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1 **The energy-signalling hub SnRK1 is important for sucrose-induced hypocotyl elongation**

2

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21

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23 performed experiments; N.S., J.K., A.F.L. and A.D. analyzed data; N.S., J.K. and A.D. interpreted
24 data and wrote the paper.

25

26 **One-sentence summary:** An energy signalling pathway, photoperiod and light intensity regulate sugar-
27 induced hypocotyl elongation.

28

29

30 **Running title:** Sucrose-induced hypocotyl elongation

31 **Abstract**

32 Emerging seedlings respond to environmental conditions such as light and temperature to optimize
33 their establishment. Seedlings grow initially through elongation of the hypocotyl, which is
34 regulated by signalling pathways that integrate environmental information to regulate seedling
35 development. The hypocotyls of *Arabidopsis thaliana* also elongate in response to sucrose. Here,
36 we investigated the role of cellular sugar-sensing mechanisms in the elongation of hypocotyls in
37 response to sucrose. We focused upon the role of SnRK1, which is a sugar-signalling hub that
38 regulates metabolism and transcription in response to cellular energy status. We also investigated
39 the role of TPS1, which synthesizes the signalling sugar trehalose-6-phosphate (Tre6P) that is
40 proposed to regulate SnRK1 activity. Under light/dark cycles, we found that sucrose-induced
41 hypocotyl elongation did not occur in *tps1* mutants and overexpressors of KIN10
42 (AKIN10/SnRK1.1), a catalytic subunit of SnRK1. We demonstrate that the magnitude of sucrose-
43 induced hypocotyl elongation depends on the day length and light intensity. We identified roles for
44 auxin and gibberellin signalling in sucrose-induced hypocotyl elongation under short photoperiods.
45 We found that sucrose-induced hypocotyl elongation under light/dark cycles does not involve
46 another proposed sugar sensor, HEXOKINASE1, or the circadian oscillator. Our study identifies
47 novel roles for KIN10 and TPS1 in mediating a signal that underlies sucrose-induced hypocotyl
48 elongation in light/dark cycles.

49

50

51 **Introduction**

52 Emerging seedlings monitor the environment to optimize their establishment and out-compete
53 neighbouring plants (Salter et al., 2003; Weinig et al., 2007; Koini et al., 2009; Keuskamp et al.,
54 2010; Crawford et al., 2012). Seedlings grow initially through cell expansion within the hypocotyl,
55 which elongates rapidly to optimize light capture by the cotyledons. Hypocotyl elongation is
56 controlled by several signalling pathways that converge upon phytohormones to regulate cell
57 expansion (Lincoln et al., 1990; Collett et al., 2000). Examples of signals that adjust hypocotyl
58 elongation include phytochrome-mediated signals concerning the ratio of red to far red light
59 (R:FR) (Casal, 2013), blue light (Liscum and Hangarter, 1991), UV-B light (Kim et al., 1998;
60 Hayes et al., 2014), temperature (Koini et al., 2009; Wigge, 2013; Mizuno et al., 2014),
61 photoperiod and the circadian oscillator (Dowson-Day and Millar, 1999; Más et al., 2003; Nusinow
62 et al., 2011). These signals are integrated by the PHYTOCHROME INTERACTING FACTOR
63 (PIF)-family of basic helix-loop-helix transcription factors. The PIFs are signalling hubs that
64 control plant development through genome-wide transcriptional alterations. One outcome of these
65 PIF-mediated transcriptional changes are the alterations in phytohormone signalling that regulate
66 hypocotyl elongation (Lorrain et al., 2008; Leivar and Quail, 2011).

67 Hypocotyl length is also increased by exogenous and endogenous sugars (Kurata and Yamamoto,
68 1998; Takahashi et al., 2003; Zhang et al., 2010; Liu et al., 2011; Stewart et al., 2011; Stewart
69 Lilley et al., 2012; Zhang et al., 2015; Zhang et al., 2016). Under light/dark cycles, exogenous
70 sugars are proposed to cause hypocotyl elongation by inducing auxin signals through the PIF-
71 mediated gene regulation (Stewart et al., 2011; Stewart Lilley et al., 2012). Under extended
72 darkness, brassinosteroid and GA phytohormones are involved in sugar-induced hypocotyl
73 elongation, which may also involve the target of rapamycin (TOR) kinase regulator of energy- and
74 nutrient-responses (Zhang et al., 2010; Dobrenel et al., 2011; Zhang et al., 2015; Zhang et al.,

2016). This elongation phenotype in darkness is thought to form a response to the starvation conditions that arise when plants are cultivated under periods of darkness exceeding the length of the daily light/dark cycle (Graf et al., 2010; Zhang et al., 2016). In comparison to these known roles for phytohormones and transcriptional regulators, the contribution of sugar sensing mechanisms to sucrose-induced hypocotyl elongation remain unknown.

Several sugar- or energy-signalling mechanisms underlie the metabolic and developmental responses of plants to sugars. One mechanism involves the Sucrose non-fermenting 1 (Snf1)-related protein kinase SnRK1 (Baena-González et al., 2007; Baena-González and Sheen, 2008), and another involves HEXOKINASE1 (Jang et al., 1997; Moore et al., 2003). SnRK1 controls metabolic enzymes directly by protein phosphorylation (Baena-González and Sheen, 2008). It also regulates > 1000 transcripts in response to carbohydrate availability, for example by adjusting bZIP transcription factor activity (Baena-González et al., 2007; Smeekens et al., 2010; Delatte et al., 2011; Matioli et al., 2011; Mair et al., 2015). Both SnRK1- and hexokinase-mediated sugar signalling involve specific sugars functioning as signalling molecules that provide cellular information concerning sugar availability. For example, SnRK1 activity is thought to be regulated by trehalose-6-phosphate (Tre6P), whose concentration tracks the cellular concentration of sucrose (Lunn et al., 2006; Zhang et al., 2009; Nunes et al., 2013; Yadav et al., 2014). Tre6P is synthesized from UDP glucose and glucose-6-phosphate, which are derived from mobilized and transported sucrose, and also directly from photosynthesis. In *Arabidopsis thaliana*, Tre6P is synthesized by trehalose-6-phosphate synthase (TPS). Of 11 TPS homologs encoded by the *Arabidopsis* genome, TREHALOSE-6-PHOSPHATE SYNTHASE1 (TPS1) synthesizes Tre6P in plants (Gómez et al., 2010; Vandesteene et al., 2010), and TPS2 and TPS4 are catalytically active in yeast complementation assays (Delorge et al., 2015). Tre6P is believed to regulate SnRK1-mediated signalling by suppressing the activity of SNF1-RELATED PROTEIN KINASE1.1

99 (KIN10/AKIN10/SnRK1.1), which is a catalytic subunit of SnRK1 that is fundamental to the
100 signalling role of SnRK1 (Baena-González et al., 2007; Zhang et al., 2009; Nunes et al., 2013).
101 Manipulation of Tre6P metabolism in plants alters developmental phenotypes. For example, *tps1*
102 knockout mutants undergo seedling developmental arrest (Gómez et al., 2006), expression of
103 bacterial Tre6P synthase (*otsA*) or phosphatase (*otsB*) affects leaf senescence (Wingler et al.,
104 2012), and Tre6P and KIN10 act within a photoperiod-response pathway that controls the induction
105 of flowering (Baena-González et al., 2007; Gómez et al., 2010; Wahl et al., 2013). Signalling by
106 Tre6P and KIN10 is also important for the regulation of growth rates. Growth is increased by
107 sucrose in the presence of Tre6P (Schluepmann et al., 2003; Paul et al., 2010), but the lack of a
108 quantitative (correlative) relationship between relative growth rates and [Tre6P] suggests that a
109 threshold [Tre6P] is required for growth to occur (Nunes et al., 2013). Therefore, it has been
110 suggested that control of KIN10/11 by [Tre6P] may ‘prime’ the regulation of growth-related genes
111 to capitalize upon increased energy availability, rather than by inducing growth directly (Nunes et
112 al., 2013). Remarkably, the impact of this pathway is sufficiently global that its manipulation can
113 increase maize yields by almost 50% (Nuccio et al., 2015) and increase the yield and drought
114 tolerance of wheat (Griffiths et al., 2016).
115 Given the importance of Tre6P metabolism and SnRK1 for growth regulation under cycles of light
116 and dark, we wished to determine whether this energy-signalling mechanism is important for the
117 regulation of sucrose-induced hypocotyl elongation. Moreover, because Tre6P signalling is
118 reported to act upon GA and auxin signalling genes (Paul et al., 2010; Li et al., 2014) and these
119 phytohormones are involved in sucrose-induced hypocotyl elongation (Zhang et al., 2010; Stewart
120 Lilley et al., 2012), we reasoned that SnRK1 might act upon these phytohormones to regulate
121 sucrose-induced hypocotyl elongation.

122 Here, we identified a novel role for Tre6P and KIN10 in the mechanisms that cause sucrose-
123 induced hypocotyl elongation. We focused upon light/dark cycles rather than conditions of
124 extended darkness (Zhang et al., 2010; Zhang et al., 2015; Zhang et al., 2016), because we wished
125 to identify mechanisms that regulate growth and development under regimes more representative
126 of real-world growing conditions that do not elicit prolonged starvation. We found that the
127 sensitivity of hypocotyl elongation to sugars depends on the photoperiod and light intensity. We
128 identified that KIN10 is important for expression of transcripts encoding auxin-induced expansins.
129 Our data reveal a new mechanistic link between carbohydrate supply, energy sensing and
130 phytohormone signalling during seedling emergence.

