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Timing the switch to phototrophic growth

A possible role of GUN1

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Abbreviations: ABA, abscisic acid; *CHI*, gene encoding chalcone isomerase; *CHS*, gene encoding chalcone synthase; *F3H*, gene encoding flavanone 3-hydroxylase; GUN1, GENOMES UNCOUPLED1; *LHCB1*, gene encoding light-harvesting chlorophyll protein associated with photosystem II; *PAL1*, gene encoding phenylalanine ammonia lyase; *RBCS*, gene encoding the small subunit of Rubisco; RT-PCR, reverse transcriptase-polymerase chain reaction

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In young Arabidopsis seedlings, retrograde signaling from plastids regulates the expression of photosynthesis-associated nuclear genes in response to the developmental and functional state of the chloroplasts. The chloroplast-located PPR protein GUN1 is required for signalling following disruption of plastid protein synthesis early in seedling development before full photosynthetic competence has been achieved. Recently we showed that sucrose repression and the correct temporal expression of *LHCB1*, encoding a light-harvesting chlorophyll protein associated with photosystem II, are perturbed in *gun1* mutant seedlings.[1] Additionally, we demonstrated that in *gun1* seedlings anthocyanin accumulation and the expression of the "early" anthocyanin-biosynthesis genes is perturbed. Early seedling development, predominantly at the stage of hypocotyl elongation and cotyledon expansion, is also affected in *gun1* seedlings in response to sucrose, ABA and disruption of plastid protein synthesis by lincomycin. These findings indicate a central role for GUN1 in plastid, sucrose and ABA signalling in early seedling development.

Arabidopsis seedlings develop in response to light and other environmental cues. In young seedlings, development is fuelled by mobilization of lipid reserves until chloroplast biogenesis is complete and the seedlings can make the transition to phototrophic growth. The majority of proteins with functions related to photosynthesis are encoded by the nuclear genome, and their expression is coordinated with the expression of genes in the chloroplast genome. In developing seedlings, retrograde signaling from chloroplasts to the nucleus regulates the expression of these nuclear genes and is dependent on the developmental and functional status of the chloroplast. Two classes of *gun* (*genomes uncoupled*) mutants defective in retrograde signalling have been identified in Arabidopsis: the first, which comprises *gun2-gun5*, involves mutations in genes encoding components of tetrapyrrole biosynthesis.[2,3] The other comprises *gun1*, which has mutations in a nuclear gene encoding a plastid-located pentatricopeptide repeat (PPR) protein with an SMR (small MutS-related) domain near the C-terminus.[4,5] PPR proteins are known to have roles in RNA processing[6] and the SMR domain of GUN1 has been shown to bind DNA,[4] but the specific functions of these domains in GUN1 are not yet established. However, GUN1 has been shown to be involved in plastid gene expression-dependent,[7] redox,[4] ABA[1,4] and sucrose signaling,[1,4,8] as well as light quality and intensity sensing pathways.[9-11] In addition, GUN1 has been shown to influence anthocyanin biosynthesis, hypocotyl extension and cotyledon expansion.[1,11]

gun1 Seedling Development is Hypersensitive to ABA and Sucrose

Norflurazon (an herbicide that results in photo-oxidation of chlorophyll) and lincomycin (an antibiotic that inhibits plastid translation) lead to a loss of photosynthesis-associated nuclear gene

expression in wild-type Arabidopsis seedlings. The *gun* mutants were identified by their ability to express photosynthesis-associated nuclear genes when grown in the presence of norflurazon,[12] but only *gun1* retained the ability to express photosynthesis-associated nuclear genes when grown in the presence of lincomycin, linking the GUN1 signaling pathway to disruption of chloroplast translation.[7] The target genes of this signaling pathway have been shown to contain an ACGT motif, which is core to both the abscisic acid (ABA) response element (ABRE) and the light-responsive G-box.[4] Screens of ABA-deficient and ABA-insensitive mutants identified *aba insensitive4* (*abi4*) as having a weak *gun* phenotype.[4] Overexpression of *ABI4*, which encodes an APETALA 2-type transcription factor, suppressed the *gun1* phenotype, suggesting that ABI4 functions downstream of GUN1.[4] ABI4 has been shown to regulate *RBCS* gene expression in response to sucrose and ABA, via an S-box motif in association with the light-responsive G-box element.[13] However, most ABA signalling mutants, including *abi4*, have been identified by their insensitivity to ABA, which mediates seedling developmental arrest in wild type, whereas *gun1* does not fall into this category. *gun1* has a hypersensitive response to ABA and displays greater developmental arrest compared to wild-type seedlings.[1,4]

abi4 and many other ABA-insensitive mutants are also sugar insensitive, establishing links between sugar and ABA signalling. However, we showed that *gun1* displays sucrose hypersensitivity: sucrose-mediated developmental arrest was more severe for *gun1* than for wild-type seedlings, predominantly at the stage of cotyledon expansion.[1] Sugar developmental-arrest screens have identified many insensitive mutants but very few that are hypersensitive,[14] and fewer still that are also hypersensitive to ABA.

