Molecular and Biochemical Triggers of Potato Tuber Development

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In the present climate of functional elucidation of entire genomes by technologies comprising mass sequencing, insertional mutagenesis, and RNA expression profiling analysis, the weed Arabidopsis has rapidly established itself as the foremost plant species among plant scientists worldwide. One of the consequences of the focus on Arabidopsis is that an old theme "what is true for *Escherichia coli* is true for the elephant" is receiving increasing popularity in its modified version "what is true for Arabidopsis is true for all plants." Although this is undoubtedly true for many aspects of plant development, there are still a substantial number of developmental processes that can only be studied in specific plant systems. The tuber life cycle of potato (*Solanum tuberosum*) plants represents an example of a developmental system that cannot be studied in model systems such as Arabidopsis.

WHY STUDY POTATO TUBERS?

Potato is one of the most important crops worldwide: ranking fourth in annual production behind the cereal species rice (*Oryza sativa*), wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*). Although in Europe and North America the consumption of potatoes is mainly in the form of processed foodstuffs such as fried potatoes and chips, in less-developed countries it represents an important staple food and is grown by many subsistence farmers. The main reasons for the increasing popularity of the potato in third-world countries are the high nutritional value of the tubers combined with the simplicity of its propagation by vegetative amplification.

In addition to its clear importance for food and feed, the tuber also represents the starting material for the next generation of plants (so-called seed tubers). It is for this reason that processes related to tuber formation, storage, and sprouting have been studied intensively over many years; but because all potato varieties are true tetraploids and display a high degree of heterozygosity, genetics have played only a minor role in studying this process. However, because the potato is a member of the Solanaceae family, it was among the first crop plants to be accessible for transgenic approaches.

A TUBER IS NOT A MODIFIED ROOT BUT A MODIFIED STEM

In contrast to the widespread misconception, potato tubers do not develop from roots but are, in fact, underground stems with shortened and broadened axes. Tubers are derived from lateral underground buds developing at the base of the main stem that when kept underground develop into stolons due to diagravitropical growth (Fig. 1). When the conditions are favorable for tuber initiation (see below for details), the elongation of the stolon stops, and cells located in the pith and the cortex of the apical region of the stolon first enlarge and then later divide longitudinally. The combination of these processes results in the swelling of the subapical part of the stolon. When the swollen portion has attained a diameter of approximately 2 to 4 mm, longitudinal division stops and is replaced by randomly oriented divisions and cell enlargement. These occur primarily in the perimedulla and continue until the tuber reaches its final mass (Xu et al., 1998). The complexity of the tuber with respect to its different tissues is significantly less than that observed in, for example, seeds. Furthermore the developmental program is much more flexible, for example the final size of the tuber may vary by more than 100-fold.

IN VITRO SYSTEMS STARTING FROM NODAL EXPLANTS OFFER SYNCHRONOUS TUBER FORMATION

Tuber development and related processes are difficult to study in the field and/or in soil-grown plants due to a low level of synchrony of the tuberization process under these conditions and to the obscuring effect of soil. To circumvent this problem, in vitro methods have been developed that allow synchronous tuber formation to occur with a high frequency (Appeldoorn et al., 1997; Coleman et al., 2001). These systems essentially consist of a single nodal stem explant that will result in the differentiation of the axillary bud into a tuber instead of a leafy shoot when placed on tuber-inducing medium (characterized by a high Suc content and in some instances supplemented with the antigibberellin CCC and a

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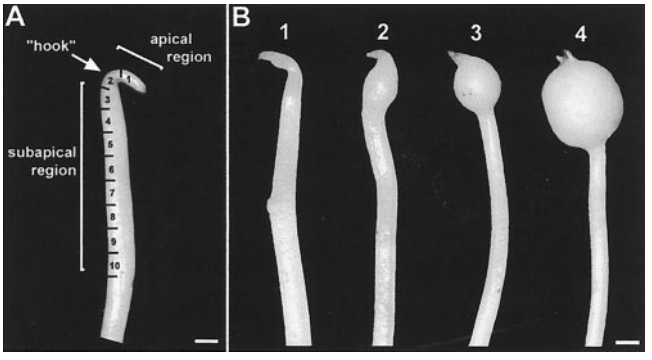

