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Isolation of a Polyubiquitin Promoter and Its Expression in Transgenic Potato Plants[1]

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A polyubiquitin clone (*ubi7*) was isolated from a potato (*Solanum tuberosum*) genomic library using a copy-specific probe from a stress-induced ubiquitin cDNA. The genomic clone contained a 569-bp intron immediately 5′ to the initiation codon for the first ubiquitin-coding unit. Two chimeric β-glucuronidase (GUS) fusion transgenes were introduced into potato. The first contained GUS fused to a 1156-bp promoter fragment containing only 5′ flanking and 5′ untranslated sequences from *ubi7*. The second transgene contained GUS translationally fused to the carboxy terminus of the first ubiquitin-coding unit and thus included the intron present in the 5′ untranslated region of the polyubiquitin gene. Both *ubi7*-GUS transgenes were activated by wounding in tuber tissue and in leaves by application of exogenous methyl jasmonate. They were also expressed constitutively in the potato tuber peel (outer 1–2 mm). Both transgenes were actively expressed in mature leaves. Exceptionally high levels of expression were observed in senescent leaves. Transgenic clones containing the *ubi7* intron and the first ubiquitin-coding unit showed GUS expression levels at least 10 times higher than clones containing GUS fused to the intronless promoter.

Ubiquitin is a small (76 amino acids), highly conserved protein found in all eukaryotes. The ubiquitin gene family contains two types of structures, polyubiquitin and ubiquitin extension protein genes. Polyubiquitin genes contain several tandem repeats of the ubiquitin-coding unit (Ozkaynak et al., 1987; Monia et al., 1990). The extension protein genes contain a single ubiquitin-coding unit fused in frame to the coding region for either of two small proteins associated with ribosomes (Finley et al., 1989; Callis et al., 1990; Garbarino and Belknap, 1994). Both types of genes are transcribed as gene fusions and translated as polyproteins (Ozkaynak et al., 1987). The polyproteins undergo rapid enzymatic hydrolysis during or soon after translation to give free ubiquitin or ubiquitin and ribosomal proteins. In plants, individual ubiquitin family members have been shown to be differentially regulated during development and by heat shock, wounding, and ethylene treatment (Gausing and Barkardottir, 1986; Garbarino et al., 1992; Genschik et al., 1992, 1994; Cornejo et al., 1993; Takimoto et al., 1994).

Several polyubiquitin genes have now been isolated from higher plants. These genes contain three to seven ubiquitin repeats. An intron is commonly found in the 5′ UTR of plant polyubiquitin genes (Binet et al., 1991; Christensen et al., 1992; Norris et al., 1993; Genschik et al., 1994; Rollfinke and Pfitzner, 1994; Callis et al., 1995). This intron has been implicated as a quantitative determinant of expression in transient assays in Arabidopsis (Norris et al., 1993). In contrast, in similar experiments using tobacco polyubiquitin constructs, deletion of the intron had no effect on expression levels (Genschik et al., 1994).

We previously reported that members of the potato (*Solanum tuberosum* L.) ubiquitin gene family are transcriptionally activated by stress in potato tubers (Rickey and Belknap, 1991). Accumulation of both ubiquitin extension protein and two polyubiquitin (*ubi7* and *ubi16*) mRNAs in tuber tissue is induced by injury and application of exogenous ethylene, but only the *ubi7* and *ubi16* polyubiquitin genes appear to be activated by heat shock (Garbarino et al., 1992). We report here the isolation of a genomic clone for one of the potato polyubiquitin genes, *ubi7*. Potato plants containing *ubi7*-GUS genes were constructed and the activity of the promoter was analyzed. The promoter retained the ability of the native gene to respond to wounding, whether or not the intron was included in the GUS construct. In addition, the promoter was active in the peel of the tuber, and very high levels of expression were observed in senescing leaves.

MATERIALS AND METHODS

Plant Materials

Potato (*Solanum tuberosum* L.) cv Lenape and cv Lemhi Russet were grown from tubers in pots in the greenhouse. For characterization of constitutive gene expression, tubers were dissected into three sections including the peel (outer 1–2 mm consisting of periderm and cortex tissue), vascular storage parenchyma (5 mm adjacent to the peel), and medullary tissue (internal core of the tuber). For determination of injury-induced gene expression in the tubers, whole tubers were cut into 1- to 4-mm cubes and either frozen

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Abbreviations: MeJas, methyl jasmonate; MU, 4-methylumbelliferone; UTR, untranslated region.

immediately in liquid nitrogen (control) or incubated in the dark in Petri dishes on filter paper moistened with water for the indicated times. For determination of injury- and phytohormone-induced gene expression in leaves, fully expanded leaflets were harvested and incubated on water in the dark. Multiple hemostat injuries were effected on the leaflets for the periods indicated prior to freezing of tissue. For treatment with MeJas (20 μM) and ABA (50 μM), excised leaves were incubated on solutions of phytohormone in the dark for 16 h.

