Sugar-mediated semidian oscillation of gene expression in the cassava storage root regulates starch synthesis

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Abbreviations: ABA, Abscisic acid; HXK, Hexokinase; SBE, Starch branching enzyme; SUT, Sucrose translocator

Running title: Oscillation of *SBE* expression in cassava

Abstract

Starch branching enzyme (SBE) activity in the cassava storage root exhibited a diurnal fluctuation, dictated by a transcriptional oscillation of the corresponding *SBE* genes. The peak of SBE activity coincided with the onset of sucrose accumulation in the storage, and we conclude that the oscillatory mechanism keeps the starch synthetic apparatus in the storage root sink in tune with the flux of sucrose from the photosynthetic source. When storage roots were uncoupled from the source, *SBE* expression could be effectively induced by exogenous sucrose. Turanose, a sucrose isomer that cannot be metabolized by plants, mimicked the effect of sucrose, demonstrating that downstream metabolism of sucrose was not necessary for signal transmission. Also glucose and glucose-1-P induced *SBE* expression. Interestingly, induction by sucrose, turanose and glucose but not glucose-1-P sustained an overt semidian (12-h) oscillation in *SBE* expression and was sensitive to the hexokinase (HXK) inhibitor glucosamine. These results suggest a pivotal regulatory role for HXK during starch synthesis. Abscisic acid (ABA) was another potent inducer of *SBE* expression. Induction by ABA was similar to that of glucose-1-P in that it bypassed the semidian oscillator. Both the sugar and ABA signaling cascades were disrupted by okadaic acid, a protein phosphatase inhibitor. Based on these findings, we propose a model for sugar signaling in regulation of starch synthesis in the cassava storage root.

Introduction

In plants, sugar sensing and signaling play critical roles in controlling many aspects of growth, metabolism and development throughout the whole plant life cycle.[1-4] Through photosynthesis, plants convert atmospheric carbon dioxide to sugars, which are transported as sucrose from sugar-exporting (source) organs, such as leaves, to sugar-importing (sink) organs, such as tubers, seeds, or storage roots. The coordinated modulation of gene expression in source and sink organs is to a large extent choreographed by the sugar status in the cells (see ref. 5 and refs therein). In general, low sugar levels promote photosynthesis and mobilization of energy reserves, whereas high sugar levels stimulate growth and storage of starch and other carbohydrates.

Development of sink organs is orchestrated by the coordinated activities of a large number of genes that encode metabolic and regulatory enzymes, as well as other proteins. Starch synthesis is catalyzed by an enzymatic machinery containing ADP-glucose pyrophosphorylase (AGPase), starch synthases (SS), starch branching enzymes (SBE), and starch debranching enzymes (DBE). Most, if not all, of these enzymes exist in two or more isoforms (see refs. 6-8 for reviews on starch biosynthesis).

Cassava (*Manihot esculenta* Crantz L.) is a perennial starch crop of major global importance, particularly in the Developing World.[9,10] Starch is synthesized and deposited in the underground tuberous storage roots, which can measure up to 1 m in length and over 10 cm in diameter. The storage root is a non-reproductive organ and can accumulate close to 85% of its total dry weight as starch.

During development of the cassava storage root, expression of the *SBEII* and *SBEI* genes, encoding, respectively, SBEII and SBEI, gradually increases from 90 to 360 days after planting (d.a.p.).[11] Accumulation of *SBEII* and *SBEI* transcripts in 360 d.a.p. plants was also found to exhibit a short-term fluctuation with a period of one day. In the work presented here, we followed the induction of *SBE* expression in isolated cassava storage root discs by sugars, sugar analogs and abscisic acid (ABA). Our results establish the presence of an endogenous semidian (12-h) oscillator in the storage root cells at the level of hexokinase (HXK). They also suggest that sugar and

ABA signaling proceeds via independent pathways, and that the sugar signaling cascade is activated by the entry of sucrose into the cell and relies on HXK activity.

Results

Diurnal control of *SBE* expression in the cassava storage root is mediated by a local, semidian oscillator

Typical 24 h expression cycles for the cassava *SBEII* and *SBEI* genes in the underground storage root from plants grown under two different day-night (LD) regimes are displayed in Fig. 1 a. Under normal growth conditions, transcription peaked at midday, corresponding to six hours into the light zone. When the onset of light was moved forward, the peak of transcription shifted accordingly. Zymogram analysis revealed that the enzymatic activity of the two proteins fluctuated in accordance with *SBEII* and *SBEI* transcript levels (Fig. 1 b). This implies that the diurnal rhythm in *SBEII* and *SBEI* expression is a regulatory phenomenon and suggests a dynamic behavior of starch synthesis in the storage root during a 24 h period. In addition to the SBEII and SBEI activities, a third activity band was visible on the zymogram. This likely corresponds to endogenous starch phosphorylase *a* (Sun et al., 2005).

There is ample evidence that sucrose stimulates expression of starch synthesis genes in many plants via a sugar-signaling pathway.[2-5,14-16] Thus a likely scenario is that the oscillation in *SBEII* and *SBEI* activity in the cassava storage root is controlled by the import of photosynthate, i.e. sucrose, from the source tissues to the underground sink. To probe this hypothesis, we monitored the time course for sucrose accumulation in the cassava storage roots from LD plants (Fig. 2). The results revealed that the onset of sucrose accumulation in the storage root coincided with the maximum levels of *SBEII* and *SBEI* transcripts, and that the maximum sucrose concentration was reached at 1 p.m., one hour after the *SBEII* and *SBEI* peak time. We interpret these findings to mean that the transcriptional machinery for the *SBEII* and *SBEI* genes in the cassava storage root is set to anticipate the incoming sucrose, i.e. the substrate for starch synthesis, from the source. The situation becomes

analogous to the circadian rhythm of photosynthesis genes, which prepares the pre-dawn photosynthetic cell for the approaching light exposure.

To further our understanding of the temporal influence of sucrose on *SBE* expression in the storage roots, we uncoupled the sink organs from source control and examined *SBEII* expression in cassava root discs from plants that had been kept in continuous darkness (DD) for 48 h to remove endogenous metabolizable sugars. The root slices were maintained under DD conditions to mimic the natural environment and transferred to sucrose medium. No *SBEII* expression could be detected in discs on non-sucrose medium (Fig. 3 a). Shifting to sucrose-containing medium efficiently increased endogenous sucrose levels and *SBEII* transcripts appeared within two hours. Surprisingly, both transcription and enzyme activity in the isolated root discs oscillated with a period of approximately 12 h (Fig. 3 b, c). Since the sucrose concentrations did not change accordingly but, rather, showed a slight decline (Fig. 3 d), the results clearly demonstrate that the observed oscillations are not governed by fluctuations in the sucrose output form the source tissues but originate from an endogenous, semidian (12-h) oscillator in the storage root itself. The oscillations persisted without any damping beyond day 2 after transfer to sucrose medium.