The interactions between ABA and sugar signalling in developing seedlings are complex. Although both ABA and sucrose can arrest seedling development, sucrose can rescue seedlings from ABA-mediated developmental arrest.[15] This observation led to the hypothesis that ABA inhibits germination and post-germinative growth by inhibiting mobilization of seed lipid reserves.[15] However, active lipid mobilization in response to ABA, leading to high levels of endogenous sucrose, has been demonstrated.[16] Thus, it is not clear why sucrose should rescue ABA-induced developmental arrest, but one possible explanation is that endogenous and exogenous sugars are perceived and affect developmental signals differently. Exogenous sugars may potentially be perceived by young seedlings as the product of photosynthesis and serve to override ABA signalling. But sugar concentrations may also play a part: 14 mM sucrose rescues ABA developmental arrest,[15] whereas 330 mM sucrose arrests seedling development.[17] We showed that medium containing 2% sucrose (85 mM) arrests seedling development and *LHCB1* expression in wild-type seedlings and to a greater extent in *gun1*.[1] However, sugar sensitivity in *gun1* seedlings appears to change during development; the addition of 7% glucose (300 mM) to the growth medium was shown to reduce *LHCB1* expression significantly when applied to 3-day-old[4] and 3-week-old[8] wild-type seedlings, but not when applied to *gun1* seedlings.

Temporal Photosynthesis Gene Expression and Anthocyanin Accumulation are Differentially Affected by Sucrose in Wild-Type and *gun1* Seedlings

We showed that the expression of *LHCB1* fluctuates during early seedling development (3–8 days old) and is different in *gun1* seedlings in comparison to wild-type.[1] Sucrose represses expression of both nuclear and plastid genes encoding photosynthetic components[18,19] and disrupts the temporal expression profile of *LHCB1* in both wild-type and *gun1* seedlings. In general, sucrose results in a decrease in *LHCB1* transcripts of around 70% in both genotypes.[1] The addition of lincomycin resulted in a further reduction of *LHCB1* transcripts in wild type, but partially released *LHCB1* from sucrose repression in *gun1*.[1] This would imply that GUN1 is required for sucrose repression of *LHCB1* expression in the absence of functional chloroplasts. To determine if a similar temporal response was observed with other nuclear genes encoding photosynthesis components, we examined the expression of the *CA1* gene (At3g01500) encoding a plastidic carbonic anhydrase, which catalyses the reversible hydration of CO_2 to form HCO_3^- and is thought to supply CO_2 for Rubisco. Transcriptome profiling identified transcripts of *CA1* as the most responsive to lincomycin treatment in wild-type plants, whereas *CA1* transcripts remained unchanged in lincomycin-treated *gun1* seedlings.[5] Analysis of *CA1* transcripts in wild-type and *gun1* seedlings over a 6-day period in the absence or presence of 2% sucrose is shown in **Figure 1**. The temporal patterns of *CA1* expression and the effects of sucrose differed between wild-type and *gun1* seedlings. In wild-type seedlings, the temporal patterns were markedly different in the absence and presence of sucrose; sucrose repressed *CA1* expression in 3–5-day-old seedlings, but had little effect in 6–8-day-old seedlings. In contrast, in *gun1* seedlings, sucrose had little effect on *CA1* transcript accumulation over the 6-day period. There was a slight repression of *CA1* expression by sucrose in 3- and 4-day-old seedlings, but in 5–8-day-old seedlings there was relatively little effect. This is different to *LHCB1* expression, which is markedly repressed by sucrose in both wild-type and *gun1* seedlings throughout the 6-day period (**Fig. 1**). These observations imply that the temporal sucrose response of *CA1*, unlike *LHCB1*, is almost entirely mediated by GUN1.