Genomic Library Screening

The *ubi7* genomic sequence was cloned from a λFIXII potato genomic library (Garbarino and Belknap, 1994). The library was first prescreened by PCR using a *ubi7*-specific primer from the 5′ UTR (CTCAATTGCCTTCAAA) and a primer complementary to sequence near the 5′ end of the first ubiquitin-coding unit (GTGATAGTCTTTCCGGT) (Garbarino et al., 1992). The library was plated in 22 aliquots of approximately 0.5×10^6 plaque-forming units, each on an *Escherichia coli* lawn. A plug was taken from each of the 22 resulting plaques, and an eluant from 11 of them was subjected to PCR. The PCR product from this screening was expected to contain the intron typically present between the 5′ UTR and the beginning ubiquitin Met of plant polyubiquitin genes. The eluants from three plugs that gave distinct PCR bands were plated and probed with ubiquitin-coding region. The λFIXII genomic clone containing *ubi7* exhibited an unusual phenotype in that infected *E. coli* formed no visible plaques. The hybridization signals thus did not correspond to any plaques. Therefore, plugs were removed from the *E. coli* lawn in locations identified by the hybridization signal. The putative λ clones in these plugs were then rescreened by PCR with the primers given above, and a clone for *ubi7* was obtained.

To prepare λ DNA a plate "lysate" procedure was used (Sambrook et al., 1989). This lysate was prepared using a high titer of the bacteriophage, and although no plaques were visible, sufficient inoculum was used to result in a low-density *E. coli* lawn. The plate was eluted with SM buffer (100 mM NaCl, 10 mM $MgSO_4$, 50 mM Tris-Cl, pH 7.5) and 1 mL of the eluant was added to 250 mL of *E. coli* (LE392) that had been grown for 2 to 3 h to a density of approximately 0.35 A_{600}. The inoculated *E. coli* were grown overnight and then 2.5 mL of chloroform were added. λ DNA was isolated using standard techniques (Sambrook et al., 1989).

DNA Sequencing

A *Sac*I fragment of approximately 7.0 kb from the λ clone that hybridized to the ubiquitin-coding region probe was subcloned into pUC19. This subclone contained a partial polyubiquitin-coding sequence and a considerable upstream sequence. This fragment and further *Sal*I subclones were used for sequencing. Sequence analysis was carried out using Sequenase version 2.0 (United States Biochemical)[2] and the Promega fmol DNA Sequencing System. The *ubi7* promoter was characterized for similarity to other control regions by screening combined data bases (GenBank and EMBL) for related sequences using the BLAST network server of the National Center for Biotechnology Information (Altschul et al., 1990). Comparison of the *ubi7* genomic sequence to the tobacco polyubiquitin gene was carried out using MacVector (Eastman Kodak).

Construction of Chimeric Genes

Two chimeric genes were constructed. The first (*ubi7*-GUS, Fig. 1) contained 1156 bp upstream from the polyubiquitin intron fused to GUS. The promoter thus contained the 5′ UTR but not the intron of the *ubi7* gene. This promoter was obtained by synthesizing a primer (Fig. 1, primer 1) 1156 bp upstream from the intron and containing an added *Bam*HI site on its 5′ end (CCGGATCCAATATTTGAAATCTCA) and a primer complementary to sequence in the 5′ UTR of *ubi7* and also containing an added *Bam*HI site (CCGGATCCTTAAAGAGAAATTTG) (Fig. 1, primer 2) (Garbarino et al., 1992). These two primers were used for a PCR of the promoter from the original *Sac*I subclone. The promoter for the second chimeric transgene gene (Fig. 1, *ubi7*-GUS-fus) was similarly amplified using the same 5′ primer as the first and a 3′ primer complementary to the 3′ end of the first ubiquitin-coding region of the polyubiquitin (Fig. 1, primer 3); this primer also contained an added *Bam*HI site (CCGGATCCACCTCCACGTAGACGG) (Garbarino and Belknap, 1994). Thus, the second promoter contained the same upstream region as the first and in addition contained the *ubi7* intron and the first ubiquitin-coding region.

