Seasonal Control of Tuberization in Potato: Conserved Elements with the Flowering Response

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day length, gibberellin, tuberization transition, stolon meristem, potato tuber development

Abstract

Fluctuations in day length determine the time to flower in many plants and in potato are critical to promote differentiation of tubers. Day length is perceived in the leaves and under inductive conditions these synthesize a systemic signal that is transported to the underground stolons to induce tuber development. Flowering tobacco shoots grafted into potato stocks promote tuberization in the stocks, indicating that the floral and tuber-inducing signals might be similar. We describe recent progress in the identification of the molecular mechanisms underlying day-length recognition in potato. Evidence has been obtained for a conserved function of the potato orthologs of the CONSTANS (CO) and FLOWERING LOCUS T (FT) proteins in tuberization control under short days (SDs). These observations indicate that common regulatory pathways are involved in both flowering and tuberization photoperiodic responses in plants.

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INTRODUCTION

Tuberization is a developmental process unique to some *Solanum* species, which under favorable conditions differentiate specialized underground propagation organs or tubers. Tubers are modified underground stems, with very short swollen internodes and scale leaves subtending the dormant axillary buds or tuber "eyes." They serve a double function to the plant, as a storage organ and as a vegetative propagation system. After a winter period of dormancy or rest, dormant axillary buds reactivate and grow out to produce a plant that is genetically identical to the mother plant, tubers thus serving as perennation organs to these annual species.

Potato tubers accumulate large amounts of starch, are low in fat, and have a protein content as high as cereals, with the added advantage of a more equilibrated composition in essential amino acids. A medium-sized potato tuber, in addition, provides about half the daily adult requirement of vitamin C. Due to these nutritional properties and ease of propagation, potato is currently the fourth most important food crop in the world, after cereals. It is used on a large scale in the chip and fries food industry, or in a processed form to obtain starch and alcohol, with the annual production of tubers approaching 300 million tons.

Tubers form in the subapical region of underground stem-like structures or stolons that develop at the base of the main stem. Under noninductive conditions, stolons grow as horitzontal stems and, if exposed to sufficient light, they become green and emerge from the soil to form a new shoot. During this process, the stolon acquires all the characteristics of a main stem, forming roots and new leaves and eventually flowering. Under low temperature or short day conditions, however, elongation growth of the stolon ceases and the tip begins to swell to form a tuber. Swelling is correlated with an expansion and radial cell division of the cells located in the pith and cortex of the subapical part of the stolon, and subsequent random cell division and expansion of the cells in the perimedullary region contribute to tuber bulking (142). These changes in cell division are accompanied by a switch in the developmental program of the stolon subapical meristem cells to a tuber fate. Meristem growth becomes determinate, and cell division ceases after a few rounds. The mechanism of sucrose unloading changes from apoplastic to symplastic, and this is accompanied by a decline in cell wall invertase activity and a

dramatic increase in the activity of the sucrose synthase and fructokinase enzymes (8, 136). During the rapid growth phase, tubers accumulate large amounts of storage compounds, mainly in the form of starch and proteins (i.e., patatin and proteinase inhibitors), which serve as a source of energy to the future plant (94). Regulatory routes leading to starch biosynthesis have been extensively studied in potato, and current knowledge on these pathways is summarized in excellent reviews (31, 35, 36).

In its natural habitat, differentiation of potato tubers occurs in autumn to early winter, depending on the potato genotype. These newly formed tubers undergo a period of inactivity or endodormancy, which is characterized by the absence of bud growth even if tubers are exposed to conditions favorable to sprouting. Such a period of physiological rest lasts for a few months and assures survival of the plant during the cool winter temperatures. Tuber sprouting is desired in potatoes that will be used as tuber seeds, but unwanted during storage, because it reduces the post-harvest life of tubers and decreases their nutritional quality. Endogenous factors determining this economically important trait are just beginning to be uncovered and have been extensively discussed in different reviews published on this subject (117, 120, 138).

DAY-LENGTH CONTROL AND GENOTYPE

Potato was brought to cultivation in the highlands of South America near the equator. In these equatorial high-altitude regions, day length remains close to 12 h (SD conditions) and temperatures are low at night. Wild Andean varieties (*Solanum tuberosum* ssp. *andigena*) are adapted to these conditions and are unable to tuberize or do so very poorly when grown under the higher temperatures of lowland tropics or the longer summer days of temperate zones. Modern cultivated potatoes derive from Chilean landraces (*Solanum tuberosum* ssp. *tuberosum*) grown in the lowlands of southern Chile and therefore are more adapted to the longer summer days of the temperate regions of Europe and North America. Repeated selection for tuberization under long days (LDs) would have favored these clones over the less-adapted Andean varieties, and Chilean landraces would have become the predominant modern breeding stock. *Tuberosum* potatoes give very low yields in the highland tropics because, under the cool temperatures and SD conditions characteristic of these regions, tubers are formed very early, before shoot growth can support a good tuber yield (30). Such a trend toward prevailing short or longer days for induction is characteristic of all *andigena* or *tuberosum* species, although there are substantial genetic differences within each group.

Short days, cool temperatures, and low rates of nitrogen supply promote tuberization, whereas tuber formation is delayed by long days, high temperatures, and nitrogen-rich fertilizers, conditions that are noninductive for tuberization (29, 75, 83). Among these environmental conditions, day length has been the most intensively investigated because of its critical effect on tuberization. SDs induce tuber formation in all potato varieties, although there is considerable variation in the degree to which this environmental cue is required for induction (118). Modern potatoes were subjected to iterative selection for early tuberization, and tuber transition in these cultivars is relatively independent of day length. However, wild potato species such as *S. tuberosum* ssp. *andigena* or *S. demissum* are strictly dependent on SDs for tuber formation (30, 76). These species tuberize only under SDs (8 h light) and do not produce tubers when grown under LD conditions (16 h light) or SDs supplemented with a night break (NB) or pulse of light in the night period (SD+NB). The inhibitory effect of a 15-min pulse of light given in the middle of the night (NB) demonstrates that it is the overall length of the night period (long nights), rather than that of the day (SDs), that induces tuber formation in these plants (**Figure 1**).

SD: short day
LD: long day
NB: night break

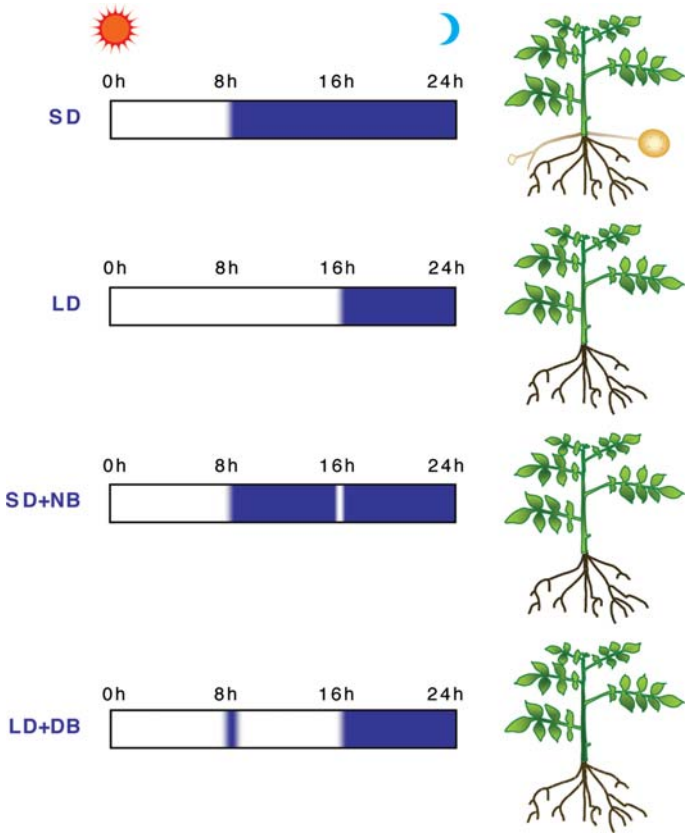

Figure 1

Day-length control of tuberization in andigena potatoes. *Andigena* species are strictly dependent on short days (SDs) (8–10 h light) for tuberization and do not tuberize under long days (LDs) (14–16 h light). Like in other SD species, the overall length of the night period rather than that of the day determines the tuberization response. Interruption of long nights with a pulse of light inhibits tuber formation, whereas interruption of long days with a dark pulse has no effect. Response to night break (NB) enables growth of induced (SD) and noninduced (SD+NB) plants under identical culture conditions except for a 15-min light interruption in the middle of the night.

Temperature is also an important environmental factor for tuberization. High temperatures inhibit tuber formation, whereas low temperatures promote tuber growth. A strong day-length response is observed at elevated temperatures, whereas day-length control is less prominent at lower temperatures (118, 129). This indicates that these environmental cues converge at some point, possibly by controlling a common component of the day-length pathway.

DAY LENGTH IS PERCEIVED IN THE LEAVES

Evidence demonstrating that the principal site of perception of the photoperiodic signal is in the leaf was obtained from grafting studies in *andigena* potatoes (20, 38). When leaves of *andigena* plants grown under inductive (8 h light) conditions were grafted onto noninduced stocks (16 h light), tubers were formed in the stock plants, whereas stocks grafted with noninduced leaves did not produce tubers. This demonstrates that inductive conditions are sensed in the leaf but not in the stolons, leading to the hypothesis that, in response to the photoperiodic signal, the leaves produce a tuberization stimulus that is transported across the graft union to the noninduced stock, where it induces tuberization.

