

*Minireview*

## Gene expression during tuber development in potato plants

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Potato tubers are modified stems that have differentiated into storage organs. Factors such as day-length, nitrogen supply, and levels of the phytohormones cytokinin and gibberellic acid, are known to control tuberization. Morphological changes during tuber initiation are accompanied by the accumulation of a characteristic set of proteins, thought to be involved in N-storage (i.e. patatin) or defense against microbial or insect attack (i.e. proteinase inhibitor II). Additionally, deposition of large amounts of starch occurs during tuber formation, which is paralleled by an increase in sucrose synthase and other enzymes involved in starch biosynthesis (i.e. ADP-glucose pyrophosphorylase, starch synthases, and branching enzyme). Potential controlling mechanisms for genes expressed during tuberization are discussed.

Tuberization; Starch metabolism; Patatin; Proteinase inhibitor II

### 1. REGULATION OF TUBERIZATION IN POTATO

The tuberization process in potato has been the subject of intensive studies at both the physiological and morphological levels. Potato tubers are underground storage organs that originate from stolons or lateral shoots developing from the axillary buds of the underground stem. They thus constitute a morphologically modified stem with reduced leaf and axillary buds, shortened internodes and a radially expanded stem axis [2].

A variety of environmental and genetic factors control the onset of tuberization. Factors such as short daylength, low temperature or low nitrogen supply favor tuber formation [6]. Plant hormones also play a central role in this process; cytokinins enhance tuber formation whereas gibberellic acid causes cessation of tuber growth [7,17].

Grafting experiments have demonstrated the occurrence of a tuberization stimulus which is formed in the leaves under short day conditions and transmitted to underground parts to induce tuber formation. The nature of this/these transmissible factor(s) is still unknown but it has been speculated that the stimulus might be related to cytokinin or abscisic acid [16]. However, some other studies suggested that, although these hormones are somehow involved in the tuberiza-

tion process, they are not the actual stimulus which triggers tuber formation. The occurrence of two acidic substances in the leaves which are very active in inducing tuberization in vitro has been reported recently [14].

### 2. TUBER SPECIFIC PROTEINS

#### 2.1. *Patatin*

The morphological changes related to the process of tuberization are accompanied by the appearance of a variety of new biochemical activities (i.e. starch accumulation) and the synthesis of a characteristic set of proteins. Up to 40% of the total soluble protein of potato tubers is represented by a family of immunologically identical glycoproteins with a molecular mass of about 40 kDa which have been given the trivial name patatin [22,28]. The high abundance of this protein in the tuber points to a function as storage compound. However, unlike most other storage proteins, patatin has been reported to have an esterase activity with a large number of lipid substrates [1,10,29,34].

Using immunocytochemical methods it has been shown that patatin is localized mainly in the vacuoles [37], which is consistent with its hydrolytic activity. Patatin is synthesized with an N-terminal signal peptide, which allows the polypeptide to enter the lumen of the endoplasmic reticulum. During transport into the endoplasmic reticulum, the signal sequence is cleaved off and the protein becomes N-glycosylated [38].

The physiological role of patatin is a subject of speculation. Besides being a storage molecule, high

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levels of its lipid-acyl hydrolase activity might have a function in the transition of the tuber from dormancy to vegetative growth [34] or, alternatively, could play a role in defense reactions against microbial infection. Thus, this lipid-acyl hydrolase, normally sequestered in the vacuole and hence biologically inactive, would be released upon mechanical damage or pathogen invasion, resulting in enzyme activation and hence in the production of toxic compounds that could inhibit the pathogen's invasion. Additionally, patatin might be involved in phytoalexin production, since it would release arachidonic acid, which is known to be a potent elicitor of phytoalexin synthesis [1].

In both greenhouse or field-grown potato plants the expression of patatin is as a rule restricted to tubers and stolons associated with growing tubers [32]. In addition, patatin is also expressed in roots, albeit at a 100-fold lower level [26]. Although tuberization is always accompanied by patatin synthesis, there are several instances where its expression is observed in non-tuberizing tissues. Patatin is expressed in leaf petioles and stems of potato plants upon removal of tubers and stolons [21]. In addition, patatin accumulates to a considerable extent in leaves of potato plantlets growing under axenic conditions on media supplied with high levels of sucrose [24]. In these cases, induction of patatin expression is independent of the tuberization process.

