Gene Expression during Transition from Dormancy to Sprouting in Potato Tubers

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ABSTRACT

This article reviews the knowledge on the genetic regulation during transition from dormancy to sprouting in potato tuber, to elucidate the molecular mechanisms underlying the physiological and cellular changes driving this complex developmental process. Extensive studies on gene expression were carried out with different tools: differential display, cDNA-AFLP, subtractive libraries, microarrays, quantitative Real Time PCR and proteomic analysis. A high proportion of sequences up- or down-regulated at the end of dormancy are still unknown or match with proteins in databases that have not yet been characterised. Other sequences encode genes involved in hormone metabolism and response, or are transcriptional factors, ribosomal and carrier proteins, or putative regulators of cell growth and division. At the end of dormancy, when the tuber enters in a source condition, a general decrease of synthesis of storage proteins and enzymes involved in biosynthetic metabolism has been observed, as well as modifications in the pattern of proteins associated to membranes. Studies conducted on transgenic plants suggest that phosphate and sucrose levels may play a crucial role in the sink-to-source transition during sprout growth, as well as the reactive oxygen species.

Keywords: EST isolation, meristem activation, sink-source transition, sprouting
Abbreviations: ABA, abscisic acid; ADH, alcohol dehydrogenase; ARF, auxin response factor; BE, bromo-ethane; DDRT-PCR, differential display reverse transcriptase-polymerase chain reaction; EST, expressed sequence tag; FLD, fluridone; GAs, gibberellins; IAA, indole acetic acid; LUC, luciferase; NCED, 9-cis-epoxycarotenoid dioxygenase; qRT-PCR, quantitative real-time PCR; TDFs, transcripts derived fragments; ZEP, zeaxanthin epoxidase

INTRODUCTION

A potato tuber is hypogaeal stem (stolon) which, under appropriate conditions, stops longitudinal growth and swells. Cells located in the pith and the cortex of the apical region of the stem first enlarge and then later divide longitudinally. In this way the subapical part of the stolon swells and subsequently, after reaching a diameter of approximately 2 to 4 mm, the longitudinal divisions stop and are replaced by randomly oriented divisions and cell enlargement (Sanz et al. 1996). During its formation, the potato tuber accumulates starch, reserve proteins, in particular the glycoprotein pata- tin, and other metabolites, becoming the strongest sink organ of the plant. Eventually, the tuber decreases its metabolic activity and as such behaves as a typical storage sink organ.

At the beginning of the swelling, the meristematic activity of the apical region of the stolon and the lateral buds of the developing tuber is repressed, and in this way, dormancy is established (Burton 1989; Claassen and Vreugdenhil 2000). At maturity, the tuber represents a compressed stem and the eyes correspond to apical and lateral axillary buds, which for a period varying from 2 to 6 months, are unable to grow. Thus, tubers can be grouped with other vegetative over-wintering organs such as seeds, corms and buds that also exhibit varying degrees of dormancy, depending on internal or external factors (Okubo 2000). Although the definition of dormancy has been controversial, it is generally accepted that dormancy refers to the physiological state in which autonomous sprout growth does not occur, when the tuber is placed under optimal growing conditions: darkness, temperature 15-20°C, relative humidity about 90% (Reust 1986; Coleman 1987; Burton 1989). Several authors (Lang et al. 1985, 1987) classified dormancy into three
classes: i) endodormancy: conditioned by internal factors of the bud; ii) paradormancy: related to plant tissues external to the bud and iii) ecodormancy: dependent on environmental conditions to which tubers are exposed. From a few days to some months after their maturity, tubers switch from a sink to a source condition, mobilizing their reserves and supplying energy and substrates to the growing sprouts. Breaking dormancy involves the resumption of cellular division in the bud meristem and mobilization of reserve substances to support growth and responses to bio-molecular and environmental triggers.

The length of dormancy is a controversial matter: it has been largely discussed because the physiological stage in which tuber seeds are planted greatly affects the yield of growing plants. Burton (1963) proposed of measuring the duration of dormancy from the date of tuber initiation, while others argued that the tip starts to swell. Dormancy is defined to have ended when the buds on the tuber, stored under optimal conditions for sprouting, start to grow. The definition for the end of the dormancy of a tuber lot is the moment when 80% of tubers have formed sprouts (Reust 1986). The presence of at least one sprout per tuber 2 mm long is conventionally taken as the indication of end of dormancy (Reust 1986; van Ittersum et al. 1992). This criterion is independent of physiological age which relates to the conditions or state of tubers, and is usually used to define the stage of development of seed tubers at planting. The main factors influencing physiological age are cultivar, environmental conditions during crop growth, time of harvest and importantly storage temperature regime (O’Brien et al. 1983; Caldiz et al. 2001).

The length of dormancy is a quantitative, genotype-dependent character; van den Berg et al. (1996) detected quantitative trait loci on nine chromosomes, either alone or through epistatic interactions.

The dormancy period varies greatly not only among potato cultivars, but also in the same lot of harvest. The length of dormancy is highly influenced by temperature and humidity in which tubers are stored (van Ittersum and Scholte 1992), by the growth conditions of mother plant such as nitrogen supply, temperature and light (van Ittersum 1992a, 1992b, 1992c). Also the size of tubers can influence the length of dormancy (Reust 1982).

The transition from dormancy to sprout development involves several physiological and biochemical changes, which are bridged by complex genetic regulatory networks. Some physiological mechanisms underlying this transition have been reviewed by Wiltshire and Cobb (1996) and by Suttle (2004a). For example, it has been noted that during dormancy meristems are metabolically active, but the efficiency of respiration, transcription and translation is reduced (Macdonald and Osborne 1988). During progression towards sprouting, tuber accumulates sugars (Wiltshire and Cobb 1996) and undergoes peroxidative damages to membrane lipids (Fauconnier et al. 2002).

The onset of sprout growth is accompanied by an increase in cell metabolism, which is strictly dependent on changes in the promoter sequences of genes (Bachem et al. 2000; Ronning et al. 2003). Other authors associated the resumption of sprout growth with changes in the methylation of meristem DNA (Law and Suttle 2003) and in histone acetylation (Law and Suttle 2004), which usually precede transcriptional activation of genes, leading to cell division and meristem growth in potato tubers.

Other dormancy-related mechanisms involve hormone changes in gene expression (Bachem et al. 2000; Ronning et al. 2003). Other genes have been found to be up-regulated in the potato tuber apical buds on dormancy release (Table 1). Expression of ARF6 was isolated by Faivre-Rampant et al. (2004a) in a subtractive hybridization library enriched in clones representing genes up-regulated in the potato tuber apical buds on dormancy release (Table 1). Expression of ARF6 is particularly high in the group of cells associated with the development of vascular tissues which lead to the meristem. The transcript was also found in the tunica and corpus. The authors provided evidence that changes in ARF6 expression occur early in the process of meristem activation and may be of

**GENE EXPRESSION AND PHYSIOLOGICAL REGULATION OF TUBER DORMANCY**

The role of hormones, ethanol and phenolic compounds, in regulation of tuber dormancy has been extensively reviewed by Suttle (2004a). The levels of five hormones (auxins, gibberellins, cytokinins, ABA and ethylene) change during storage and during the transition from dormancy to sprouting, suggesting that they may all be involved. Unfortunately, the genes involved in the metabolism of hormones, as well as their target genes are poorly characterised in potato tuber and until now little information is available on the regulatory network during dormancy and bud growth resumption. In this review, only those compounds for which some genetic determinants have been described will be discussed: a summary is given in Table 1.