Tuber induction is associated with several morphological changes in the plant (30). Leaves become larger, thinner, and a paler green, and acquire a flatter angle to the stem. Axillary branching is suppressed, flower buds abort more frequently, and senescence is accelerated, coinciding with the rapid growth phase of the tubers. Such alterations in morphology, together with transmission of the inducing signal across a graft, led to the belief that the stimulus was of hormonal nature. Several hormones, i.e., gibberellin (100, 143), cytokinin (56, 86), jasmonic and tuberonic acids (70, 130) or abscisic acid (83, 143), reportedly play a role in tuberization, and changes in the endogenous levels of these hormones could be correlated with the tuberization onset. However, as discussed below, effects of many of these hormones are likely exerted directly at the level of the stolon, and these growth regulators then play a role in tuberization transition, but are not involved in the production of the inducing stimulus derived from the leaves.

Analysis of gene expression during tuber growth has been hampered by the lack of synchronicity of the tuberization process. To overcome this problem, in vitro tuberization systems based on single-node stem cuttings were developed, in which tuber formation can

be synchronously induced in response to day length (29) or high levels of sucrose in the media (56). Single-node cuttings taken from noninduced plants will develop axillary shoots when buried in soil and kept humid, whereas formation of a stolon, a tuber subtended by a stolon, or a sessile tuber is observed in leaf cuttings taken from plants grown under shorter photoperiods, depending on the strength of induction. Tuberization is uniformly induced and swelling is observed within 4–5 days of cutting which makes these a useful tool in tuberization studies (29).

Synchronized tuber induction is also observed in stem-node cuttings cultured in vitro in high (8%) sucrose medium (27, 34), such an in vitro tuberization system is being widely used for production of virus-free certified seed potatoes or propagation of stock cultures. Microtubers obtained in vitro show identical changes in the activities of enzymes involved in sucrose unloading and starch biosynthesis to those observed in field-grown tubers (7, 10, 133), and are therefore a valuable model system in tuberization research. However, although a day-length response is still observed in potato plantlets cultured in high sucrose (56, 112), photoperiodic control is not as strict as in soil-cultured plants, as tuberization is also observed in continuous light. Therefore, these in vitro systems are not useful for studying tuberization control by day length, and *andigena* species are more suitable for these studies. In these species, stolons develop under noninductive LD or SD+NB conditions, but differentiation of these stolons into tubers is not observed unless plants are transferred to SDs. Transfer to inductive SD conditions induces a synchronous growth of tubers, with tuber swelling observed after 8–12 days of transfer to SDs. These plants offer an excellent model system in which to study production of the mobile-inducing signal derived from the leaves, and development of stable transgenic lines for both up or downregulated gene expression has substantially contributed to our understanding of the molecular mechanisms underlying production of the mobile-inducing signal in these cultivars. Here we focus on recent discoveries concerning the inhibitory function of gibberellins (GAs) on tuberization and the involvement of a conserved CONSTANS/FLOWERING LOCUS T (FT) pathway in SD-dependent control of tuber formation. An emerging topic of this work is that despite divergence of the flowering and tuberization responses, plants rely on the same signal transduction components to synchronize these two distinct developmental transition processes to seasonal changes in day length. Hence, it is feasible that similarly conserved day length–related pathways regulate other seasonal responses such as leaf abscission in fall, bud dormancy, or annual rings of cambial activity in trees.

GA: gibberellin

***FT*:** flowering locus T

PHY: phytochrome

DAY-LENGTH DURATION IS SENSED BY THE LIGHT RECEPTOR PHYTOCHROME

Red light (R) is most effective for interrupting long nights. A far red light (FR) pulse given immediately after the pulse of red light reverses the inhibitory effect of the R night break (13), indicating that length of the night is sensed by phytochromes. In potato, two phytochrome-encoding genes (PHYA and PHYB) have been characterized (52, 53). Of these, only PHYB stably accumulates in green leaves, and is thus likely involved in sensing day-length duration. By transforming *andigena* plants with an antisense construct for this gene, we showed that PHYB does play a crucial role in day-length perception and in regulation of the SD pathway of tuberization. Plants in which PHYB expression is downregulated are almost day-length insensitive and tuberize very early under LD, SD+NB, or SD conditions (59). These lines actually behave as though they are strongly induced to tuberize, forming sessile tubers that are directly attached to the main stem. These observations are consistent with a regulatory model in which PHYB functions under noninductive SD+NB or LD conditions

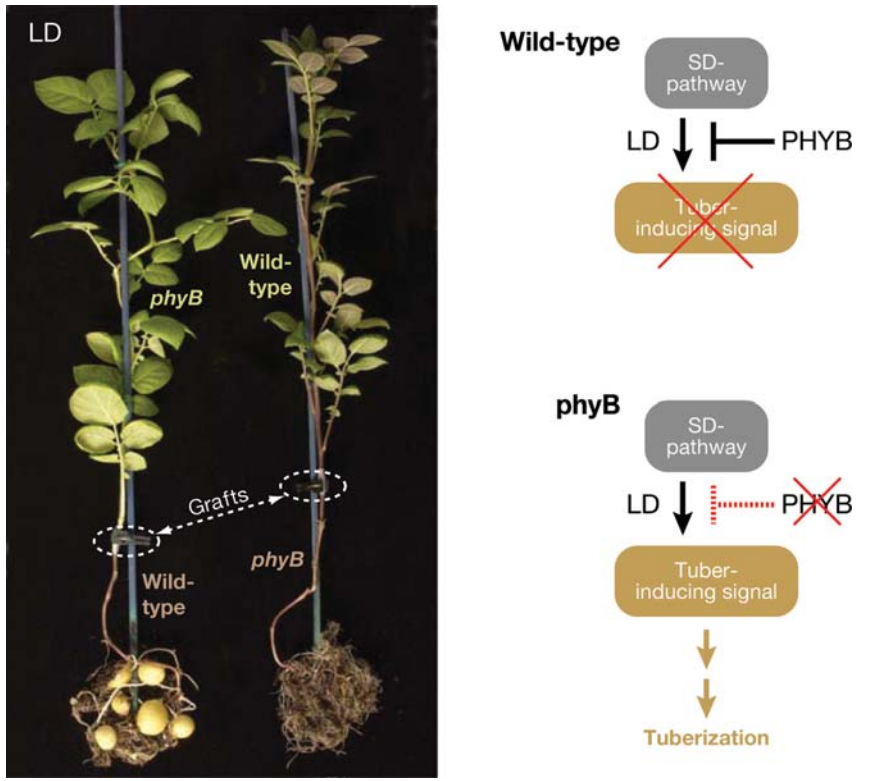

Figure 2

PHYB represses tuber induction in long days (LDs). Day length is perceived by phytochromes. PHYA resets the endogenous rhythm and PHYB senses the length of the day and night intervals. Plants in which PHYB expression is downregulated (*phyB*) show a constitutive activation of the short day (SD)-dependent tuberization pathway and are day-length insensitive. When these plants are grafted to wild-type stocks, they induce tuber formation under LDs in the stock, providing evidence for constitutive production of the tuberization stimulus in the leaves. The reverse graft of a wild-type scion into the *phyB* stock does not tuberize in LDs because production of the graft transmissible signal is repressed under noninductive conditions in wild-type leaves.

to repress a SD regulatory pathway promoting tuber formation. This inducing pathway is constitutively activated in the *phyB* mutants, and these plants then tuberize under any day-length condition. In line with this observation, *phyB* scions grafted onto wild-type plants induced the wild-type stocks to tuberize in LDs, whereas tuber formation is not observed in reciprocal grafts of wild-type scions onto *phyB* stocks (61). This agrees with constitutive production of the tuberization stimulus in these leaves, and the idea that PHYB represses synthesis of this stimulus under noninductive conditions (**Figure 2**).

Evidence for a function of PHYA in day-length control was also obtained in antisense lines with reduced levels of expression of this photoreceptor (54). A 6-h extension of the day with FR+R light caused a delay in tuber formation in wild-type plants, but not in antisense *PHYA* lines, providing evidence for a role of this light-labile photoreceptor in day-length perception. Exposure to 5 h FR or FR+R light at the end of the night, before transfer to constant (free-running) light conditions, advances the phase of circadian movement of the leaves in wild-type potato or *PHYB* antisense lines but not in plants with

reduced levels of PHYA accumulation. Blue light still causes a phase shift in these lines, thus showing a role for PHYA in the input to the clock but not as an integral part of the circadian clock (146). Concerted action of PHYA, PHYB, and blue light receptors then seems to mediate photoperiodic control of tuber formation, PHYA and cryptochromes having a role in setting the phase of the internal circadian rhythm, whereas PHYB would make tuberization responsive to the photoperiod by sensing the presence/absence of light during the sensitive phase of the rhythm.

Besides loss of photoperiodic control of tuberization, the antisense *phyB* lines exhibit an elongated phenotype that is reminiscent of plants treated with saturating doses of GAs. Similar to the *slender* mutants, these plants have paler leaves and very elongated internodes, indicative of either increased GA synthesis, altered feedback regulation, or a constitutive activation of the response to GAs. The *StGA20ox1* transcript encoding GA 20-oxidase was upregulated (62) and GA content was elevated in *phyB* shoots (79), which contrasts with the recognized inhibitory effect of GAs on tuberization. Nevertheless, we obtained evidence indicating that the inhibitory effects of GAs are directly exerted at the stolon level, and increased aerial levels of GAs not only do not inhibit tuberization but likely play a role in modulating production of the tuberization stimulus.