131 **Results**

132 *KIN10 and TPS1 are required for sucrose-induced hypocotyl elongation in light/dark cycles*

133 We investigated whether KIN10 and TPS1 contribute to sucrose-induced hypocotyl elongation
134 under light/dark cycles (Kurata and Yamamoto, 1998; Takahashi et al., 2003; Stewart et al., 2011;
135 Stewart Lilley et al., 2012). We studied hypocotyl elongation in transgenic Arabidopsis where
136 KIN10 activity was manipulated by overexpressing the catalytic subunit of KIN10 (KIN10-ox)
137 (Baena-González et al., 2007). Although KIN10 activity is regulated post-translationally by Tre6P
138 (Zhang et al., 2009), KIN10 overexpression alone alters the abundance of energy-response
139 transcripts in protoplasts (Baena-González et al., 2007). We used KIN10 overexpression rather
140 than knockouts, because KIN10/11 double knockouts disrupt pollen production and are lethal
141 (Zhang et al., 2001; Baena-González et al., 2007). We also used hypomorphic TILLING (targeted
142 induced local lesions in genomes) mutants with reduced TPS1 activity (*tps1-11*, *tps1-12*) (Gómez
143 et al., 2006; Gómez et al., 2010), which is preferable to *tps1* loss-of-function mutants that cause
144 seedling developmental arrest (Gómez et al., 2006).

145 First, we investigated the effect of exogenous sucrose upon hypocotyl elongation in a variety of
146 photoperiods (Fig. 1). Under 4 h and 8 h photoperiods, sucrose supplementation of wild type
147 seedlings caused a significant increase in hypocotyl length relative to the sorbitol control (2.1-fold
148 and 2.3-fold relative to sorbitol controls, under 4 h and 8 h photoperiods respectively) (Fig. 1A-E).
149 In comparison, under 16 h photoperiods and constant light conditions exogenous sucrose did not
150 promote hypocotyl elongation (Fig. 1A-E).

151 Next, we investigated roles of KIN10 in sucrose-induced hypocotyl elongation under light/dark
152 cycles. Under 8 h photoperiods, the hypocotyls of two KIN10-ox lines (Baena-González et al.,
153 2007) did not elongate significantly in response to exogenous sucrose relative to the MS control
154 (Fig. 1B). Both KIN10-ox lines elongated 1.5-fold in response to sucrose relative to the sorbitol
155 control (Fig. 1B). Exogenous sucrose caused no significant increase in the hypocotyl length of
156 KIN10-ox seedlings under 4 h photoperiods (Fig. 1C). Hypocotyls of the *L. er.* background and
157 KIN10-ox appeared shorter when supplemented with exogenous sucrose in constant light and 16 h
158 photoperiods. However, this could be an osmotic effect rather than a sucrose response because
159 hypocotyl elongation responded identically to sucrose and the sorbitol control (Fig. 1B).

160 Since KIN10 activity is thought to be regulated by Tre6P (Zhang et al., 2009), we investigated the
161 role of the Tre6P biosynthetic enzyme TPS1 in sucrose-induced hypocotyl elongation under
162 light/dark cycles. In two *tps1* TILLING mutants under 8 h photoperiods, sucrose supplementation
163 caused a significant 2.3-fold increase in hypocotyl length in the wild type relative to the sorbitol
164 control, compared with 1.6-fold and 1.3-fold increases in hypocotyl length in *tps1-11* and *tps1-12*
165 respectively (Fig. 1D). Under 4 h photoperiods, sucrose caused a significant 2-fold increase in
166 hypocotyl length of the wild type relative to the sorbitol control, compared with no significant
167 increase in length in *tps1-11* and a significant 1.5-fold increase in hypocotyl length in *tps1-12* (Fig.
168 1E). Together, these experiments with KIN10 overexpressors and *tps1* mutants indicate that TPS1

169 and KIN10 are involved in one or more mechanisms that increase hypocotyl length in response to
170 exogenous sucrose. This suggests that SnRK1-mediated energy signalling regulates hypocotyl
171 elongation in response to sucrose supplementation.

172 *HEXOKINASE1 is not required for sucrose-induced hypocotyl elongation under light/dark cycles*
173 Hexokinase is thought to function as a sugar sensor that regulates development in response to the
174 concentration of glucose (Jang et al., 1997; Moore et al., 2003), so we investigated whether
175 hexokinase-based signalling also contributes to sucrose-induced hypocotyl elongation. For this, we
176 measured the elongation of hypocotyls in response to exogenous sucrose in the *glucose insensitive2*
177 (*gin2-1*) mutant of HEXOKINASE1. Overall, *gin2-1* hypocotyls were slightly shorter than the wild
178 type under all conditions tested (Fig. 1F). Exogenous sucrose caused a significant increase in
179 hypocotyl length of wild type and *gin2-1* seedlings, producing hypocotyls 63% and 67% longer
180 than the osmotic control in the wild type and *gin2-1*, respectively (Fig. 1F). Therefore, sucrose
181 caused a similar magnitude of hypocotyl elongation in *gin2-1* and the wild type. This suggests that
182 interconversion of sucrose to glucose, and therefore hexokinase-based glucose signalling, does not
183 contribute to sucrose-induced hypocotyl elongation in short photoperiods.

184 *Relationship between day-length, light intensity and sucrose-induced hypocotyl elongation*

185 Our data suggest that the magnitude of the sucrose-induced increase in hypocotyl length depends
186 upon the photoperiod or the quantity of light received. In the wild type, sucrose increased
187 hypocotyl length under short (4 h or 8 h) but not long (16 h or constant light) photoperiods under
188 photosynthetically active radiation (PAR) of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 1B-E, Fig. 2A). In addition,
189 sucrose caused significantly greater hypocotyl elongation under 4 h photoperiods compared with 8
190 h photoperiods of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 2A). We reasoned that these varying responses to sucrose
191 might arise from differences in total daily PAR received under each of these conditions, or

192 alternatively from the sensing of photoperiod length. To investigate this we compared the
193 magnitude of sucrose-induced hypocotyl elongation under the same total daily integrated PAR,
194 under longer photoperiods (16 h at $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 8 h at $80 \mu\text{mol m}^{-2} \text{s}^{-1}$) and under shorter
195 photoperiods (8 h at $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 4 h at $80 \mu\text{mol m}^{-2} \text{s}^{-1}$). Under a 16 h photoperiod at
196 $40 \mu\text{mol m}^{-2} \text{s}^{-1}$, sucrose caused a significant increase in hypocotyl length (Fig. 2B, C). This
197 contrasts a 16 h photoperiod at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$, where sucrose did not promote hypocotyl
198 elongation (Fig. 1, Fig. 2A). This suggests that the quantity of light received influences the
199 sensitivity of hypocotyl elongation to sucrose. Under 8 h photoperiods, sucrose caused greater
200 hypocotyl elongation under $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ (mean 4.1 mm increase) than under $80 \mu\text{mol m}^{-2} \text{s}^{-1}$
201 (mean 3.3 mm increase), which also suggests that hypocotyl elongation is more responsive to
202 sucrose under lower light conditions (Fig. 2B, D). When daily integrated PAR was the same under
203 4 h and 8 h photoperiods, there was no difference in the increase in hypocotyl length caused by
204 sucrose (Fig. 2D, E). These responses suggest that daily integrated PAR influences the magnitude
205 of sucrose-induced hypocotyl elongation. However, the magnitude of sucrose-induced hypocotyl
206 elongation was significantly less under 16 h photoperiods at $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ than 8 h photoperiods
207 at $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 2B, C), suggesting that under long photoperiods, the magnitude of sucrose-
208 induced hypocotyl elongation could be also determined by a photoperiod-response mechanism
209 acting independently from daily integrated PAR. These data provide the insight that the
210 photoperiod-sensitivity of sucrose-induced hypocotyl elongation is determined by both the absolute
211 photoperiod and the amount of light received.

212 *Interaction between hypocotyl elongation by exogenous sucrose and the circadian oscillator*

213 The circadian oscillator regulates hypocotyl elongation because the accumulation of PIF proteins is
214 restricted to the end of the night (Nozue et al., 2007; Nusinow et al., 2011). Since the circadian

215 oscillator responds to exogenous and endogenous sugars (Dalchau et al., 2011; Haydon et al.,
216 2013) and KIN10 overexpression can lengthen circadian period (Shin et al., 2017), we investigated
217 whether sucrose-induced increases in hypocotyl length under short photoperiods involve the
218 circadian oscillator. First, we tested whether the circadian oscillator components CIRCADIAN
219 CLOCK ASSOCIATED1 (CCA1), LATE ELONGATED HYPOCOTYL (LHY) and TIMING OF
220 CAB2 EXPRESSION1 (TOC1) are required for sucrose-induced hypocotyl elongation using the
221 *cca1-11 lhy-21 toc1-21* triple mutant (Ding et al., 2007). *cca1-11 lhy-21 toc1-21* causes circadian
222 arrhythmia under constant light and temperature, and disrupts rhythms of oscillator transcripts,
223 including evening complex components that regulate hypocotyl elongation (Ding et al., 2007).
224 Under 4 h photoperiods, the magnitude of the sucrose-induced increase in hypocotyl length was
225 unaltered in *cca1-11 lhy-21 toc1-21* (Fig. 3A; Fig. S1). Under 4 h photoperiods the hypocotyls of
226 *cca1-11 lhy-21 toc1-21* were of similar length to the wild type (Fig. 3A), whereas under 8 h
227 photoperiods, *cca1-11 lhy-21 toc1-21* has longer hypocotyls than the wild type (Ding et al., 2007).
228 We also investigated whether two proteins that confer sugar sensitivity to the circadian oscillator,
229 GIGANTEA (GI) and PSEUDO-RESPONSE REGULATOR7 (PRR7) (Dalchau et al., 2011;
230 Haydon et al., 2013), contribute to sucrose-induced hypocotyl elongation under short photoperiods.
231 We tested this because the *prp7-11* mutation renders the oscillator insensitive to sugar signals that
232 entrain the oscillator (Haydon et al., 2013), and the *gi-11* mutation alters oscillator responses to
233 long-term exposure to exogenous sucrose (Dalchau et al., 2011). In all cases, *gi-11* had longer
234 hypocotyls than the wild type (Fig. 3B), but the magnitude of the sucrose-induced increase in
235 hypocotyl length was unaltered in *gi-11* relative to the wild type (Fig. 3D). Likewise, the *prp7-11*
236 mutant also did not alter the magnitude of sucrose-induced increases in hypocotyl length (Fig. 3C,
237 D).