Sugar-induced semidian oscillation of *SBE* expression relies on sucrose sensing at the plasma membrane and HXK activity

Employing different sugars and sugar analogs potentially can yield valuable clues regarding the mechanisms of the sugar-signaling pathway. This is particularly true since exogenously supplied sucrose can readily convert to hexoses in the apoplast or cytosol, obscuring interpretations as to whether the sensed sugar is sucrose or some downstream metabolite. Palatinose (α–glucose-1,6-β-fructose) and turanose (α–glucose-1,3-β-fructose) are both isomers of sucrose (α–glucose-1,2-β–fructose). From the plants so far studied, it is generally considered that palatinose and turanose can neither be recognized by plant sucrose transporters (SUTs), nor be metabolized by plant enzymes.[17-20] However, although they are not taken up by plant cells, work on barley embryos,[21] potato tubers[17] and tobacco leaves[22,23] shows that palatinose and turanose can modulate sugar signaling, and it was suggested that they are perceived extracellularly, possibly at the plasma membrane, by disaccharide sensors distinct from SUTs.[18,21] Also, at least in one case, for the main phloem-loading SUT in

Arabidopsis (AtSUC2), it was shown that turanose could serve as a transported SUT substrate.[24] Uptake of turanose was also reported for leaves of garden cress[25] although it is not clear if a SUT or some other transporter was engaged.

As is demonstrated in Figure 4, addition of turanose to cassava storage roots induced *SBE* expression to almost the same extent as sucrose. Remarkably, turanose also mimicked sucrose by sustaining the semidian oscillation of *SBE* transcript levels (Fig. 4 b). Glucose and fructose also stimulated *SBE* expression but were weaker inducers than sucrose. Mannose, which is taken up by the cells and phosphorylated by HXK,[18,26,27] as well as mannitol and 3-*O*-methyl-glucose, which are taken up by the cells but are poor substrates for HXK, had no effect. Also trehalose, which has been implicated in plant sugar signaling, [2,4] had no effect.

The inability of mannose to induce *SBE* expression implies that HXK-dependent phosphorylation of an incoming substrate does not suffice to initiate a signaling event, contrary to the phosphotransferase system in bacteria. This by itself, however, does not disqualify HXK as an important element in the intracellular signaling pathway. A possible path would be that the signal initiated at the level of a SUT, or a designated sucrose sensor, in the plasma membrane results in activation of HXK, with subsequent phosphorylation of endogenous glucose. In the intact plant, glucose will be plentiful in developing storage roots but in our experimental system it would likely be derived from mobilization of starch reserves in the amyloplast. To assess the requirement for HXK in sucrose induction of *SBE* expression, we investigated the effects of sucrose and turanose in the presence of glucosamine, a well-documented HXK inhibitor.[2,4,18] The results revealed that induction by turanose was completely abolished and that of sucrose severely diminished after addition of the inhibitor (Fig. 4 c). Thus the signal that emanates from sucrose sensing at the plasma membrane depends on HXK activity for downstream transduction.

The finding that the sugar-signaling pathway was disrupted by HXK inhibition, suggested that hexose phosphates, such as glucose-1-P or glucose-6-P, might be potent inducers of *SBE* expression. To our knowledge, plant plasma membranes do not contain hexose phosphate translocators and, therefore, delivery of extracellular hexose phosphates to storage root slices is expected to be poor. However, we found that the effect of exogenous glucose-1-P on *SBE* induction was stronger than for glucose and comparable to that of sucrose (Fig. 4 *a*). If the efficient uptake of glucose-1-P points to a plasma membrane glucose transporter with broad substrate

specificity, or to some other facilitated transport mechanism is not known. Glucose-1-P and glucose-6-P are equilibrated in the cell by the action of phosphoglucomutase, and so we do not know which of them is the signaling agent, or if they both are equally effective. A noteworthy difference between glucose and glucose-1-P was that glucose, just like sucrose and turanose, supported oscillation of *SBE* expression, whereas glucose-1-P did not (Fig. 4 *b*).

Based on these findings, we arrive at the following conclusions concerning the sugar-signaling cascade that regulates the semidian *SBE* expression in the cassava sink cells:

1. The main trigger for the signaling event operates at the level of sucrose perception or transport, i.e. the arriving sucrose molecule from the source does not need to be hydrolyzed by cell wall invertase or cytosolic enzymes.

2. HXK activity is crucial for the intracellular signaling pathway, and it is the hexose phosphates, not the phosphorylation step, that mediate the signal.

3. HXK is activated by sucrose and, possibly, glucose transport at the plasma membrane.

4. The oscillator that brings about the semidian rhythmicity of *SBE* expression is located upstream of glucose-1-P/glucose-6-P but downstream of glucose, i.e. at the level of HXK.

ABA promotes induction of *SBE* expression but bypasses the oscillator

Sugar signaling does not operate in splendid isolation but rather is integrated in cellular regulatory networks. Most notably, there is a tight interaction between sugar and hormonal signaling, particularly for ABA.[2,4,28-30] Work on *Arabidopsis* has shown that ABA stimulates the accumulation of storage reserves, such as starch,[28] and from studies on *Arabidopsis* leaves it was reported that ABA enhances sucrose-induction of genes encoding SBE2.2 and the small subunit of AGPase.[28,31]

Addition of ABA to cassava storage root discs effectively induced *SBE* expression (Fig. 5 *a*). Induction occurred both in the absence or presence of added sucrose, and the effects were additive. Importantly, ABA-induced *SBE* expression in cassava sink cells was constitutive and did not oscillate (Fig. 5 *b*). Thus induction of *SBE* expression by both ABA and glucose 1-P bypasses the semidian oscillator. Taken together, these results also tentatively place the influence of ABA in the sugar-

hormonal regulatory network downstream of HXK, a notion supported by the insensitivity of ABA induction to glucosamine (Fig. *5 c*).

Not surprisingly, several studies have provided evidence for the involvement of reversible phosphorylation events in ABA signaling.[14] Two of the genes implicated in ABA signaling turned out to encode protein phosphatases[32] and inhibition of protein phosphatase 1 and 2A by okadaic acid was found to alter ABA-induced gene expression.[33] As is shown in Fig. 5 *d*, okadaic acid inhibited ABA induction of *SBE* expression. Furthermore, okadaic acid inhibited also the induction by sucrose, turanose, glucose and glucose-1-P (Fig. 5 *e*), suggesting that protein phosphatase 1 or 2A might be a common regulator for the sugar and ABA signaling pathways in the cassava storage root.