Patatin is encoded by a gene family with 10–18 members per haploid genome, depending on the

cultivar [42]. Several cDNA and genomic clones encoding patatin have been isolated and characterised [4,18,19,25,33,42]. Their coding regions are 4–5 kb in length and are interrupted by six introns. Based on sequence comparison of the 5'-untranslated regions of the clones, the genes can be separated into two types, which differ by the presence (class II) or absence (class I) of a 22 nucleotide insertion just upstream of the initiation codon. Class I transcripts are tuber specific, whereas class II genes are expressed in both tubers and roots, albeit at a much lower level than class I ones [26].

Chimeric genes consisting of the 5'-upstream regulatory regions of class I and class II patatin genes fused to the *E. coli*  $\beta$ -glucuronidase (GUS) gene [11] have been transferred to potato plants and their respective modes of expression studied (Fig. 1) [15,31,42,44]. A 1.5 kb 5'-upstream promoter region of a class I gene was able to confer a high level of tuber-specific GUS activity, on average 100- to 1000-fold higher in tubers than in either leaves, stems or roots. Histochemical analysis revealed this activity being present in parenchymal tissue but not in the peripheral phellem cells of transgenic tubers. A 2.7 kb promoter region of a class II gene showed an expression pattern clearly different from that of the class I gene. In this case, the strong GUS activity was restricted to a few defined cells within the potato tuber and to a small zone just behind the meristem in roots.

In contrast to class II genes, class I genes can be activated in leaves by conditions that promote the ac-

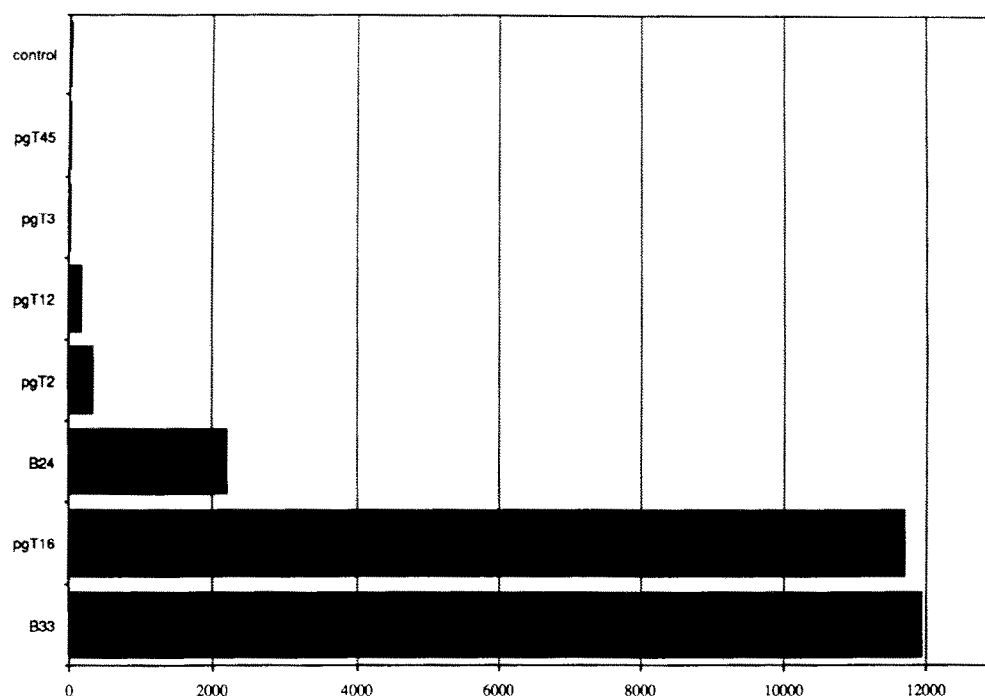


Fig. 1. Tuber-specific activity of the different patatin promoters. Class I (pgT16, B24 and B33) and class II (pgT2, pgT3, pgT12 and pgT45) promoters were fused to the bacterial  $\beta$ -glucuronidase gene to compare their relative strength in tubers. GUS activity is indicated in pmol 4-methylumbelliferone production/mg tuber protein/min.

cumulation of starch in storage organs, i.e. high sucrose concentration. High levels of externally supplied sucrose or glucose, but not sorbitol, induce patatin gene expression in leaves [31,45]. A similar response can be observed when the GUS gene is driven by a class I, but not a class II, promoter. Whereas the expression of the class I promoter is very low in leaves from plantlets supplied with 2% sucrose, an increase of GUS activity up to 100-fold is found in leaves from plants supplied with 7% sucrose. This activation in leaves is cell specific since it is observed in the mesophyll and epidermal cells but not in vascular tissue nor guard cells.