238 These experiments indicate that two mechanisms providing sugar inputs to the circadian oscillator
239 (Dalchau et al., 2011; Haydon et al., 2013) and three core oscillator components do not contribute
240 to sucrose-induced increases in hypocotyl length under short photoperiods.

241 *Phytohormone signalling and sucrose-induced hypocotyl elongation under light/dark cycles: auxin*
242 Sucrose-induced hypocotyl elongation in the light involves auxin and GA signalling (Zhang et al.,
243 2010; Stewart Lilley et al., 2012). We investigated the involvement of phytohormones in sucrose-
244 induced hypocotyl elongation under light/dark cycles, and their relationship with SnRK1-mediated
245 signalling. First, we examined the effect of the inhibitor of polar auxin transport 1-N-
246 naphthylphthalamic acid (NPA) upon sucrose-induced hypocotyl elongation. NPA inhibited sucrose-
247 induced hypocotyl elongation in a concentration-dependent manner, such that 10 μ M NPA
248 completely abolished sucrose-induced elongation (Fig. 4A). Consistent with previous work
249 (Stewart Lilley et al., 2012), this indicates that under light/dark cycles sucrose-induced hypocotyl
250 elongation is auxin-dependent. Next, we examined the responses of auxin- and PIF-dependent
251 expansin transcripts to sucrose. Expansins are a large family of cell-wall modifying enzymes that
252 allow turgor-driven cell expansion, and some expansin transcripts are upregulated by auxins in a
253 PIF-dependent manner during hypocotyl elongation (Li et al., 2002; Miyazaki et al., 2016;
254 Gangappa and Kumar, 2017). We examined *EXPANSIN A4 (EXPA4)*, *EXPA8* and *EXPA11*
255 transcripts, which are auxin-induced in seedlings (Goda et al., 2004; Esmon et al., 2006; Winter et
256 al., 2007; Lee et al., 2009). *EXPA8* and *EXPA11* transcripts were upregulated by conditions of
257 constant darkness, which also increases hypocotyl elongation (Fig. S2A) (Boylan and Quail, 1991),
258 and downregulated by 10 μ M NPA, which suppresses hypocotyl elongation (Fig. S2B) (Stewart
259 Lilley et al., 2012). *EXPA4* was unaltered by these conditions (Fig. S2). Therefore, *EXPA8* and
260 *EXPA11* transcript abundance was increased by conditions that promote hypocotyl elongation, and

261 reduced by conditions that suppress hypocotyl elongation. Next, we monitored the change in
262 abundance of these two expansin transcripts in response to sucrose under 4 h photoperiods. In the
263 wild type, *EXPA11* transcripts were upregulated by 3% sucrose, whereas *EXPA8* transcripts were
264 not upregulated by sucrose relative to the controls (Fig. 4B-E). In KIN10-ox, where sucrose does
265 not promote hypocotyl elongation under light/dark cycles, *EXPA8* and *EXPA11* transcripts were
266 not increased by sucrose (Fig. 4B-E). *EXPA8* was sucrose-induced relative to the controls in *tps1-*
267 *11*, but not in *tps1-12* (Fig. 4B, C). *EXPA11* transcripts were sucrose-induced in both *tps1-11* and
268 *tps1-12* (Fig. 4D, E). The induction of these two expansin transcripts by sucrose in *tps1* mutants
269 was unexpected, because both KIN10-ox and *tps* mutants suppress sucrose-induced hypocotyl
270 elongation under short photoperiods (Fig. 1). We also examined several other transcripts associated
271 with auxin biosynthesis or responses, but the osmotic controls caused substantial alterations in
272 transcript abundance that prevented interpretation of their regulation by sucrose (Fig. S3).

273 *Phytohormone signalling and sucrose-induced hypocotyl elongation under light/dark cycles:*
274 *gibberellins*

275 We tested whether GA signalling also contributes to sucrose-induced hypocotyl elongation under
276 short photoperiods. After germination, wild type seedlings were transferred to media containing
277 3% sucrose or an osmotic control, supplemented with combinations of the GA biosynthesis
278 inhibitor paclobutrazol (PAC), GA, or a carrier control. Consistent with previous studies, wild type
279 seedlings grown on media supplemented with PAC or PAC and GA had significantly shorter
280 hypocotyls than controls (Fig. 5A) (Cowling and Harberd, 1999; Liu et al., 2011). PAC abolished
281 sucrose-induced hypocotyl elongation, with a small hypocotyl length rescue occurring when GA
282 was supplied in combination with PAC (Fig. 5A). We confirmed that the GA was active by

283 demonstrating that, consistent with previous reports (Cowling and Harberd, 1999), hypocotyl
284 length is increased by GA supplementation (Fig. S4).

285 GA increases growth by causing degradation of DELLA growth repressor proteins, and also
286 through DELLA-independent mechanisms (Peng et al., 1997; Fu et al., 2002; Cheng et al., 2004;
287 Cao et al., 2006). Therefore, we investigated the involvement of DELLA proteins in sucrose-
288 induced hypocotyl elongation under light/dark cycles. The *gai-1* mutant harbours a deletion within
289 the DELLA domain of *GIBBERELLIC ACID INSENSITIVE* (*GAI*), which prevents GA-induced
290 proteasomal degradation of *GAI* (Peng et al., 1997; Fu et al., 2002). Under 4 h photoperiods,
291 sucrose supplementation increased hypocotyl length in *gai-1*, but the magnitude of sucrose-induced
292 elongation in *gai-1* was reduced compared with the wild type (hypocotyls became 36.5% longer in
293 *gai-1* in response to sucrose, compared with 59.2% longer in the wild type) (Fig. 5B). Under 16 h
294 photoperiods, sucrose did not induce hypocotyl elongation in the wild type or *gai-1* (Fig. 5B),
295 which is consistent with Fig. 1B, C. We also examined the effect of a mutant lacking all five
296 DELLA proteins upon sucrose-induced hypocotyl elongation under light/dark cycles (Koini et al.,
297 2009). Under short photoperiods, sucrose-induced hypocotyl elongation was unaltered in this
298 mutant (Fig. 5C). Interestingly, under long photoperiods sucrose promoted hypocotyl elongation in
299 the DELLA global mutant, whereas sucrose was without effect upon wild type hypocotyls (Fig.
300 5C). The partial attenuation of sucrose-induced hypocotyl elongation in *gai-1* (Fig. 5B) combined
301 with the derepression of sucrose-induced hypocotyl elongation under long photoperiods in the
302 DELLA global mutant (Fig. 5C) suggests that DELLA-mediated GA signalling contributes to, but
303 does not exclusively control, sucrose-induced hypocotyl elongation.

304 *Phytohormone signalling and sucrose-induced hypocotyl elongation under light/dark cycles:*

305 *abscisic acid*

306 ABA suppresses seedling development (Belin et al., 2009) and several studies have linked Tre6P
307 and abscisic acid (ABA) signalling (Avonce et al., 2004; Ramon et al., 2007; Gómez et al., 2010;
308 Debast et al., 2011). Therefore, we investigated whether ABA signalling contributes to sucrose-
309 induced hypocotyl elongation under light/dark cycles. Sucrose-induced hypocotyl elongation was
310 unaffected by the ABA receptor quadruple mutant *pyr1-1 pyl1-1 pyl2-1 pyl4-1*, which is highly
311 ABA-insensitive (Park et al., 2009) (Fig. S5). This suggests that PYR/PYL-mediated ABA
312 signalling does not participate in the mechanisms underlying sucrose-induced hypocotyl elongation
313 under light/dark cycles.

314 **Discussion**

315 *KIN10 and TPS1 contribute to sugar-induced hypocotyl elongation under light/dark cycles*

316 Here, we make the new finding that a mechanism involving KIN10 activity and Tre6P metabolism
317 regulates sucrose-induced hypocotyl elongation under light/dark cycles. Whilst hypocotyl
318 elongation arises from cell expansion rather than growth through increases in cell number
319 (Gendreau et al., 1997), our data are consistent with studies demonstrating that Tre6P metabolism
320 is a crucial regulator of growth responses to sucrose. For example, Arabidopsis seedlings
321 overexpressing the bacterial Tre6P phosphatase *otsB*, which reduces [Tre6P], accumulate less
322 biomass compared with the wild type when supplemented with sucrose (Schluepmann et al., 2003).
323 The converse is also true; *otsA* (TPS) overexpressors, in which [Tre6P] is increased, accumulate
324 more biomass than the wild type when supplemented with sucrose (Schluepmann et al., 2003).
325 Therefore, our data using *tps1* mutants as a proxy for altered Tre6P metabolism provide new

326 evidence to support the notion that Tre6P promotes growth under conditions of increased sucrose
327 availability (Schluepmann et al., 2003; Zhang et al., 2009).

328 Overexpression in Arabidopsis of the bacterial Tre6P synthase *otsA* has been reported to produce
329 seedlings having shorter hypocotyls than the wild type (Paul et al., 2010). The sucrose-insensitivity
330 of hypocotyl elongation in *tps1* mutants (Fig. 1) and the shorter hypocotyls in seedlings with
331 increased [Tre6P] (*otsA-ox*) may appear to conflict with each other (Paul et al., 2010). However,
332 the experiments are not directly comparable. We found that exogenous sucrose only caused
333 hypocotyl elongation under short photoperiods or lower light conditions (Fig. 2). In comparison,
334 the *otsA-ox* experiments involved 16 h photoperiods at higher PAR ($150 \mu\text{mol m}^{-2} \text{s}^{-1}$) and shaking
335 liquid culture (Zhang et al., 2009), both of which could mask the hypocotyl elongation response
336 that we investigated.