Discussion

The findings in this work are summarized in a model of the sugar-induced signal transduction pathway that controls *SBE* expression in the cassava storage root sink (Fig. 6). Sucrose arriving in the sieve elements is translocated to the sink cells via a SUT. Some of the sucrose might be converted to glucose and fructose by apoplastic invertase, with subsequent uptake of the hexoses through monosaccharide transporters (MSTs). The SUT-substrate complex transmits a signal to HXK, activating the enzyme above a basal constitutive level. A similar signal might originate at the engaged MST. In both cases, the rationale is to signal to the cell that carbon has been allocated from the source to the sink for starch synthesis, and that glucose-6-P is in demand for transport to the amyloplast. From glucose-6-P, or a downstream metabolite, the signal eventually leads to activation of a protein phosphatase. The ensuing events are likely to involve migration of a transcription factor to the nucleus, followed by activation of the *SBE* genes.

Whereas the sugar-signaling pathway serves to regulate *SBE* expression in response to photosynthate status, ABA signaling would communicate stress conditions. It is known that environmental factors such as temperature and length of water deficit affect root expansion in cassava, and it has been proposed that ABA plays a critical role in the regulatory network that is activated by drought.[9,34] Under

such circumstances, or when the sink cell experiences osmotic stress due to high influx of sugars, conditions are not favorable for continued photosynthesis and vegetative growth and the plant would downregulate source activities and upregulate sink activities. From their work on regulation of starch synthesis in *Arabidopsis* leaves,[31] suggested a model where ABA signaling increases the sensitivity of metabolic processes to a separate sugar signal. High sugar levels and other high osmotic conditions result in ABA accumulation and induces a "storage mode", while in the absence of ABA the plant switches to a "mobilization mode". In our model, the cross talk between sugar and ABA signaling merges at the protein phosphatase. Since induction of *SBE* expression by sucrose and ABA in combination was higher than for sucrose alone, it is quite possible that ABA boosts the sugar signal by interacting with the protein phosphatase. The placement of ABA activity downstream of HXK follows from the observation that signaling by both ABA and glucose-1-P circumvented the oscillator, and from the fact that ABA induction was insensitive to HXK inhibition.

The nature of the semidian oscillator is unknown but we hypothesize that it is functionally linked to HXK; semidian rhythmicity of *SBE* expression was obtained after induction with sucrose, turanose and glucose but not glucose-1-P or ABA. Conceivably, an oscillation in HXK activity could be dictated by a transcriptional rhythm for a HXK-interacting protein in very much the same way as described by Jones and Ort[35] for the circadian regulation of sucrose phosphate synthase (SPS) activity in tomato leaves. The authors showed that the activity of SPS was dependent on reversible phosphorylation and subject to both diurnal and circadian control, and they inferred that an endogenous rhythm in the transcription of a protein phosphatase promoted the circadian oscillation of the SPS phosphorylation state. Interestingly, a 12-h endogenous rhythm for SPS activity was reported for soybean leaves.[35,36] The diurnal behavior of *SBE* transcription and SBE activity in the storage roots of intact cassava plants and the existence of a local semidian oscillator in the storage root cells suggest that the temporal regulation of *SBE* expression comprises multiple levels of modulation.

Arguably, our studies on isolated storage root discs suffer from several shortcomings with regards to transposing the results to the whole plant level. For example, we do not know if phloem unloading in cassava is apoplastic or symplastic and, hence, to what extent the apoplastic uptake of sucrose that occurs in the root discs represents the normal transfer of solutes in cassava. In potato, phloem unloading

shifts from essentially apoplastic during stolon elongation to predominantly symplastic during tuberization.[37,38] Obviously, it will be important to determine the principles for phloem unloading in the cassava plant.

Another issue concerns the physiological significance of the semidian (and diurnal) fluctuations of *SBE* gene expression in the cassava storage root. It has been shown recently that SBE activity in wheat amyloplasts and chloroplasts is regulated by phosphorylation.[39] If this is the same in cassava, and how such an allosteric regulation would cooperate with the semidian oscillation of *SBE* expression during development of the cassva storage root, remains to be investigated.

There are also questions with regards to the metabolic fluxes proposed in Fig. 6 that need to be addressed in future experiments. Although it is well established that UGPase is involved in production of Glu-1-P[40-42], in their work on transgenic potatoes Zrenner et al.[43] suggested that only a minor portion of UGPase activity is required for normal carbon metabolism and starch synthesis in tubers. Thus to what extent UGPase contributes to Hx-P pool levels in the cassava storage root is not yet clear. Further, we are assuming in our model that the predominant form of carbon uptake in cassava amyloplasts is Glu-6-P as is the case in potato tubers, and not ADP-Glu as in cereals seeds (see recent review by Geigenberger and Fernie[44]). That, of course, needs to be firmly established.

In conclusion, we have uncovered a novel feature in the regulation of starch synthesis, an endogenous semidian sink oscillator, and present a model of the sugar-signaling network that controls *SBE* expression in the developing cassava storage root. We hope the model provides a framework for further studies in plant sugar signaling and sink-source communication.

Materials and methods

Plant material and growth conditions

Manihot esculenta Crantz cv. MH95/0414, officially designated as NASE 12 in Uganda, was grown in a glasshouse under a 14-h light, 23 °C/10-h dark, 18 °C (LD) regimen. Unless stated otherwise, growth conditions were as described.[11] Cassava

storage roots were sampled at 360 d.a.p. for transcript, protein and carbohydrate analyses.

RNA gel blot analysis

Total RNA extraction from cassava storage roots and RNA gel blot analysis was carried out as described by Baguma et al.[11]

Zymogram analysis

Zymograms for SBE activity were performed as described by Sun et al.[12]

Sugar and starch analyses

Sugars were extracted by the ethanol method.[13] Estimation of sugar and starch content was done by measurement of changes in NADPH concentration at A_{340} using the Enzytec™ Kits (Scil Diagnostics GmbH, Sweden).

Induction of *SBE* expression

360-day-old plants were entrained to dark conditions for 48 h. In series, cassava storage roots were harvested, sliced into 5 mm discs and depleted of endogenous sugars in 3% mannitol (v/v) for 24 h. The discs were subsequently transferred to a stack of Whatman papers saturated with 200 mM sugars or sugar analogs, and/or 5 μM ABA. Glucosamine (0-100 mM) or okadaic acid (1μM) were added as indicated.

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Figure legends

Figure 1. Activity rhythms in the cassava storage root from plants grown under LD conditions.