The PCR products were digested with *Bam*HI and ligated into the binary vector pCGN1547 (McBride and Summerfelt, 1990), into which the GUS (Jefferson et al., 1987) coding region and a nopaline synthase polyadenylation signal (Bevan et al., 1983) had previously been cloned. The orientation of the promoter was confirmed by PCR analysis. The promoter-GUS junctions were then sequenced using a primer complementary to the 5′ end of the GUS-coding region.

Plant Transformation

The chimeric ubiquitin-GUS transgenes were introduced into potato cv Lenape and cv Lemhi Russet using the *Agrobacterium tumefaciens* strain PC2760 (An et al., 1985) harboring disarmed Ti plasmid pAL4404 (Hoekema et al., 1983) and binary transformation vector pCGN1547 (McBride and Summerfelt, 1990). Potato transformation was carried out as described previously (Snyder and Belknap, 1993), except that the Suc concentration in stage I and stage II was reduced from 3 to 2%.

[2] References to a company and/or product by the USDA is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others that may also be suitable.

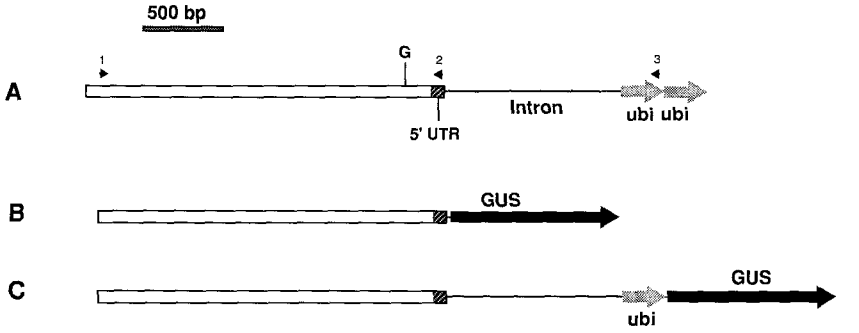

Figure 1. Structures of the endogenous potato *ubi7* gene, including the first two ubiquitin monomers and 5′ flanking sequence, and transgene constructs. A, Structure of the *ubi7* gene. Numbers (arrowheads) and black arrows indicate positions of PCR primers used in transgene construction; G indicates the position of the G box; the hatched area shows the position of the 5′ UTR; and the first two ubiquitin-coding regions are indicated by the shaded arrows. B, Structure of the *ubi7*-GUS transgene in which the GUS-coding region (black arrow) is fused directly to UTR of *ubi7*. C, Structure of the *ubi7*-GUS-fus transgene in which the GUS-coding region is introduced as a translational fusion to the first ubiquitin-coding region (ubi).

GUS Assays

Fluorescent GUS assays were performed essentially as described by Jefferson et al. (1987). Samples for GUS assays were frozen in liquid nitrogen and stored at −80°C. Approximately 0.2 to 0.5 g of tissue was ground in 300 μL of extraction buffer containing 50 mM phosphate buffer (pH 7.0), 10 mM EDTA, 0.1% Triton X-100, 3.6 mM Sarkosyl, and 0.07% β-mercaptoethanol. The sample was centrifuged and 1 to 4 μL of the supernatant were added to 300 μL of assay buffer containing 1 mM methyl umbelliferyl-β-D-glucuronide. An aliquot (1–10 μL) was withdrawn and added to 1 mL of 0.2 M sodium carbonate after 5, 10, 15, and 20 min of incubation at 37°C. Protein concentrations were determined using a Coomassie blue-binding assay (Bio-Rad).

Northern Analysis

Total RNA was isolated using a small-scale procedure described by Verwoerd et al. (1989). Northern analyses were performed as described previously (Rickey and Belknap, 1991). The *ubi7* 3′ specific probe was labeled by PCR as described previously (Garbarino et al., 1992) and the probe for GUS was generated using a random primer oligolabelling kit (Pharmacia).