337 Our experiments suggest that increased KIN10 activity might attenuate the elongation response of
338 hypocotyls to exogenous sucrose under light/dark cycles. The KIN10-ox lines that we used
339 overexpress the catalytic subunit of SnRK1 (Baena-González et al., 2007). KIN10 overexpression
340 downregulates transcripts associated with anabolic processes and upregulates transcripts associated
341 with energy starvation (Baena-González et al., 2007). Therefore, in our experiments KIN10
342 overexpression may have stopped seedlings from taking advantage of the greater energy
343 availability caused by sucrose supplementation, so preventing sucrose-induced hypocotyl
344 elongation in KIN10-ox (Fig. 1).

345 *Photoperiod-dependency of sugar-induced hypocotyl elongation*

346 We made the new finding that under relatively high light, exogenous sucrose increases hypocotyl
347 length in photoperiods of 8 h and shorter, but not under long photoperiods or constant light (Fig. 1,
348 Fig. 2). These data reconcile differences between previous studies of sucrose-induced hypocotyl

349 elongation. Previous studies reporting sucrose-insensitivity of hypocotyl elongation in the light
350 were conducted in continuous light (Zhang et al., 2010), in which we also found sucrose to be
351 without effect upon hypocotyls (Fig. 1B, Fig. 2A). In comparison, studies reporting that sucrose
352 does promotes hypocotyl elongation in the light were conducted under 8 h photoperiods (Stewart et
353 al., 2011; Stewart Lilley et al., 2012), where we likewise found that sucrose causes hypocotyl
354 elongation (Fig. 1B, Fig. 2). Therefore, the sensitivity of hypocotyls to sucrose-induced elongation
355 depends upon the photoperiod or the amount of light received each day.

356 One explanation for this response could be that the daily quantity of light determines the magnitude
357 of sucrose-induced hypocotyl elongation through the accumulation of photosynthetic metabolites.
358 Our experiments indicate that under shorter photoperiods, the sensitivity of hypocotyl elongation to
359 sucrose depends upon the total amount of daily light (Fig. 2A, D, E). Furthermore, sucrose-induced
360 hypocotyl elongation under long photoperiods only occurred when the seedlings were under lower
361 light conditions (Fig. 2A, B, C). One interpretation is that under long photoperiods and higher light,
362 cells are replete with sugars (Sulpice et al., 2014) therefore supplementation with exogenous
363 sucrose has a relatively small effect upon the hypocotyl length of already sugar-rich seedlings. In
364 contrast, under short photoperiods or lower light the background level of endogenous sugar is
365 lower (Sulpice et al., 2014), so supplementation with exogenous sucrose has a greater effect upon
366 hypocotyl length.

367 An alternative interpretation is that PIFs integrate light signals derived from photoreceptors with
368 SnRK1-mediated sugar signals to modulate the sensitivity of elongating hypocotyls to sucrose,
369 because PIFs are required for sucrose-induced hypocotyl elongation (Stewart et al., 2011; Stewart
370 Lilley et al., 2012). This might explain the PAR-independent reduction in sucrose-induced
371 hypocotyl elongation that occurred under long photoperiods (Fig. 2C). In the future, it will be
372 informative to resolve the relative contributions of these mechanisms to sucrose-induced hypocotyl

373 elongation, given that Tre6P can regulate expression of both PIFs and auxin signalling genes (Paul
374 et al., 2010). This could provide insights into the nature of the coupling of SnRK1-mediated sugar
375 signalling and growth regulation by PIFs (Paul et al., 2010; Stewart et al., 2011; Stewart Lilley et
376 al., 2012).

377 *Involvement of phytohormone signals in sucrose-induced hypocotyl elongation under light/dark*
378 *cycles*

379 Auxin, GA and brassinosteroids are reported to mediate sucrose-induced hypocotyl elongation,
380 with a role for auxin identified under light/dark cycles and roles for GA and brassinosteroids
381 identified under extended darkness (de Lucas et al., 2008; Zhang et al., 2010; Liu et al., 2011;
382 Stewart et al., 2011; Stewart Lilley et al., 2012; Zhang et al., 2015; Zhang et al., 2016). Consistent
383 with this, our data indicate that auxin signalling has a major role in sucrose-induced hypocotyl
384 elongation under light/dark cycles (Fig. 4A), with GA signalling also contributing to this process
385 (Fig. 5B, C). We suggest two possible reasons why paclobutrazol completely abolished sucrose-
386 induced hypocotyl elongation (Fig. 5A), whereas the *gai-1* mutant only led to partial inhibition of
387 this phenotype (Fig. 5B). One possibility is that DELLA-independent GA signalling contributes to
388 sucrose-induced hypocotyl elongation, since DELLA proteins control around 40-60% of GA-
389 regulated transcripts (Cao et al., 2006). An alternative possibility is that these were off-target or
390 ectopic effects of paclobutrazol, because the paclobutrazol-induced attenuation of hypocotyl
391 elongation was not rescued fully by GA supplementation (Fig. 5A).

392 Auxin-induced expansins that are upregulated during hypocotyl elongation were also induced by
393 sucrose supplementation (Fig. 4B-E; Fig. S2). Whilst *EXPA11* was induced strongly by sucrose,
394 the small response of *EXPA8* to sucrose in the wild type makes it difficult to interpret the responses
395 of *EXPA8* to sucrose in KIN10-ox and the *tps1* mutants (Fig. 4B, C). Interestingly, sucrose

396 induction of *EXPA11* was abolished in KIN10-ox, suggesting a role for KIN10 in expansin gene
397 expression within elongating hypocotyls. In comparison, these expansins were sucrose-inducible in
398 *tps1-11* and *tps1-12* (Fig. 4B-E). One possible explanation is that KIN10-ox causes a much greater
399 level of SnRK1 activity compared with the *tps* mutants, which are hypomorphic alleles that
400 harbour reduced Tre6P concentrations (Gómez et al., 2010) and are not completely deficient in
401 sucrose-induced hypocotyl elongation (Fig. 1D, E).

402 An alternative and speculative explanation for the different behaviour of expansin transcripts in
403 KIN10-ox and *tps* mutants could relate to Tre6P-KIN10 regulating growth through two broad
404 processes- firstly, though direct signalling effects upon growth (e.g. by regulating auxin signals),
405 and secondly through metabolic effects, such as growth constraints due to altered nocturnal
406 catabolism. This could point to TPS1 and SnRK1 making independent contributions to sucrose-
407 induced hypocotyl elongation under light/dark cycles, potentially through separate signalling and
408 metabolic effects, rather than acting in series. Our data suggest that sucrose-induced hypocotyl
409 elongation under light/dark cycles includes a signalling effect, previously proposed to occur
410 through PIF-regulated auxin signals (Stewart et al., 2011; Stewart Lilley et al., 2012). On the other
411 hand, the unexpected behaviour of expansin transcripts in *tps1* mutants (Fig. 1D, E) suggests that
412 mechanisms additional to auxin/GA signalling might contribute to sucrose-induced hypocotyl
413 elongation under light/dark cycles. These additional mechanisms could involve brassinosteroid
414 and/or TOR signalling, which are required for sucrose-induced increases in hypocotyl length under
415 extended darkness (Zhang et al., 2015; Zhang et al., 2016). It would be informative in future to
416 investigate the crosstalk between SnRK1 and TOR energy signalling during hypocotyl elongation,
417 to gain insights into the relative importance of these energy management pathways to the below-
418 ground (darkness) and above-ground (light/dark cycles) stages of seedling establishment.

419 *Conclusions*

420 We identified a novel role for the SnRK1 energy signalling hub in the regulation of sucrose-
421 induced hypocotyl elongation under light/dark cycles. We propose that KIN10 could be positioned
422 upstream from the auxin and GA signals that lead to sucrose-induced hypocotyl elongation in the
423 light (Liu et al., 2011; Stewart et al., 2011; Stewart Lilley et al., 2012). A question for future
424 investigation concerns the functional organization of this pathway. In one scenario, KIN10-
425 mediated energy signalling regulates hypocotyl elongation by acting upon phytohormone
426 signalling, potentially through PIFs (Stewart Lilley *et al.*, 2012). In a different and non-exclusive
427 scenario, SnRK1-mediated alterations in metabolic enzyme activity and growth-related transcripts
428 prime hypocotyls to capitalize upon increased sucrose availability (Nunes *et al.*, 2013a). This is an
429 interesting question in the case of hypocotyl elongation, which arises from cell expansion rather
430 than growth through cell division and biomass accumulation *per se* (Gendreau *et al.*, 1997). These
431 two possibilities are non-exclusive, because the phenotypic differences that we report between
432 KIN10-ox lines and *tps1* mutants (e.g. expansin transcript accumulation; Fig. 4) could implicate
433 more than one mechanism in sucrose-induced hypocotyl elongation.

434 A further question for future investigation is of the nature of the interplay between KIN10/Tre6P,
435 TOR and brassinosteroids in the regulation of hypocotyl elongation in response to sugars. One
436 speculative hypothesis is that under conditions of starvation, such as when a developing below-
437 ground seedling is exhausting its seed-based energy store, brassinosteroid signalling produces a
438 strong elongation cue to drive seedling emergence into the light (Zhang et al., 2015; Zhang et al.,
439 2016). Then, once the seedling has emerged into the daily cycles of light and dark, KIN10/Tre6P
440 adjusts the elongation of hypocotyls to allow optimal seedling establishment under local light
441 conditions (Fig. 1, Fig. 2). It is possible that increased SnRK1 activity under conditions of
442 transiently low light, for example due to unpredictable changes in the weather, operates alongside
443 phototransduction pathways to prevent inappropriate etiolation following seedling emergence.

444 Therefore, one potential function of the mechanism that we identified might be to adapt the rate of
445 seedling development to optimize the use of seed and photosynthetic resources under fluctuating
446 light environments.