Sucrose also induces anthocyanin accumulation in young seedlings in a distinct temporal fashion.[20] We showed that anthocyanins reach a peak 1 day later in *gun1* seedlings in comparison to wild-type, and that the amounts accumulated were lower in *gun1*.[1] Lincomycin and norflurazon treatments disrupted both the temporal profile and decreased sucrose-induced anthocyanin levels in both genotypes. We demonstrated that GUN1-mediated regulation of anthocyanin biosynthesis was via transcriptional control of the "early" anthocyanin biosynthesis genes (*PAL*, *CHS*, *CHI* and *F3H*). Whereas ABA-mediated seedling developmental arrest can be rescued by

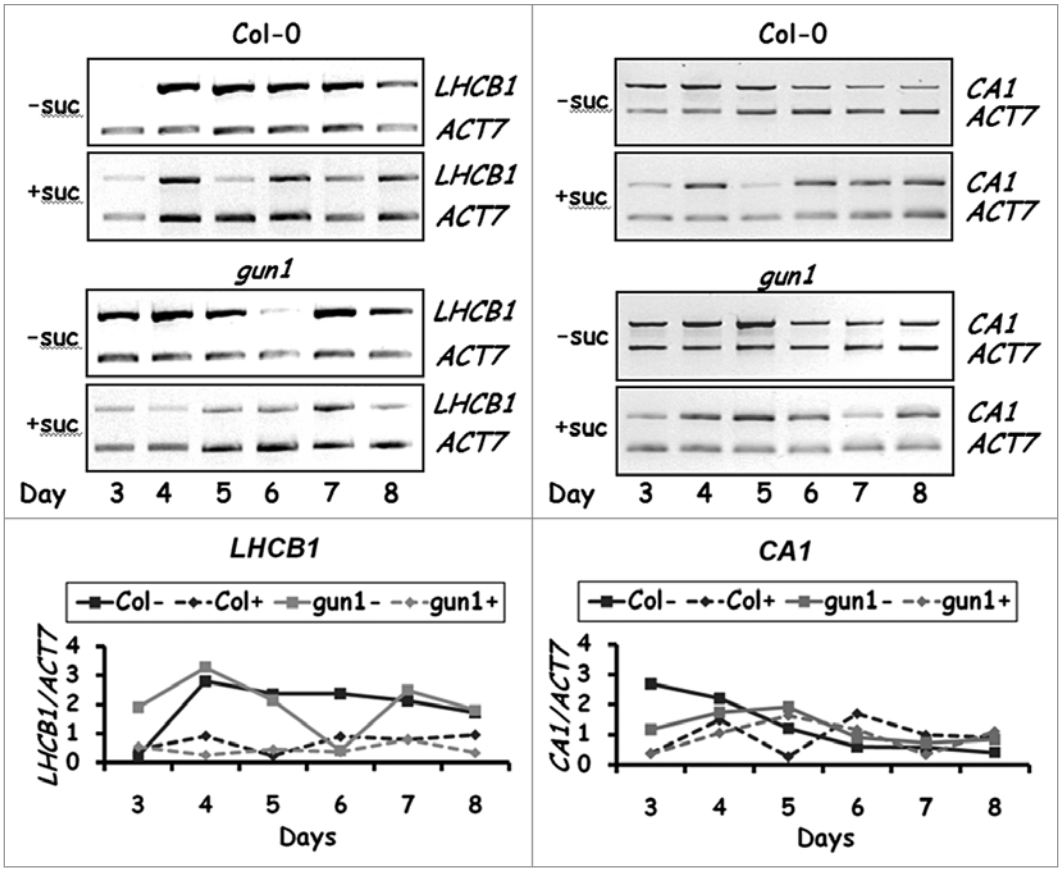

Figure 1. Time course of *LHCB1* and *CA1* transcript abundance in wild-type and *gun1* seedlings. RT-PCRs were performed on RNA extracted from 3- to 8-day-old wild-type (Col-0) and *gun1-1* seedlings grown on 0.5x MS-agar medium, ±2% sucrose (suc). The PCR products separated by electrophoresis in 1% agarose gels are shown in the upper part of the figure. The amounts of *LHCB1* and *CA1* PCR products normalized to those of *ACT7* are shown in the lower part of the figure.

sucrose, ABA has been shown to have a synergistic effect on sucrose-induced anthocyanin accumulation in wild-type Arabidopsis seedlings.[21] This appears not to involve ABI4, as 3-day-old *abi4* seedlings accumulate more anthocyanin in response to sucrose than wild-type seedlings and *CHS* transcripts were shown to be increased.[22] ABI4 may have a role in negatively regulating "early" anthocyanin biosynthesis genes, in contrast to GUN1 which appears to have a positive regulatory role.