**Auxins**

The first studies, started in the 1940s, were conducted to elucidate the role of free and esterified auxins in regulation of dormancy. Pioneering studies indicated that the endogenous levels of auxins were low in tissues of dormant tubers and increased during early sprout growth. At relatively high doses, indole acetic acid (IAA) and the more stable 1-naphthalene acetic acid (NAA) were found to be potent inhibitors of sprout growth when supplied exogenously (Denny 1945). Extremely low concentrations of auxin stimulated the growth of non-dormant sprouts, but had no discernable effects on dormant eyes (Heming 1949). Studies using high performance liquid chromatography (HPLC) coupled with fluorometric detection report no increase in free IAA content until the end of dormancy (Sukhova et al. 1993). More recent data describe the progressive increase in both free and conjugated IAA levels in tuber apical meristems during dormancy, which rapidly fall on sprouting (Sorce et al. 2000). A continuous increase of IAA has been observed also in tuber slices stored at 4°C during progression toward sprouting (Reverberi et al. 2001).

The role of auxins in the regulation of gene expression has been poorly characterised. In potato, there are two families of proteins which have been found to be key regulators of auxin-responsive gene expression in other plants: the auxin/indole-3-acetic acid (Aux/IAA) proteins and the auxin response factors (ARFs), (Hagen and Guilfoyle 2002; Liscum and Reed 2002; Wei et al. 2006). ARFs are regulatory proteins that bind with the amino terminal of a specific sequence in the promoters of auxin-responsive genes (Ulmason et al. 1999a). These factors can act as repressors or activators of transcription depending on their structure: glutamine-rich central domains activate transcription whereas proline-serine/threonine-rich central domains confer repressor activity (Ulmason et al. 1999b). Aux/IAA proteins do not bind DNA, but interact with ARFs, modulating their activity (Guilfoyle et al. 1998; Wei et al. 2006). CDNA high sequence similarity to Arabidopsis thaliana ARF6 was isolated by Faivre-Rampant et al. (2004a) in a subtractive hybridization library enriched in clones representing genes up-regulated in the potato tuber apical buds on dormancy release (Table 1). Expression of ARF6 is particularly high in the group of cells associated with the development of vascular tissues which lead to the meristem. The transcript was also found in the tunica and corpus. The authors provided evidence that changes in ARF6 expression occur early in the process of meristem activation and may be of
primary importance. In particular, ARF6 expression level decreases upon tuber initiation, when stolon growth changes from a longitudinal to a lateral expansion and there is a marked decline in the mitotic index in the shoot apical meristem. On the other hand, ARF6 expression is strongly induced in buds upon endodormancy release, when meristematic activity recommences. Expression of ARF6 increases strongly in auxillary buds on release from the effects of apical dominance (paradormancy or correlative inhibition). A high level of ARF6 transcript was found in leaf tips, supporting a correlation with high meristematic activity.

In Arabidopsis, ARF6 functions as an activator of gene expression (Tiwari et al., 2003), if the same is the case in potato, the ARF6-induced changes in gene expression may be a key process in the control of dormancy release.

**Abscisic acid**

Originally isolated and characterised from abscising lupin (Lupinus albus et luteus), cotton (Gossypium hirsutum) fruit and dormant sycamore (Platanus occidentalis) buds and leaves, ABA has been considered to play a pivotal role in the regulation of plant dormancy (Addicott and Cairns 1983). The ABA synthesis in vascular plants is described in Fig. 1A (Nambara and Marion-Poll 2005). In the potato tuber, the 8'-hydroxylase pathway leading to phaseic acid (PA) and its metabolite dihydrophaseic acid (DPA) is the predominant route of ABA degradation (Fig. 1B) (Suttle 1995; Nambara and Marion-Poll 2005).

In the potato tuber, ABA levels are generally highest after harvest, during endodormancy, and decline as sprouting commences (van den Berg et al., 1991), but its role in regulating tuber dormancy is rather controversial: endogenous ABA level rises at the end of dormancy when tubers are stored at low temperatures (Coleman and King 1984).

Suttle and Hulstrand (1994) have studied the role of ABA in maintaining dormancy in potato microtubers generated in vitro. Microtubers grown in the presence of the herbicide fluridone (FLD), an inhibitor of ABA synthesis, exhibited premature spraying. When accompanied with the simultaneous application of exogenous racemic ABA, the result is a dose-dependent increase in endogenous ABA and a concomitant decrease in premature sprouting, suggesting a role of this hormone in dormancy maintenance. However, Sorce et al. (1996) reported that ABA decreases in the whole tuber after the loss of dormancy, but in bud eyes ABA concentration falls before the mother plant senescence, whereas a gradual rise accompanies overcoming of dormancy during storage.

To further elucidate the role of ABA in dormancy regulation, Destefano-Beltrán et al. (2006a) measured the endogenous ABA content in tuber meristems and their surrounding periderm and underlying cortical tissue during postharvest storage. The ABA content was found to be relatively low and constant in the periderm up to 100 days of storage and declined further. In contrast, the ABA content of meristems rose to about 43% during the first 27 days of storage, to decline definitively. A similar trend, although with lower values, was observed in the cortical tissue.

One gene sequence (SiZEP) encoding the biosynthetic enzyme zeaxanthin epoxidase (ZEP) (Fig. 1A), two putative sequences (SiNCED1 and SiNCED2) coding for 9-cis-epoxy-carotenoid dioxygenase (NCED) (Fig. 1A) and three sequences coding for the catalytic enzyme ABA-8'-hydroxylase (SCYP707A1, SCYP707A2, SCYP707A3) (Fig. 1B) were monitored to quantify the relative abundance of their mRNAs in the three specific tuber tissues mentioned above.

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**Table 1** Genes involved in hormonal regulation of tuber dormancy.