RESULTS

Structure of the Potato *ubi7* Genomic Clone

We previously reported the isolation of a potato cDNA (*ubi7*) coding for polyubiquitin (Garbarino et al., 1992). We have now isolated a genomic clone for *ubi7* and obtained approximately 1800 bases of upstream sequence. The structure of the 5′ portion of the gene is shown in Figure 1A. The sequence homologous to the cDNA is interrupted by a 569-bp intron. Similar to other reported plant polyubiquitin genes (Binet et al., 1991; Christensen et al., 1992; Norris et al., 1993; Genschik et al., 1994; Rollfinke and Pfitzner, 1994), the intron is located adjacent to the translation start codon for the first ubiquitin-coding unit (Fig. 1A). The sequence of the potato intron is AT rich and highly repetitive in nature (data not shown).

Comparison of the *ubi7* 5′ sequence to other plant polyubiquitin genes revealed significant similarity in only a single case, the polyubiquitin gene from tobacco recently described by Genschik et al. (1994). A comparison of the *ubi7* and tobacco sequences 5′ to the ubiquitin-coding region revealed a single region of similarity (Fig. 2). This similar domain, approximately 260 bp in length, includes several positive regulatory elements identified by Genschik

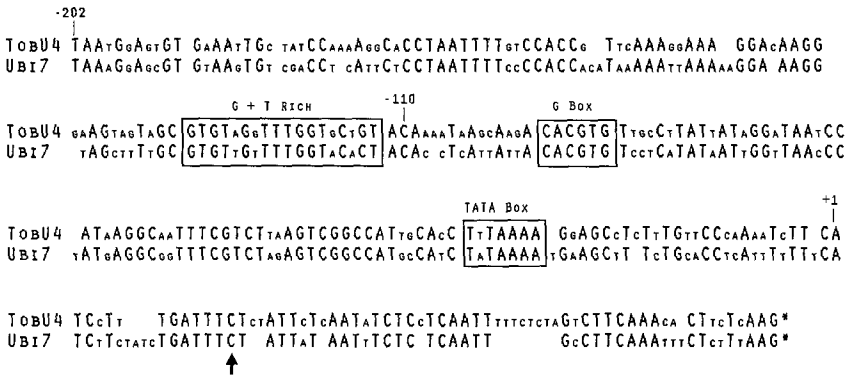

Figure 2. Sequence homology between the promoter regions of potato *ubi7* and tobacco polyubiquitin *Ubi.U4* (Genschik et al., 1994) (TobU4). Positions of the G box, TATA box, and G + T rich box are indicated. The transcription start site identified by Genschik et al. (1994) is indicated by +1. The black arrow shows the 5′ end of the previously described *ubi7* cDNA (Garbarino et al., 1992).

et al. (1994) and extends to the intron boundary. Two key positive regulatory domains, the G box and G + T rich box identified in the tobacco promoter, are highly conserved both in sequence and in relative position (Fig. 2). The G box element has been demonstrated to bind nuclear proteins in a wide variety of plant genes (Foster et al., 1994). The G + T rich box is contained within a 49-bp region shown to contain a positive regulatory domain (Genschik et al., 1994). Given the degree of sequence conservation observed in Figure 2, it is striking that sequences both 5′ and 3′ (intron) to this domain show no significant similarity. Screening of the available data bases using the BLAST network server of the National Center for Biotechnology Information (Altschul et al., 1990) did not reveal significant similarity of the potato *ubi7* promoter to any other plant polyubiquitin genes.

ubi7 Transgene Constructs

In previous studies characterizing the effects of the 5′ intron and ubiquitin-coding sequences on transgene expression from polyubiquitin promoters, transient assays of gene expression have been used (Norris et al., 1993; Genschik et al., 1994). To determine potential environmental and developmental regulatory effects associated with the intron- and ubiquitin-coding sequences, two transgenes were constructed and introduced into potato by *Agrobacterium*-mediated transformation. The first construct, *ubi7*-GUS (Fig. 1B), contained the marker gene (GUS) fused directly to the polyubiquitin 5′ UTR region. The second transgene, *ubi7*-GUS-fus, contained GUS as a translational fusion to the carboxy terminus of the first ubiquitin-coding region (Garbarino and Belknap, 1994) (Fig. 1C) and contained the 5′ intron. In the ubiquitin-GUS fusion construct, the marker gene product is synthesized as a polyprotein, which is hydrolyzed in the plant to give free GUS protein (Garbarino and Belknap, 1994).