447

448 **Materials and Methods**

449 *Plant material and growth conditions*

450 *Arabidopsis* (*Arabidopsis thaliana* (L.) Heynh.) seeds were surface-sterilized and sown on half-
451 strength Murashige & Skoog basal salt mixture (Duchefa, Netherlands) (0.5 MS) with 0.8% (w/v)
452 agar (Noordally et al., 2013). Seeds were then stratified (3 days at 4 °C) and germinated and grown
453 for 7 days under 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of white light at 19 °C, except Fig. 2B-E where PAR was
454 reduced. Media was supplemented with either 3 % (w/v) sucrose (87.6 mM) or 87.6 mM sorbitol as
455 an osmotic control, according to the experiment. For experiments investigating gibberellin
456 signalling, media was supplemented with 20 μM paclobutrazol (PAC) and 100 μM gibberellic acid
457 (GA₃ form) (both Sigma-Aldrich) with a methanol carrier. Paclobutrazol is effective for studies of
458 GA signalling during development at the concentration of 20 μM (Penfield et al., 2004; MacGregor
459 et al., 2015). For experiments investigating auxin signalling, media was supplemented with 1-N-
460 naphthylphthalamic acid (NPA, Sigma-Aldrich) at up to 10 μM with a dimethylsulfoxide (DMSO)
461 carrier. Controls were supplemented with the appropriate carrier at the same concentration as
462 treatment media (0.1% (v/v) DMSO for NPA; 0.12% (v/v) methanol for PAC and GA).
463 To transfer growing seedlings to media containing GA or PAC, surface sterilized and stratified
464 seeds were pipetted onto 1 μm pore-diameter nylon mesh (Normesh, UK), on top of 0.5 MS 0.8%
465 (w/v) agar, and allowed to germinate for 3 days. Seedlings were then transferred to 0.5 MS
466 supplemented with either 3% (w/v) sucrose (87.6 mM) or 87.6 mM sorbitol, plus 20 μM PAC, 100

467 μ M GA or both PAC and GA. Hypocotyls were measured after 5 days growth on treatment plates.
468 For experiments with circadian oscillator mutants, we did not use arrhythmic CCA1-ox plants
469 because overexpression of CCA1 causes very long hypocotyls (Wang and Tobin, 1998), which
470 would confound investigation of the role of sugars in hypocotyl elongation.
471 Genotypes used were *tps1* TILLING mutants (Gómez et al., 2010), KIN10-ox (Baena-González et
472 al., 2007), *gin2-1* (Moore et al., 2003), *gai-1* (Koorneef et al., 1985), DELLA global mutant (Koini
473 et al., 2009), *pyr1 pyl1 pyl2 pyl4* (Park et al., 2009), *cca1-11 lhy-21 toc1-21* (Ding et al., 2007), *gi-*
474 *11* (Richardson et al., 1998) and *prr7-11* (Yamamoto et al., 2003; Nakamichi et al., 2005). In the
475 KIN10-ox lines, *KIN10* transcript abundance was 17-fold greater than the wild type in elongating
476 hypocotyls (Fig. S6A). In the *tps1-11* and *tps1-12* alleles, *TPS1* transcript abundance was
477 unchanged (*tps1-11*) or slightly increased (*tps1-12*) compared with the wild type (Fig. S6B). This
478 result for the *tps1* alleles was unsurprising because these are mis-sense mutants rather than
479 insertion mutants (Gómez et al., 2010).

480 *Hypocotyl measurement*

481 Seedlings were grown on square petri dishes within temperature-controlled growth chambers
482 (Panasonic MLR-352). Plates were angled at about 45 degrees to allow hypocotyls to elongate
483 without touching lids. Hypocotyls were measured by positioning 7 day-old seedlings on the surface
484 of 1% (w/v) agar for photography (Nikon D50) and subsequent measurement using the ImageJ
485 software (<https://imagej.nih.gov/ij/>).

486 *RNA extraction and qRT-PCR*

487 RNA was extracted according to (Noordally et al., 2013), using the Machery-Nagel Nucleospin II
488 plant RNA extraction kit incorporating DNase I treatment (Thermo-Fisher), except approximately
489 60 seedlings were used per RNA sample. cDNA was synthesized using the High Capacity cDNA

490 Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems), according to manufacturer's
491 instructions. cDNA was analyzed using an MXPro 3005 real time PCR system (Agilent) with
492 Brilliant III Ultra-Fast SYBR qPCR mastermix (Agilent) (primers in Table S1). At least two
493 technical repeats were performed for each qRT-PCR reaction. Data were analyzed using the $\Delta\Delta C_t$
494 method, with *PROTEIN PHOSPHATASE 2A SUBUNIT A3 (PP2AA3)* as a reference transcript.

495 *Accession numbers*

496 Arabidopsis Genome Initiative identifiers for the genes mentioned in this study are: *KIN10*
497 (At3g01090), *TPS1* (At1g78580), *HEXOKINASE1* (At4g29130), *CCA1* (At2g46830), *LHY*
498 (At1g01060), *TOC1* (At5g61380), *GI* (At1g22770), *PRR7* (At5g02810), *EXPA4* (At2g39700),
499 *EXPA8* (At2g40610), *EXPA11* (At1g20190), *YUCCA8* (At4g28720), *YUCCA9* (At1g04180),
500 *CYP79B3* (At2g22330), *IAA29* (At4g32280), *SAUR15* (At4g38850).

501

502 **Supplemental Material**

503 **Figure S1.** The *cca1-11 lhy-21 toc1-21* triple mutant does not alter sucrose-induced hypocotyl
504 elongation (direct repeat of Figure 3A).

505 **Figure S2.** Selection of expansin transcripts for experimentation.

506 **Figure S3.** Sucrose supplementation of growth media did not alter abundance of auxin biosynthesis
507 transcripts or auxin-responsive transcripts relative to osmotic controls.

508 **Figure S4.** Efficacy of GA₃ used for study.

509 **Figure S5.** ABA signalling is not required for sucrose-induced hypocotyl elongation under short
510 photoperiods.

511 **Figure S6.** *KIN10* and *TPS1* transcript abundance in *KIN10-ox* and *tps1* TILLING mutants.

512 **Table S1.** qRT-PCR primer sequences.

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523

524 **Figure legends**

525 **Figure 1.** KIN10 and TPS1 participate in sucrose-induced hypocotyl elongation. (A)
526 Representative images of *L. er.* wild type, KIN10-ox and *tps1* seedlings cultivated under a variety
527 of photoperiods, with and without supplementation with 3% sucrose. All panels scaled identically.
528 Images are a subset of seedlings used to generate data in (B-E). (B-E) Lengths of hypocotyls of
529 seedlings grown under (B, D) constant light, 16 h and 8 h photoperiods, and (C, E) 4 h
530 photoperiods. Photoperiods are indicated underneath graphs. (F) Effect of sucrose supplementation
531 upon *gin2-1* hypocotyl length. S.E.M. is small under continuous light (0.03 – 0.05 mm), so not
532 visible on graphs. Data were analysed with ANOVA and Tukey's post-hoc tests ($n = 10$ (B-E) or n
533 $= 20$ (F) seedlings in three independent experiments, \pm S.E.M). Different letters indicate
534 statistically significant differences between means, specifically within each light condition ($p <$
535 0.05). (B-E); MS is half-strength MS media, and Suc and Sor are 0.5 MS supplemented with 3%
536 (w/v) sucrose or equimolar sorbitol (87.6 mM osmotic control), respectively.

537

538 **Figure 2.** Day-length dependency of sucrose-induced hypocotyl elongation in wild type seedlings.

539 (A) Increase in hypocotyl length caused by sucrose under range of photoperiods (data derived from
540 Fig. 1, plotted relative to sorbitol control). (B-E) Comparison of (B, D) absolute hypocotyl length
541 and (C, E) proportional increase in hypocotyl length caused by sucrose supplementation under
542 specified photosynthetically active radiation (PAR) and photoperiod. Mean \pm S.E.M; (A, C-E)
543 $n = 10$ seedlings in two independent experiments (B) $n = 20$ seedlings. Data analysed using
544 ANOVA followed by post-hoc Tukey test. Different letters indicate statistically significant
545 differences between means ($p < 0.05$).

546

547 **Figure 3.** The circadian oscillator does not participate in sucrose-induced hypocotyl elongation
548 under short photoperiods. Sucrose-induced change in hypocotyl length of (A) a circadian oscillator
549 triple mutant (*cca1-11 lhy-21 toc1-21*, background *Ws-2*) and (B, C) two oscillator components
550 participating in sucrose regulation of the circadian oscillator. (D) Change in hypocotyl length
551 caused by sucrose supplementation in *gi-11* and *prr7-11*, expressed relative to 0.5 MS control. MS
552 is 0.5 MS media, and Suc and Sor are 0.5 MS supplemented with 3% (w/v) sucrose and sorbitol
553 (87.6 mM, osmotic control), respectively. Data are mean \pm S.E.M ($n = 10 - 16$), analysed with (A-
554 C) ANOVA and post-hoc Tukey tests and (D) two-sample t-test comparing mutant with wild type
555 for each treatment. Data show one of three independent repeats of the experiment, conducted under
556 4 h photoperiods. Different letters indicate statistically significant differences between means ($p <$
557 0.05).

558

559 **Figure 4.** Auxin signalling underlies sucrose-induced hypocotyl elongation and KIN10 regulates
560 expansin gene expression. (A) Hypocotyl length of seedlings cultivated with a range of

561 concentrations of the inhibitor of polar auxin transport 1-N-naphthylphthalamic acid (NPA), under
562 4 h photoperiods (mean \pm S.E.M; $n = 20$). (B-E) Sucrose-induced changes in expansin transcript
563 abundance in elongating wild type, *tps1* and KIN10-ox seedlings under 4 h photoperiods. (B, D)
564 Indicate *EXPA8* and *EXPA11* transcript abundance relative to *PP2AA3* (mean \pm S.E.M; $n = 3$). (C,
565 E) Indicate the magnitude of sucrose-induced change in transcript abundance in each genotype
566 relative to the osmotic control. Data analysed with ANOVA and post-hoc Tukey tests, and with
567 statistical significance indicated using starring (N.S. = not significant $p > 0.05$; * = $p \leq 0.05$; ** =
568 $p < 0.01$; *** = $p < 0.001$).