<table>
<thead>
<tr>
<th>Gene/Sequence</th>
<th>Protein</th>
<th>Function (established or presumed)</th>
<th>Regulation/expression</th>
<th>Transgenic potato lines</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARF6</td>
<td>Homologous to auxin responsive factor</td>
<td>Transcriptional factor</td>
<td>Up regulated on release from dormancy</td>
<td></td>
<td>Faivre-Rampant et al. 2004</td>
</tr>
<tr>
<td>SiZEP</td>
<td>Zeaxanthin epoxidase</td>
<td>ABA biosynthesis</td>
<td>See Fig. 2</td>
<td></td>
<td>Destefano-Beltrán et al. 2006a, 2006b</td>
</tr>
<tr>
<td>SiNCED1</td>
<td>9-cis-epoxycarotenoid dioxygenase</td>
<td>ABA biosynthesis</td>
<td>See Fig. 2</td>
<td></td>
<td>Destefano-Beltrán et al. 2006a, 2006b</td>
</tr>
<tr>
<td>SiNCED2</td>
<td>9-cis-epoxycarotenoid dioxygenase</td>
<td>ABA biosynthesis</td>
<td>See Fig. 2</td>
<td></td>
<td>Destefano-Beltrán et al. 2006a, 2006b</td>
</tr>
<tr>
<td>SCYP707A1</td>
<td>ABA-8-hydroxylase</td>
<td>ABA catabolism</td>
<td>See Fig. 2</td>
<td></td>
<td>Destefano-Beltrán et al. 2006a, 2006b</td>
</tr>
<tr>
<td>SCYP707A2</td>
<td>ABA-8-hydroxylase</td>
<td>ABA catabolism</td>
<td>See Fig. 2</td>
<td></td>
<td>Destefano-Beltrán et al. 2006a, 2006b</td>
</tr>
<tr>
<td>Stgan</td>
<td>Short-chain alcohol dehydrogenase</td>
<td>ABA biosynthesis</td>
<td></td>
<td>Antisense: premature sprouting</td>
<td>Bachem et al. 2001</td>
</tr>
<tr>
<td>RD22</td>
<td>Homologous to RD22 of Arabidopsis thaliana</td>
<td>Seed development</td>
<td>ABA responsive gene, down-regulated at the end of dormancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TUBBY</td>
<td>Homologous to TUBBY protein of Arabidopsis thaliana</td>
<td>ABA signalling</td>
<td>Down-regulated at the end of dormancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cysteine protease inhibitors</td>
<td>Inhibitors of proteases</td>
<td>ABA responsive gene, down-regulated at the end of dormancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>StG20ox1</td>
<td>GA 20-oxidase</td>
<td>Gibberellic biosynthesis</td>
<td>Constitutive over expression: shorter dormancy</td>
<td></td>
<td>Carrera et al. 2000</td>
</tr>
<tr>
<td>IPT</td>
<td>Isopentenyltransferase</td>
<td>Cytokinin biosynthesis</td>
<td>Constitutive over expression: premature spraying</td>
<td></td>
<td>Galis et al. 1995</td>
</tr>
<tr>
<td>Sho</td>
<td>Isopentenyltransferase of Petunia hybrida</td>
<td>Cytokinin biosynthesis</td>
<td>Constitutive over expression: premature spraying</td>
<td></td>
<td>Zubko et al. 2005</td>
</tr>
<tr>
<td>dcs</td>
<td>Bacterial 1-deoxy-D-xylulose 5-phosphate synthase</td>
<td>Isoprenoid biosynthesis</td>
<td>Constitutive over expression: premature spraying</td>
<td></td>
<td>Morris et al. 2006</td>
</tr>
</tbody>
</table>
Fig. 1 ABA metabolism in vascular plants and metabolic genes cloned and studied in *Solanum tuberosum*. (A) Key steps and enzymes involved in ABA biosynthesis: zeaxanthin epoxidase (ZEP) catalyzes conversion of zeaxanthin to violaxanthin. A reverse reaction occurs in chloroplasts catalyzed by violaxanthin de-epoxidase, (VDE). In the box the gene *StZEP* coding for ZEP in *Solanum tuberosum* is indicated. The formation of cis-isomers of violaxanthin and neoxanthin requires an isomerase and the enzyme neoxanthin synthase (NSY). Violaxanthin and neoxanthin are cleaved by a family of 9-cis-epoxycarotenoid dioxygenases (NCED). *StNCED1/2*, indicated in the box are two cDNAs cloned in *Solanum tuberosum* coding for NCED. Xanthoxin is then converted by a short-chain alcohol dehydrogenase (ABA2) into abscisic aldehyde, *Stgan* is a sequences coding for a short-chain alcohol dehydrogenase homologous to ABA2. Abscisic aldehyde is finally oxidized into ABA by an abscisic aldehyde oxidase (AAO3). (B) Key steps and enzymes involved in ABA catabolism: ABA is hydroxylated in three different pathways, but the 8'-hydroxylation, catalyzed by ABA-8-hydroxylase is thought to be the predominant pathway. *StCYP707A1/2/3*, are three cDNAs coding for ABA-8-hydroxylase in *Solanum tuberosum*. Reprinted from: Nambara E, Marion-Poll A (2005) Abscisic acid biosynthesis and catabolism. *Annual Review of Plant Biology* 56, 165-185, with kind permission of the authors and Annual Reviews, ©2005.
Expression of the genes involved in ABA metabolism was analyzed in different potato tuber tissues sampled at different times of storage. The expression of *StZEP* was found to be relatively constant in meristems, exhibiting a transient increase during early and mid-to-late storage in periderm and peaks in the cortex during mid-storage. A lack of correlation between ZEP activity and ABA content was also observed in other experiments. Transgenic tubers with both a sense and antisense construct of ZEP, under the control of a tuber-specific promoter, accumulated high levels of ZEP activity, but the amount of ABA was the same as in the controls.

The expression of *StNCED1* and *StNCED2* increases transiently in the early storage in the meristem, mirroring the ABA content. This effect is more pronounced for *StNCED2* suggesting that this gene has a key role in the ABA biosynthesis in this tissue. The situation in the periderm is less clear: despite constant or decreasing levels of ABA, expression of both genes is biphasic, with transient increases at early and late storage. In the cortex, expression of *StNCED1* correlates with ABA content, with transient increases during early and late storage, while *StNCED2* transcript abundance declined significantly during storage.

All three catabolic genes *StCYP707A* are expressed in meristems during storage: the expression of *StCYP707A1* increases at the end of storage, *StCYP707A2* rises steadily, while *StCYP707A3* does not change significantly. An increase in ABA catabolism, therefore, is correlated with the expression of these genes. In the periderm, expression of *StCYP707A1* is not detectable, while *StCYP707A2* and *StCYP707A3* are up-regulated in late storage, along with a decline in ABA content. In the cortex, expression of *StCYP707A3* is non-detectable, whereas *StCYP707A1* and *StCYP707A2* are down-regulated during storage.

The interpretation of these data is complicated due to the natural asynchrony in sprouting, to minimise these differences, the experiments were repeated on meristems treated with the synthetic dormancy-terminating agent bromoethane (BE) that induces a rapid and highly synchronous sprouting of dormant tubers. The endogenous ABA content in tuber meristems increases 2-fold 24 hours after BE treatment and then declines dramatically. Seven days after harvest, the expression of *StZEP* does not mirror changes in ABA content in any of the tissues examined: it is relatively constant in meristems, exhibits a transient increase during early and mid-to-late storage in periderm and peaks in the cortex during mid-storage. A lack of correlation between ZEP activity and ABA content was also observed in other experiments. Transgenic tubers with both a sense and antisense construct of ZEP, under the control of a tuber-specific promoter, accumulated high levels of ZEP activity, but the amount of ABA was the same as in the controls.