GIBBERELLINS DELAY TUBERIZATION

GAs have long been implicated in the regulation of tuber development (137). Tuber initiation is delayed by applying GA_3 (115, 143), whereas adding inhibitors of GA biosynthesis such as tetcyclacis, chlorocholine chloride, paclobutrazol, or ancymidol enhances tuber formation in stem-node cuttings (1, 42, 83, 115, 136) and in greenhouse-grown plants (12, 60). There is multiple evidence for an inhibitory function of GAs on tuber formation. First, tuber initiation is correlated with a sharp decrease in the endogenous GA_1 content in the stolon (143). Second, modifying endogenous GA levels due to overexpression or antisense inhibition of the GA 20-oxidase *StGA20ox1* gene results in delayed or advanced tuberization in SDs, showing a correlation between GA levels and tuberization onset (18). Third, the *andigena ga1* dwarf mutant, apparently blocked in the 13-hydroxylation biosynthetic step, can form tubers in LD conditions, although it requires cultivation for several months under these noninducing conditions (131). Transfer to SDs induces rapid tuberization (within three to four days) in these dwarf potatoes, indicating that although SD requirement is less severe than in wild-type plants, tuberization in these mutants is still under day-length control. This indicates two independent pathways of tuberization: a SD pathway and a GA-dependent pathway, the balance between the inducing and inhibitory effects of these pathways determining tuberization onset.

GA 20-oxidase and GA 3-hydroxylase catalyze the two last steps in the GA biosynthetic pathway, and GA 2-oxidase catalyzes degradation of bioactive GAs into inactive catabolites (47). These enzymes encode key regulatory steps for GA biosynthesis and homeostasis and thus are subjected to transcriptional feedback control by the end product GA_1 (48), and are regulated in response to day-length conditions or phytochrome (66). Studies concerning potato GA biosynthetic activities have focused mainly on these genes, as they are the best candidates to mediate the changes in GA levels observed in induced stolons. Analysis of expression of three different transcripts encoding potato GA 20-oxidase activity did not show significant differences in the levels of accumulation of any of these mRNAs in SD as compared to LD conditions (17). In LDs, an extended interval of accumulation of these mRNAs was detected during the supplementary hours of light, but transcript levels were not higher than in SDs, indicating that, in contrast to *Arabidopsis* or spinach, expression of these genes in potato is not differentially

regulated by day length. Important changes in transcript levels were, however, observed for gene *StGA3ox2* encoding potato GA 3-hydroxylase (J. Bou, J.L. García-Martinez & S. Prat, submitted). In plants entrained to LD conditions, this mRNA accumulates to low levels in the stem but is abundantly expressed in the stolon. Transfer to SD conditions leads to upregulated expression of this gene in the apex and stem nodes, but to a complete repression of gene expression in the stolon, suggesting an important role of this gene in downregulated GA synthesis during tuberization transition. Microarray RNA-profiling analysis of tuber development has also identified a GA 2-oxidase transcript that is upregulated in induced stolons (69). Expression of this gene is activated very early after transfer to SD conditions, and transcript accumulation is observed before any visible swelling of the stolon, preceding accumulation of the tubulin *TUB8* mRNA (125). The *Stgan* transcript encoding a short-chain alcohol dehydrogenase was also identified in amplified fragment length polymorphism (AFLP)-based RNA–finger-printing analysis of genes that were upregulated in the induced stolons (11). Antisense lines for the *Stgan* gene show a taller growth habit and elevated levels of GAs, indicating that this steroid dehydrogenase-related gene functions in breaking down GAs through an alternative route to GA 2-oxidases (11). Changes in the GA 3-hydroxylase, GA 2-oxidase, or *Stgan* transcripts are among the earliest changes in gene expression observed during tuber differentiation. This indicates that local drop in GA_1 levels is a key event in tuberization transition. What remains to be determined is how this GA-dependent signal is integrated with the SD-inducing signal derived from the leaves.

GIBBERELLIN SYNTHESIS IN AERIAL TISSUES

To analyze further the role of potato GA 3-oxidase in tuberization, we overexpressed the *StGA3ox2* gene in *andigena* potato plants. Transgenic lines exhibiting high levels of expression of this transgene showed a taller phenotype and increased shoot GA_1 content, which correlated with the upregulated levels of expression of the transgene. However, contrary to expectations, these lines tuberized earlier in SD conditions and showed a higher tuber yield than the untransformed controls (J. Bou, J.L. García-Martínez & S. Prat, submitted for publication). This observation appears to indicate a negative effect on tuberization of increased levels of the GA_{20} precursor, as in GA 20-oxidase overexpressers (18), but a tuberization-promotive effect of an increased rate of GA_1 synthesis in the shoot, as in the GA 3-oxidase lines. A possible explanation for this contradictory result is that GA_{20} is more active at inhibiting tuberization than GA_1, or, alternatively, that these two GAs are not equally transported to the stolon. It is possible that GA_{20} is rapidly transported throughout the plant, thus inhibiting tuber transition, whereas GA_1 remains in the vicinity of the cells where it is produced, exerting its effect mainly on these cells. Evidence for a different mobility of these two GA molecules was in fact obtained in grafting experiments with the pea *Le* and *Na* mutants, blocked respectively in the GA 3-oxidase and *ent*-kaurenoic acid oxidase biosynthetic steps (23). In these studies, *Na* scions grafted on wild-type stocks grew normally, due to efficient conversion of GA_{20}, mobilized from the wild-type stock, into bioactive GA_1 by endogenous GA 3-oxidases in the mutant. However, *Le* scions grafted on wild-type stocks remained dwarf because of the poor transport of GA_1 from the wild-type stock (95). Such a preferential transport model would actually explain the early tuberization phenotype of the 3-oxidase overexpressers, as an increased rate of GA_{20} to GA_1 conversion in these plants would bring a reduction in the concentration of GA_{20} available to be transported to the stolon.

Although it is accepted that tuberization induction in SDs correlates with a reduced rate of synthesis of bioactive GAs in the

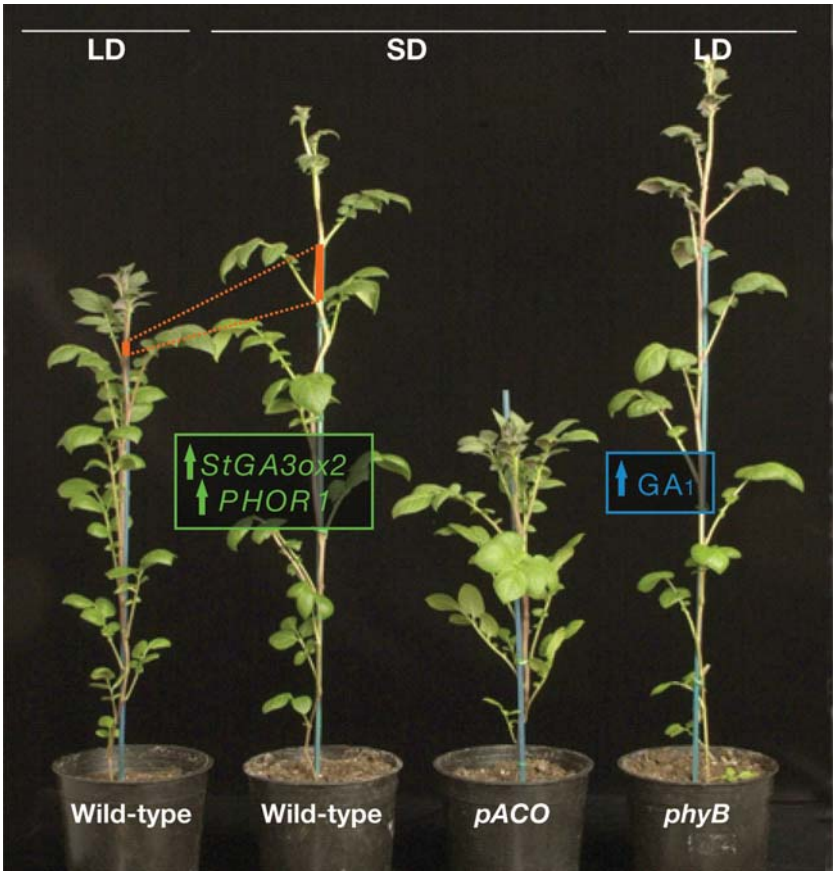

Figure 3

Elongation of the stem observed after transfering the plants to short days (SDs), indicating an increased synthesis of GA_1 in the shoot. Transfer of *andigena* plants to SD conditions induces tuber formation but also a visible elongation of the stem. This is observed only in plants exposed to SDs (8 h), but not in plants exposed to SDs with a 15-min interruption of the night (SD+NB, noninductive conditions), and correlates with increased levels of expression of the *StGA30x2* and *PHOR1* transcripts (*green box*). pACO lines expressing the *Arabidopsis CO* gene do not show this response and tuberize very late in SDs. A constitutive elongation of the stem is observed in the *phyB* lines that are insensitive to day length and behave as though strongly induced to tuberize. Elevated levels of GA_1 were observed in these lines (*blue box*).

leaves (65, 74, 93, 100), we have consistently observed that transfer to SD conditions induces elongation of the youngest internodes of the stem (**Figure 3**). Stem growth is rapidly induced after transfer to SD conditions, and these plants show a stronger resemblance to the *phyB* antisense lines than plants kept under LDs.