If ABI4 regulates photosynthesis-associated nuclear gene expression in response to GUN1-mediated retrograde signalling, it is not clear how it manifests contrasting seedling developmental phenotypes of *gun1* in response to ABA and sucrose. A weak *gun* phenotype has been reported for 4-day-old *abi4* seedlings on medium containing sucrose and lincomycin.[4] However, this phenotype would appear to be transient, as 7-day-old *abi4* seedlings do not display a *gun* phenotype, whereas 7-day-old *gun1* seedlings do (**Fig. 2**). Lincomycin treatment completely abolished *CA1* expression in wild-type and *abi4* seedlings, but had no effect on *CA1* transcripts in *gun1* seedlings. However, because of the fluctuations in the amounts of *LHCB1* and *CA1* transcripts during early seedling development and because sucrose disrupts this temporal expression (see **Fig. 1**), observations at a single specific time point on medium containing sucrose may be potentially misleading. ABI4 may act downstream of GUN1 in response to sucrose, but chloroplast translation-related signaling may act, at least in part, independently of ABI4.

The greatest difference in sucrose-induced anthocyanin accumulation between *gun1* and wild-type seedlings was observed on day 4 in the presence of lincomycin, when *gun1* contained only 47% of wild-type levels.[1] This coincides with a distinct developmental difference between the genotypes: *gun1* had expanded cotyledons and extended hypocotyls, whereas wild-type seedlings did not. This phenotype was seen in seedlings grown on medium containing lincomycin with or without sucrose, but was not observed in seedlings grown on norflurazon or on media without inhibitors. This provides further evidence that signaling of the chloroplast developmental/functional status and sucrose signaling may be operating at least partially independently through GUN1. Sucrose inhibited cotyledon opening in *gun1* seedlings but not in the absence of functional chloroplasts, whereas in wild-type seedlings both sucrose and decreased chloroplast translation delayed cotyledon opening.

It would appear that GUN1 regulates expression of photosynthesis-related nuclear genes, seedling development

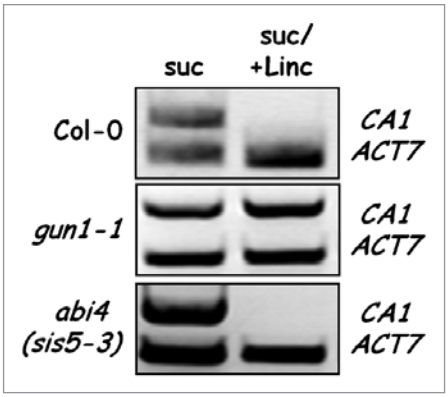

Figure 2. Effect of sucrose and lincomycin on *CA1* transcripts in wild-type, *gun1 and abi4* seedlings. RT-PCRs for carbonic anhydrase 1 (*CA1*) and actin (*ACT7*) transcripts were performed on RNA extracted from 7-day-old wild-type (Col-0), *gun1-1* and *abi4-1* seedlings grown on 0.5x MS-agar medium, 2% sucrose (suc) and ±0.5 mM lincomycin (Linc). The PCR products were separated by electrophoresis in a 1% agarose gel.

and anthocyanin biosynthesis in young seedlings in response to developmental and environmental stimuli before and up until photosynthetic competence has been established. However, in *gun1* seedlings these responses are not usually completely abolished, but they are diminished or delayed, suggesting that GUN1 may serve to optimise the timing of a crucial developmental event in seedlings.

GUN1 may Optimize the Switch to Photoautotrophic Growth

In the developing Arabidopsis seedlings, lipid reserves in the form of triacylglycerol (TAG) are mobilized in the form of sucrose; this requires the bypass of the decarboxylative reactions of the tricarboxylic acid (TCA) cycle and is achieved by post-transcriptional regulation of NAD^+-isocitrate dehydrogenase.[23] Carbon flow through the full TCA cycle is delayed until the seedling is photosynthetically competent and no longer dependent on stored reserves. In the absence of exogenous sugars, the switch from autotrophic to phototrophic growth occurs in 2–3-day-old seedlings,[23] but in the presence of sucrose, lipid mobilization is retarded[24] and metabolic switching may be delayed. However, the effects of sucrose on lipid mobilization are not seen if sucrose is applied after 3 days.[24] Several of the components of GUN1 signaling appear to affect target photosynthesis-related nuclear genes only at an early stage of seedling development. Inhibitors of plastid translation, such as lincomycin and chloramphenicol, are effective in regulating photosynthesis genes only if applied to seedlings younger than 3 days old,[25,26] and responsiveness to sucrose changes in older seedlings (see **Fig. 1**).