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after treatment, ABA content in the meristem declined by >80%, and exogenous ABA was readily metabolized by isolated meristems to PA and DPA, showing how the BE treatment can almost double the rate of ABA metabolism (De- stefano-Beltrán et al. 2006b). In these meristems, a correlation was found between the increase in ABA content and expression of SnCDE2, while a decrease in ABA content was correlated with an increased expression of the three SacyP7 genes (Fig. 2B). These results confirm that NCED and the catabolic ABA-8'-hydroxylase are key enzymes in ABA metabolism in tuber meristems. Therefore, the ABA content in the tuber balances between synthesis and metabolism that increasingly favours catabolism as dormancy ends.

The biosynthetic enzyme ABA2 which catalyzes the conversion of xanthoxin to abscisic aldehyde (Fig. 1A) is a short-chain alcohol dehydrogenase (Gonzales-Guzman et al. 2002) belonging to a family of short proteins involved in many developmental processes (Wu et al. 2007). cDNA, referred to as Sgan that shared a high homology with a short-chain alcohol dehydrogenase, was isolated in potato by Bachem et al. (2001). Transgenic tubers expressing the antisense Sgan gene showed a premature sprouting and elevated levels of biologically active gibberellins and their relative precursors (Bachem et al. 2001) (Table 1). The levels of ABA in these transgenic tubers have not yet been determined, but these data suggest once more that biosynthesis of this hormone is crucial in maintaining the dormancy.

Termination of dormancy is associated with decreased expression of some ABA-responsive genes, isolated by high throughputscreening and reviewed by Campbell et al. (2005) (Table 1). Of interest is the down-regulation of cDNAs coding for D22, a protein first characterised in Arabidopsis thaliana, expressed during the early and middle stages of seed development (Yamaguchi-Shinozaki et al. 1993). The termination of dormancy in meristems also resulted in the down regulation of a putative TUBBY protein. In A. thaliana a TUBBY-like protein has been shown to be involved in ABA signalling (Lai et al. 2004). Also cysteine protease inhibitors, whose expression is downregulated at the end of dormancy, are induced by ABA (Seki et al. 2002). These observations are consistent with the fact that dormancy is regulated, at least partially, by ABA (Suttle and Hulstrand 1994) and support the existence of a connection between the dormant state and the level of ABA-related transcripts in the potato tuber.

Gibberellins

Typically, treatment with GA3 induces rapid sprouting in seed tubers when required. Bioassays have demonstrated the presence of GA-like activities in tuber extracts and indicated that endogenous levels of certain GAs increased as sprout growth commences (Suttle 2004a). At present, over 100 GAs have been identified in seed plants. The biosynthetic pathway of GAs, reviewed by Hedden and Phillips (2000) is shown in its principal components in Fig. 3; it involves three cellular compartments: plastids, endomembranes and cytoplasm.

In all species of Solanaceae examined, the predominant GAs are members of the early 13-hydroxylation pathway leading to GA1 (Fig. 3); in addition, the occurrence of GA4 in certain potato tissues has also been reported (Jones et al. 1988; van den Berg et al. 1995; Carrera et al. 2000). Although exogenous GA3 is able to terminate dormancy, the role of active GAs in regulating dormancy is not well clarified. Quantification of endogenous GAs by gas chromatography-mass spectrometry (GC-MS) revealed a dynamic pattern of GAs content during progression of tuber dormancy (Suttle 2004b). The concentration of GAs is initially relatively high, declines during postharvest storage and rises again as sprout growth commences. Surprisingly, the content of GAs is higher in deeply dormant tubers than in tubers beginning to exit dormancy. The content of GA1, GA19, and GA20 is greatest in tubers exhibiting robust sprout growth. These observations suggest that endogenous GAs play a role in the regulation of sprout growth after the termination of dormancy, but not in the progression of tuber dormancy.

Suttle (2004b) explored the ability of exogenous gibberellin precursors or members of the early 13-hydroxylations pathway to interrupt dormancy. These compounds exhibited a time and dose-dependent ability to prematurely terminate tuber dormancy. As expected, GA1 which is the most active hormone is the most efficient, followed by GA3 and GA20. Earlier members in this pathway (kaurene and GA12) are ineffective. Injection of GA3 had no effect on tuber dormancy, but enhances sprout growth of non-dormant tubers. Although levels of GA1 and GA20 rose modestly as sprout growth commences, GA19 levels increased significantly at the late storage. This suggests that conversion of GA19 to GA20 is the rate-limiting step in the GA biosynthetic pathway in tubers. This metabolic step is catalyzed by GA20-oxidase, a multifunctional enzyme, whose levels and activities are tightly regulated in many plant tissues. Three cDNAs encoding potato GA 20-oxidase (SgA20x1/2/3) were isolated (Carrera et al. 1999) (Table 1). To investigate the role of this enzyme in tuber dormancy and other developmental processes, transgenic potato lines that over- and down-regulate these genes were produced. Two lines, with constitutive (expressing the transgene in the whole plant) and leaf-specific over-expression of the gene were selected. Tubers harvested from both types of transgenic plants exhibited a shorter dormancy period and formed longer and thinner sprouts than the control tubers. Shortening of dormancy was greater in the constitutive over-expressing lines than in the leaf-specific over-expressors, indicating that dormancy was probably regulated by GAs synthesized directly in the tuber rather than by GAs imported from the leaves. Transgenic lines with the antisense SgA20x1 showed a reduction in the level of GA1. Tubers harvested from these lines developed shorter sprouts than those of untransformed lines, but no effect on dormancy duration was observed (Carrera et al. 2000).

The application of growth retardants that inhibit GA biosynthesis on in vitro tubers was used to study the role of GAs on tuber dormancy. The application of growth retardants resulted in a premature dormancy release different to the expected result if GA biosynthesis was required for breakage of dormancy (Suttle 2004b). Studies conducted with tubers of dwarf mutants, Solanum tuberosum, spp. andigena, showed that endogenous levels of GA3 were below the limit of detection during the entire period of postharvest dormancy. In the same tubers, sprout growth was severely reduced as compared to the growth of the wild-type potato, but the duration of dormancy remained unchanged (Suttle 2004b). These results are consistent with observations in transgenic lines by Carrera et al. 2000 (see above) and illustrate how the reduction of GA3 affects sprout growth, but not the duration of dormancy.

Although the role of GAs in dormancy regulation remains controversial, it has been observed that their artificial increase, either by exogenous injection or by ectopic expression of biosynthetic genes, typically results in premature sprouting of tubers. The endogenous level increases only when sprout growth commences; the GAs, therefore, seem to act preferentially in promoting the growth of non-dormant sprouts rather than in interrupting dormancy.

The studies of Bachem et al. (2001), discussed earlier, suggest that the levels of GAs are affected by the metabolism of ABA.

Cytokinins

Natural cytokinins are adenine derivatives and can be classified by the configuration of their N6-side chain as isoprenoid or aromatic cytokinins (Mok and Mok 2001).