The photoperiod responsive 1 (*PHOR1*) gene was identified in a search for genes showing upregulated expression in SD leaves and with a possible function in tuberization control (5). *PHOR1* encodes a U-box domain protein with an arm-repeat region homologous to the *Drosophila* segment polarity gene, *armadillo*. Antisense repression of PHOR1 led to a reduction in stem length and resulted in early tuberization in SDs and partial insensitivity to applied GAs. Subcellular localization studies using a fusion to the green fluorescent

***PHOR1*:** photoperiod responsive 1

SCF: Skp1 Cullin F-box/ring-H2

***gai-1*:** GA-insensitive 1

protein showed that PHOR1 is localized to the cytosol but rapidly migrates to the nucleus upon treatment with GAs. PHOR1 was recently shown to exhibit E3-ligase activity and to ubiquitinate the DELLA repressor proteins to target them for degradation by the 26S proteasome (A. Espinosa, J.M. Iglesias, and S. Prat, unpublished results) in a reaction independent of that mediated by the SLEEPY1 F-box protein SCF complex (26). It is therefore possible that upregulated expression of this E3 ligase in aerial tissues functions by relieving DELLA-mediated growth restraint in response to day-length conditions, thus promoting the increase in stem growth observed in SDs. Consistent with this observation, increased levels of expression of the *StGA3ox2* transcript were observed in the apex and nodes of SD plants, suggesting an increased rate of GA_1 synthesis in the aerial tissues of these plants. Such increase in shoot GA_1 synthesis is mimicked in the GA 3-oxidase overexpresser lines, which resemble SD-induced plants by exhibiting a taller phenotype and tuberizing earlier than the controls. A similar diversion mechanism can also explain the somehow contradictory phenotype of the *phyB* antisense plants, in which a four- to sixfold increase in GA_1 content in the shoot paradoxically coexists with a strong induction to tuberize. Therefore, contrary to the general idea that GAs inhibit tuberization, our observations indicate that high levels of GAs in the stolons inhibit tuberization, whereas a high rate of GA_{20} to GA_1 conversion in the shoot would favor tuber formation, likely by lowering the levels of GA_{20} in the aerial tissues and thus transport of this precursor to the stolon.

GIBBERELLINS EXERT A LOCAL EFFECT IN THE STOLON

How do GAs regulate tuberization transition? The observation that the andigena *ga1* dwarf mutant, with a block in the 13-hydroxylation step, can form tubers in LDs seemed to point to a function of these hormones in day-length signaling (131); however, several lines of evidence, including the sharp drop in GA_1 levels in the stolon tip coinciding with the onset of tuberization (143), support a local inhibitory function of these hormones in the stolon. Evidence for a main function of GAs in repressing stolon differentiation was obtained in a recent study in which wild-type and *phyB* potato lines blocked in the GA-response pathway were obtained by expression of the *gai-1* dominant allele of *Arabidopsis* (M. Rodriguez-Falcon & S. Prat, submitted for publication). *gai-1* encodes a DELLA repressor protein with a 17 amino acid deletion within the DELLA domain that renders the protein unresponsive to GA-dependent degradation and leads to a severe dwarf phenotype that does not respond to exogenous GA (25, 88, 126). Expression of this allele caused a GA-insensitive dwarf phenotype in both genetic backgrounds, the strength of this effect being directly correlated with the levels of expression of the transgene. Although GA response was compromised, tuberization time was not affected in these transformants, which tuberized about at the same time as the respective controls, thus suggesting an independent function for both GA and day-length pathways.

GA insensitivity could not overcome the SD requirement of wild-type *andigena* plants to tuberize. After several months of culture in LD conditions, tubers were observed in the *ga1*-deficient mutant but not in the GA-insensitive *gai-1* transformants, indicating that the GA-response pathway is still required for tuberization in LDs. It is possible that increased GA_1 synthesis in aerial tissues not only is involved in diverting GA_{20} from the stolon, but also in promoting tuberization by activating one of the genes involved in tuberization control. However, such a positive control function of GAs needs further investigation.

Tubers developed by the dwarf lines were highly irregular in shape and looked more like swollen stolons than tubers. An accumulation of tuber-specific transcripts and massive amounts of starch were observed in the

basal internodes subtending these tubers, thus showing unrestricted differentiation of all stolon internodes to a tuber fate. In the most severely dwarf lines, swelling of the buried stem was also observed, suggesting tuber differentiation of the whole belowground region. These results are consistent with a function of GAs in restricting tuber transition to the subapical region of the stolon, the drop in GA_1 levels at the onset of tuberization thus playing a role in defining the domain of cells that will undergo transition to a tuber fate. In the *gai-1* transformants, a block in the GA response will likely expand the domain of tuberization competence to the whole stolon and main belowground stem, as tuber differentiation is also observed in these organs.

GAs promote stolon elongation by inducing a transverse alignment of cortical microtubules to the long axis of growing cells (114). Reduced GA levels after treatment with the GA synthesis inhibitor uniconazol were reported to cause a reorientation of the stolon cell cortical microtubules to longitudinal or oblique directions, thus initiating longitudinal division of these cells and radial expansion of the tuber (33). The sharp decrease in GA levels observed in the cells located at the subapical region of the stolon is thus likely directly involved in signaling the change in the plane of cell division of these cells during differentiation to a tuber fate. However, the observation that the entire stolon and belowground stem undergo tuber fate transition in the *gai* lines would indicate an additional function of low GAs in differentiation of the cells in the subapical meristem to make them competent to respond to the tuberization-inducing signal derived from the leaf. This leads to a regulatory model in which tuber differentiation is regulated by the concerted action of two opposing signals: a GA-dependent signal that represses tuber growth and a leaf-derived signal that promotes tuber transition. How these signals are integrated at the molecular level is at present unknown. Recent identification of the rice *GIBBERELLIN INSENSITIVE DWARF1* (*GID1*) gene as a soluble receptor for gibberellin (127) will provide a better tool for GA-response modification than the *gai* dominant allele. ESTs coding for homologs of this gene have been reported in potato and could be used to design specific RNAi constructs aimed to impair GA signalling in the stolons or the leaves, thus allowing to analyze the effects on tuber differentiation of a tissue specific block in GA perception.

KNOX: Knotted-like homeobox

***POTH1*:** potato homeobox 1

BEL: BEL-type homeodomain factor

THE KNOX GENES MAY SUPPRESS GIBBERELLIN ACTIVITY IN THE STOLON MERISTEM

Several studies have implicated the KNOX genes in repression of GA synthesis in the shoot apical meristem. Overexpression of the tobacco *NTH15* homeobox gene, for example, leads to a decrease in bioactive GA levels by repressing GA 20-oxidase gene expression (109, 123). The *Arabidopsis* homeobox protein SHOOTMERISTEMLESS (STM) excludes expression of the *AtGA20ox1* gene from the shoot apical meristem, as repression of GA synthesis is crucial to maintaining the indeterminate state of the meristem corpus cells (43). In potato, overexpression of the homeobox *POTH1* gene resulted in enhanced tuberization in vitro, under both SD and LD photoperiods, together with a mouse ear leaf phenotype. Accumulation of the *GA 20-oxidase1* transcript is repressed in the overexpresser lines, and GA_1 levels reduced by one half (107), suggesting a role of this KNOX regulatory protein in repression of GA synthesis. In line with this observation, GA application suppressed the leaf mouse ear phenotype of the overexpressers, giving a more wild-type leaf shape. POTH1 interacts with BEL5, a member of the BEL-1 family of TALE homeodomain proteins that is upregulated both in leaves and stolons in SDs (21). Ectopic *BEL5* expression also leads to enhanced tuber formation and decreased levels of *GA 20-oxidase1* mRNA in the stolon tips, but in contrast to POTH1 does not affect

ABA: abscisic acid
JA: jasmonic acid
TA: tuberonic acid

leaf shape (21). Members of the KNOX/BEL families of transcription factors interact selectively, the different heterodimer combinations apparently regulating different downstream genes. In this regard, the heterodimer POTH1/BEL5 binds the *GA 20 oxidase1* gene promoter with higher affinity than the POTH1 or BEL5 homodimers and is more effective at suppressing the activity of this promoter (22). These observations reinforce the idea that the POTH1-BEL5 heterodimer may play a role in tuberization by suppressing bioactive GA synthesis in the stolon tip, thus creating a reduced GA regime favorable for meristematic activity and enabling undifferentiated cells to respond to the leaf-derived inducing signal. Generating transgenic lines with increased levels of expression of both transcription factors will be crucial to prove interaction of these homeodomain proteins in vivo and to reveal to which extent reduced GA synthesis mediates tuberization control by these regulatory factors, thus further contributing to our understanding of the mechanistic basis underlying tuber fate determination in response to low GAs.

OTHER HORMONAL SIGNALS

Besides GAs, several other phytohormones have also been implicated in tuberization. A possible role of ABA in this process, for example, was demonstrated by the higher number of tubers, earlier tuberization, and sessile tubers obtained after ABA application (83). Similar effects have been reported in vitro, with earlier tuberization and formation of sessile tubers observed in high sucrose + ABA. Application of ABA can stimulate tuber formation also in low sucrose, or in the presence of GAs, thus counteracting the inhibitory effect of these hormones (143). Tuberization, however, is not associated with an increase in the endogenous levels of ABA, as a similar decrease in ABA levels was observed in noninduced or induced stolons during development (74, 143). In line with this evidence, the ABA-deficient *droopy* mutant of *S. phureja* tuberized normally (99), indicating that ABA synthesis is not required for induction. Thereby, it was concluded that ABA would not play a main role in tuberization and that the stimulatory effects of this hormone are due to the antagonistic effect of ABA and GAs (143).