Figure 1. Tuberizing stolon tips taken from a single plant. A number of distinct stages in swelling process are apparent, ranging from no swelling (1) to prominent subapical swelling (4). Note the progressive incorporation of the apical bud. Bar = 1 mm. Reprinted from Viola et al. (2001).

cytokinin) and incubated in darkness. At least at the levels of ultrastructure and the relative activities of enzymes of carbohydrate metabolism (Veramendi et al., 1999), this system has been shown to be comparable to soil-grown tubers. Although the usefulness of these in vitro systems is undisputed, it is important to keep in mind that by their nature they show little or no response to photoperiod, the factor that most influences tuberization.

Tuber induction, initiation, enlargement, dormancy, and sprouting represent the typical life cycle of a potato tuber. In the following paragraphs we will follow this developmental scheme to outline our present understanding of the various processes with respect to biochemical and molecular triggers. Some emphasis will be given to transgenic approaches.

INDUCTION OF TUBERIZATION INVOLVES A MULTITUDE OF ENVIRONMENTAL AND ENDOGENOUS FACTORS

It has been long known that various environmental and endogenous factors influence tuberization. Thus induction of tuberization is favored by long nights (short photoperiods), cool temperatures, low rates of nitrogen fertilization, and more advanced "physiological age" of the seed tuber (Fig. 2; for a detailed review, see Jackson, 1999).

With respect to the involvement of hormones, there are many reports in the literature describing the importance of gibberellic acid (GA), cytokinin, jasmonic acid and related compounds, or abscisic acid (ABA) for tuber induction. These data are in places somewhat contradictory, however, one clear and substantiated observation is that GA levels decline during tuber induction. Furthermore, in plants in which tuberization has been environmentally stimulated (for example by elongating the length of the dark period), tuberization can be prevented by exogenous application of GA. These observations are in line with elegant transgenic studies in which modulation of the endogenous gibberellin oxidase activity resulted in plants displaying altered GA content. Elevation of GA by the overexpression of the GA oxidase led to transgenic potato plants that required a longer duration of short-day photoperiods to form tubers, whereas antisense inhibition of this enzyme resulted in plants that tuberize earlier than control plants (Carrera et al., 2000) when grown under short days though they still did not tuberize under long days. When taken together, the results of these different approaches suggest an unequivocal role for GA in tuber induction though it may still not be the whole story.

Exogenous applications of cytokinin either to in vitro system or by direct application to stolons of developing potato plants has also been reported to increase speed of tuber induction. However, when the levels of this hormone were elevated by transgenesis, the resultant transformants displayed an increased endogenous level of cytokinin but were characterized by a complex developmental pattern with differences in both tuber morphology and sprouting (Galis et al., 1995). The newest kids on the block of phytohormones and tuber induction are jasmonic acid and its derivatives. Tuberonic acid, which is chemically very similar to jasmonic acid, was observed to have strong tuber-inducing properties during in vitro conditions and as such was favored for a long time to be the tuber-inducing signal (Jackson, 1999). This hypothesis has been tested by elevating the endogenous levels of jasmonic acid either by the expression of an allene oxidase cyclase (Harms et al., 1995) or by direct application of jasmonic acid onto potato leaves (Jackson, 1999). It is surprising that neither approach had any effect on tuber induction. Transgenic potato plants displaying a reduced activity of one isoform of lipooxygenase were, however, characterized by a much lower number of distorted tubers and by a failure to respond to conditions normally favorable for tuber induction using leaf bud cuttings from intact plants (Kolomiets et al., 2001). It is unfortunate that no biochemical analysis of the effect of the reduction of lipooxygenase activity on the composition of possible products of the LOX pathway was performed, although, irrespective of

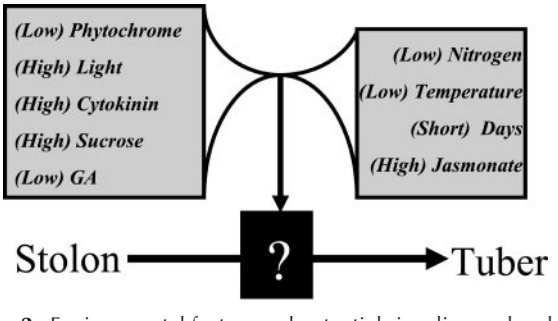

Figure 2. Environmental factors and potential signaling molecules in the induction of tuberization. The qualifying term given in brackets identifies the condition favoring tuber induction.

this shortcoming, these data suggest an involvement of some of the lox-derived metabolites in the induction of tuber formation/tuber enlargement. The evidence that ABA has a role in tuber induction is less convincing than for cytokinins and jasmonic acid derivatives (Jackson, 1999).