It is thought that cytokinins stimulate cell division in plant tissues by releasing the G1 cell cycle block (Francis and Sorrell 2001). All cells in the dormant tuber meristem are arrested in the G1 phase of the cell cycle, and thus a role of...
of cytokinins in the termination of dormancy has been suggested. Studies have demonstrated that the application of cytokinins to dormant tubers may reduce the dormant period and elicit early sprouting. Endogenous levels of isopentenyladenine (IP) and trans-zeatin-type cytokinins increase in tubers prior to the onset of sprout growth (Turnbull and
Hanke 1985b; Suttle 1998). Moreover, injection of IP or trans-zeatin in dormant tubers accelerates the termination of dormancy and the start of sprout growth (Suttle 1998). Indeed the efficiency of these supplements depends on their concentration and on the physiological stage of the test tubers. When added immediately after harvest or during the initial period of storage, exogenous cytokinins are unable to stimulate sprouting. The effect is better documented when they are supplied during late storage; in this case the still-dormant tubers exhibit a dose-dependent premature sprouting (Turnbull and Hanke 1985a; Suttle 1998).

The role of these hormones in the regulation of dormancy is also supported by the observation that cytokinin overproducing plants expressing the gene for isopentenyltransferase (IPT), an enzyme involved in cytokinin biosynthesis, exhibit very early sprouting (Galis et al. 1995). Zubko et al. (2005) obtained similar results with potato transformed with the gene (Sho) coding for IPT that was isolated in Petunia hybrida. Overexpressing Sho resulted in an increase in the level of total cytokinins and in particular of \( \text{N}^6-(\text{–isopentenyl}) \text{ adenine (2iP).} \) Transgenic lines with the most pronounced cytokinin biosynthesis produced tubers with a reduced dormancy period and in some cases, with no dormancy at all (Table 1).

Morris et al. (2006) examined the effect of the expression of the bacterial gene \( \text{dxs,} \) encoding 1-deoxy-D-xylulose 5-phosphate synthase (DXS) in potato. This is a key enzyme in the isoprenoid biosynthetic pathway which produces a wide range of metabolites, including cytokinins. Transgenic tubers expressing the \( \text{dxs} \) gene showed a significant increase in the levels of trans-zeatin, associated with a reduction in the length of dormancy (Table 1).

Ethanol

The first evidence that ethanol can break dormancy was demonstrated with Jerusalem artichoke (Helianthus tuberosus) (Petel et al. 1993). Claassens et al. (2005) conducted a detailed analysis of the action of ethanol on dormancy and sprouting in potato tubers, studying the effects at the level of visible sprouting and gene activity. The experiments were conducted on \textit{in vitro} tubers grown in a medium containing 0.5% ethanol and with 1% or 8% of sucrose. Ethanol treatment in a medium with 1% of sucrose induced the growth of sprouts. Ethanol in combination with 8% of sucrose induced the growth of secondary tubers. In both cases, ethanol terminated dormancy, whereas the nature of the growth pattern was determined by the level of sucrose.

The effect of ethanol on sprout growth persisted even after the tubers were transferred to an ethanol-free medium. Ethanol vapours were able to induce sprout growth as well, and tubers stored under anaerobic conditions accumulated ethanol and showed premature sprouting. The effect of ethanol on \textit{in vivo} gene expression during the termination of dormancy was studied using potato luciferase (LUC) reporter lines. Particular attention was paid to genes involved in the regulation of the cell cycle and to storage-related genes. Reporter plants containing the promoter of the cell-cycle genes \( \text{cycB1;1} \) and \( \text{CDC2a} \) and the storage-related genes \( \text{AGPaseS} \) and \( \text{αPat21} \), involved in the synthesis of the storage protein patatin, fused to the coding sequence of the LUC reporter gene, were used.

In microtubers the expression of these genes, rapidly declined after the addition of ethanol with both 1% and 8% of sucrose in the medium (Fig. 4). This means that processes like cell division and synthesis of reserves, which are normally associated with tuber development, are blocked by ethanol. Ethanol affects the expression of the cell cycle and...
storage-related genes early, when there is no detectable effect on sprout growth. Down-regulation of gene expression is visible within 10 h after ethanol addition, whereas the growth of sprouts starts only 2-3 days after treatment.

In the apical bud, an up-regulation of cell cycle-related genes is observed when tubers are placed in the presence of ethanol with 8% of sucrose in the medium, which promotes the growth of secondary tubers, but not in the presence of ethanol and with 1% of sucrose which promotes sprout growth. Thus, a high activity of the cell cycle genes appears related to tuber development with no effect on resumption of sprout growth. In the presence of ABA, treatment with ethanol is not able to promote sprouting, and the activity of the cell cycle genes is not affected.

The shortening of dormancy in tubers stored in anaerobic conditions, with consequent accumulation of ethanol, suggests that alcohol dehydrogenase (ADH) may play a role during dormancy. Treatment of tubers with 4-methylpyrazole (4-MP), an inhibitor of ADH, inhibited sprouting and prevented ethanol-induced down-regulation of storage and cell cycle genes. These data suggest that ADH activity is important for breaking dormancy. However, the products of ADH have no effect: treatments with acetaldehyde or acetic acid do not induce sprouting or affect gene expression in the tubers. Moreover, the conversion of ethanol in aldehydes by ADH decreases the NADH level, which, as observed in other species (Gallais et al. 1998), promotes sprouting.

Reactive oxygen species

Generation of reactive oxygen species (ROS), including superoxide anions (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (OH$^-$), is ubiquitous in biological systems, and occurs either as unavoidable by-products of metabolic reactions or through signal-regulated processes under normal and stress conditions (Bolwell 1996, Reverberi et al. 2001). In plants, ROS levels are determined by some enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT); this last, in particular, is considered responsible for removal of excess of H$_2$O$_2$.

In potato tubers, dormancy release is accompanied by a transient but remarkable increase in H$_2$O$_2$ content, moreover the application of inhibitors of CAT or of exogenous H$_2$O$_2$ results in a reduction of the dormancy period and in rapid and synchronised sprouting (Bajji et al. 2007). The effects of CAT inhibition on potato tuber dormancy and sprouting were evaluated using the transgenic technology. Plants partially repressed in their CAT activity by antisense mRNA showed acceleration in sprouting kinetics in transgenic tubers compared to those from the wild type, confirming that accumulation of H$_2$O$_2$ plays a role in breaking dormancy (Bajji et al. 2007). It has been suggested that H$_2$O$_2$ may favour the oxidative pentose phosphate pathway (Fontaine et al. 1994) or would yield oxygen for respiration and other oxidation processes (Robert 1969) and for monooxygenases implicated in gibberellin biosynthesis (Fontaine-Roux et al. 1997). Other effects of H$_2$O$_2$ in cellular mechanisms involved in germination cannot however be excluded, in fact the endogenous level of H$_2$O$_2$ modulates the expression of many genes and is involved in the control of growth and differentiation (Penel 1997). In a recent review, Bailly (2004) has reported that the control of dormancy by hormones such as ABA and ethylene could be connected to H$_2$O$_2$ signalling and such kind of interplay constitutes a challenge for future research in this area.