569

570 **Figure 5.** Gibberellin signals contribute to sucrose-induced hypocotyl elongation under short
571 photoperiods. (A) The GA biosynthesis inhibitor paclobutrazol (PAC) at 20 μ M inhibits sucrose-
572 induced hypocotyl elongation. Seedlings were germinated on MS agar and transferred to treatment
573 media after germination; carrier control was 0.12% (v/v) methanol. (B) Sucrose-induced hypocotyl
574 elongation was attenuated in *gai-1* mutant seedlings. (C) Sucrose-induced hypocotyl elongation
575 was unaltered in a DELLA global knockout mutant. Experiments performed under 4 h
576 photoperiods. Data are mean \pm S.E.M ($n = 20$) from one of two independent repeats, analysed with
577 ANOVA and post-hoc Tukey tests. Different letters indicate statistically significant differences
578 between means ($p < 0.05$). Osmotic control was 87.6 mM sorbitol.

579

580 Supplemental Figure Legends

581

582 **Figure S1.** The *cca1-11 lhy-21 toc1-21* triple mutant does not alter sucrose-induced hypocotyl
583 elongation under light/dark cycles. This is a direct repeat of the experiment in Figure 3A where
584 data approach statistical significance. (A) Comparison of hypocotyl length of Ws-2 background

585 and *cca1-11 lhy-21 toc1-21* grown on 0.5 MS media (MS) and 0.5 MS media supplemented with
586 3% (w/v) sucrose (Suc); (B) Increase in hypocotyl length of wild type and *cca1-11 lhy-21 toc1-21*
587 caused by exogenous sucrose, relative to 0.5 MS control. Data are mean \pm S.E.M; $n = 10$; statistical
588 significance from two-sample t-tests comparing mutant and wild type for each treatment; N.S. = no
589 significant difference ($p \geq 0.05$).

590

591 **Figure S2.** *EXPA8* and *EXPA11* transcripts were (A) up-regulated by conditions that promote
592 hypocotyl elongation (constant darkness) and (B) down-regulated by the auxin transport inhibitor
593 NPA (mean \pm S.E.M.; $n = 3$). Transcript abundance was relative to *PP2AA3* reference transcript
594 and used 7-day old *L. er.* seedlings. Data analysed with ANOVA followed by post-hoc Tukey test.
595 Different letters indicate statistically significant differences between means ($p < 0.05$).

596

597 **Figure S3.** Sucrose supplementation did not alter the abundance of auxin biosynthesis transcripts
598 or auxin-responsive transcripts relative to osmotic controls, due to responses of osmotic controls.
599 Data indicate relative abundance of three auxin biosynthesis transcripts (*YUCCA8*, *YUCCA9*,
600 *CYP79B3*) and two auxin-responsive transcripts (*IAA29*, *SAUR15*) in two backgrounds, using
601 *PP2AA3* as the reference transcript. Seedlings (60 per replicate) were grown on 0.5 MS, 3% (w/v)
602 sucrose, or 87.6 mM sorbitol as osmotic control, and harvested for RNA 4 days and 7 days after
603 germination (indicated on x axis). Two background lines were used to evaluate whether there were
604 ecotype-specific phenotypes. Data are mean \pm S.E.M; $n = 2$ independent biological repeats.
605 Analyzed by ANOVA ($p \geq 0.05$ in all cases, i.e. not significant).

606

607 **Figure S4.** Confirmation of activity of GA₃. 100 μ M GA₃ increased hypocotyl length relative to
608 the carrier control in both *L. er.* and Col-0 backgrounds, under 4 h and 16 h photoperiods.

609 Seedlings were germinated and grown in presence of GA. Data were collected during methods
610 development and are not directly comparable with other experiments. Data expressed as mean \pm
611 S.E.M. ($n = 20$) and analysed with ANOVA followed by post-hoc Tukey test. Different letters
612 indicate statistically significant differences between means ($p < 0.05$).

613

614 **Figure S5.** ABA signalling is not required for sucrose-induced hypocotyl elongation under short
615 photoperiods. The *pyr1-1 pyl1-1 pyl2-1 pyl4-1* quadruple mutant incorporates Col-0 and *L. er.*
616 backgrounds (Park et al., 2009), both of which are included as controls. Data indicate mean
617 hypocotyl lengths of seedlings grown on 0.5 MS supplemented with 3% sucrose or an osmotic
618 control (87.6 mM sorbitol), under 4 h photoperiods. Data are mean \pm S.E.M.; $n = 20$ (background
619 lines); $n = 3 - 9$ depending on treatment for *pyr1-1 pyl1-1 pyl2-1 pyl4-1* (low replicate numbers
620 due to poor mutant germination). Data are from one of two independent repeats. Statistical
621 significance from independent-samples Kruskal-Wallis analysis of variance on ranks and post-hoc
622 Dunn tests comparing mutant and wild type for each treatment; *** = $p < 0.001$; N.S. = no
623 significant difference ($p \geq 0.05$).

624

625 **Figure S6.** *KIN10* and *TPS1* transcript abundance KIN10-ox and *tps1* TILLING mutants. (A)
626 *KIN10* transcript abundance in two independent KIN10-ox lines (Baena-González et al., 2007), its
627 *L. er* background, and also Col-0. Transcript abundance is relative to *PP2AA3* reference. (B) *TPS1*
628 transcript abundance in *tps1-11* and *tps1-12* (Gómez et al., 2010), alongside the *L. er* and Col-0
629 backgrounds. Transcript abundance was measured in 7 day old seedlings and is relative to the
630 *PP2AA3* reference transcript. Data expressed as mean \pm S.E.M ($n = 3$) and analyzed with ANOVA
631 followed by post-hoc Tukey test. Different letters indicate statistically significant differences
632 between means ($p < 0.05$).

633

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635

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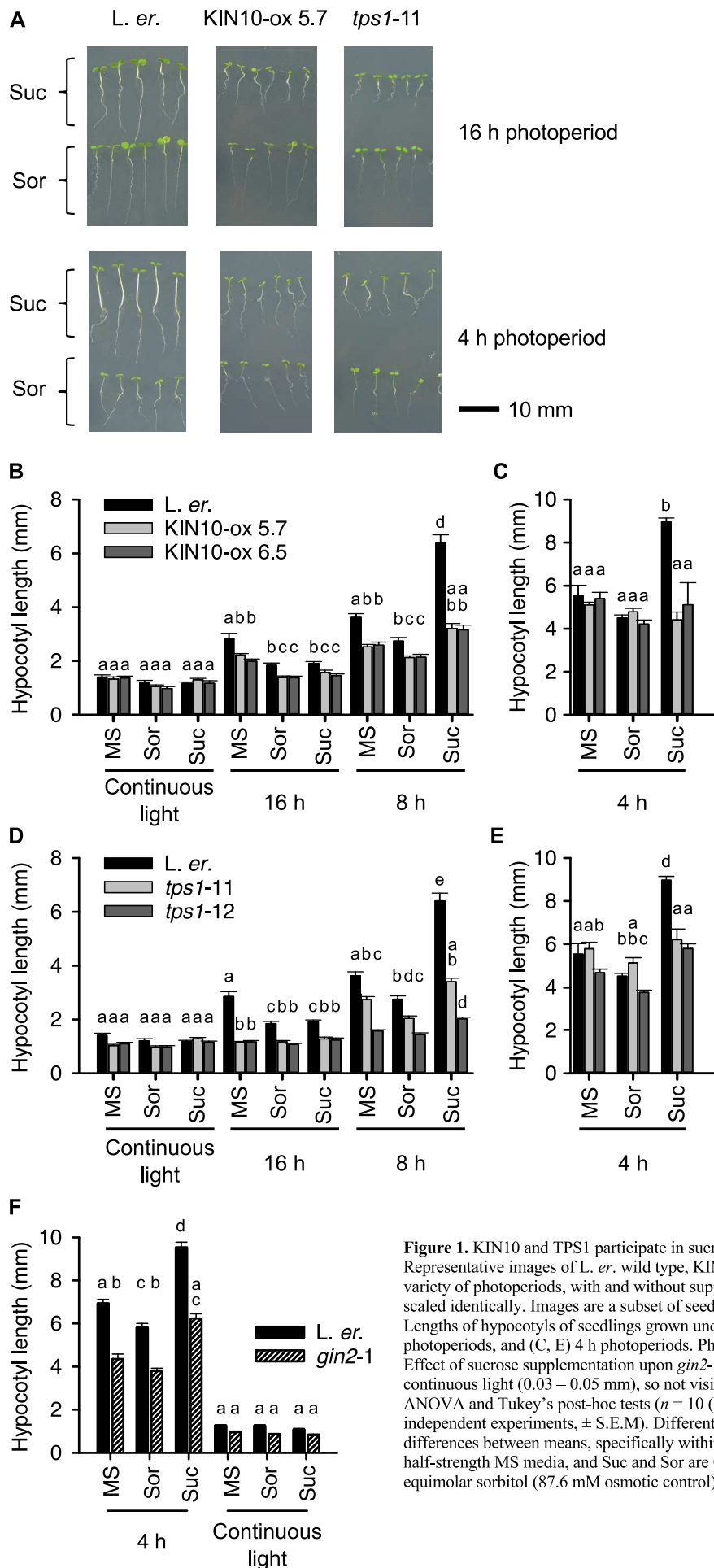


Figure 1. KIN10 and TPS1 participate in sucrose-induced hypocotyl elongation. (A) Representative images of *L. er.* wild type, KIN10-ox and *tps1* seedlings cultivated under a variety of photoperiods, with and without supplementation with 3% sucrose. All panels scaled identically. Images are a subset of seedlings used to generate data in (B-E). (B-E) Lengths of hypocotyls of seedlings grown under (B, D) constant light, 16 h and 8 h photoperiods, and (C, E) 4 h photoperiods. Photoperiods are indicated underneath graphs. (F) Effect of sucrose supplementation upon *gin2-1* hypocotyl length. S.E.M. is small under continuous light (0.03 – 0.05 mm), so not visible on graphs. Data were analysed with ANOVA and Tukey's post-hoc tests ($n = 10$ (B-E) or $n = 20$ (F) seedlings in three independent experiments, \pm S.E.M). Different letters indicate statistically significant differences between means, specifically within each light condition ($p < 0.05$). (B-E); MS is half-strength MS media, and Suc and Sor are 0.5 MS supplemented with 3% (w/v) sucrose or equimolar sorbitol (87.6 mM osmotic control), respectively.