Another important aspect of the induction of tuberization is the long-standing observation that the stimulus is received in the leaves of the plant and is graft-transmissible (Gregory, 1956). Furthermore, the tuber-inducing stimulus and the flowering stimulus must be related or at least similar, because grafting of a flowering-induced plant of tobacco onto a potato scion leads to the formation of tubers. Although the precise nature of this signal is as yet unknown, there is very convincing evidence from transgenesis studies that phytochrome B is involved in the production of a graft transmissible inhibitor of tuberization (Jackson et al., 1998). Another member of the phytochrome family, Phy A, has recently been demonstrated, via the use of transgenic potato plants, to be involved in resetting the circadian clock and delaying tuber formation under noninducing conditions (Yanofsky et al., 2000). Thus, it appears that a concerted action of both phytochromes is involved in the repression of tuber induction. The requirement for high levels of Suc for successful tuber induction in in vitro systems suggests that it may also play a role in the induction process. It is however a difficult task to resolve the importance of Suc for tuber induction using a genetic approach. Although plants with inhibited Suc transporter activity show a significantly reduced level of tuber formation, this effect cannot be attributed to a direct consequence of lowered Suc delivery. The reduced amount of carbohydrate availability leads also to a reduced development of other sink organs of the plant (Riesmeier et al., 1994).

TUBER INITIATION AND ENLARGEMENT

In addition to the changes in morphology and cell division that occur in early phases of the stolon-tuber transition, tuber initiation and enlargement are accompanied by massive changes in the physiology and metabolism. During enlargement tubers become the largest sink of the potato plant storing massive amounts of carbohydrates (mainly starch) and also significant amounts of protein. Furthermore tubers decrease their general metabolic activity and as such behave as typical storage sinks.

CHANGES ON THE PROTEIN LEVEL

About 2% of the fresh weight of a potato tuber is present as protein whereas between 15% and 25% is represented as starch. The protein composition changes dramatically during stolon-tuber transition resulting in the formation of a much-simplified protein complement consisting of only a few highly abundant proteins such as patatin. A number of experiments aimed at the identification of proteins expressed specifically during early stages of tuber development and being responsible for, or at least causally linked to, tuber development. Early experiments involving the most abundant proteins including patatin and various proteinase inhibitors clearly ruled out a role for these proteins in tuber initiation. More recently, extensive cDNA amplified fragment-length polymorphism-based analysis of various stages of tuber enlargement has led to the identification of numerous genes that may play a role in tuber enlargement (Bachem et al., 1996). Transgenic plants in which the expression of two of these candidate genes, one exhibiting homology to steroid dehydrogenases and the other to α-soluble *N*-ethylmaleimide-sensitive factor attachment protein, have been independently altered, however, show either only minor changes in their tuber development or rather pleiotropic changes that make it difficult to assess their role in tuber development (Bachem et al., 2000a, 2001).

CARBOHYDRATE METABOLISM

Starch Biosynthesis Is Not Needed for Tuber Formation

In addition to changes in the protein composition, the most pronounced change observed during very early stages of tuber initiation and enlargement is the massive formation of starch, which in the mature tuber typically represents 20% of the fresh weight. Given this massive change in metabolism and considering the fact that a high supply of carbohydrates such as Suc to the developing stolons has been identified as a condition favoring tuber induction, it is not too surprising that for a long time starch formation was another parameter discussed as being required for tuber initiation and enlargement. Mainly based on transgenic approaches it is, however, clear that starch formation is not required for these processes. The most direct data result from potato tubers in which the ADP-Glc pyrophosphorylase was reduced by antisense repression and that display a significantly reduced starch level. These plants display normal tuber formation. The only change observed is that the number of tubers increased and the size of the individual tuber decreased, which might indicate a change in the competition between various sinks (Müller-Röber et al., 1992).