METABOLIC EVENTS INVOLVED IN DORMANCY AND SPROUTING

As mentioned in the introduction, dormancy is strictly associated with tuber initiation and enlargement. One of the assumptions is that bud rest starts with the first phases of tuberisation when the tuber exhibits a high capacity to accumulate metabolites. As reported by Viola et al. (2001), tuberisation is accompanied with a switch from apoplastic to symplastic phloem unloading of sucrose. Symplastic unloading dramatically increases the intracellular concentration of sucrose with a strong impact on the metabolic and developmental processes. A high concentration of sucrose induces the transcription of several genes involved in tuber storage metabolism (Müller-Röber et al. 1990; Visser et al. 1994), which allows for the conversion of soluble assimilates (sugars and amino acids) into starch (starch and proteins). On the other hand, the apical bud of the stolon, synchronously isolated from the rest of the tuber, results in a reduction in cell division (Viola et al. 2001). These functional differences between apical bud and swelling tuber are consistent with differences in sugar distribution and acid invertase activity along the axis of tuberisation. A cell-wall acid invertase was strictly localised in the apical bud region of the stolon, whereas the soluble acid invertase was more abundant in the sub-apical region. The same authors proposed that sucrose is unloaded by symplastic communication in the swelling tuber and is then hydrolysed in the cytoplasm, whereas in the apical bud, sucrose hydrolysis occurs in the intercellular space. This hypothesis is supported by the expression of the gene invGE, coding for an apoplastic invertase, in the stolon bud (Viola et al. 2001).

A recent study, Viola et al. (2007) demonstrated that the availability of sugars is low in dormant buds, but increases greatly at the onset of sprouting. The availability of sugars during sprouting may be related with the establishment of symplastic connections between growing sprouts and the rest of tuber. Therefore, buds are symplastically isolated from the tubers during dormancy and this limits the sugar flux. At the end of dormancy, the symplastic connections between tuber parenchyma and buds are re-established, thus the nutrients can flow and growth starts. So far, changes in transcript profiles directly associated to sugar flux have not been found in growing sprouts.

Degradation of starch accumulated in the tuber parenchyma is the main source of energy for growing sprouts. However, not all the enzymes involved in starch degradation are equally involved in sprouting. Tubers of transgenic plants with a significant reduction of the R1 enzyme, involved in starch degradation, showed normal sprouting behaviour (Lorberth et al. 1998). On the other hand, transgenic plants in which the cytosolic isoform of a degrading starch phosphorylase was inhibited were not affected in their metabolism of carbohydrates, but displayed an increase in the number of sprouts and a shorter dormancy (Duwenig et al. 1997).

The sucrose formation and starch breakdown can be linked with the cellular level of inorganic pyrophosphate. Transgenic plants expressing an additional inorganic pyrophosphatase (Farré et al. 2001; Fernie and Willmitzer 2001) driven by a tuber-specific promoter, exhibited a shortened dormancy by 6 to 7 weeks. The inorganic pyrophosphatase would enhance the conversion of glucose-1-phosphate, resulting from starch breakdown, to UDP-glucose through UDP-glucose pyrophosphorylase by the removal of the inorganic pyrophosphate. This would increase the sucrose and cell wall biosynthesis required by the rapidly growing sprouts. However, it should be stressed that although these results were reproducible over a large number of tubers, premature sprouting was only observed over moderate enzymatic activity. Transgenic plants strongly expressing pyrophosphatase displayed the opposite phenotype, and in extreme cases they never sprouted (Hajirezaei et al. 1999) (Fig. 5). This would increase the sucrose and cell wall biosynthesis required by the rapidly growing sprouts. However, it should be stressed that although these results were reproducible over a large number of tubers, premature sprouting was only observed over moderate enzymatic activity. Transgenic plants strongly expressing pyrophosphatase displayed the opposite phenotype, and in extreme cases they never sprouted (Hajirezaei et al. 1999). It has been suggested that in this case the lack of sprouting was due to a complete shut-down of glycolysis for the inhibition of the pyrophosphate-dependent phosphofructokinase. Sugar availability is, therefore, crucial as an energy supply which drives sprout growth but the studies concerning metabolic regulation of sprouting are very few. Hajirezaei et al. (2003) suggest that sucrose level may act as a signal triggering the mobilisation of reserve compounds in storage parenchyma according to the sink demand of growing sprouts. Due to increased suc-
Based on these results, it was suggested that a low sucrose sequence, reserve mobilization in the transgenic tubers was impaired. A yeast invertase in phloem cells (Sonnewald et al. 2001) control of potato tuber sprouting. Trends in Plant Science 6, 333-335, with kind permission of Elsevier, Ltd., ©2001.

The removal of inorganic pyrophosphate (PPi), with formation of inorganic phosphate (Pi) catalyzed by an inorganic pyrophosphatase (PPase), is critical in two steps: in the conversion of glucose-1-phosphate (G1P) to UDP-glucose (UDPG) and in the conversion of fructose-6-phosphate (F6P) into fructose-1,6-bisphosphate (FBP). In presence of low level of PPi and high level of inorganic phosphate (Pi) FBP is converted to F6P and Pi to PPi and glycolysis is inhibited. Other abbreviations: ADPG: ADP-glucose; G6P: glucose-6-phosphate. Reprinted from Sonnewald U (2001) Control of potato tuber sprouting. Trends in Plant Science 6, 333-335, with kind permission of Elsevier, Ltd., ©2001.

Sucrose fluxes into phloem towards the bud (black arrows), but it is converted into hexoses by the invertase and it could not be utilized for sprouting. In consequence, although a high catabolic activity is present in the tuber the tuber converts proteins into aminoacids and starch into sucrose. The sucrose demand in developing sprouts, soluble sugar level decreases in storage parenchyma cells and this may act as a signal to drive the catabolism of reserves. To study the possible regulatory role of sucrose in potato tuber metabolism, the phloem transport of sucrose was blocked by the expression of a yeast invertase in phloem cells (Fig. 6). As a consequence, reserve mobilization in the transgenic tubers was highly accelerated, but sprouting was strongly impaired. Based on these results, it was suggested that a low sucrose level may trigger starch mobilisation, but sucrose flux toward buds is necessary to promote sprout growth.