ubi7 Chimeric Gene Expression in Transgenic Plants

When the chimeric transgenes were introduced into potato plants, GUS activity could be detected in both tubers and leaves (Fig. 3). Fluorometric assays of the transformed plants showed that GUS activity in both leaves and tubers was approximately 10 times higher for the construct containing the intron and ubiquitin monomer than for the promoter alone. Although the two constructs used here do not allow differentiation of the effects of the intron and the ubiquitin protein fusion in increasing expression levels, it is clear that inclusion of the intron and monomer greatly enhance transgene expression. These results are similar to those previously obtained using constructs of a ubiquitin-ribosomal protein gene (*ubi3*) in potato (Garbarino and Belknap, 1994) as well as other systems (Butt et al., 1989; Ecker et al., 1989). In Figure 4 mRNA levels for transgenic clones containing *ubi7*-GUS and *ubi7*-GUS-fus are shown. Densitometer scans of autoradiographs indicate relative GUS mRNA levels of approximately 1, 1.4, 2, and 0.4 for clones 1C, 11C, 34A, and 55B, respectively (data not shown). Similarly, scans of the blot probed for endogenous *ubi7* mRNA show mRNA relative levels of approximately 1, 4, 2, and 2.5 for clones 1C, 11C, 34A, and 55B, respectively. It is clear from these data that the increased levels of GUS activities observed in the *ubi7*-GUS-fus relative to the *ubi7*-GUS clones are not associated with a commensurably higher level of GUS mRNA (Stanford et al., 1990; Morelli et al., 1994).

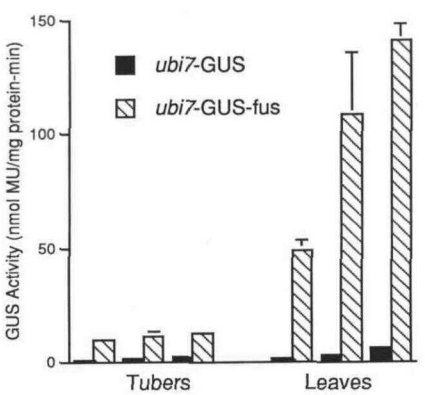

Figure 3. Comparison of the GUS activities in potato plants containing the *ubi7*-GUS and *ubi7*-GUS-fus transgenes. Assays were performed on six independent transformants for each transgene. Leaflets (fifth or sixth from meristem) and mature tuber tissues were used. Values shown are the means ± SD of three tubers or leaflets.

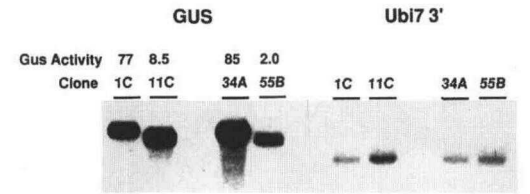

Figure 4. Steady-state transcript levels and GUS activities in leaves of transgenic potato plants containing the *ubi7*-GUS and *ubi7*-GUS-fus transgenes. Total RNA (10 μg/lane) was prepared from leaves from two *ubi7*-GUS (55B and 1C) and two *ubi7*-GUS-fus (34A and 11C) transgenic clones, and duplicate blots were prepared and hybridized to probes for GUS and the 3′ noncoding region of potato *ubi7*. Leaf GUS activities (as in Fig. 3) are indicated in nmol MU mg^{-1} protein min^{-1}.

Expression from the *ubi7* promoters within the tubers was not uniform. As shown in Figure 5, transgene mRNA was significantly higher in the peel of the tuber than in internal tissues. Densitometer scans of autoradiographs

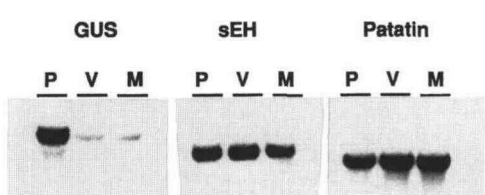

Figure 5. Steady-state transcript GUS levels in tuber tissues of transgenic potato plants containing the *ubi7*-GUS transgene. Total RNA (10 μg/lane) was prepared from tuber peel (P, outer 1–2 mm), vascular storage parenchyma (V, 5 mm of tissue adjacent to the peel), and medullary (M, internal core) tissues of tubers from Lemhi Russet clone 10.2. RNA was hybridized to probes for GUS, soluble epoxide hydrolase (sEH), and patatin.

