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AKIN10 Activity as a Cellular Link Between Metabolism and Circadian-Clock Entrainment in *Arabidopsis thaliana*.

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Addendum to:

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Abstract

AKIN10, the catalytic subunit of the Snf1 (sucrose non-fermenting 1)-related kinase 1 (SnRK1) complex, acts as an energy sensor in plants. We showed that *AKIN10*-induced expression affects the pace of the circadian clock and particularly the phase of expression of *GIGANTEA (GI)*. The AKIN10 effect on period length required *TIME FOR COFFEE* (*TIC*), a circadian-clock component with developmental and metabolic roles. Here we expand on the possible interactions between *AKIN10*, whose activity is involved in transcriptional reprogramming, and clock elements *GI* and *TIC*. We hypothesize how they could participate in clock entrainment through a metabolic signal derived from carbon pools and starch metabolism. Additionally, we consider further the role of cellular energy status to the clock through the formation of a hypothetical protein complex. We also demonstrate the role of AKIN10, but not its sequence-related kinase AKIN11, on clock periodicity. Altogether we present a model of action of these elements in metabolic-related clock entrainment.

TEXT

Recently we have shown that *AKIN10* can work in the circadian clock [1]. This gene encodes a catalytic subunit of the SnRK1 with known roles in homeostasis, particularly energy metabolism [2]. *AKIN10* overexpression dramatically lengthens circadian clock periodicity in the presence of light, but not under darkness [1]. This result, together with the global transcriptional reprogramming of stress pathways [2], suggests that *AKIN10* activity can be linked to light transitions that occur at dawn and dusk. Notably these are the times when levels of metabolites, such as starch, soluble glucose and sucrose, as well as amino-acid pools, reach their maximum and minimum levels [3]. As both photosynthesis and starch metabolism are under circadian control and *AKIN10* expression and activity is responsive to sugars [4-6], SnRK1 could act as a sensor of carbon pools or derived trehalose signaling and contribute to circadian clock entrainment by a metabolic signal. However, *AKIN10* would require a still unknown "light factor" that specifically triggers its activity under light conditions, a process required for clock entrainment [7, 8].

AKIN10 overexpression did not affect the circadian rhythm *per se* as robust oscillations endured, but its induction caused an increase in period length [1]. We described a delay in the *GIGANTEA* (*GI*) expression rhythm under constant light, and a stark phase shift under diurnal conditions [1]. This particular effect is interesting as *GI* encodes for a protein that participates in several developmental and physiological processes, such as starch metabolism, growth and flowering time, circadian clock control and oxidative stress tolerance [9] and has been proposed as a carbon sensor that mediates the long-term response to sucrose [10]. Therefore we suggest that AKIN10 activity triggered by low ATP/AMP ratios mediates the short-term response to changes in carbon pools and affects the circadian clock under light conditions and thus participates in

clock entrainment towards dawn. GI would act as a long-term response factor under darkness. Circadian clock entrainment by sugars derived from photosynthesis has been demonstrated [11]. However how internal carbon sources entrain the clock is not yet fully understood. AKIN10 could be a key element that participates in the circadian-clock resetting either by direct protein interaction or phosphorylation of circadian-clock genes or associated targets or either by an indirect action through the phosphorylation of another metabolic-regulatory element resulting in changes of cellular energy status that feeds back to the circadian clock.

Considering the *AKIN10* effect on circadian clock period [1], we evaluated if the isoform kinase *AKIN11* would also alter circadian clock periodicity. For this, we evaluated the circadian-clock driven rhythms of wild type, *AKIN10*, and *AKIN11* transgenic lines harboring the *CCA1:LUC* construct. After transcriptional induction with β-estradiol [see [1, 12] for methods], *AKIN11* induction had no significant effect on period and these lines resembled the wild-type *CCA1:LUC* rhythm. *AKIN10* increases led to period lengthening, as expected. This result is consistent with the wide spatio-temporal expression and activity of *AKIN10* compared to that of *AKIN11* [13]. It remains to be seen if AKIN11 could have a minor and specific role in the circadian clock during plant development. Thus the induction of *AKIN10*, but not *AKIN11*, triggered a lengthening of clock periodicity (Figure 1).