The glucoside of 12-OH jasmonic acid (JA), tuberonic acid (TA), was isolated from shoots of potato plants induced to tuberize (70). Both JA and TA stimulate in vitro tuber formation when added to the agar medium (122, 130), and increased levels of JA were detected in the stolons at tuberization onset (2). JA itself, however, does not seem to be involved in tuberization control, as application of JA on noninduced *andigena* leaves did not induce tuberization even though it did activate wound-induced gene expression (58). Preferential accumulation of the 11-OH JA and 12-OH JA derivatives in leaflets of *S. demissum* plants grown under SD conditions led to the postulation that the respective hydroxylating enzymes are activated in SD conditions, and these hydroxylated forms are then glucosidated and transported out of the leaves to initiate tuber formation (49). However, attempts to demonstrate a causal link between these hydroxylated JAs and tuber formation have failed, as tuber formation is still observed in plants treated with the LOX inhibitor salicylhydroxamic acid (SHAM) to repress JA synthesis. Further evidence against a role of these hydroxylated JAs in tuberization control comes from the recent isolation of a gene encoding hydroxyjasmonate sulfotransferase from *Arabidopsis* (37). Characterization of this hydroxylating activity demonstrates that 12-OH JA synthesis is not restricted to tuberizing species, suggesting that hydroxylated JAs are probably involved in developmental processes other than tuber formation. These observations would exclude a function of jasmonates as components of the tuberization-inducing signal synthesized in the leaves and point more to a specific action of these compounds in the stolon (122). JAs were reported to cause a similar change in the orientation of cortical microtubules as that observed in response to the

inhibitor of GA synthesis *uniconazol* (3, 81); thus high JA levels are likely to contribute to induced radial cell expansion during tuberization onset. Transcripts encoding lipoxygenase activity are upregulated during tuberization transition (9), and evidence for the involvement of a Lox1-class lipoxygenase, designated *POTLX-1*, in tuber growth was also obtained (72). Antisense lines repressed in expression of this tuber-specific 9-LOX showed a strong reduction in tuber yield, and stem-node cuttings taken from these plants failed to tuberize. Sprouts from these transgenic tubers were shorter than sprouts from the controls and developed multiple branched shoots, demonstrating an additional role of this LOX activity in sprout elongation (72).

Cytokinins (CK) have long been suggested to play a prominent role in tuberization. They are predominantly used for microtuber induction in vitro (27, 56) and promote tuberization when directly applied to isolated stolons cultured in vitro (28, 86). Attempts to induce tuber formation by applying CK to the leaves, however, have produced ambiguous results as these treatments were unable to induce tuberization in *andigena* plants grown under noninducing conditions. Also, even endogenous CK levels increase in stolon tips during induction (82) and later stages of tuber growth (85), the promotive effects of these hormones could only be observed in the presence of high (above 4%) concentrations of sucrose (86). These observations suggest that CK may function to control tuber enlargement and growth, but would not signal transition to a tuber fate. Cytokinins can activate cell division by stimulating Cyclin D expression (101) and thus may be involved in cell proliferation during the early phases of tuber growth. These hormones might also control sink strength by activating the expression of genes implicated in assimilate partitioning, such as invertases, sucrose synthase, and hexose transporter genes (103), thus pointing to a role of increased CK levels in sink identity acquisition during the storage phase of tuber growth. Evidence for a function of CK in sink regulation was obtained in transgenic tobacco lines expressing the cytokinin *ipt* biosynthetic gene in axillary buds (40). Local overproduction of CK in these lines caused a morphological alteration characterized by the development of very short lateral branches, with small narrow-scale leaflets and swollen internodes, which accumulate large amounts of starch. Localized *ipt* expression thus appears to be sufficient to confer tuber-related identity to the cytokinin-accumulating cells, also in species that normally do not tuberize as tobacco. Increased cytokinin levels due to antisense suppression of the potato MADS box gene *POTM1* also result in increased starch accumulation and active cell division in specific regions of the meristem and the leaves (106). These observations indicate an important function of these hormones in sink-source regulation and starch accumulation, although further research is still required to dissect the exact mechanisms involved in this response. In the meantime, it is important to note that in addition to a reduction in GA levels, levels of cytokinin were elevated by four- to fivefold in the transgenic lines overexpressing the KNOX *POTH1* or *BEL5* genes (21, 107). This result implies a possible involvement of these homeodomain proteins in triggering tuber formation through a modification of both GA and cytokinin levels. KNOX activity was recently shown to activate the CK biosynthetic genes *isopentenil transferase5* and -7 (*AtIPT5* and *AtIPT7*) and the early CK-activated gene *ARABIDOPSIS RESPONSE REGULATOR5* (*ARR5*), and to lead to a dramatic increase in CK levels (63, 144). KNOX proteins were shown as well to regulate GA catabolism, by increasing expression of the GA 2-oxidase *AtGA2ox2* and *AtGA2ox4* genes, thus preventing GA influx into the meristem and confining GA activity to the differentiating leaf primordia (63). Thereby, KNOX proteins seem to promote meristem activity by increasing CK and lowering GA activity, both high CK and low GA levels being a requisite for meristem development.

***POTLX-1*:** potato Lox1-class lipoxygenase

CK: cytokinin

***POTM1*:** potato MADS box 1

CO: constans
GI: gigantea
CRY: cryptocrome

THE DAY-LENGTH REGULATORY PATHWAY

Interspecific grafting experiments between tobacco and potato plants show that the flowering signal produced in the tobacco leaves is similar to or identical to the signal that induces tuberization in potato (19). In these studies, shoots of tobacco species with different photoperiodic requirements (SD, LD, or day neutral) for floral induction were grafted onto *andigena* potatoes, and tuber formation was analyzed in the potato stocks. It was clear that when scions were obtained from tobacco plants induced to flower, they induced tuberization in the potato stock, but when they were taken from nonflowering plants they did not. Identical results were obtained with LD, SD, or day-neutral tobacco species. *N. silvestrys* species induced tuberization in the potato stock only if they were exposed to LDs, whereas *N. tabacum* Mammoth induced a similar tuberization response only if it was exposed to SDs. These observations suggest that the stimulus for flowering in tobacco could be the same as the stimulus inducing tuberization in potato, and that the nature of this stimulus is equivalent in day-neutral, SD, or LD plants.

Day-Length Control of Flowering Time

The molecular mechanisms by which plants recognize day length to induce flowering have been best characterized in *Arabidopsis*. In this facultative LD plant, LDs promote flowering, whereas flowering is delayed in SDs. Mutations in the *gigantea* (*gi*), *constans* (*co*), and *flowering locus T* (*ft*) genes cause late flowering in LDs but do not affect flowering in SDs, indicating a role of these genes in the LD flowering pathway (73). *GI* encodes a large nuclear protein of unknown function, which is required for the activation of CO transcription (32, 87). *CO* expression is reduced in the *gi* mutants, and overexpression of *CO* overcomes the late-flowering phenotype of these mutants. Mutations in the *GI* gene also cause additional defects due to faster cycling of circadian rhythms, indicating a function of this gene close to the endogenous clock. *CO* encodes a nuclear CCT domain zinc-finger protein with a central role in day-length control of flowering (97, 119). This transcription factor functions as an output to the clock and directly activates expression of the downstream floral regulator genes *FT* and *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*, also known as *AGL20*). *FT* encodes a RAF-kinase inhibitor-like protein and *SOC1* encodes a MADS-box transcription factor, and overexpression of these genes causes extreme early flowering (14, 67, 111). They are also regulated in response to the vernalization or autonomous flowering pathways thus being proposed to function as floral integrators (46, 98, 147).

CO is cyclically expressed with a broad biphasic peak of expression in LDs. Under LDs, expression of *CO* coincides with the light at dawn and in the afternoon, causing *FT* expression and floral induction. In SDs, the *CO* peak is narrower and occurs only in the night. *FT* is then not expressed and flowering is delayed (**Figure 4**). Light regulates *CO* activity by suppressing degradation of the CO protein (128). In the dark, CO is ubiquitinated and degraded by the proteasome complex. The CRY1/CRY2 and PHYA photoreceptors stabilize the CO protein in blue light and FR light, respectively, and PHYB promotes CO degradation in R. These results explain why the cryptochrome and *phyA* mutants flower later in blue and FR light conditions, whereas the *phyB* mutant flowers earlier than wild-type plants in R, and also provide a molecular basis to the external coincidence model for day-length perception (90). This model proposes that the circadian clock generates an internally regulated rhythm controlling flowering and is sensitive to light at a particular phase of the rhythm. When the plant is exposed to light at this particular phase, flowering is induced in LD plants or delayed in SD plants.