(a) RNA gel blot analysis. Storage root RNA was extracted from plants grown under two different LD regimens at indicated times during the day and assayed for *SBEII* and *SBEI* gene activity.

(b) Zymogram analysis. Protein was extracted from storage roots at indicated times during the day and analyzed for starch branching enzyme activity.

Times are indicated as follows: 24, midnight; 06, 6 am; 12, noon; 18, 6 pm; 21, 9 pm. SPa, starch phosphorylase *a*.

Figure 2. Changes in carbohydrate levels in the cassava storage root from plants grown under LD conditions.

(a) Starch and sugars were extracted at indicated times during the day. Lv-suc, leaf sucrose; Lv-starch, leaf starch; Lv-glu, leaf glucose; SR-suc, storage root sucrose; SR-glu, storage root glucose.

(b) *SBEII* and *SBEI* gene activity between 11 am (11) and 1 pm (13).

Times are indicated as follows: 9, 9 am; 10, 10 am; 11, 11 am; 12, noon; 13, 1 pm; 14, 2 pm; 15, 3 pm.

Figure 3. Sucrose induction of *SBE* expression and SBE activity in isolated discs of cassava storage roots kept under DD conditions.

(a) RNA gel blot analysis of *SBE* expression. Root discs from dark-adapted plants (360-d-old) were depleted of endogenous sugars for 48 h (48-h-DD) and then transferred to sucrose medium. RNA was extracted at indicated times after sugar induction and assayed for *SBEII* and *SBEI* activity.

(b) RNA gel blot analysis of *SBE* expression. A detailed time-course for *SBEII* and *SBEI* gene activity in the interval 12 – 24 h after sugar induction.

(c) Zymogram analysis. Protein was extracted from storage root discs at indicated times after sugar induction and analyzed for starch branching enzyme activity. (For identification of the activity bands, see Sun et al., 2005).

(d) Sucrose content. Sugars were extracted from storage root discs depleted of endogenous sugars for 48 h (48-h-DD) and at indicated times after addition of sucrose or H_2O.

SPa, starch phosphorylase *a*, Suc, sucrose

Figure 4. RNA gel blot analyses of *SBE* expression after application of sugars or sugar analogs to isolated discs of cassava storage roots kept under DD conditions.

(a) *SBE* expression after addition of sucrose (Suc), glucose (Glu), fructose (Fru), glucose-1-P (Glu-1-P), trehalose (Tre), mannose (Man), mannitol (Mnt), 3-*O*-methyl glucose (3-O-m-glu), or turanose (Tur).

(b) *SBE* expression at different time points after addition of turanose, glucose, or glucose-1-P (Glu-1-P). Times are indicated as in Fig. 1.

(c) Sucrose or turanose-induced *SBE* expression in the presence of 0-100 mM glucosamine (Glu-NH_2).

Figure 5. RNA gel blot analyses of *SBE* expression after application of sucrose, glucose, turanose and/or abscisic acid (ABA) to isolated discs of cassava storage roots kept under DD conditions.

(a) Induction of *SBE* expression by ABA, sucrose, or ABA + sucrose.

(b) *SBE* expression at indicated times after induction with ABA.

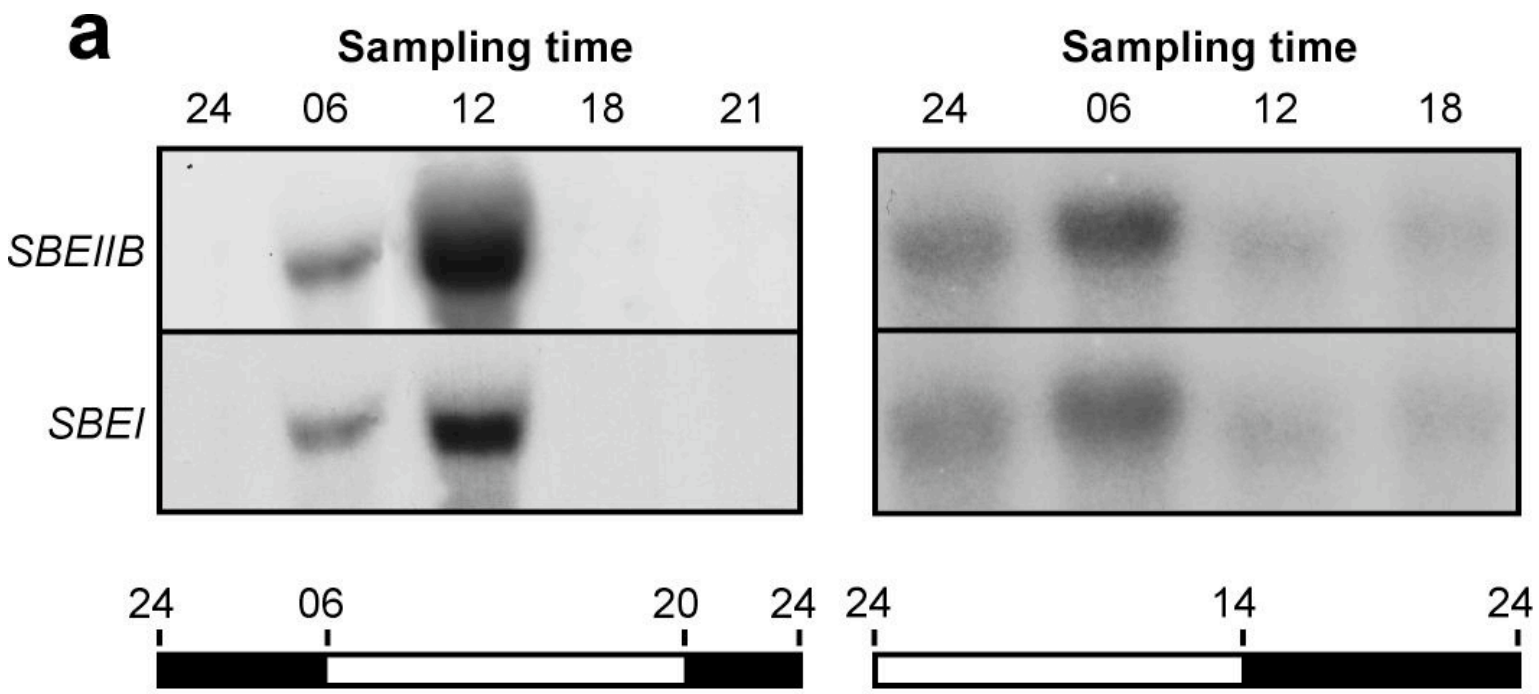
(c) Induction of *SBE* expression by ABA in the presence or absence of 100 mM glucosamine (Glu-NH_2).