Deletion analysis of a class I gene promoter region suggests that sequences directing tuber-specific expression are located downstream to position -195. The sucrose inducibility of the different truncated promoters indicates that a sucrose responsive element must be located in close vicinity to the tuber-specific element (Liu, X.-J. et al., submitted for publication). It will be interesting to see whether tuber specificity and inducibility by metabolic signals is conferred by the same *cis*-elements.

## 2.2. Proteinase inhibitor II

Plant storage organs such as seeds or tubers very often contain considerable amounts of inhibitors of proteolytic enzymes [35]. The inhibitory activity of these proteins is mostly directed towards proteolytic enzymes from microorganisms and insects, but only rarely against proteinases of plant origin [30]. They are consequently thought to play a role in the defense reaction of the plant against microbial and insect attack.

Proteinase inhibitor II constitutes about 5% of the total protein in potato tuber. The expression of this protein is under the control of both developmental and environmental factors. In non-wounded potato plants the expression of proteinase inhibitor II is restricted to tubers and young floral buds (Peña-Cortés, in preparation). However, wounding of the plant, as a consequence of mechanical damage or insect attack, triggers the accumulation of the protein in the aerial organs. This accumulation is not restricted to the site of the injury; rather, it is also observed in non-wounded organs apart from the wound site. This indicates that an inducing factor or wound hormone is released upon wounding and rapidly transported to other tissues of the plant, thereby inducing the expression of these genes. We have recently obtained strong evidence for the involvement of the phytohormone, abscisic acid, as mediator in this wound response [23].

Both cDNAs as well as genomic clones encoding potato proteinase inhibitor II have been isolated and characterised. They encode a 154 amino acids polypeptide, which is highly homologous to its tomato counterpart. Sequence comparison of the genomic and cDNA clones reveal the presence of an intron located in the region coding for the N-terminal part of the protein.

This region corresponds to the signal peptide which directs the co-translational import of the protein into the endoplasmic reticulum, from where it is transported to its final destination in the vacuole.

A chimeric gene consisting of 1.3 kb of the 5'-regulatory region fused to GUS was introduced into potato and tobacco plants, where it showed both constitutive expression in stolons and tubers of non-wounded potato plants, as well as systemic, wound-inducible expression in stem and leaves of potato and tobacco. In a similar way, a chimeric gene consisting of the PI-II promoter region fused to CAT showed wound-induced expression in transgenic tobacco plants [40]. Histochemical analysis indicate an association of the potato proteinase inhibitor II promoter activity with the vascular tissue in leaves, both wounded and systematically induced, petioles, stems and developing tubers [12]. These data show that one single member of the PI-II gene family contains *cis*-active elements able to respond to both developmental and environmental signals.

The analysis of progressive 5' deletions of the promoter revealed the requirement of a far upstream region (position -1300 to -700) for high-level expression. Additional deletion of sequences from -700 to -514 abolished the low level of wound-induced gene expression observed in the -700 truncated promoter [13]. Similar effects were found in the tuber-specific expression. The proteinase inhibitor II promoter seems therefore to differ in this respect from patatin and other plant genes, where less than 500 bp upstream of the transcription start site are sufficient for full promoter activity.

## 3. ENZYMES INVOLVED IN STARCH BIOSYNTHESIS

Carbohydrates are produced during photosynthesis in mature leaves, thus representing source tissues [41]. They have to be delivered at the sites of carbohydrate demand, the so-called sink tissues.

The most abundant form of transport sugar is sucrose which is most likely exported from the leaf by transferring it to the apoplast, and then by active loading into the phloem [41]. Upon arrival in potato tubers sucrose is mainly converted to starch.