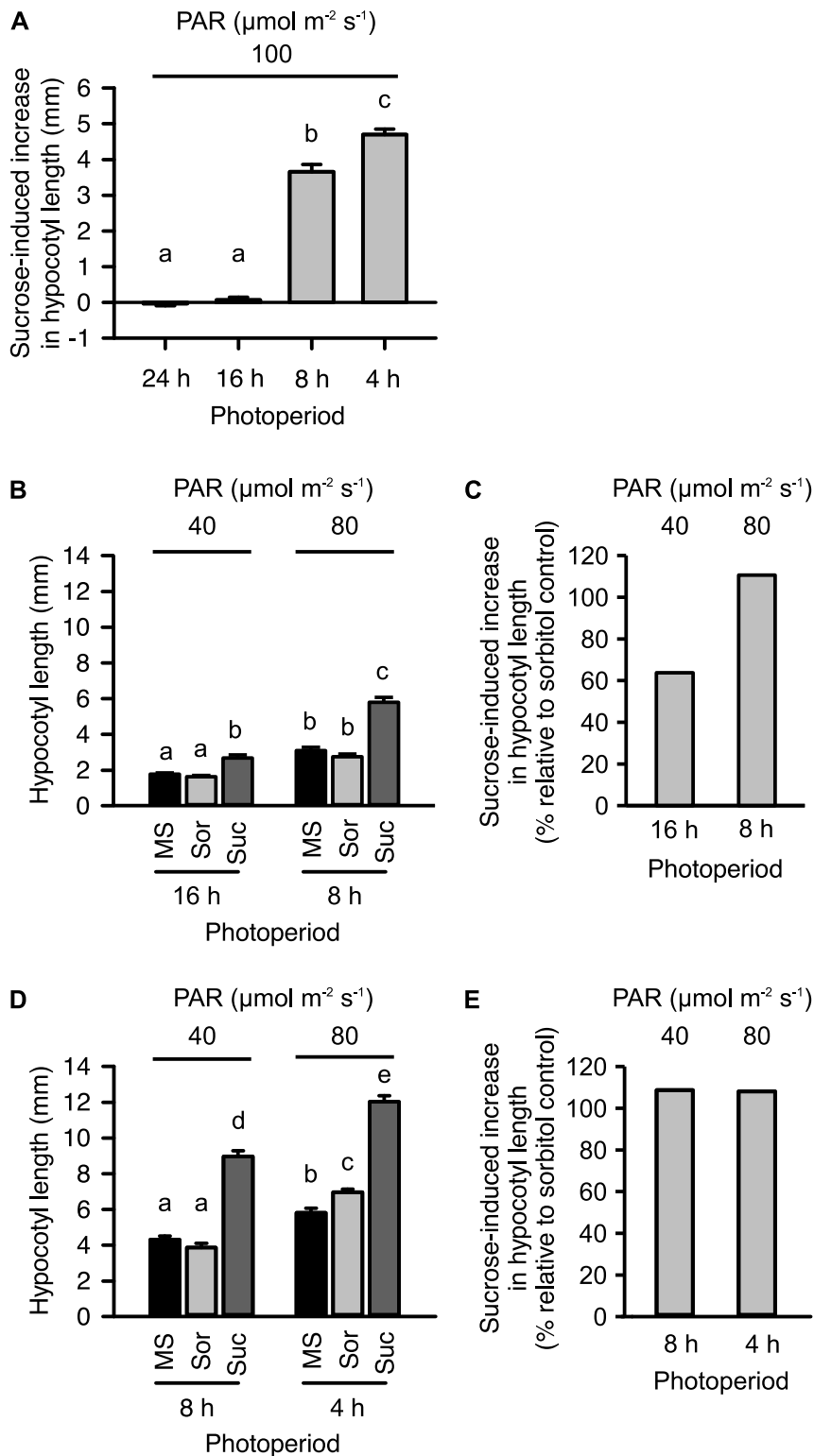


Figure 2. Day-length dependency of sucrose-induced hypocotyl elongation in wild type seedlings. (A) Increase in hypocotyl length caused by sucrose under range of photoperiods (data derived from Fig. 1, plotted relative to sorbitol control). (B-E) Comparison of (B, D) absolute hypocotyl length and (C, E) proportional increase in hypocotyl length caused by sucrose supplementation under specified photosynthetically active radiation (PAR) and photoperiod. Mean \pm S.E.M; (A, C-E) $n = 10$ seedlings in two independent experiments (B) $n = 20$ seedlings. Data analysed using ANOVA followed by post-hoc Tukey test. Different letters indicate statistically significant differences between means ($p < 0.05$).

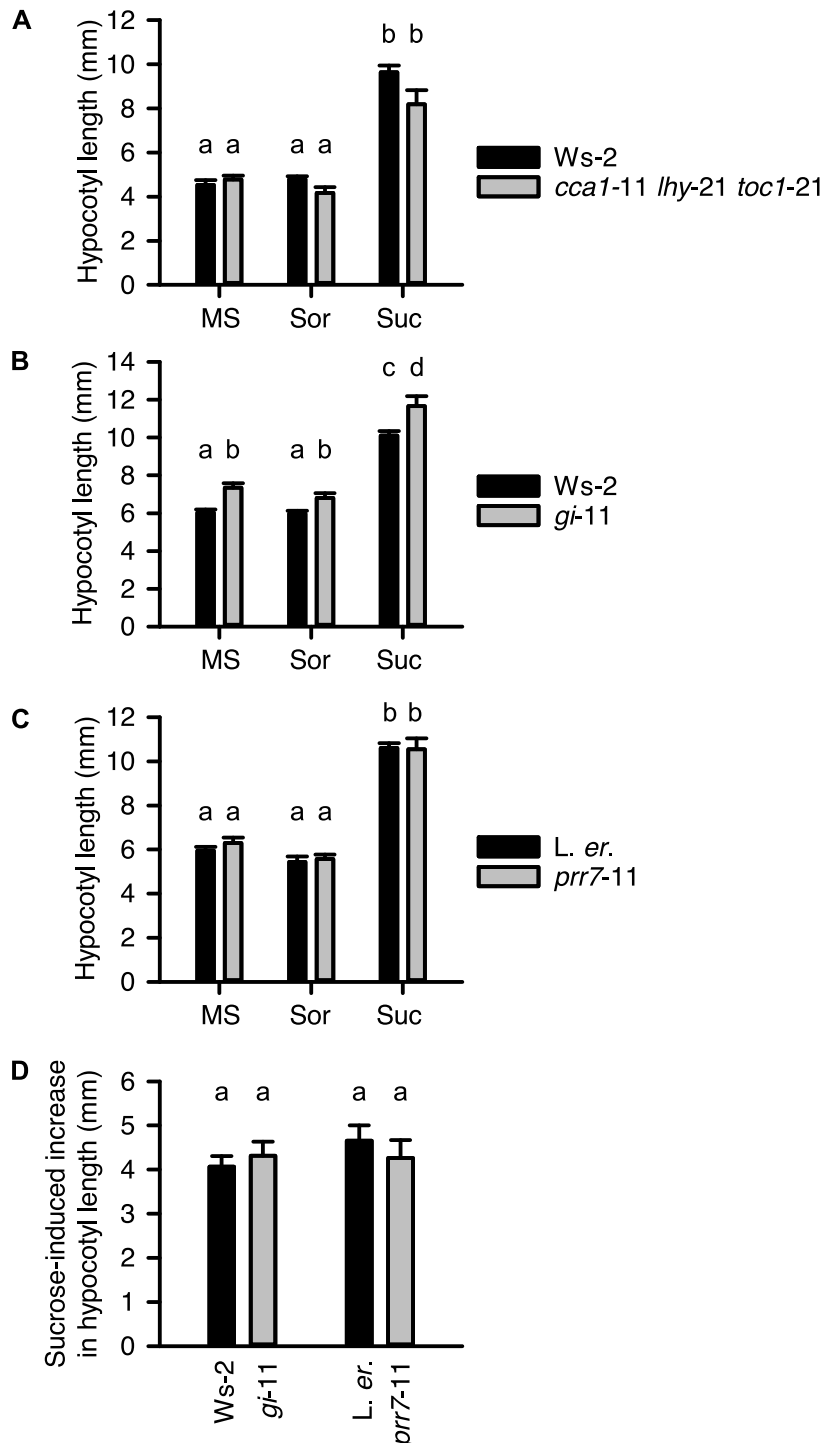


Figure 3. The circadian oscillator does not participate in sucrose-induced hypocotyl elongation under short photoperiods. Sucrose-induced change in hypocotyl length of (A) a circadian oscillator triple mutant (*cca1-11 lhy-21 toc1-21*, background Ws-2) and (B, C) two oscillator components participating in sucrose regulation of the circadian oscillator. (D) Change in hypocotyl length caused by sucrose supplementation in *gi-11* and *prp7-11*, expressed relative to 0.5 MS control. MS is 0.5 MS media, and Suc and Sor are 0.5 MS supplemented with 3% (w/v) sucrose and sorbitol (87.6 mM, osmotic control), respectively. Data are mean \pm S.E.M ($n = 10 - 16$), analysed with (A-C) ANOVA and post-hoc Tukey tests and (D) two-sample t-test comparing mutant with wild type for each treatment. Data show one of three independent repeats of the experiment, conducted under 4 h photoperiods. Different letters indicate statistically significant differences between means ($p < 0.05$).