The requirement of a functional *TIME FOR COFFEE* (*TIC*) for *AKIN10* effect on the period lengthening of the circadian clock is compelling. Not only because the AKIN10 and TIC both work to lengthen periodicity [1, 14, 15], but also because the *tic* clock is faulty just prior to dawn [16]. This is the time at which metabolism switches from catabolism, including starch degradation, to anabolism by products derived from photosynthesis. Previously we have shown that *tic* presents a

starch-excess phenotype [13], which is similar to that of the *gi* mutant [9, 17]. This result is consistent as *AKIN10/AKIN11* RNA interference (RNAi) lines were unable to break down starch during the night [2]. However, TIC epistasis over AKIN10 within the circadian clock may not apply to the starch excess phenotype of *tic* and *AKIN10* silenced lines because both SnRK1 have been reported to be necessary for starch synthesis in *Phsycomitrella patens* and in higher plants [18, 19]. Furthermore induction of the *DARK INDUCED GENES* (*DIN*), which are activated upon stress, requires AKIN10/AKIN11. The *tic* transcript profile showed that *DIN1/SEN1*, *DIN4*, *DIN6/ASN1* and *DIN10* were overexpressed at dawn [13]. Thus the expression of these genes may not require a functional interaction between AKIN10 and TIC, as was the case for circadian periodicity. The *tic* transcriptome profile suggests that TIC-AKIN10 interaction may be specific to the oscillator and that AKIN10 does not require a functional TIC in order to perform other metabolic activities, such as regulation of *DIN* gene expression. The TIC-AKIN10 signaling interaction in relation to carbohydrate metabolism appears complex.

TIC encodes for a protein without known functional domains, whereas GI has been described as a protein that stabilizes ZEITLUPE (ZTL) under blue light due to its chaperone activity [9, 20]. Nonetheless both genes share alterations in similar metabolic and physiological processes, such as carbohydrate metabolism, growth, circadian-clock control and oxidative stress [9, 13, 14, 21]. Therefore it is plausible that these proteins function independently from AKIN10 in governing the timing of starch metabolism. It remains to be shown how all of these factors coordinate circadian-clock entrainment, plant development and carbon metabolism.

TIC exerts its time-specific function within the circadian clock by a still unknown mechanism as its mRNA and protein do not oscillate through the day [14]. In one hypothetical

scenario, a metabolic event at dawn could trigger TIC activation and consequently it would display its circadian-clock function. In a second hypothetical scenario, TIC would be constitutively active and be attenuated by a rhythmic factor. The epistatic relationship of *TIC* to *AKIN10* could imply that the former "disables" AKIN10. Following this line of thought, it is plausible that the AKIN10 effect on the circadian clock is promoted by TIC through a previously proposed protein complex [15].

Based on the requirement for *TIC* in *AKIN10* effect on circadian period, we hypothesize that AKIN10 stimulates TIC clock activity. Perhaps the role of AKIN10 in the period lengthening is reciprocally promoted by TIC through regulated formation of a protein complex [15]. Previously we have demonstrated that TIC is necessary for MYC2 proteasomal degradation in the jasmonic-acid response pathway [22]. Considering AKIN10 interacts with SKP1 (S-phase kinase associated protein 1) and mediates its proteasomal binding of an ubiquitin ligase [23], the regulated formation of a protein complex to alter protein half life that mediates clock entrainment to metabolites is plausible. Such a hypothesis waits to be tested *in vitro* and demonstrated by a biological effect *in vivo*.

In summary, the genetic interaction of *TIC-AKIN10* and their effect on circadian periodicity suggested a mechanism through which TIC could exert its clock function (Figure 2). Additionally, it opens a possible link between metabolism and energy signaling in regards to oscillator entrainment. Clarifying and establishing these mechanisms will require further research in the area.

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Figure Legends

Figure 1

The induction of *AKIN10*, but not *AKIN11*, lengthened the circadian clock period under constant light conditions

A) *CCA1:LUC* luminescence rhythms of wild type (wt), *pER8::AKIN10* and *pER8::AKIN11* transgenics under constant light without β-estradiol treatment. B) *CCA1:LUC* luminescence rhythms of wt, *pER8::AKIN10* and *pER8::AKIN11* under constant light after 5μM β-estradiol was exogenously supplied. Induction of *AKIN10* (white circles), but not *AKIN11* (gray triangles), caused a lengthening effect on clock periodicity. Black diamonds Col-0 wild type, white circles and grey triangles *AKIN10* and *AKIN11* transgenic lines, respectively. C) Period estimations of luminescence rhythms from B by FFT-NLLS of wt and *pER8::AKIN11*.