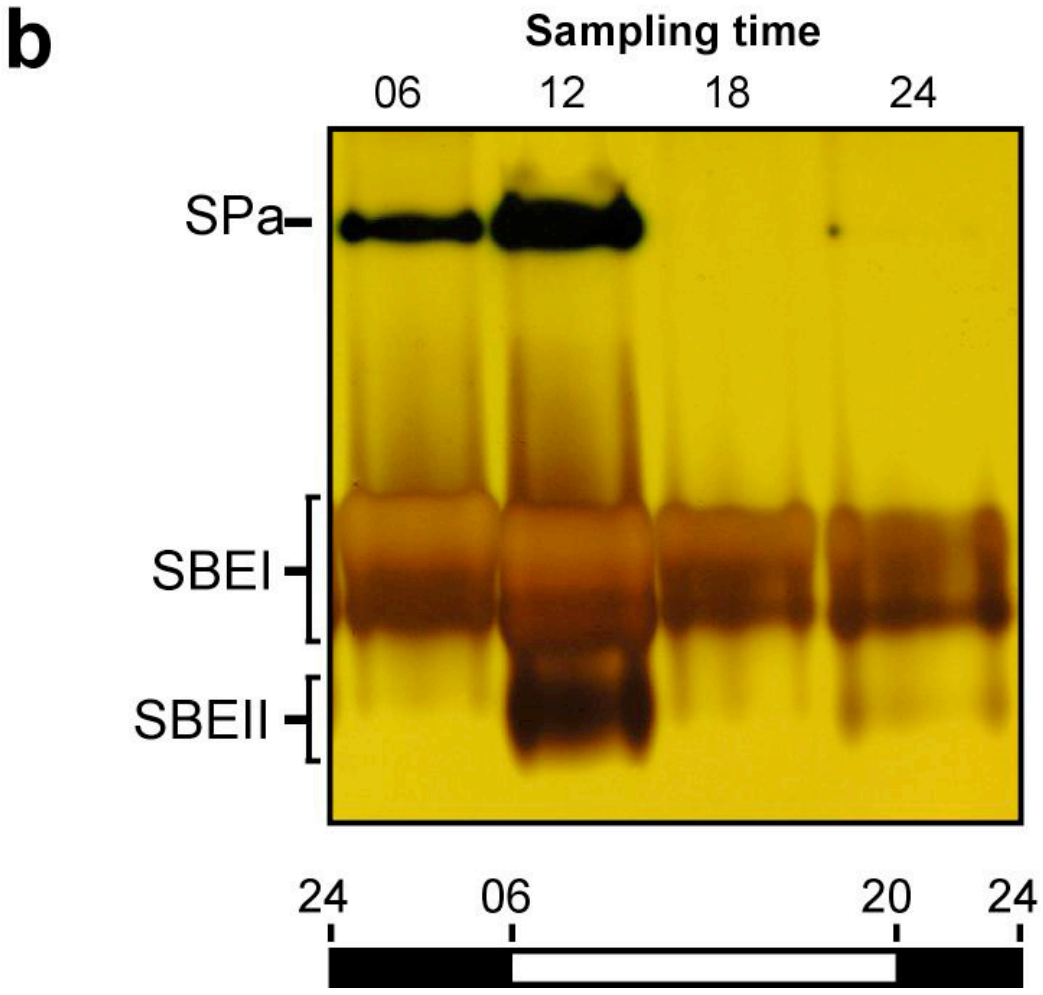
(d) Induction of *SBE* expression by ABA in the presence or absence of okadaic acid (OA).

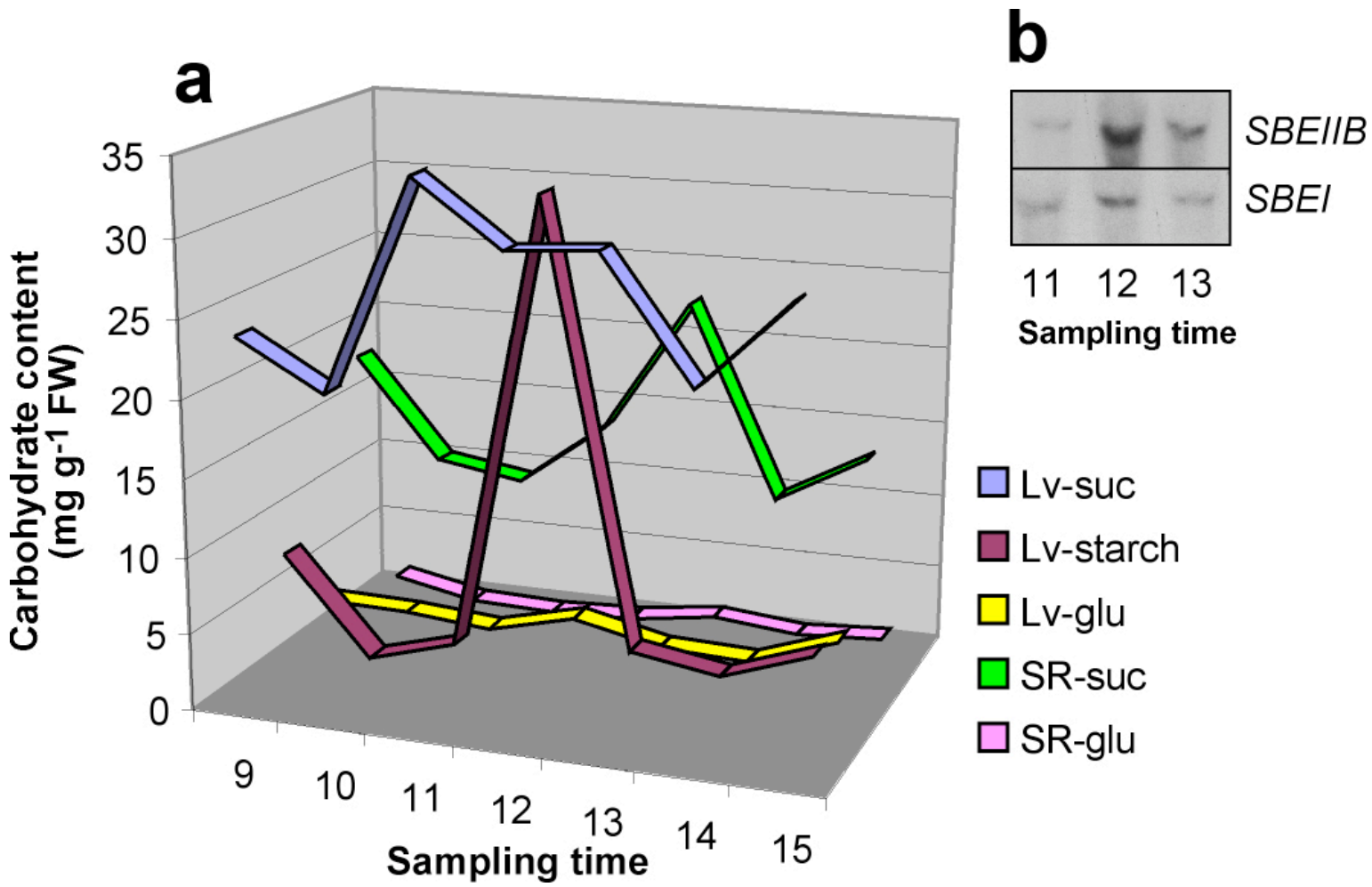
(e) Induction of *SBE* expression by sucrose, turanose, glucose or glucose-1-P (Glu-1-P) in the presence or absence of okadaic acid.