Table I. *Comparison of ubi7 transgene activity in tuber peel versus tuber storage tissue*

Tubers from three independent transformants were assayed for GUS activity. Values are the means ± SD of three tubers from each plant. Peel was the outer 1 to 2 mm of the tuber.

Construct	GUS Activity		Fold Increase
	Storage tissue	Peel	
	nmol MU mg^{-1} protein min^{-1}		
ubi7-GUS 24	1.0 ± 0.3	6.4 ± 0.6	6.4
ubi7-GUS 28	1.6 ± 0.3	4.5 ± 0.4	2.8
ubi7-GUS-fus 18	15.6 ± 3.8	32.0 ± 3.7	2.1

indicate relative GUS mRNA levels of approximately 1, 0.12, and 0.12 for the peel, vascular storage parenchyma, and medullary tissues, respectively (data not shown). Similarly, for both constructs, the observed GUS activity (Table I) was higher in the tuber peel than in internal tissues. A similar distribution was not observed with either patatin or soluble epoxide hydrolase (Stapleton et al., 1994) mRNA. The relatively high level of GUS activity observed in the internal tissues (Table I) in relation to the level of GUS mRNA (Fig. 5) is a phenomenon that has been previously observed for wound-regulated GUS transgenes in potato tubers (Stanford et al., 1990; Morelli et al., 1994).

Previous experiments (Garbarino et al., 1992) indicated that the endogenous *ubi7* genes were expressed at approximately the same levels in meristems, immature leaves, and mature leaves. Characterization of the *ubi7* transgenic clones reveals that this promoter is highly active in senescent leaves (Table II). Similar to the above results, *ubi7*-GUS-fus transgenic lines had considerably higher activity than constructs lacking the intron and ubiquitin monomer. The levels of GUS activity in senescent (yellowing) *ubi7*-GUS-fus leaves were more than 20-fold higher than observed in mature leaves of transgenic clones expressing GUS from a cauliflower mosaic virus 35S promoter (Stanford et al., 1990). Although GUS protein has been observed to have a long half-life in transgenic plants, previous data (Garbarino and Belknap, 1994) indicate that GUS stability alone cannot account for the large increase in GUS activity observed in senescing leaves.

Table II. *Comparison of ubi7 transgene activity in young versus old leaves*

Leaflets from two independent transformants of each promoter construct were assayed for GUS activity. Young leaflets were approximately the fifth leaf from the meristem. Senescent leaves were yellowing. Values are the means ± SD of three leaflets.

Construct	GUS Activity		Fold Increase
	Young leaflet	Senescent leaflet	
	nmol MU mg^{-1} protein min^{-1}		
ubi7-GUS			
58	1.2 ± 0.3	26.7 ± 2.0	22
55	2.3 ± 0.5	26.6 ± 9.4	11
ubi7-GUS-fus			
50	48.8 ± 4.8	449 ± 46	9.2
48	142 ± 7.0	533 ± 72	3.8

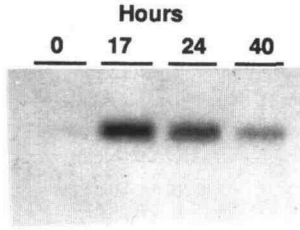

Figure 6. Injury-induced accumulation of GUS mRNA in tubers of transgenic potatoes containing the *ubi7*-GUS transgene. Total RNA (10 μg/lane) was prepared from tubers (samples include peel as well as internal tissues) from Lemhi Russet clone 2.6 at 0, 17, 24, and 40 h following cutting injury and was hybridized to a probe for GUS.

Wound-Induced Expression of *ubi7*-GUS Transgenes

As previously reported (Garbarino et al., 1992), tuber mRNA levels from the endogenous potato *ubi7* genes accumulate in response to wounding and application of exogenous ethylene. Similarly, transcript levels for the *ubi7*-GUS chimeric transgene increase following wounding of tuber tissue (Fig. 6). A time course for wound induction of GUS activity in both tuber and leaf tissue for a transgenic line containing *ubi7*-GUS with a relatively high level of basal expression is shown in Figure 7. These data indicate that wound induction is not dependent on the presence of the 5′ intron. A comparison of the wound-induced GUS enzyme levels in *ubi7*-GUS and *ubi7*-GUS-fus plants is shown in Table III. GUS activities increase approximately 2- to 6-fold in both cases. However, the levels of expression in transgenic lines containing the *ubi7*-GUS-fus construct are approximately 10-fold higher than observed in the *ubi7*-GUS clones.