The initial step in sucrose breakdown is catalysed by either invertases or sucrose synthase. Since starch accumulation in potato tubers is paralleled by an increase in sucrose synthase activity, sucrose synthase is believed to be the most important enzyme for sucrose catabolism [3]. After sucrose hydrolysis the resulting carbohydrates are transported into the amyloplast either as hexose units or as three carbon phosphate-esters. The most direct way from sucrose to starch would be the import via hexoses. This would locate the major branch point between starch accumulation and respiration at

the reactions catalyzed by the phosphofructokinase (PFK), pyrophosphate:fructose-6-phosphate phosphotransferase (PFP) and fructose-1,6-bisphosphatase (FBPase).

Several enzymes are involved in the biosynthesis of starch, i.e. ADP-glucose pyrophosphorylase, starch synthases, and the branching enzyme [29]. The first enzyme synthesizes ADP-glucose, which is the substrate of the starch synthase. Starch synthases catalyse the formation of new  $\alpha$ -1,4 bonds between preexisting primers and glucose units. Branching enzyme, also called Q enzyme, catalyses the formation of the  $\alpha$ -1,6 branch point linkages in amylopectin. These enzymes are confined to the amyloplast [20]. In contrast to the enzymes involved in starch biosynthesis, all sucrose synthesizing enzymes are located in the cytoplasm [5].

Regulation of starch synthesis is exerted at the level of ADP-glucose pyrophosphorylase [39]. As mentioned above, this enzyme catalyses the synthesis of ADP-glucose, the glucosyl donor of starch synthase, from glucose-1-P and ATP. ADP-glucose synthesis is markedly influenced by the levels of the small effector molecules, 3-PGA and  $P_i$ , which allosterically activate and inhibit, respectively, enzyme activity. 3-PGA at high concentrations reverses or overcomes the inhibition caused by  $P_i$  [9].

A marked and rapid increase in ADP-glucose pyrophosphorylase activity coincides with the initiation and very early development of potato tubers, showing a good correlation between enzyme activity and capacity of starch synthesis.

In contrast to the genes encoding patatin or proteinase inhibitors, very few potato genes coding for enzymes involved in starch synthesis have been described. A potato cDNA clone encoding for sucrose synthase has been described recently [36]. Using heterologous probes from maize, we have isolated cDNA clones encoding two different potato ADP-glucose pyrophosphorylase polypeptides (AGPase S and AGPase B; Müller et al., submitted for publication). Northern blot experiments show that the two genes differ in their expression pattern in different organs analysed. Furthermore, one of the genes (AGPase S) is strongly inducible by metabolizable carbohydrates (e.g. sucrose) on the RNA level. The accumulation of AGPase S mRNA was found to be always accompanied by an increase in starch content, suggesting a linkage of AGPase S expression to the state of a tissue, being either a sink or a source for carbohydrates. In contrast, expression of AGPase B remains nearly unchanged under various experimental conditions. Whereas expression of AGPase B is similar in tuber and mature leaf, sucrose synthase and AGPase S show a much stronger expression in tubers than in leaf, root and stem. Furthermore, similarly to patatin mRNA, both genes are clearly inducible in petiole experiments by high levels of metabolizable carbohydrates (e.g.

sucrose). Increase in the steady-state level of mRNA is accompanied by the accumulation of starch.

#### 4. CONCLUSION

Tuber-specific genes are normally expressed in tubers. Nevertheless, under certain conditions they can be induced to high levels of expression in other organs. In all cases, the accumulation of tuber-specific proteins closely correlates with starch formation. It is conceivable that the availability of starch or one of its precursors acts as a signal, not only to initiate the morphological changes typical for tuberization but also for the activation of the 'tuber-specific' genes. Alternatively, the coincidence of both processes could be explained by assuming a common regulation for both of them. Expression in leaves is closely related to whether these organs are currently a sink tissue, which depends on carbohydrate imported from other regions of the plant. Removal of potato tubers, for example, results in the accumulation of patatin and starch in above-ground stems and petioles where they do not normally occur. In the absence of the normal storage tissue, therefore, other vegetative tissues assume a storage role. In a similar way, elevated levels of the major transport sugar sucrose, results in an increase of the transcripts in leaf and petiole. These results suggest that the accumulation of these proteins is regulated by the immediate need for storage, rather than strict developmental control.

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