Genetic studies in rice show that this flowering control pathway is highly conserved in

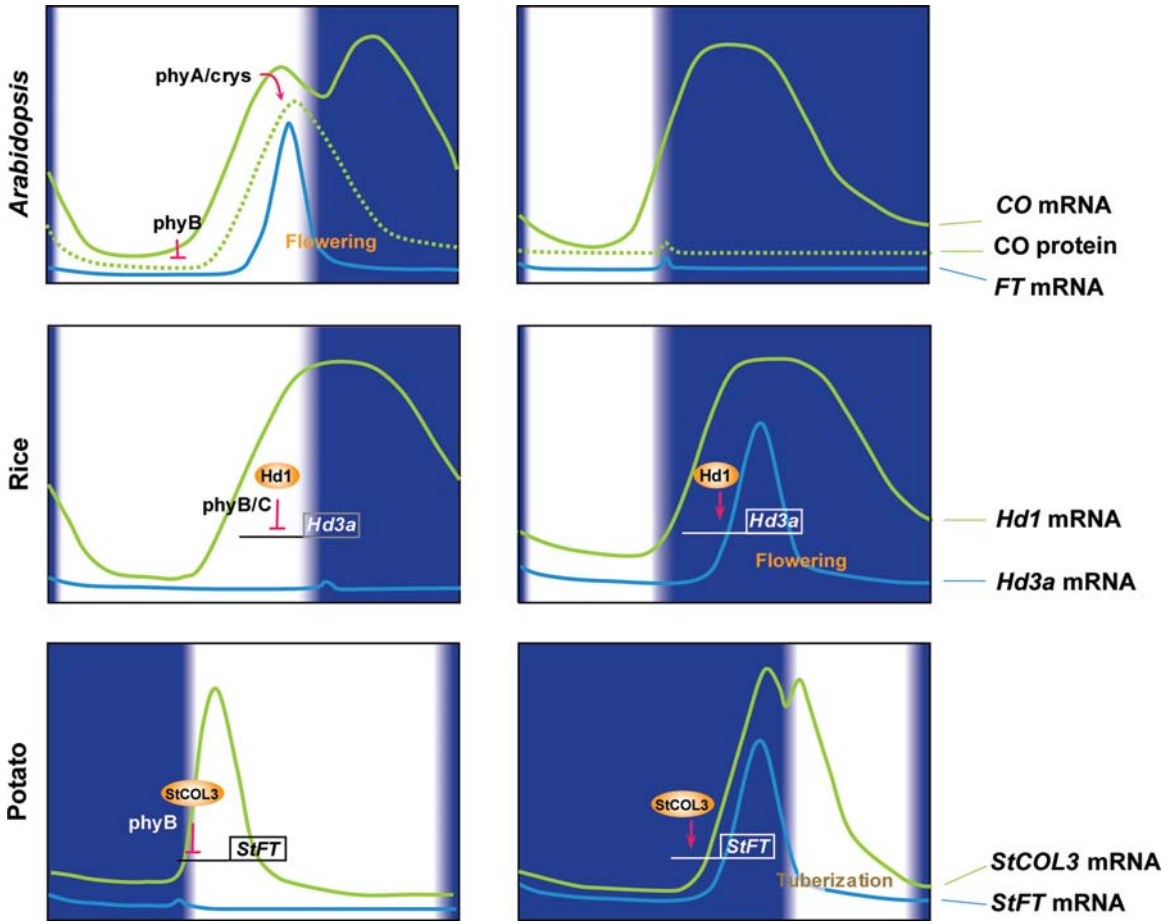

Figure 4

Diurnal rhythm of expression of the CONSTANS (CO) homolog in potato. *StCOL3* mRNA exhibits a diurnal rhythm of expression that peaks at dawn and therefore has a different phase than those of *CO/Hd1* in *Arabidopsis* and rice. *StCOL3* accumulates at the end of the night to midday under long days (LDs), and the coincidence of *StCOL3* expression with light suppresses tuberization under these conditions. In short days (SDs), *StCOL3* accumulates only during the night, and tuberization is then promoted. Hence, regulation of tuberization by *StCOL3* is reversed with respect to the light, as observed for *Hd1*. In rice, phytochrome modifies Hd1 function to inhibit expression of the *FT*-homolog *H3da*. The rice *se5* mutant defective in phytochrome activity shows early flowering irrespective of day length because Hd1 is always in the dark form. Function of PHYB might be similar in potato, with the *phyB* lines showing early tuberization under all day-length conditions. Dark areas represent the night period and light areas represent the interval of light.

this SD plant. *OsGI*, *Heading date 1* (*Hd1*, the ortholog of *CO*) and *Heading date 3a* (*Hd3a*, the ortholog of *FT*) regulate day-length-dependent flowering by acting in a genetic pathway closely related to the one described in *Arabidopsis* (44, 71, 145). However, overexpression of *OsGI* in transgenic rice causes late flowering under both SD and LD conditions. In these transgenic lines, expression of the *CO* ortholog *Hd1* is increased, but expression of *Hd3a* is suppressed (45). This suggests that regulation of the *FT* gene by *CO* is reversed in rice, and *Hd1* suppresses flowering under LD conditions. Consistent with

***Hd1*:** Heading date 1

***Hd3a*:** Heading date 3a

COL: constans-like

this hypothesis, loss-of-function mutations in the *Hd1* gene caused early flowering under LDs and late flowering under SDs (145), and this was correlated with an increase in *Hd3a* transcript levels in LDs but decreased levels of this transcript in SDs (71). *Hd1* shows a diurnal rhythm with a pattern similar to that of *CO* (45, 71). Under LDs, *Hd1* is expressed at high levels at the mid- to end of the day (**Figure 4**). Interaction of *Hd1* with light is dependent on phytochrome because high levels of *Hd3a* expression and early flowering in LDs is observed in the *se5* mutants, with a lesion in a heme oxygenase gene required for chromophore synthesis (57). Therefore, it was proposed that in LDs *Hd1* is expressed at the end of the day and is somehow modified by phytochrome, repressing *Hd3a* expression and inhibiting flowering. In SDs, *Hd1* is only expressed during the night when phytochrome is in an inactive form, which allows *Hd1* to induce *Hd3a* expression, thereby inducing flowering. These results demonstrate that *Arabidopsis* and rice utilize the same genetic pathways to recognize day length and promote flowering, although specific differences to these LD and SD species, involved in mediating opposite regulation of *FT* (*Hd3a*) expression by the CO and Hd1 proteins, need to be studied in further detail.

A CONSTANS Homolog is Involved in Tuberization Control

Evidence for a role of the CO protein in day-length control of tuberization was also obtained in transgenic *andigena* plants expressing the *CO* gene from *Arabidopsis* (80). Lines overexpressing the *AtCO* gene (pACO lines) were smaller than the controls and in SD conditions tuberized much later than wild-type plants. Whereas control plants started to form tubers after two weeks of transfer to SD-inducing conditions, the pACO overexpressers required more than nine weeks under SDs to tuberize, thereby demonstrating a negative effect of *AtCO* on the photoperiodic control of tuberization.

Analysis of the diurnal rhythm of expression of the *StCOL*-1 gene, encoding a zinc-finger regulatory protein with homology to *CO*, showed that oscillation of this transcript was not affected in the pACO overexpresser lines. Thus, CO overexpression did not seem to affect diurnal rhythm of expression of clock-regulated genes, but did interfere with a further downstream step in day-length regulation, either by blocking the production or transport of the tuberization-inducing signal or the response of the stolons to this inductive signal. Grafting combinations of controls and pACO lines were critical to demonstrate that the negative effect of *AtCO* on tuberization is caused by an interfering function in the leaves. In these studies, wild-type scions grafted onto pACO stocks tuberized normally, but pACO scions grafted onto wild-type stocks showed a similar delay in tuber formation as that observed for the pACO lines (80). This excluded an effect of *CO* in the stolon but pointed to a specific function of the *Arabidopsis* protein in the leaves, possibly by interfering with SD function of the endogenous potato *CO* ortholog and thus with generation of the inducing signal. These findings indicate a function of *CO* in the regulation of the synthesis or the transport of this systemic inducing signal, thus showing a close association of this regulatory protein with the mobile signal produced in the leaves.

A detailed study of the pattern of expression of the *Arabidopsis CO* gene, using a promoter fusion to the GUS marker gene, showed that *CO* is expressed in the vascular tissue of the hypocotyl, the cotyledons, and the leaves and is detected at the apex and young leaves (6, 121). Expression of *CO* under control of the *AtSUC2* or *rolC* promoters driving specific expression to the phloem companion cells was able to complement the *co* mutation, but such a complementation was not observed when *CO* was directly expressed in the meristem. Interestingly, phloem expression of *CO* did not induce early flowering in the *ft* mutants, indicating that the flowering promoting effects of *CO* are mediated by *FT*.

CO was not able to move from the phloem to adjacent cells, which indicates that the non-cell autonomous flowering-promoting effects of *CO* require movement of the *FT* transcript or protein, or activation of a signaling step downstream of FT. In line with this evidence, *FT* complements the late-flowering phenotype of the *co* mutants when expressed in the phloem, but also when expressed in the meristem or meristem epidermal cells, demonstrating that either the RNA or the protein can move between cells. In transgenic lines carrying a heat shock inducible *Hsp:FT* construct, heating a single leaf was found to be as effective at inducing flowering as heating the whole plant. After 6 h of leaf induction, an increase in transgenic *FT* transcript could be detected in the shoot apex, showing that the transgenic *FT* mRNA is able to move from the leaf to the shoot apex (55). These results demonstrate that the *FT* mRNA is part of the long-distance inducing signal although they do not exclude subsequent movement of the FT protein or the possibility that FT might still induce another transcript or compound that moves together with its own transcript.