Similar to a variety of other wound-induced plant genes (Sembdner and Parthier, 1993), the *ubi7*-GUS mRNA also accumulates in response to MeJas (Fig. 8). No similar induction by ABA was observed.

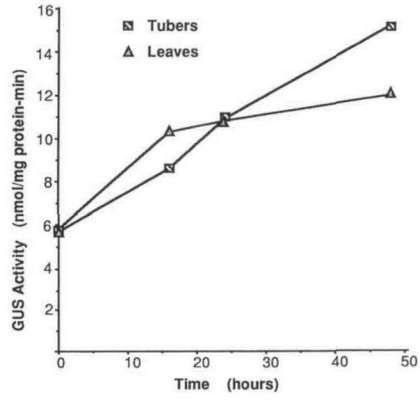

Figure 7. Time course for induction of GUS activity in leaves and tubers of a transgenic potato clone containing the *ubi7*-GUS transgene. Leaves from Lemhi Russet clone 2.6 were wounded with multiple hemostat injuries, and tubers from the same clone were cut into 1- to 2-mm slices for the period indicated prior to determination of GUS activities.

Table III. *Wound activation of ubi7 transgenes in tuber storage tissue*

Tubers of three independent transformants of each promoter construct were assayed for GUS activity. Tubers were cut and either frozen immediately or allowed to sit for 48 h as described in "Materials and Methods." Values are the means ± SD; $n = 3$.

Construct	GUS Activity		Fold Increase
	Unwounded	Wounded	
	nmol MU mg^{-1} protein min^{-1}		
ubi7-GUS			
24	1.0 ± 0.3	6.6 ± 3.4	6.6
28	1.6 ± 0.3	2.6 ± 0.2	1.6
39	0.28 ± 0.1	1.2 ± 0.2	4.3
ubi7-GUS-fus			
12	12.3 ± 1.7	31.4 ± 2.4	2.6
23	9.3 ± 0.9	30.1 ± 5.9	3.2
18	11.1 ± 2.3	29.8 ± 1.7	2.7

DISCUSSION

The potato ubiquitin gene family contains several members that are independently regulated in response to physical stress and phytohormones (Rickey and Belknap, 1991; Garbarino et al., 1992; Garbarino and Belknap, 1994). We previously described the polyubiquitin cDNA *ubi7*, the mRNA of which accumulates in response to injury, heat shock, and treatment with exogenous ethylene (Garbarino et al., 1992). Here we have characterized the corresponding *ubi7* genomic clone.

The potato *ubi7* gene has structural features similar to other plant polyubiquitin genes, in particular the presence of an intron immediately 5′ to the initiation codon for the first ubiquitin monomer. The location of this intron shows a high degree of conservation among different plant species. The introns vary in size from 300 bp in Arabidopsis (Norris et al., 1993) to more than 1 kb in tomato (Rollfinke and Pfitzner, 1994) and maize (Christensen et al., 1992). The sequences of the introns in the separate species also show limited homology.

A comparison of the *ubi7* genomic sequence to previously reported plant polyubiquitin genes revealed a region of significant homology to a tobacco gene. The region of homology is limited to approximately 250 bp located 5′ to the intron (Fig. 2), with very little homology between the intronic sequences. Genschik et al. (1994) characterized a homologous region in the tobacco promoter and identified several important positive regulatory elements that are conserved in both sequence and location in the two control elements (Fig. 2). However, the sequence divergence in regions flanking this conserved domain suggests that *ubi7* is not the potato homolog of the tobacco polyubiquitin gene but rather represents a similarly regulated member of this gene family. We previously identified additional wound-regulated potato polyubiquitin cDNAs (Garbarino et al., 1992).

Introns have been shown to play an important role in the expression of many endogenous (Callis et al., 1987; Buchman and Berg, 1988; Luehrsen and Walbot, 1991; Jonsson et al., 1992) and heterologous (Mascarenhas et al., 1990; Maas et al., 1991; Jonsson et al., 1992) eukaryotic genes. Although transcriptional control elements have been localized to introns (Jeffers and Pellicer, 1992; Fahrner et al., 1993), in other cases it is the processing of the intron that affects the efficiency of gene expression (Luehrsen and Walbot, 1991; Korb, 1993). The evidence suggests that in many cases the efficiency of intron processing directly affects steady-state mRNA levels. The conserved 5′ location of the polyubiquitin intron in plants suggests a possible role in regulation of expression. Previous experiments using transient assays suggest that the intron enhances expression in Arabidopsis (Norris et al., 1993) but not in tobacco (Genschik et al., 1994). To evaluate the potential for improved transgene expression, transgenic lines containing GUS fused directly to the promoter were compared to lines in which GUS was introduced as a translational fusion to the first ubiquitin monomer. The developmental (Tables I and II) and stress-induced (Table III) responses of the two transgene types were similar. This suggests that inclusion of the intron and first ubiquitin monomer sequence results in an increase in expression without altering the overall pattern.