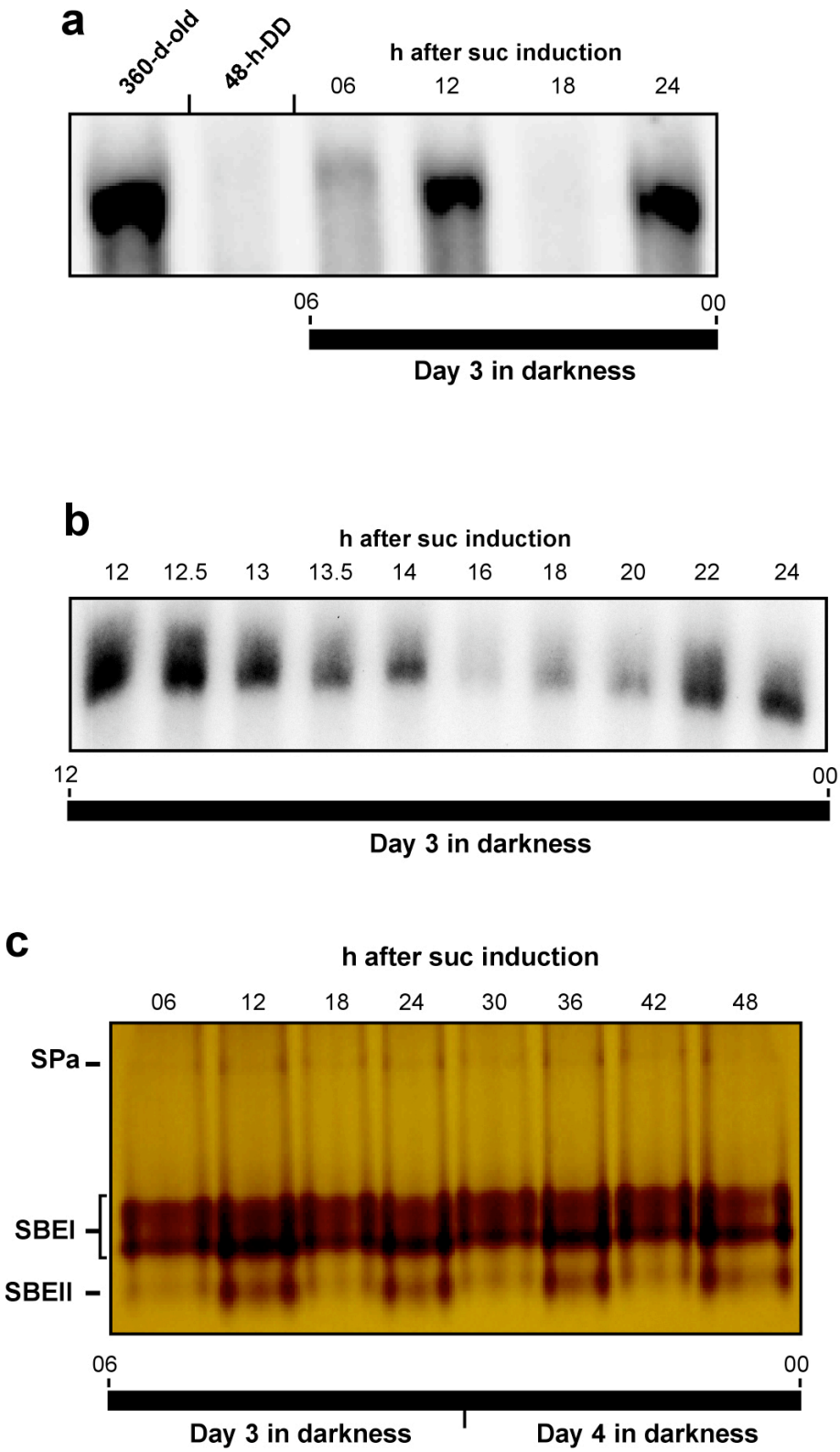
Figure 6. Model showing sugar and ABA signaling transduction (dashed purple arrows) during regulation of *SBE* gene expression in the cassava storage root.