Potato might be a good model system to test long-range phloem transport of *FT* because of the larger size of phloem cells and ease of grafting experiments. Emerging evidence from transgenic studies seems to indicate that the genetic mechanisms controlling photoperiodic tuberization in potato are closely related to those mediating flowering time control in *Arabidopsis* and rice. Preliminary evidence indicates that a *GIGANTEA* homolog of potato might be implicated in photoperiodic control of tuber formation (D. Hannapel, personal communication). Three *CO* homologs also have been identified in potato, and evidence for a role in tuberization control has been obtained for one of these genes, designated *StCOL3* (N. González-Schain, S. Prat & P. Suárez-López, unpublished results). *StCOL3* is cyclically expressed with a biphasic peak of expression at the end of the night (**Figure 4**). Under SDs, *StCOL3* expression rises during the second half of the night and is still high during the first day hours. In LDs, the peak is narrower and occurs only during the day. Hence, this transcript peaks at a different time of the day than observed for the *CO/Hd1* transcripts in *Arabidopsis* or rice. Despite such a difference in the timing of expression, *StCOL3* accumulation seems to fit with a similar model as that described in rice, and tuberization is promoted when StCOL3 is expressed during the night but delayed when expression of this protein coincides with light. Therefore, it will be interesting to compare the orthologs from potato, rice, or the SD plant *Pharbitis nil* (78) with the CO *Arabidopsis* protein, and to search for conserved domains that might explain the differential regulatory function of the SD proteins.

SP: self-prunning

FT Homologs May Function as Tuberization Integrators

FT is one of the targets of *CO* and functions as an integrator of the different flowering regulatory pathways. *FT* induces early flowering not only when expressed in the phloem, but also when directly expressed in the meristem cells or in the meristem epidermis (6). Grafting of tobacco shoots into potatoes showed that the flowering stimulus produced in tobacco leaves induces tuberization in the potato stock (19). Hence, an important issue is whether *FT* also plays a role at tuberization induction, as would be expected for a function of this transcript as part of the inducing stimulus. In tomato, *FT* is a member of a small gene family comprised by six genes designated as *SP* (*SELF-PRUNNING*), based on the first member of the gene family to be isolated (16, 91). This gene is the ortholog of *TERMINAL FLOWER 1* (*TFL1*) and is involved in tomato sympodial growth after floral transition (91). A seventh additional *FT* homolog is found in the TIGR potato expressed sequence tag (EST) database. Phylogenetic analysis placed two of these gene homologs into the same clade as *Arabidopsis FT* and rice *Hd3a*, thus pointing to a function of these genes as orthologs of *FT*.

A single amino acid change was recently demonstrated to be sufficient to convert the *TFL1* repressor in an activator of flowering (15, 41). This residue is always His in those members shown to have *TFL1*-like function and Tyr in those with *FT*-like function. The two potato *FT* homologs exhibit a Tyr residue in this position, consistent with a likely *FT/Hd3a* ortholog function of these genes. Expression analysis showed that one of these genes is strongly induced under SD conditions but not under LDs (A. López & S. Prat, unpublished results). Abundance of this mRNA was much higher in the *phyB* antisense lines than in wild type, and this transcript is repressed in the pACO lines, in which tuberization induction is delayed. Hence, we have observed a strong correlation between levels of expression of this transcript and tuberization induction, pointing to a function of this gene as an *FT* ortholog. These results indicate that genetic mechanisms controlling photoperiodic flowering in rice and *Arabidopsis* and tuberization in potato are closely related (**Figure 5**). Control of tuberization time by *StCOL3* is then likely mediated by regulation of *FT* activity, perhaps through a PHYB-dependent mechanism of regulation similar to that reported in rice (57). According to this model, interaction of PHYB with *StCOL3* in the presence of light would repress *FT* expression in LDs, whereas in SDs, *StCOL3* accumulates in the dark, thus activating *FT* expression. In the *phyB* mutants, PHYB is repressed and interaction with *StCOL3* in the light is abolished. These plants then accumulate the *FT* transcript and tuberize in LDs. Further studies are required to confirm this hypothetical model and to determine how *FT* expression promotes tuberization transition. The mechanism of action of these proteins was explored by means of protein-protein interaction studies in a yeast two-hybrid system, which led to identification of several interacting partners (92). One of such partners, the bZIP transcription factor FD, is expressed at the shoot apex before floral induction and forms a complex with FT that activates transcription of the floral identity gene *APETALA1* (*AP1*) (4, 141). It will be interesting to investigate whether similar transcription factors act as partners of FT in the stolon subapical meristem, and which are the possible tuber identity genes regulated by this complex.

GENE EXPRESSION DURING TUBER DEVELOPMENT

Which genes act downstream of *StFT* to trigger tuberization transition? Molecular mechanisms underlying early phases of tuber development are still poorly understood. Previous gene-expression analyses mainly focused on the later phases of tuber growth, in which starch accumulation and storage protein synthesis take place. In these studies, lipoxygenase and proteinase inhibitor transcripts, such as proteinase inhibitor I, proteinase inhibitor II, or Kunitz-type proteinase inhibitors, were abundant, raising the question of whether these inhibitors may function as storage proteins in addition to providing protection against pest attack. The major storage protein of potato tubers is patatin, which makes up to 40% of the total tuber protein. On the other hand, starch is the primary constituent of potato tubers, accounting for 80% of the tuber dry matter. Genes involved in starch synthesis and degradation are therefore abundant during the later stages of tuber growth.

More recent RNA-profiling studies of earlier stages of tuber development show a common expression profile, characterized by a strong up- or downregulation of several genes, illustrating highly coordinated pathways of transcriptional control during tuber initiation and growth (69). One of the earliest genes to be upregulated was GA 2-oxidase, as reported above. Upregulation of this gene was observed prior to visible swelling, indicating that a decrease in GA levels precedes other transcriptional changes during tuber transition. Genes involved in starch synthesis were strongly upregulated from the swelling

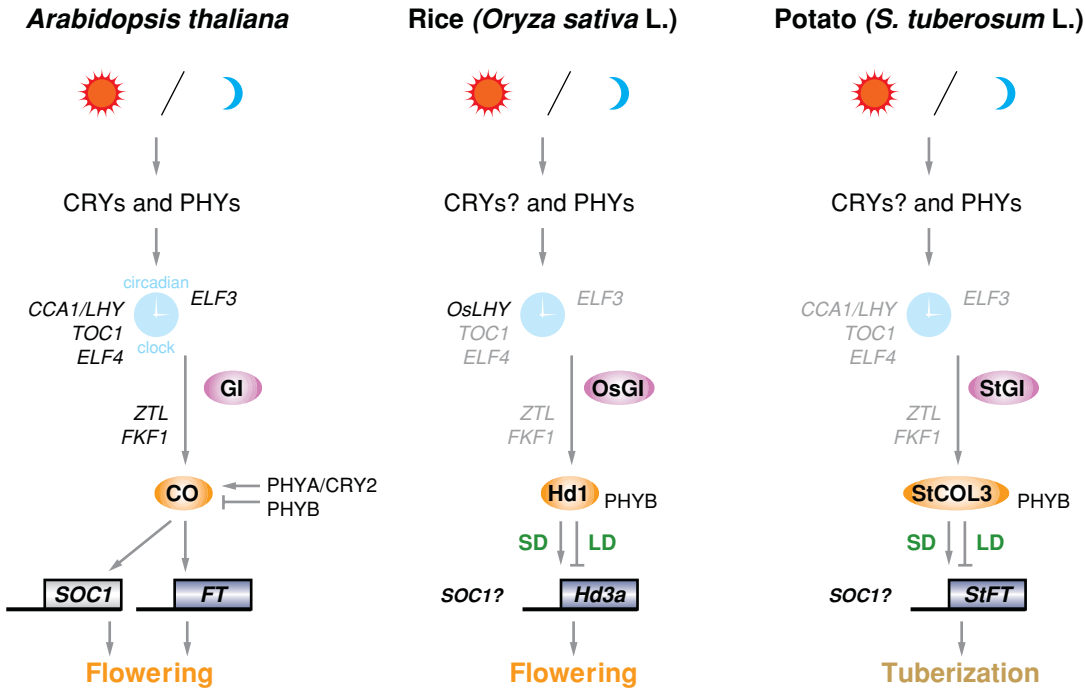

Figure 5

Short-day (SD) pathway controlling tuberization in potato: conservation with the day-length flowering pathways in *Arabidopsis* and rice. The circadian clock is reset by phytochromes and cryptochromes and is the central component of the day-length measuring mechanism. The clock regulates abundance of GIGANTEA (*GI*), which positively regulates transcription of the nuclear zinc-finger proteins constans (CO) and Heading date 1 (Hd1). These transcription factors in turn regulate expression of flowering locus T (FT) and Heading date 3a (H3da), two RAF-kinase inhibitor-related proteins that strongly promote flowering. CO/Hd1 activity is regulated by light in a post-transcriptional manner. Whereas CO activates transcription of *FT* in the light, Hd1 appears to activate *H3da* expression in the dark, but negatively control expression of this floral integrator in the light. Interaction of Hd1 with phytochrome appears to mediate *H3da* repression in the light. Homologs of *GI* (*StGI*), *CO* (*StCOL3*), and *FT* (*StFT*) have been identified in potato and preliminary evidence indicates that they are implicated in tuberization control. *StFT* mRNA accumulation correlates with the peak of *StCOL3* transcript during the night, suggesting a light-reversed mechanism of regulation, as reported in rice. Components of the clock autoregulatory feedback loop such as *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*), *TIMING OF CAB EXPRESSION1* (*TOC1*), and *EARLY FLOWERING4* (*ELF4*) have not been characterized in potato. Genes involved in the light input to the clock, such as *EARLY FLOWERING 3* (*ELF3*) or the F-box kelch repeat proteins *FLAVIN-BINDING KELCH REPEAT F-BOX1 (FKF1)* and *ZEITLUPE* (*ZTL*), also remain to be identified.

stage onward. Expression of genes involved in sucrose cleavage changed during the early stages of tuber development, correlating with the switch from apoplastic to symplastic sucrose unloading (8, 135). Sucrose synthase (*SUSY*) and fructokinase increased dramatically, whereas transcripts for soluble acid invertase were downregulated. Plastidic phosphoglucomutase was strongly upregulated, whereas levels of the cytosolic isoforms did not change, thereby reflecting an active import of glucose-6-phosphate into the amyloplast (124). ADP-glucose pyrophosphorylase (*AGPase*), with a key regulatory function

in starch synthesis (84), and transcripts for soluble starch synthase (*SSS*), granule-bound starch synthase (*GBSS*), and starch branching enzyme (*BE*), were strongly upregulated at tuber organogenesis and further tuber development, consistent with the role of these enzymes in starch synthesis (31, 35).