**SEARCH FOR NEW GENES INVOLVED IN DORMANCY REGULATION**

Transcriptome analysis has been used to mine genes involved in potato dormancy. A schematic representation of the different methodologies used and the results are shown in Fig. 7. Agrimonti et al. (2000) made use of Differential Display Reverse Transcriptase PCR (DDRT-PCR) as described by Liang and Pardee (1992) to isolate genes that are up- or down-regulated during dormancy and sprouting in potato tubers: two cDNA clones were isolated and named G1-1 and A2-1 (Table 2). These cDNAs hybridize with mRNAs expressed at a low level in the tuber; G1-1 is turned on at the end of dormancy, while A2-1 is turned off. Sequence analysis of G1-1 shows some similarities with an expressed sequence tag (EST clones) of the Solanaceae family. Agrimonti et al. (2007). In *silico* analysis showed that A2-1 encodes for a putative ATPase with the Walker box ATP domain that is characteristic of a superfamily of proteins displaying a remarkable diversity of functions, such as active transport of low-molecular-weight compounds and plasmid partitioning in bacteria (Koonin 1993). Transgenic tubers, in which G1-1 and A2-1 were inactivated by antisense technology, were produced and analyzed. Statistical analysis on the length of dormancy in transgenic lines for the antisense G1-1 gene showed a significant increase. Conversely, A2-1 antisense plants did not reveal any significant change of dormancy (Marmiroli et al. 2000). Analysis with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) performed on G1-1 antisense transgenic tubers revealed that two small proteins, of 4292 and 4371 Da, disappeared after inactivation (Careri et al. 2003). The G1-1 expression increased in tuber buds during the first stages of sprouting, whereas almost no transcript was found in the parenchyma (Agrimonti et al. 2007). The highest levels of expression were detectable in sprouts and in developing sink leaves. In *situ* hybridization on potato tissues showed that the G1-1 transcript was mainly localized in the tunica, corpus and pro-cambium tissues of growing sprouts (Fig. 8). To get more insight into the function of the G1-1 gene, the authors chose a correlative approach by comparing the expression of its tomato homologues (LeG1-1 and LeG1-2) during seed germination. The expression of these sequences was highest after the primordial roots emerged from the teguments. Indeed, LeG1-1 and LeG1-2 transcript levels were low in meristematic structures formed ex novo in the *in vitro* culture of tomato seeds. These results suggest that G1-1 and its homologues may be responsible for activation and growth of pre-existing meristems, but not for their *de novo* formation (Agrimonti et al. 2007).

Faivre-Rampant et al. (2004a) used suppression subtractive hybridization to produce a library of genes enriched with those up-regulated on the release of dormancy. A total of 385 different sequences was isolated: approximately 12% of which were similar to ribosomal proteins, which may reflect a resumption of biosynthetic activity on release from dormancy. Heat shock proteins or those with similarity to chaperonines made up some 9% of the sequences. A relatively high percentage of carrier proteins (4%) and transcription factors (3%) were also identified within these sequences. The possible role of an auxin responsive factor, isolated in the subtractive library was discussed earlier. The remaining 36% of these sequences were unknown or matched with unknown proteins.

An EST containing TCP bHLH, a domain characteristic of a family of transcription factors, TCP (Cubas et al. 1999) was isolated within a BAC clone (Faivre-Rampant et al. 2004b). The steady state level of mRNA for this sequence, called *step1*, was higher in dormant than in sprouting buds and therefore its expression pattern correlates with inactivity of both apical and axillary meristems in potato.
Genetic regulation of dormancy and sprouting. Agrimonti and Marmiroli (2000) used cDNA-AFLP methodology (Bachem et al. 1996) to isolate genes differentially expressed during the life cycle of a tuber in a tuberisation and dormancy synchronised system. Three transcripts derived fragments (TDFs), related to the tuber life cycle showed homology to plant nsLTP genes, coding for a class of proteins capable of binding lipid compounds in plant tissues (Horvath et al. 2002). The function of these proteins remains substantially unknown; Sterk et al. (1991) suggests that they possibly have a role in transferring lipophylic compounds in the apoplast for epicuticular wax formation.

The expression profile of the three nsLTP related potato TDFs shows a short induction in the stolon just prior to tuber formation and increased expression during sprout development. Histological analysis of transgenic tubers, carrying the GUS gene fused with the promoter of one of the three related nsLTP TDFs, showed that GUS activity was concentrated in the vascular bundles that lead to the eyes. The activity increased in the vascular system of growing sprouts after the end of dormancy. These genes may have a role in maintaining dormancy and in regulating sprouting, but in potato plants expression of their antisense RNA was not associated with phenotypic differences, as compared to the untransformed controls, and thus the real function of nsLTP is still undetermined.

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Analysis of the expression profiles during the life cycle of the potato tuber, reported by Trindade et al. (2004), led to the isolation of 412 TDFs from dormant tubers. Eighty eight percent were expressed in the early stages of dormancy, 84% during the late dormancy and 78% during sprouting. The majority of TDFs was expressed during the first phase of dormancy (28%), followed by last period of dormancy (19%) and only 7% were differentially expressed during sprouting. The conclusion from these studies was that the number of expressed genes decreases as the life cycle progresses, with a slight increase during sprouting, but most of these genes showed a profile of constant expression. This agrees with the idea that a large number of genes are required for the metabolic processes occurring during the potato life cycle, and also with the notion that more genes are expressed during tuber induction and tuber growth than during dormancy and sprouting. Expression of several genes during tuber formation is altered by the addition of gibberellic acid, the effect resulted in a different expression profile and/or level, and in other cases a delayed expression of some sequences (Trindade et al. 2004).

More recently, a large number of genes that govern the developmental characteristics of potato have been isolated.
using a high throughput approach. Two big projects to isolate and sequence ESTs were conducted by the Institute for Genomic Research (TIGR) (Ronning et al. 2003) and by the Canadian Potato Genome Project (CPGP) (Flinn et al. 2005). Sequenced libraries represented the transcripts of tubers and flowers at different stages of development, leaves, late and sequence ESTs were conducted by the Institute for genomic research (TIGR) (Ronning 2005). Sequenced libraries represented the transcripts of Canadian potato genome project (CPGP) (Flinn et al. 2008). Campbell et al. (2008) utilised TIGR microarray analysis and quantitative real-time PCR (qRT-PCR) to examine gene expression changes in potato tuber meristems both allowed for the termination of dormancy spontaneously after storage, or exposed to the synthetic inducer BE.

Natural progression of dormancy to sprouting resulted in a greater number of down-expressed than up-expressed cDNAs. Even though the interpretation of this difference is problematic, it is substantially consistent with the observations of Trindade et al. (2004), discussed earlier. Characterisation of the cDNAs by putative function indicates that there are no large functional differences between down- or up-regulated transcripts during dormancy termination (Fig. 9). It appears conceivable that there is no global metabolic shift associated with the end of dormancy in tuber meristems, consistent with the observation of Bachem et al. (2000) who also failed to observe any gross changes in gene expression in whole microtubers during the progression of dormancy. However, changes observed in the expression of genes related to the accumulation and mobilization of the storage molecule e.g. patatin, are indicative of the shift of tuber metabolism from sink-to-source condition. In growing sprouts mobilisation of storage proteins is accompanied by an increase in expression of cDNAs encoding cysteine protease in growing sprouts (Ronning et al. 2003) along with a decrease in the expression of protease inhibitors of metallo-carboxypeptidase, cysteine protease, aspartic protease, as well as in a number of other unspecified protease inhibitors. Thus, termination of dormancy in tuber meristems is associated with a decreased expression of inhibitors of all major classes of plant proteases (Callis 1995; Schaller 2004). Other genes induced by ABA are down regulated at the end of dormancy and they have been discussed in the paragraph.