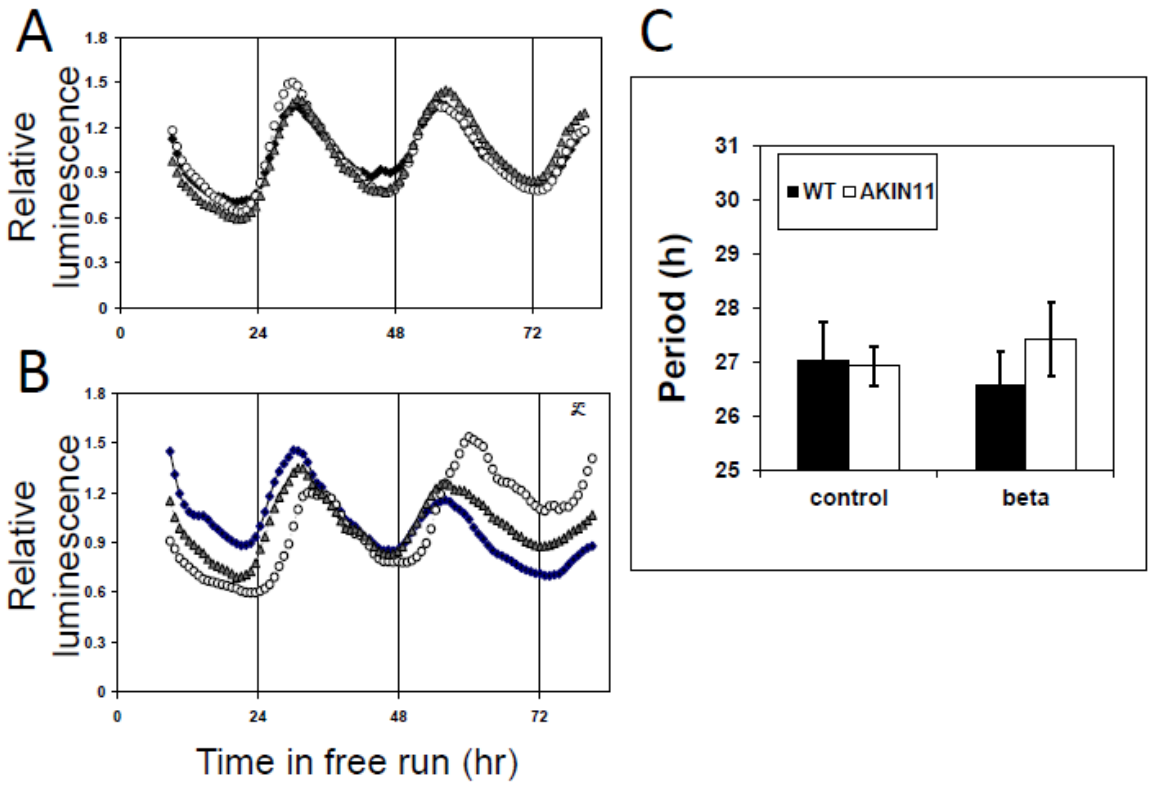
A
B
C
Relative luminescence
Time in free run (hr)
Period (h)
WT
AKIN11
control
beta

Figure 2

Proposing that *AKIN10* and *TIC* act as a sensor hub regarding circadian-clock entrainment and circadian clock periodicity

TIC and AKIN10 signaling interaction could serve as a sensor of carbon pools, cellular energy levels, stress and/or light to provide a resetting signal for the circadian clock to a new day. This is dawn entrainment. Such signals trigger reprogramming by either coordinating the expression of genes controlled by AKIN10 and/or gene expression via the circadian clock. Cellular energy status sensed either as sucrose and glucose levels, trehalose signaling or ATP/AMP ratios (depicted as a sucrose molecule for simplicity) by AKIN10, hypothetically would translate into circadian clock entrainment at dawn by a signaling interaction with TIC and a "light factor." Interaction with other proteins for particular sensing of an external signal as sucrose by *GIGANTEA* (*GI*) is also hypothesized in the cartoon. The time-specific relations between TIC and AKIN10 are depicted at the night-to-day transition. Thus the signal provided by *AKIN10-TIC* could be integrated in the core of the oscillator at the transition from *LHY* and *CCA1* repression to induction. AKIN10 Sucrose non-fermenting related kinase a subunit; TIC, TIME FOR COFFEE; GI, GIGANTEA; LHY LATE ELONGATED HYPOCOTYL; CCA1 CIRCADIAN AND CLOCK ASSOCIATED 1; TOC1, TIMING OF CAB EXPRESSION 1. The thunderbolt represents the photic entrainment as well as the "light factor" that promotes AKIN10-derived period lengthening. The clock face represents the circadian machinery that control rhythms and periodicity (sinusoidal waves). Solid lines represent stimulus, effects or interactions demonstrated previously. Dashed lines symbolize the signals from *TIC* and *AKIN10* toward the circadian clock in regard to entrainment.