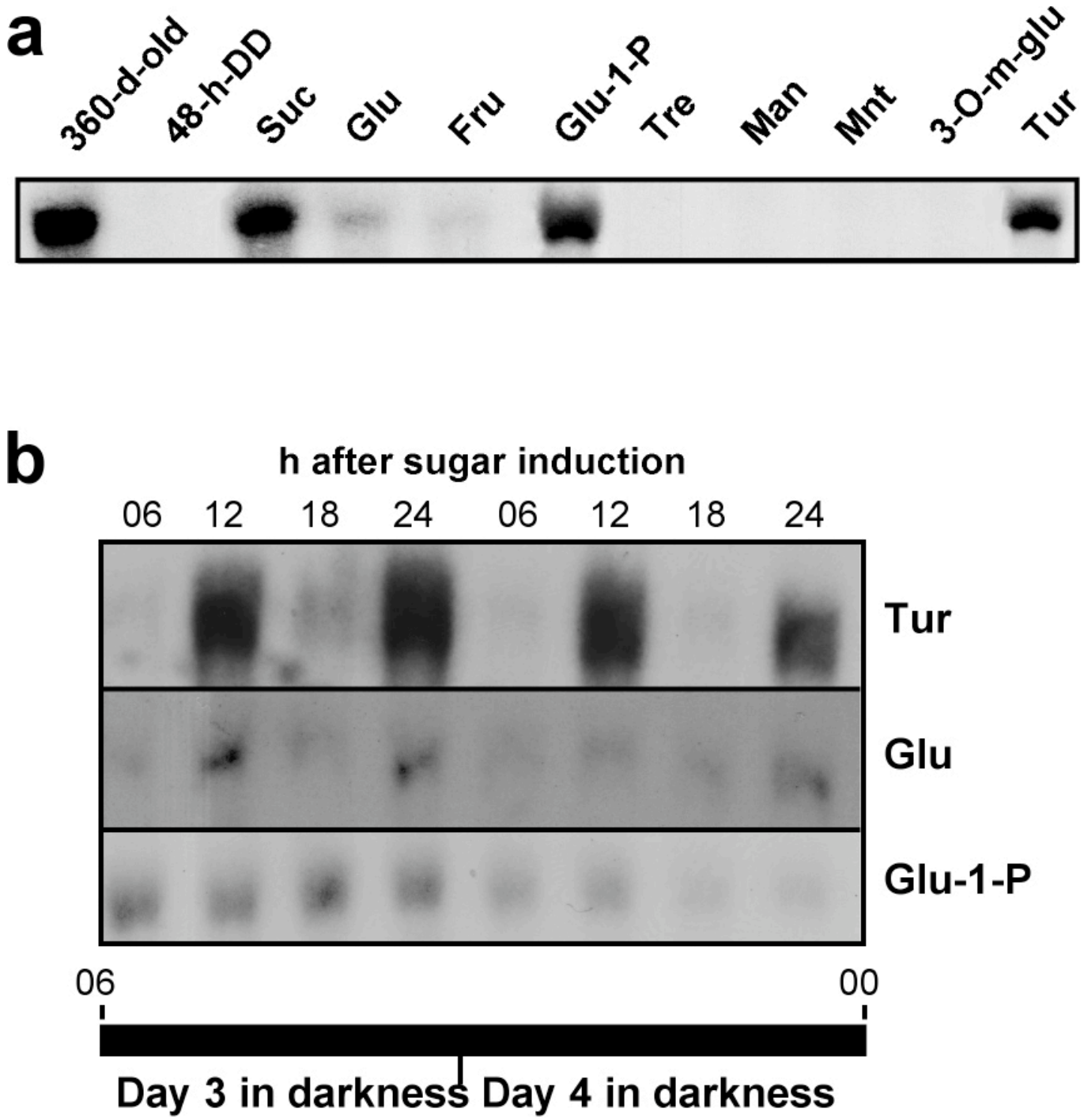
Sugar signaling is predominantly activated by the entry of sucrose. A signal is transmitted from the SUT to glucose-6-P, or a downstream regulator, via HXK. The sugar and ABA signaling pathways intersect at a protein phosphatase. The semidian oscillator (circled squiggle) is functionally associated with HXK. ABA, abscisic acid; Fru, fructose; Glu, glucose; HPT, hexose phosphate transporter; HXK, hexokinase; Hx, hexose; Hx-P, hexose-phosphate; INV, apoplastic invertase; MST, monosaccharide transporter; PGM, phophoglucomutase; PP, protein phosphatase; PP_i, pyrophosphate; SBE, starch branching enzyme; SSE, starch synthesizing enzymes; SUSY, sucrose synthase; SUT, sucrose transporter; UGPase, UDP-glucose pyrophosphorylase; X, unknown protein.

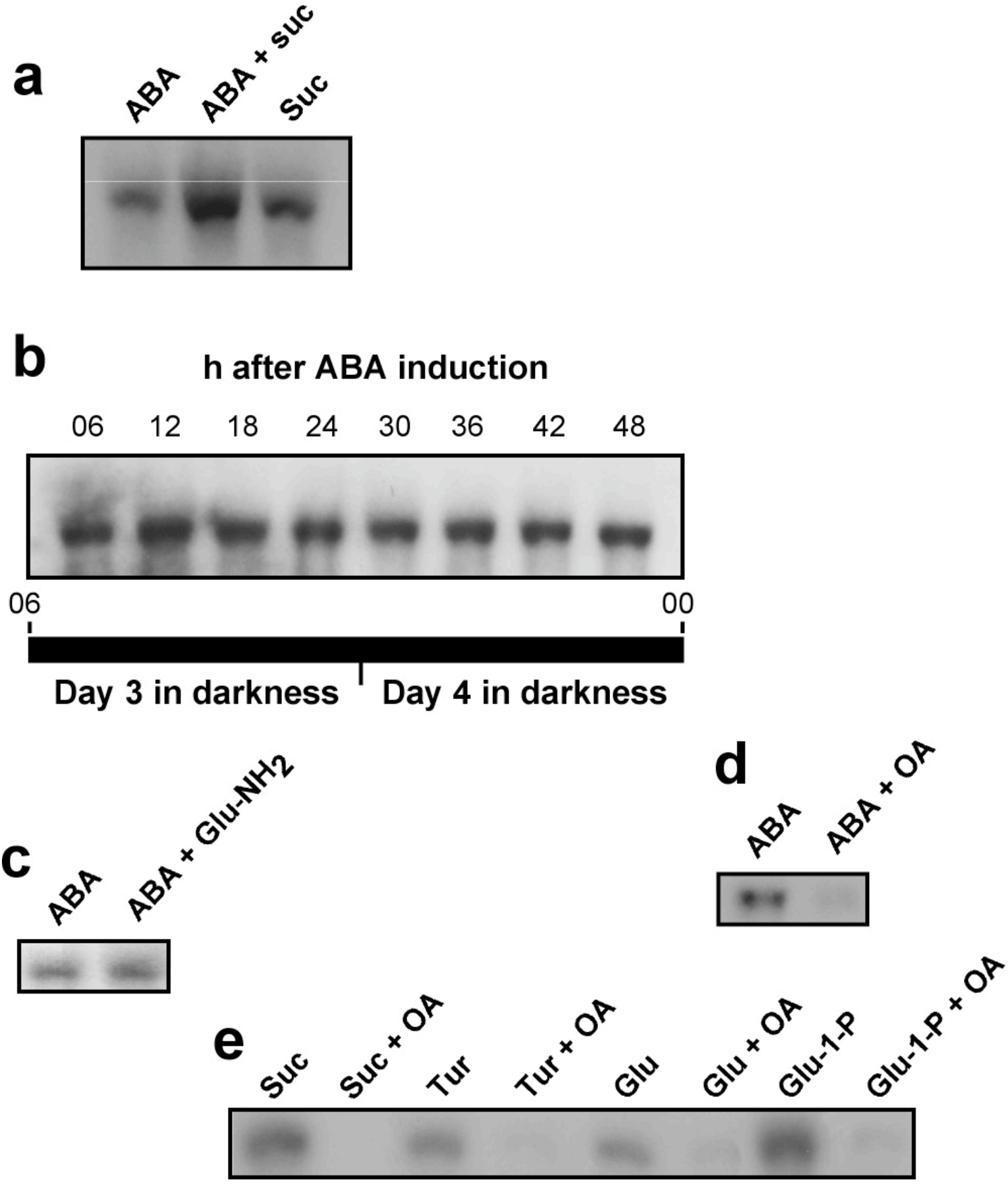

a
Sampling time
24
06
12
18
21
SBEIIB
SBEI
Sampling time
24
06
12
18
24
06
20
24
24
14
24