Fig. 8 In situ hybridization of G1-1 gene on potato tuber tissues during transition from dormancy to sprouting. A purple-brown stain indicates the presence of G1-1 transcript. (A) Apical bud of dormant tuber (T0); (B) Apical bud of tuber stored for 3 weeks at 20°C (T1); (C) Apical bud 1 mm long; (D) Apical bud 1 mm long hybridized with sense riboprobe; (E) sprout apex; (F) sprout apex hybridized with sense riboprobe. Samples E and F were visualized in dark-field microscopy. Abbreviations: AM: apical meristem; AXM: axillary meristem; LP: leaf primordia, PC: procambium; P: parenchyma. Reprinted from: Agrimonti C, Visioli G, Bianchi R, Torelli A, Marmiroli N (2007) G1-1 and LeG1-1/LeG1-2 genes are involved in meristem activation during breakage of dormancy and early germination in potato tubers and tomato seeds. Plant Science 173, 533-541, with kind permission of Elsevier, Ltd., ©2007.

Fig. 9 Results of high throughput expression analysis of sequences isolated during transition from dormancy to sprouting. Functions encoded by cDNAs that exhibit twofold increase or decrease in expression during transition from dormancy to sprouting in tubers under storage conditions are indicated. Data obtained from: Campbell M, Segear E, Beers L, Knauber D, Suttle J (2008) Dormancy in potato tuber meristems: chemically induced cessation in dormancy matches the natural process based on transcript profiles. Functional and Integrative Genomics 8, 317-328.
above.

In comparison to down-regulated cDNAs, there are fewer genes exhibiting an increase in expression after dormancy termination. During dormancy, tuber meristems are arrested in the G1 phase of the cell cycle, while a resumption of cell cycle activity and the resultant cell division accompanies the onset of sprout growth (Campbell et al. 1996). A number of cDNAs, including ovule/fiber elongation, protein-degradation, actin, B2, H3 and H4, map kinase, and γ-tubulin encode proteins that have possible functions in cell division and growth, and all exhibited increased expression in sprouting meristems. These data support previous studies that have shown that acetylation patterns of histone proteins change as potato dormancy terminates (Law and Suttle 2004) and suggest that altered histone acetylation may be indicative of de novo synthesis of histone proteins.

A cDNA encoding for an oxoglutarate-dependent dioxygenase (GDO) exhibits significant increase in expression at termination of dormancy. GDO is a large group of proteins involved in the incorporation of molecular oxygen during catabolic and anabolic pathways (de Carolis and de Luca 1994). The ODO cDNA found in potato meristems has a high similarity to Solanum chacoense 2-oxoglutarate-dependent dioxygenase (SP22) and Lycopersicon esculentum 2-oxoglutarate-dependent dioxygenase (GAD2). SP22 encodes a transcript that is expressed during the pollen pistil developmental response (Lantin et al. 1999). GAD2 is regulated by GA and ABA interactions in tomato, and therefore, the expression of this homologue may be associated with a hormonal shift during dormancy termination (Jacobsen and Ne 1996).

To better understand the molecular aspects of transition from dormancy to sprouting, the analysis of specific protein synthesis during the process has been performed. Borgmann et al. (1994) analysed the protein pattern in potato tubers during the sink-to-source transition by two-dimensional electrophoresis (2-DE). Of 1072 polypeptide detected, 296 (28%) were specific to the sink tubers, 185 (17%) were uniquely associated with the source tubers and 591 (55%) were common to both developmental stages. Some of the sink specific proteins were identified as patatin isoforms and as sucrose synthase, a key enzyme involved in carbohydrate metabolism. Conversely, source specific proteins resulted associated with the membranes suggesting that the sink to source transition may be related to different transport characteristics of “sink” as compared with “source” tubers. These results were substantially confirmed by Espen et al. (1999) who analysed protein profiles in the soluble and microsomal fraction of parenchymatic tissues of tubers during the final period of growth and during storage. Many of changes in polypeptide profile were detected in the microsomal fraction, suggesting that the changes in cell membrane functionality during the different phases of the tuber life cycle could be linked also to the presence of specific proteins on cell membranes. As the sugar unloading in sink tubers seems to occur symplastically and the apoplastic loading in source involves active transport mediated by carrier proteins (Wright and Oparka 1989; Viola et al. 2001) changes in the proteins associated with membranes may be expected.

Some of the polypeptides that were no longer detected in mature tuber appeared again during storage, suggesting that the dormancy, characterised by the drop of some metabolic activities might be due to a lack of synthesis of specific proteins.

Significant changes in protein synthesis during the potato tuber life cycle, including development, dormancy, storage and sprouting, were also recently reported by Le- hesranta et al. (2006). Proteomic profiling conducted by 2-DE combined with multivariate analysis showed that the different stages of tuber life are characterised by peculiar sets of proteins. Noticeably, sprouting tubers are characterised by a very peculiar profile. The identification of polypeptides, conducted by high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) confirmed the results mentioned above, highlighting the active synthesis of storage proteins and enzymes related to primary and secondary metabolism in developing tubers. The synthesis of these proteins decreases during storage and sprouting, according to a general drop out of biosynthetic metabolism.

CONCLUSIONS

The identification of genes that regulate the transition from dormancy to sprouting has increased our understanding of the physiological bases of the process. As expected, some genes are involved in hormone metabolism and response, and others are involved in mobilisation of reserve substances. Recently, the application of new tools has resulted in an overall picture of the transcription profile during tuberisation, dormancy and sprouting. Comparisons of the results obtained by different authors have identified some common features: 1) transcriptional activity is highest during tuberisation but decreases during dormancy and sprouting; 2) the end of dormancy is not accompanied by a significant increase in transcriptional activity; 3) many genes, up- and down-regulated at the end of dormancy, are unknown or coded for unknown proteins; 4) no known genes related with the cell cycle appear regulated during the resumption of bud growth, even though modifications in methylation and acetylation of histones, events that usually precede the transcriptional activation of genes leading to cell division and meristem growth, have been observed.

Proteomic analysis of the potato tuber life cycle evidenced a general decrease of proteins related to active metabolism in the source tubers with respect to sink tubers, reflecting a general decrease in biosynthetic metabolism after tuber maturation. The sink-to-source transition is associated with changes of membrane proteins, probably related to the modification of metabolite flux in the different conditions.

In general all the gene sequences and proteins isolated provide a robust background for future research, but the comprehension of the overall process of dormancy and sprouting is still far because many of these sequences have unknown functions or encode for proteins apparently not related to the process. Silencing or over-expression of these sequences in transgenic plants does not always give clear results. Surely, integration of all the “omics” approaches is required: transcriptomics and metabolomics as well as proteomics together with the transgenic approach can help in understanding the role of these sequences. Additionally, genetic approaches based on identification of genes underpinning QTL should not be neglected to understand the global process of dormancy and sprouting regulation.

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