class I) and class II patatin transcripts by primer extension. (A) Extension products of a primer complementary to the 5' untranslated sequence of the cDNA pGM01 just upstream of the initiation codon. (B) Extension products of a primer complementary to a 22 nucleotide insertion (relative to the cDNA) present in a number of genomic clones. (A and B) Lane 1, primer only, no RNA. Lane 2, 0.2 micrograms of tuber poly A⁺ RNA. Lane 3, 2.0 micrograms of root poly A⁺ RNA. Sizes of extension products are shown. The occurrence of faster migrating bands, appearing as a ladder in Figure 6A, lane 2, was variable between experiments, and are apparently non-specific stops.

use of these two oligos to map the 5' ends of patatin mRNAs suggests that the cap site is the same for both classes of transcripts (Figure 7), and is located 25 nucleotides downstream of the presumptive TATA box. Though we did not size the primer extension products beside a sequencing ladder, non-specific stops during primer extension yielded a ladder of one nucleotide increments culminating in strong signals corresponding to the mRNA 5' ends (see Figure 6). Therefore we feel the cap site assignments have been made accurately since the primer extension reactions provided an internal sizing ladder.

Based on the data presented above, we conclude that the genomic clone recently described by Bevan *et al.* (1986) is a class I gene, while the two apparent pseudogenes described by Pikaard *et al.* (1986) and the clone described by Rosahl *et al.* (1986) are class II patatin genes.

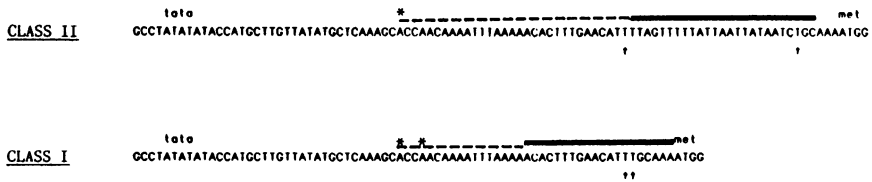


Figure 7. Summary of the primer extension experiments showing the cap site and surrounding sequences to be highly homologous for class I and class II patatin transcripts. Consensus sequences are shown for both class I and class II genes (from references 7, 8, 9, and Mignery *et al.*, in preparation), and the sequences are aligned relative to the TATA box. The regions complementary to the primers are identified by solid lines above the sequence. Dashed lines represent regions reverse transcribed during primer extension, and asterisks denote the apparent cap sites. The nucleotides flanking the 22 nucleotide insertion in class II genes are identified by vertical arrows.

DISCUSSION

Patatin is the major protein species in potato tubers, but has previously not been detected in other tissues. In this report we have demonstrated that the patatin multigene family is differentially regulated and expressed in tubers and in roots.

It is curious that previously published studies have failed to detect patatin in roots. Paiva *et al.* (3), using the cultivar "Superior", did not detect significant amounts of patatin in roots, leaves, or stems by ELISA (enzyme-linked immunosorbent assay). However, we have used the identical antibody preparation and the same potato cultivar in the present study. One possible explanation for this discrepancy stems from our experience with western blots. Using Paiva's antibody raised against tuber patatin purified by the method of Racusen and Foote (1), we found significant background staining of polypeptide bands over the entire length of the lanes on the western blots. This background non-specific staining was eliminated by purifying the antibody further by affinity chromatography over a column of CNBr-linked SDS-PAGE purified tuber patatin (see Figure 2B). We estimate that patatin purified according to Racusen and Foote is about 99% pure by SDS-PAGE and western blotting, and the antibody raised against this protein preparation is probably 99% anti-patatin. However, the remaining 1% impurity made detection of patatin in roots difficult to distinguish above background on western blots. Therefore it is possible that ELISA was similarly unable to distinguish patatin levels in roots significantly above background in the study of Paiva *et al.* Compounding the problem of detection by ELISA is the fact that root and tuber patatin do not react the same with antibody raised against tuber patatin (see fused-

rocket, Figure 1 of this paper). In a recent study, Rosahl *et al.* (20) did not detect patatin by nucleic acid hybridization techniques in roots, stems, or leaves in the potato genotypes they studied. It is possible that genotypic differences, as well as environmental factors, may influence the level of patatin gene expression in roots. We have obtained the same qualitative results presented in this paper with the cultivars "Superior" and "Kennebec" grown in the field or as micropropagated plantlets *in vitro*. However, the quantitative levels of patatin in roots does seem to vary during the growing season in these and several other cultivars (Hannapel and Park, in preparation).