Suc Is the Major Form of Photo-Assimilates Delivered to the Tuber

As described above, soluble carbohydrates, most notably Suc, have convincingly been described to be strong inducers of tuberization. However, whether the path of Suc delivery was via symplastic or apoplastic unloading has been controversially discussed over many years. In a series of very elegant studies

using a combination of fluorescent dyes and radioisotope labeling, Viola et al. (2001) obtained clear evidence that both symplastic and apoplastic unloading play a role for Suc delivery into the developing tuber. Using this approach they were able to demonstrate that concomitant with the first visible sign of tuber initiation, there is a switch from predominantly apoplastic unloading into stolons that are undergoing extension growth toward predominantly symplastic unloading into tubers. Thus the early stages require a specific unloading mechanism such as an active Suc transporter whereas in the later stages an active transport mechanism is not required. In the case of symplastic unloading Suc should be metabolized within the cytosol. A detailed study of the activity of the two potential sucrolytic activities, invertase and Suc synthase, unequivocally showed that whereas acid invertase predominates during early stages of tuberization, Suc synthase becomes the major sucrolytic activity once starch formation becomes the major sink for the incoming Suc (Appeldoorn et al., 1997). This switch in sucrolytic activities appears to closely parallel the switch in unloading mechanism. The import and subsequent metabolism of carbon into the tuber during tuber initiation and enlargement is summarized in Figure 3, A and B, respectively. That Suc synthase is the predominant sucrolytic activity in the developing tuber makes it a reasonable assumption that its activity is crucial for the further enlargement of the tuber. This assumption was clearly confirmed in transgenic potato plants displaying a reduced activity of the major isoform of Suc synthase (Zrenner et al., 1995) resulting in reduced tuber number and dry weight.

On the subsequent pathway to starch formation, the importance, with respect to tuber development and/or starch formation, of several more enzymes has been tested via transgenesis. It is intriguing that the only other protein that was shown to exert massive effects on tuber development was the ATP/ADP translocator, a protein localized in the inner membrane of plastids. Reducing its activity in transgenic plants led to a considerable reduction in tuber number, starch content, and a massive change in tuber morphology (Tjaden et al., 1998).

In stark contrast to the weak response of tuber development with respect to reductions in the expression levels of most of the proteins involved in catalyzing the Suc-starch transition are the observations made in transgenic plants in which Suc mobilization was modulated. Increasing Suc mobilization by the expression of heterologous invertase or Suc phosphorylase in the cytosol of transgenic potato tubers led to massive changes in metabolism characterized by a strong increase in glycolysis and respiration. Furthermore, expression of the same invertase gene behind an apoplastic targeting sequence resulted in a reduced tuber number but a dramatic increase in tuber size (Sonnewald et al., 1997). Further data suggest that the cytosolic level of Suc in the tuber is an important parameter for the development of the potato tuber to become a storage sink, and any changes brought about by lowering this level result in massive rearrangements of the metabolism of the tuber. A further class of chemicals that have long been postulated to play a role during tuber development are the polyamines. The results of a recent transgenesis study in which *S*-adenosyl-Met decarboxylase was increased leading to an increase in the