The two constructs used here do not allow differentiation of the relative contributions of intron- and ubiquitin-coding sequences to the increased GUS expression observed in *ubi7*-GUS-fus relative to *ubi7*-GUS (Fig. 1; Tables I-III). However, the increased expression observed in the transgenic lines containing the GUS translation fusion may reflect an increase in translational efficiency rather than higher levels of transcript accumulation (Fig. 4). In the case of *ubi3*-GUS transgenes (Garbarino and Belknap, 1994), which do not contain a large 5′ intron, the incorporation of GUS as a translation fusion to the ubiquitin monomer resulted in a 5- to 10-fold higher enzyme expression as well as increased levels of detectable GUS mRNA. Although a strict correlation between GUS mRNA and activity levels would not be expected in these transgenic lines (Stanford et al., 1990; Morelli et al., 1994), the increase in GUS activity seen with the intron/monomer-containing *ubi7* construct was not associated with a similar increase in steady-state levels of GUS mRNA (Fig. 4).

The *ubi7* data presented here are consistent with previously published results in *E. coli* and yeast indicating that expression of transgenes as ubiquitin fusions results in increased levels of expression (Butt et al., 1989; Ecker et al., 1989). A potential source of the observed increase in expression in the translational fusion is the differences in sequences surrounding the translational initiation codon (Kozak, 1986). The sequence surrounding the initiation codon in the ubiquitin monomer, representing an authentic potato transcription start site, has the potential to confer

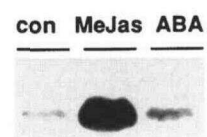

Figure 8. Regulation of the *ubi7*-GUS transgene by MeJas. Total RNA (10 μg/lane) was prepared from leaves incubated in the dark for 16 h in water (con), 20 μM MeJas, or 50 μM ABA and hybridized to a probe for GUS.

the observed significantly improved translatability to the GUS fusion transgene (Kozak, 1986).

Two developmental cues were found using the GUS transgenes. First, expression within the tuber tissue is not continuous across the organ. Significantly higher levels of GUS mRNA (Fig. 5) and activity (Table I) are found in areas of the tuber that include periderm and cortex tissue than are found in the internal regions. Second, very high levels of GUS activity are observed in senescing leaves (Table III). This observation is consistent with a role for the *ubi7* gene products in targeting specific proteins for degradation during the senescing process (Hershko, 1988).

Similar to the endogenous *ubi7* gene (Garbarino et al., 1992), the transgenes exhibit increased mRNA accumulation in response to injury of the tubers (Fig. 6). A wound-induced increase in GUS activity is also observed in leaf tissue (Fig. 7). Although many mRNAs that are MeJas regulated also respond to ABA (Hildmann et al., 1993; Sembdner and Parthier, 1993), wound-induced transcripts that respond to exogenous MeJas, but not ABA, have also been reported (Mason et al., 1990; Bell and Mullet, 1991; Mason et al., 1993; Stapleton et al., 1994). The G box domain present in the *ubi7* promoter (Fig. 2) has been associated with MeJas regulation in other plant genes, including the soybean vegetative storage protein genes *vspA* and *vspB* (Mason et al., 1990) and potato proteinase inhibitor II (gene Pi-II) (Kim et al., 1992). Similar to other wound-regulated plant genes, the transgenes described here also respond to the application of exogenous MeJas (Fig. 8).

The unique expression pattern observed using the *ubi7* transgenes suggests multiple practical applications of this promoter, e.g. in expression of introduced genes designed to protect tubers from invading bacteria and fungi. Their constitutive expression would allow deposition of gene product around the outside of the tuber, providing prophylactic protection. Deposition of transgene product would also occur at sites of injury. In addition, the unusually high *ubi7* expression observed in senescing leaves may indicate a role for this promoter in expressing foreign proteins in these tissues.

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