Previous work in this laboratory demonstrated that patatin could be induced to accumulate in petioles (where it is not detectable under normal conditions) in stem cuttings from which the axillary bud had been removed (3). The patatin which accumulated was immunologically identical to tuber patatin, and showed the typical tuber patatin isoform pattern on coomassie-stained SDS-polyacrylamide gels. The level of patatin in these petioles was equivalent to the levels found in tubers on a per cent of fresh weight basis. In addition, primer extension of the mid-exon 1 oligo used in this report showed that both class I and class II transcripts are present in these petioles, as in tubers (Pikaard and Park, unpublished observations). Therefore the stem cutting system appears to be a valid system for the study of patatin gene expression as it occurs in tubers. Patatin in roots, however, is immunologically distinct from tuber patatin, has a different migration pattern on SDS-polyacrylamide gels, and is present only in trace amounts. In addition, we have shown in this paper that only one of the two patatin gene classes is expressed in roots (class II). Precise quantitation of root patatin protein levels is not possible by immunological methods such as rocket immunoelectrophoresis or ELISA since root and tuber patatin do not react identically with antibody raised against tuber patatin (Figure 1). However, the fact that the root patatin isoforms are not readily apparent in coomassie stained gels of root protein (Figure 2A) allows us to estimate that they are present, at most, at 50-100 fold lower levels than in tubers, or about 0.4-0.8% of the total protein.

Western blots of total protein extracted from tuber and root and subjected to SDS-PAGE show different patatin molecular weight distributions in the two organs (Figure 2B). However, the *in vitro* translation products of tuber and root poly A+ RNA immunoprecipitated by patatin antibody are of similar molecular weight (Figure 2C). These data suggest that post-translational modifications of patatin precursors may differ in tubers and roots. However,

it is possible that the polypeptide sequence of tuber and root patatins are different enough that identical post-translational mechanisms (such as glycosylation of the same amino acids) in both tissues turn out distinct products after processing.

S1 mapping of tuber and root poly A+ RNA using a probe derived from the extreme 5' end of the full-length cDNA pGM01 gave the first suggestion that the class of patatin transcripts expressed in roots (class II) was a subset of the patatin transcripts expressed in tubers (classes I and II). Based on hybridization stringency experiments coupled to S1 nuclease analysis (Figure 4), we estimate class I and class II patatin transcripts to be approximately 97% homologous in nucleotide sequence.

Primer extension analysis of mRNA from roots and tubers also showed the root (class II) transcripts to be an apparent subset of tuber patatin transcripts, in agreement with the S1 data. The 5' untranslated leader sequence of class II transcripts is 22 nucleotides longer than class I transcripts due to the presence of a 22 nucleotide insertion just upstream of the initiating methionine codon. This insertion is present in the same location relative to the cap site as in several genomic clones that have been described (7,8). As a result, the cap site and surrounding sequences between the TATA box and the insertion are highly homologous for both class I and Class II patatin genes.

Patatin mRNA is about the same size in tubers and roots as shown on the northern blot in Figure 3. We estimate that patatin mRNA, like patatin protein, is about 100 fold less abundant in roots than in tubers. An apparent discrepancy in the data concerns the relative abundance of class I and class II patatin mRNAs in tubers. Examination of the S1 data suggests that the two classes are approximately equal in abundance (Figure 4), whereas primer extension from the oligonucleotide complementary to the middle of exon 1 suggests that class I is more abundant. We feel that the S1 data is more reliable for quantitation since a longer probe is used, and would therefore be expected to be less sensitive to point mutations that could decrease the stability of the resulting hybrid. In contrast, the 21 nucleotide mid-exon 1 oligo used for primer extension (Figure 5) may be more homologous to class I than to class II RNAs which could skew the results due to hybrid stability. Another point in favor of the S1 data is that class II transcripts in roots appear to be approximately 50-fold less abundant than class II transcripts in tubers. If class II transcripts comprise about 50% of the total patatin mRNA present in tubers as suggested by S1 analysis, then the amount of patatin mRNA

in roots should be about 100-fold lower than the total amount in tubers. This is in agreement with the northern blot results.

Though the class II transcripts of both tubers and roots appear qualitatively the same by primer extension, S1 mapping, and hybridization stringency experiments, we have no direct evidence that the identical class II genes are expressed in both organs. Sequence comparison of class II clones isolated from both tuber and root cDNA libraries will be required to address this question. If indeed they turn out to be the same genes, it becomes of interest to understand the fifty-fold difference in relative class II transcript abundance in tubers and roots as well as the molecular basis for tuber-specific expression of class I patatin genes.

The physiological role of patatin remains unknown, but the occurrence of high levels of patatin has thus far been associated with the occurrence of massive amounts of starch, both in tubers, and in petioles of cultured stem cuttings (3). It will be of interest to determine if patatin in roots is predominantly localized in the region behind the root cap where starch granules accumulate.

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