In addition to a steady down- or upregulated trend, observed for genes involved in sucrose-to-starch synthesis or storage proteins, a transient change in gene expression was also detected for several additional genes (69). Such up- or downregulated peak of expression occurred at stolon swelling and tuber initiation, thus suggesting a function of these genes in cell division and cell expansion (142). Gene-expression analyses using the luciferase reporter system showed that cell division and protein storage and starch synthesis do not occur in a fixed order during tuber development (134). In vivo analyses using promoter fusions of the *AGPaseS*, the patatin *Pat21* and cell-cycle genes *cycB1* and *CDC2a*, showed that whereas induction of the *CDC2a* and *cycB1* genes always coincided with visible swelling, activation of the *AGPaseS* and *Pat21* genes was highly variable. Activation of these promoters oscillated from 4 days before to 10 days after onset of swelling, and occasionally they were also upregulated in stolons. This suggests that the signals responsible for activating starch and storage protein accumulation are different from those inducing swelling, and therefore that two independent signaling pathways are involved at the control of these genes during tuber development.

Although no clear indication of the molecular mechanisms involved in tuber transcriptional control has been obtained to date, there is no doubt that sucrose is an important metabolic signal coordinating gene expression during tuber initiation and growth. In vitro tuberization is highly dependent on sucrose (115, 143), suggesting a specific role of sucrose in triggering storage sink function. The switch from apoplastic to symplastic sucrose unloading during tuberization is correlated with a decrease in hexose and a concomitant increase in sucrose levels in the stolon subapical cells. Such a rise in cellular sucrose likely mediates increased starch synthesis and storage protein gene expression in the tuberizing stolons (51, 108). Consistent with this function, genes involved in sugar metabolism and starch synthesis, including *SUSY* and *AGPase*, are activated by sucrose (84, 110), and sucrose also mediates post-translational regulation of AGPase (50), thereby providing an additional short-term level of control in starch synthesis. Sucrose also upregulates expression of the patatin and proteinase inhibitor II genes in organs different from the tubers (64, 89, 139). Tuber-specific and sucrose-inducible *cis* elements were identified in the patatin promoter (39, 68), and a DNA-binding protein designated Storekeeper (STK) was identified, which binds to these elements and apparently mediates tuber-specific and sucrose-inducible expression of this gene (148). In addition to cytokinin, high sucrose was also correlated with increased levels of expression of the cell-cycle D-type cyclin transcripts (24, 101), suggesting that sucrose signaling might also play an important role in modulating cell division and stolon swelling during tuber differentiation. Together, these observations point to a central role of sucrose in promoting storage cell differentiation and source-sink regulation, with interaction between the sugar, gibberellin, and cytokinin signal transduction pathways likely involved in such control (102).

Components involving sugar sensing and sugar signaling are starting to be unveiled. A regulatory role for hexokinase (HXK) as a glucose sensor controlling multiple plant growth and developmental responses, for example, has been demonstrated (113, 116). Isolating glucose insensitive (*gin*) mutants showed that several of these mutations were allelic to ABA signaling genes, pointing to a close interaction between the glucose and ABA signaling pathways (104). Sucrose nonfermenting 1-related protein kinases (SNF1) also function as mediators of sucrose-specific signals and are induced in response to high sucrose. Antisense

potato lines with reduced levels of expression of the SNF1-related protein kinase PKIN1 showed decreased sucrose synthase gene expression and failed to induce expression of this gene in response to high sucrose, thus providing clear evidence for a role of PKIN1 in the sucrose signaling pathway regulating carbohydrate metabolism (96). Sucrose-specific signaling pathways also repress translation of the *Arabidopsis* ATB2 bZIP transcription factor, through a conserved upstream open reading frame in the 5'-UTR of the gene (105, 140). However, future studies will be required to demonstrate a function of these sugar signaling components in tuber differentiation and define how transition to a tuber fate is integrated with this sugar signaling pathway.

A pending issue is the identification of the genes conferring tuber identity. As opposed to flowering, tuber formation does not involve organogenesis. Tubers are comprised by the storing parenchyma cells, vascular bundles, and the peridermis, but there is no differentiation in specialized organs. Therefore, floral homeotic genes involved in whorl organ formation have not been identified in tubers. This, together with the observation that tuber storage proteins can accumulate in aerial organs, brought the belief that tuber formation is under less strict control than flowering. Pathways involved in day-length control tuber transition, however, are closely related to those controlling flowering. Upregulated expression of *FT*-ortholog genes was also correlated with tuberization induction, which indicates that genes acting downstream of these integrators may be directly involved in tuberization transition by promoting differentiation of stolon meristem cells to a tuber fate. Aside from a possible implication of the KNOX/BEL tandem of transcription factors, no clear clues exist about these tuber identity genes. The expression of inducible constructs for the *StFT* integrators will be instrumental to the identification of such important genes.

CONCLUSIONS AND PERSPECTIVES

Most potato varieties are tetraploid, which adds considerable difficulty to genetic studies in these plants. Transformation methods are, however, well established in cultivated and *andigena* species, and transgenic approaches substantially contributed to our understanding of the molecular mechanisms underlying day-length control of tuberization. This research has provided evidence for two main pathways of tuberization control: a GA-dependent and a SD-dependent pathway, with concerted action of these two signaling pathways regulating tuberization transition of the stolon subapical meristem cells.

GAs have long been considered inhibitory to tuberization. Modification of GA levels or block of the response to GAs by expression of the *Arabidopsis gai-1* dominant allele showed that GA signaling has a negative role in tuberization transition, by repressing differentiation of the stolon cells to a tuber fate. Another important finding was that increased levels of these hormones in the shoot were not only not detrimental to tuber formation but seemed to be required for induction. Strong tuberization induction in *phyB* lines, for example, coexist with a fourfold increase in endogenous levels of GA_1, whereas the potato *CO* overexpresser lines (pACO) show delayed tuberization in SDs, and a characteristic semidwarf phenotype indicative of a reduction in GA levels. These observations suggest some sort of cross-talk regulation between the SD and GA pathways, although further studies will be necessary to clarify the mechanisms underlying such interaction.

Evidence for a role of *CO* in tuberization transition was obtained by expression of the *Arabidopsis* gene in *andigena* potato plants. Orthologs of the *GIGANTEA*, *CO*, and *FT* genes were recently identified in potato and preliminary evidence points to a conserved role of these genes in tuberization control. Hence, regulatory pathways involved in day-length control of tuberization appear to be closely

related to those controlling flowering time in *Arabidopsis* or rice. Transcription of the potato *CO* ortholog *StCOL3* peaks during the day in LDs, but accumulates in the dark in SDs. This indicates that in potato, as in rice, expression of the target *StFT* genes is repressed by *CO* in the light and induced by this regulatory protein in the dark. Thus, reverse regulation of these genes might be a general feature of SD plants and be the principal mechanism underlying differential response to day length in SD as compared to LD species. How this different regulation is generated is not yet understood. Direct comparison of the SD or LD orthologs will help to shed light on such differential mechanisms of transcriptional control.

Quantitative trait loci (QTL) analyses have identified 11 loci affecting tuberization in reciprocal backcrosses between *Solanum tuberosum* and *S. berthaultii*, a major QTL being mapped on chromosome 5 (132). The *Arabidopsis* MADS box protein *SUPPRESSOR OF OVER-EXPRESSION OF CO1* (SOC1) is another target of CO with a function as flowering time integrator (77, 111), and at least one *SOC1* homolog has been identified in potato, although the function of this homolog in tuberization control has not been tested. Sequencing of the complete tomato genome will undoubtedly aid the identification of additional flowering time homologs, and isolation of the corresponding potato orthologs is relatively straightforward due the high-nucleotide sequence conservation between tomato and potato. Hence, combining comparative genomics and reverse genetics studies should contribute in the future to our understanding of the complex genetic networks controlling tuber development in potato and to the identification of the gene targets of these genetic pathways involved in triggering stolon-to-tuber transition and thereby a function as tuber identity genes.

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Using grafts of tobacco scions into *andigena* potato stocks, the authors demonstrate that the flowering signal produced in tobacco induces tuberization in the potato stock.

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