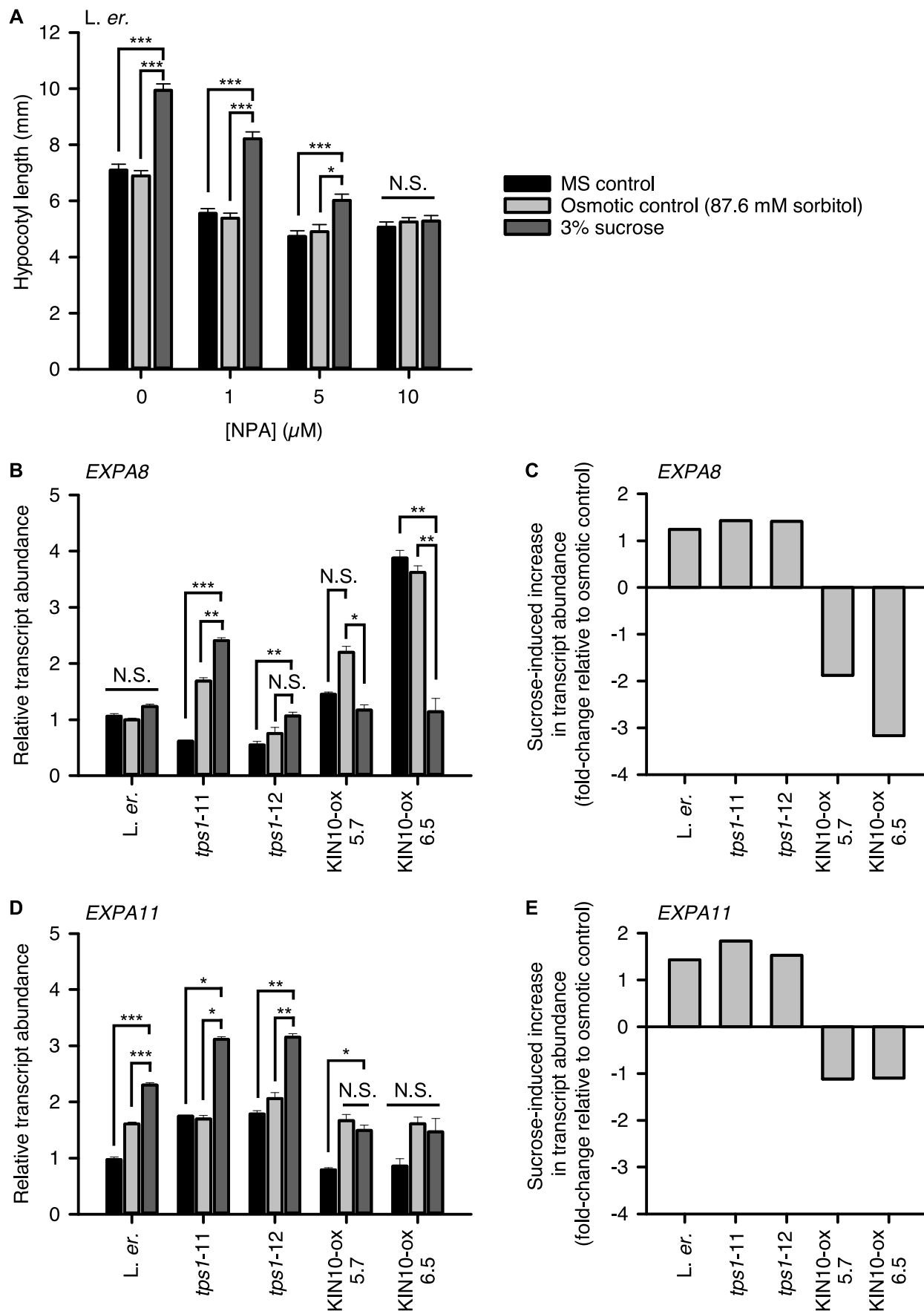


Figure 4. Auxin signalling underlies sucrose-induced hypocotyl elongation and KIN10 regulates expansin gene expression. (A) Hypocotyl length of seedlings cultivated with a range of concentrations of the inhibitor of polar auxin transport 1-N-naphthylphthalamic acid (NPA), under 4 h photoperiods (mean \pm S.E.M.; $n = 20$). (B-E) Sucrose-induced changes in expansin transcript abundance in elongating wild type, *tps1* and KIN10-ox seedlings under 4 h photoperiods. (B, D) Indicate *EXPA8* and *EXPA11* transcript abundance relative to *PP2AA3* (mean \pm S.E.M.; $n = 3$). (C, E) Indicate the magnitude of sucrose-induced change in transcript abundance in each genotype relative to the osmotic control. Data analysed with ANOVA and post-hoc Tukey tests, and with statistical significance indicated using starring (N.S. = Not Significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Downloaded from <http://www.plantphysiol.org/> on November 16, 2017. Published by www.plantphysiol.org. Copyright © 2017 American Society of Plant Biologists. All rights reserved.

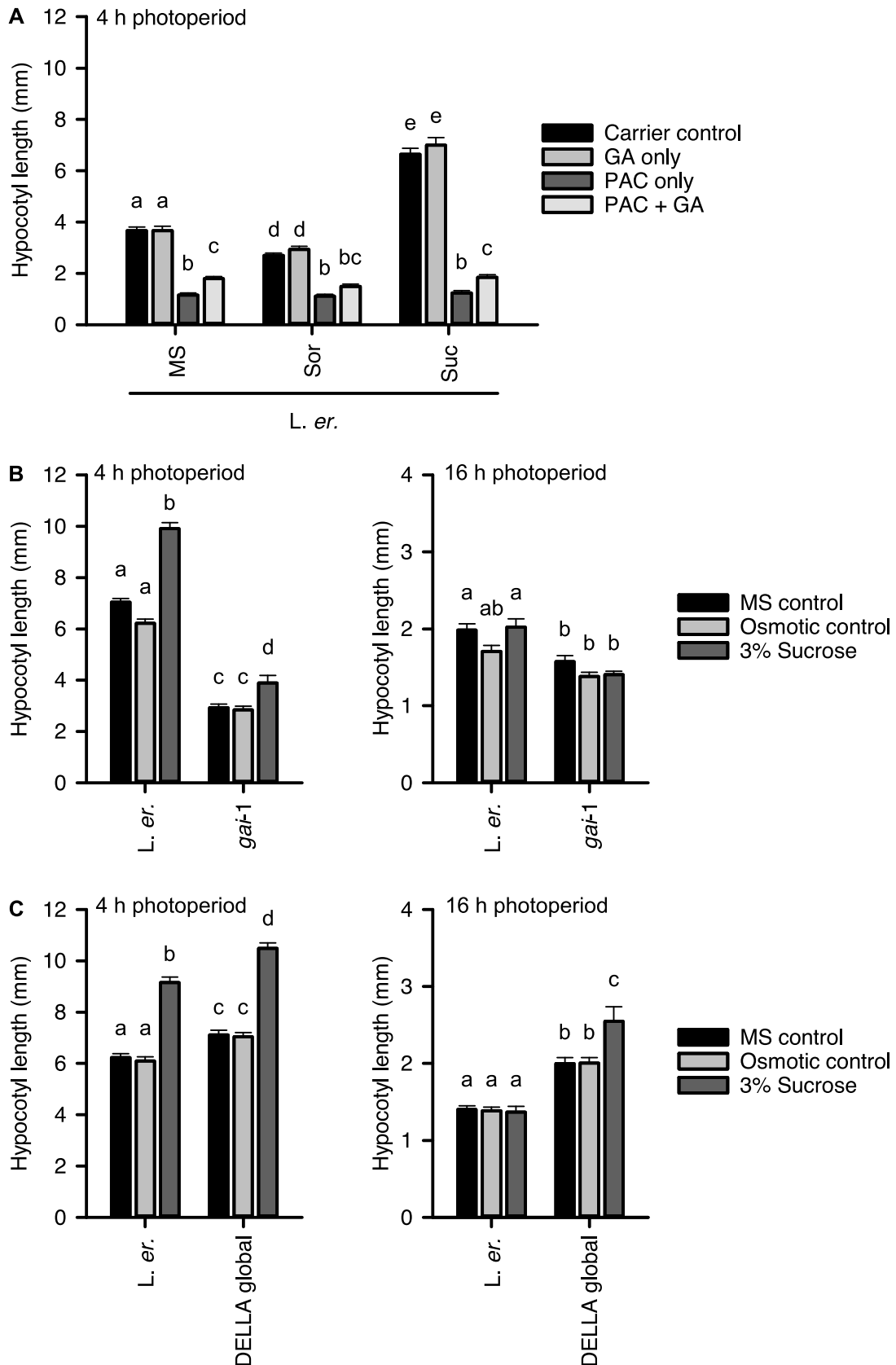


Figure 5. Gibberellin signals contribute to sucrose-induced hypocotyl elongation under short photoperiods. (A) The GA biosynthesis inhibitor paclobutrazol (PAC) at 20 μ M inhibits sucrose-induced hypocotyl elongation. Seedlings were germinated on MS agar and transferred to treatment media after germination; carrier control was 0.12% (v/v) methanol. (B) Sucrose-induced hypocotyl elongation was attenuated in *gai-1* mutant seedlings. (C) Sucrose-induced hypocotyl elongation was unaltered in a DELLA global knockout mutant. Experiments performed under 4 h photoperiods. Data are mean \pm S.E.M ($n = 20$) from one of two independent repeats, analysed with ANOVA and post-hoc Tukey tests. Different letters indicate statistically significant differences between means ($p < 0.05$). Osmotic control was 87.6 mM sorbitol.

Parsed Citations

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