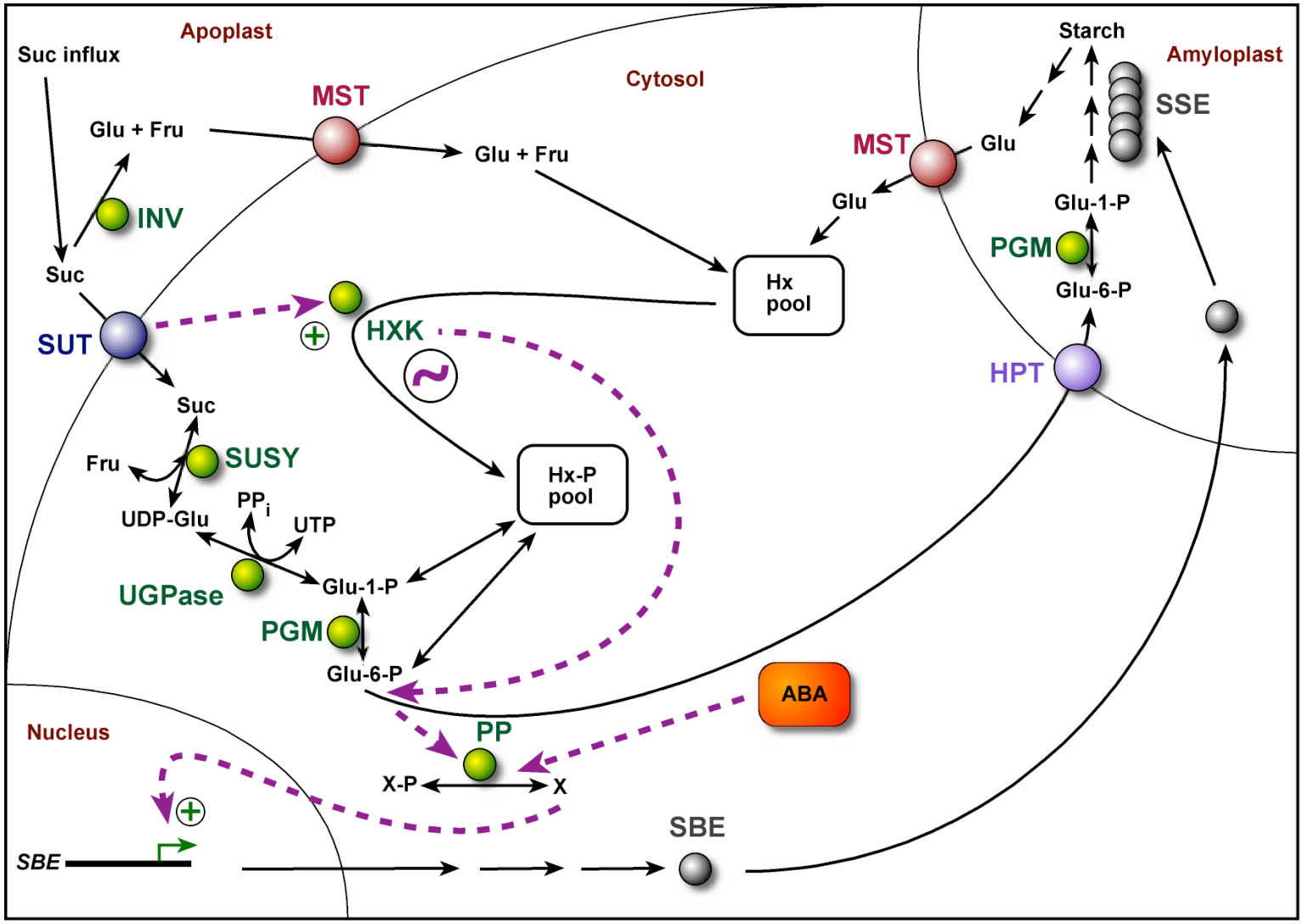

b
Sampling time
06
12
18
24
SPa
SBEI
SBEII
24
06
20
24


a
b
Carbohydrate content (mg g-1 FW)
35
30
25
20
15
10
5
0
9
10
11
12
13
14
15
Sampling time
Lv-suc
Lv-starch
Lv-glu
SR-suc
SR-glu
SBEIIB
SBEI
11
12
13
Sampling time


a
360-d-old
48-h-DD
h after suc induction
06
12
18
24
06
00
Day 3 in darkness
b
h after suc induction
12
12.5
13
13.5
14
16
18
20
22
24
12
00
Day 3 in darkness
c
h after suc induction
06
12
18
24
30
36
42
48
SPa
SBEI
SBEII
06
00
Day 3 in darkness
Day 4 in darkness
d
Sucrose content (mg g-1 FW)
20
15
10
5
0
-5
No treatment
48-h-DD
6 h suc
12 h suc
18 h suc
24 h suc
6 h H2O
12 h H2O
18 h H2O
24 h H2O


a
360-d-old
48-h-DD
Suc
Glu
Fru
Glu-1-P
Tre
Man
Mnt
3-O-m-glu
Tur
b
h after sugar induction
06
12
18
24
06
12
18
24
Tur
Glu
Glu-1-P
06
00
Day 3 in darkness
Day 4 in darkness
c
0 mM
50 mM
100 mM
Suc + Glu-NH2
Tur + Glu-NH2


a
ABA
ABA + suc
Suc
b
h after ABA induction
06
12
18
24
30
36
42
48
06
00
Day 3 in darkness
Day 4 in darkness
c
ABA
ABA + Glu-NH2
d
ABA
ABA + OA
e
Suc
Suc + OA
Tur
Tur + OA
Glu
Glu + OA
Glu-1-P
Glu-1-P + OA


Apoplast
Suc influx
Glu + Fru
INV
Suc
SUT
MST
Glu + Fru
Cytosol
Hx pool
HXK
Suc
Fru
SUSY
UDP-Glu
PPi
UTP
UGPase
Glu-1-P
PGM
Glu-6-P
Hx-P pool
PP
X-P
X
ABA
Nucleus
SBE
SBE
Starch
Amyloplast
SSE
MST
Glu
Glu
Glu-1-P
PGM
Glu-6-P
HPT