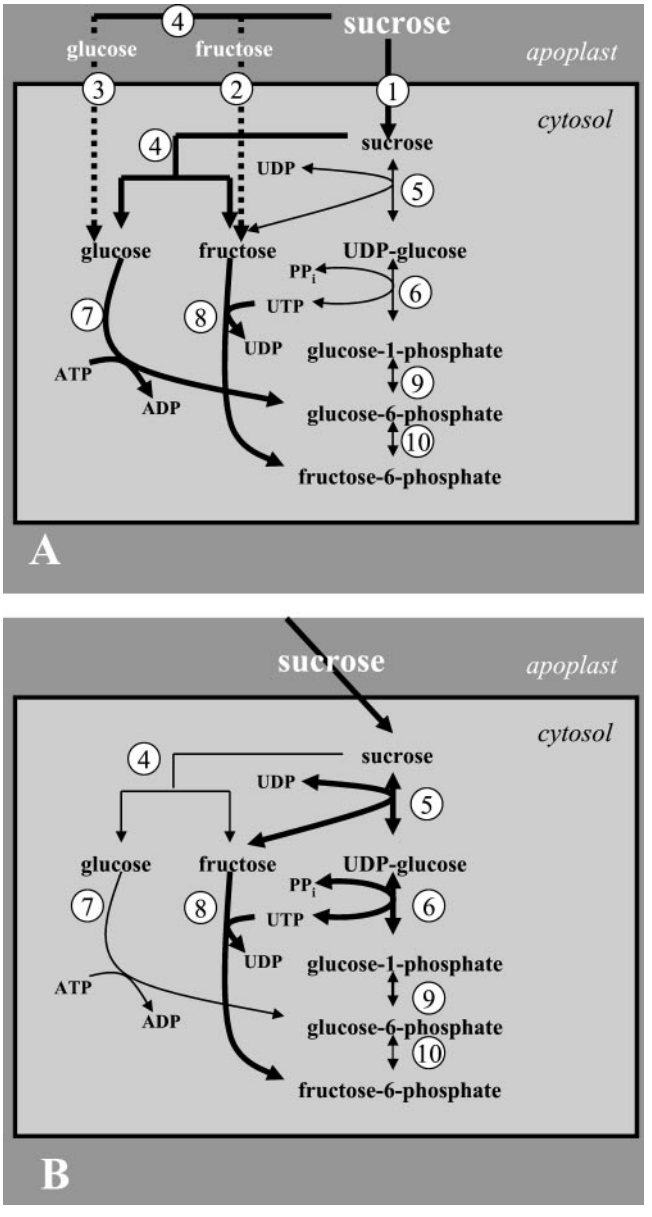

Figure 3. The predominant route of Suc unloading and subsequent mobilization. A, Prior to tuber initiation. B, During tuber enlargement. The numbers denote the following enzymes: 1, Suc transporter; 2 and 3, hexose transporter(s); 4, invertase; 5, Suc synthase; 6, UDP-Glc pyrophosphorylase; 7, hexokinase; 8, fructokinase; 9, phosphoglucomutase; and 10, phospho-Glc isomerase. The thickness of the arrow indicates the predominant flux.

level of polyamines accompanied by an increased tuber number and a decrease in tuber size are consistent with these postulates (Pedros et al., 1999).

DORMANCY AND SPROUTING

As described in the introduction, the life cycle of a potato tuber is characterized by initiation and growth followed by a period of dormancy and finally sprouting resulting in the next (vegetative) generation. Here we will treat the dormancy and sprouting period jointly as many aspects and parameters influencing dormancy will either directly or indirectly influence sprouting.

It is important to note that the period of dormancy cannot cleanly be separated from tuber initiation and enlargement. Rather, one has to realize that tuber initiation already depends upon the apical meristem becoming dormant as soon as the longitudinal cell division in the stolon tip arrests and is replaced by cell divisions in the fourth to the eighth node. The buds in the eyes of the tuber become dormant successively with the apical eye being the last one to join (Xu et al., 1998, and refs. therein). Thus, dormancy is a process that largely parallels tuber enlargement.

Given this fact, it is not too large a surprise that of the various factors that are discussed with respect to influencing tuber induction, many have also been described to influence dormancy and sprouting. Because a very detailed review of these factors has appeared recently (Claassens and Vreugdenhil, 2000) we will only shortly summarize the main findings of this review and then concentrate on more recent and more neglected observations. With respect to macroparameter, as a rule, low temperatures lead to longer periods of dormancy. Furthermore, dormancy periods are influenced by the history of the plant that produced the tubers (photoperiod) and by its genotype. As is the case for tuber initiation, the clearest role of phytohormones in dormancy can again be assigned to gibberellins. There is very convincing evidence from many laboratories that dormancy of tubers during storage can be broken by exogenous application of GA. This is in keeping with observations of premature sprouting from tubers of transgenic plants overexpressing a GA-20-oxidase and thus displaying increased levels of gibberellins (Carrera et al., 2000). ABA, the well-known antagonist of GA, has long been studied; however, there is conflicting evidence as to its importance for tuber dormancy.

The main observations that imply a role of ABA in dormancy are correlative evidence, supplied by several groups, that at the end of the dormancy period, levels of ABA decline. These data suggest that at a certain level, ABA is important to maintain dormancy and its role is therefore analogous to that during seed development. Furthermore, this observation is in keeping with the finding that ABA synthesis inhibitors lead to precocious sprouting. The potential role of ABA is further supported by the observation that three of eight quantitative trait loci mapped as influencing dormancy behavior also influence ABA levels (Claassens and Vreugdenhil, 2000). Thus, ABA might play a role in reaching full dormancy, but in contrast to the clear action of GA action, the exact role of ABA remains somewhat mysterious. Cytokinins are reported to have the ability to break dormancy, however the tubers might only be competent within a certain time window. The supposed role of cytokinins is in agreement with the observation that cytokinin overproducing plants that express the ipt gene are characterized by very early sprouting (Galis et al., 1995). Much less attention has been paid to the role of auxins in these processes, although again some correlative evidence has been reported between sprouting and IAA levels. A role for the hormone ethylene has been postulated, however, on the basis of studies involving application of ethylene synthesis inhibitors such as $AgNO_3$ or applying exogenous ethylene, which led to the conclusion that ethylene is involved in establishing and keeping tuber dormancy (Claassens and Vreugdenhil, 2000). Jasmonic acid has also recently been discussed as being important for sprouting; however, the generation of transgenic plants with modulated levels of JA did not provide any support for this hypothesis.

The other main area discussed in connection with sprouting and dormancy addresses the concomitant changes in carbohydrate. Thus, starch degradation has been discussed as an important event related to the induction of sprouting. Although there is no doubt that a sprouting tuber needs to obtain energy from the mother tuber (most of which is derived from starch degradation), it is worth mentioning that transgenic potato plants that show a significantly reduced expression of the R1 enzyme, which has been demonstrated to be involved in starch degradation, show a normal sprouting behavior (Lorberth et al., 1998). The importance of activities of enzymes associated with carbohydrate polymers was, however, highlighted by the phenotype of transgenic plants in which the cytosolic isoform of starch phosphorylase was inhibited characterized by increased number of sprouts and also reduced dormancy (Duwenig et al., 1997).

The most compelling, albeit surprising, result with respect to dormancy and sprouting was observed when analyzing transgenic plants expressing an additional inorganic pyrophosphatase. When driven by a tuber-specific promoter and within a certain expression level of pyrophosphatase, these plants sprouted 6 to 7 weeks earlier than control plants (Farré et al., 2001). The reason underlying this dramatic and significant change is presently unclear. One hypothesis is based upon the central role pyrophosphate plays by linking Suc formation and starch breakdown. The presence of an inorganic pyrophos-

phatase would enhance the conversion of Glc-1-phosphate resulting from starch breakdown to UDP-Glc through UDP-Glc pyrophosphorylase by the removal of the inorganic pyrophosphate formed. This would lead to enhanced Suc and/or cell wall biosynthesis required by the rapidly growing sprout. It should be stressed that although these results were very clear and stable over three generations the phenotype is seemingly only observed over a specific range of activity. Transgenic plants strongly expressing an pyrophosphatase under a different promoter display the opposite phenotype, i.e. they never sprouted (Hajirezaei and Sonnewald, 1999). The authors suggest that in this instance the lack of sprouting was due to a complete shut down of glycolysis due to the inhibition of the action of the pyrophosphate-dependent phosphofructokinase. In addition to Suc and starch, hexoses have been discussed as being of importance to sprouting. Anecdotal evidence based on the observation of transgenic lines expressing a cytosolic invertase is in accord with this supposition. However, because these transgenic plants display rather pleiotropic changes in their metabolism, it is difficult to reconcile their earlier sprouting to their altered hexose content. It is equally possible that these plants might sprout earlier because they have a much-increased general metabolic activity characterized by a massive increase in glycolysis and respiration.

In summary, although the last few years have seen considerable advances in the understanding of the processes underlying tuber development, and in the identification of the key players orchestrating these, our knowledge remains far from complete. It is likely that the application of recently developed multiparallel technologies to access and describe the levels of transcripts, proteins, and metabolites (Bachem et al., 2000b; Roessner et al., 2001a, 2001b) will allow further elucidation of what is clearly a very complex and highly specific process.

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