These changes in responsiveness appear to coincide with attainment of photosynthetic competence. GUN1 may function in the timing of this event, possibly influenced by inputs from sugar sensing, which may be able to distinguish between sugars generated from lipid mobilization and those produced by photosynthesis. GUN1 also appears to orchestrate seedling development to coincide with the transition to phototrophic growth; this necessitates cotyledon opening to maximise photosynthesis and anthocyanin accumulation to protect new and developing chloroplasts.

References

1. Cottage A, Mott EK, Kempster JA, Gray JC. The Arabidopsis plastid-signalling mutant *gun1* (*genomes uncoupled1*) shows altered sensitivity to sucrose and abscisic acid and alterations in early seedling development. J Exp Bot 2010; 61:3773-86.
2. Mochizuki N, Brusslan JA, Larkin R, Nagatani A, Chory J. Arabidopsis genomes uncoupled 5 (GUN5) mutant reveals the involvement of Mg-chelatase H subunit in plastid-to-nucleus signal transduction. Proc Natl Acad Sci USA 2001; 98:2053-8.
3. Larkin RM, Alonso JM, Ecker JR, Chory J. GUN4, a regulator of chlorophyll synthesis and intracellular signaling. Science 2003; 299:902-6.
4. Koussevitzky S, Nott A, Mockler TC, Hong F, Sachetto-Martins G, Surpin M, et al. Signals from chloroplasts converge to regulate nuclear gene expression. Science 2007; 316:715-9.
5. Cottage AJ, Mott EK, Wang JH, Sullivan JA, MacLean D, Tran L, et al. *GUN1* (*GENOMES UNCOUPLED1*) encodes a pentatricopeptide repeat (PPR) protein involved in plastid protein synthesis-responsive retrograde signaling to the nucleus. In: Allen JF, Gantt E, Golbeck JH, Osmond B, eds. Photosynthesis. Energy from the Sun. Springer-Verlag, Berlin: 14th International Congress on Photosynthesis, 2008:1205-11.
6. Schmitz-Linneweber C, Small I. Pentatricopeptide repeat proteins: a socket set for organelle gene expression. Trends Plant Sci 2008; 13:663-70.
7. Gray JC, Sullivan JA, Wang JH, Jerome CA, MacLean D. Coordination of plastid and nuclear gene expression. Phil Trans R Soc Lond 2003; 358:135-45.
8. Zhang ZW, Yuan S, Xu F, Yang H, Zhang NH, Cheng J, et al. The plastid hexokinase pHXK: a node of convergence for sugar and plastid signals in Arabidopsis. FEBS Lett 2010; 584:3573-9.
9. McCormac AC, Terry MJ. The nuclear genes Lhcb and HEMA1 are differentially sensitive to plastid signals and suggest distinct roles for the GUN1 and GUN5 plastid-signaling pathways during de-etiolation. Plant J 2004; 40:672-85.
10. Ruckle ME, DeMarco SM, Larkin RM. Plastid signals remodel light signaling networks and are essential for efficient chloroplast biogenesis in Arabidopsis. Plant Cell 2007; 19:3944-60.
11. Ruckle ME, Larkin RM. Plastid signals that affect photomorphogenesis in *Arabidopsis thaliana* are dependent on GENOMES UNCOUPLED 1 and cryptochrome 1. New Phytol 2009; 182:367-79.
12. Susek RE, Ausubel FM, Chory J. Signal transduction mutants of Arabidopsis uncouple nuclear CAB and RBCS gene expression from chloroplast development. Cell 1993; 74:787-99.
13. Acevedo-Hernandez GJ, Leon P, Herrera-Estrella LR. Sugar and ABA responsiveness of a minimal RBCS light-responsive unit is mediated by direct binding of ABI4. Plant J 2005; 43:506-19.
14. Leon P, Sheen J. Sugar and hormone connections. Trends Plant Sci 2003; 8:110-6.
15. Garciarrubio A, Legaria JP, Covarrubias AA. Abscisic acid inhibits germination of mature Arabidopsis seeds by limiting the availability of energy and nutrients. Planta 1997; 203182-7.
16. Pritchard SL, Charlton WL, Baker A, Graham IA. Germination and storage reserve mobilization are regulated independently in Arabidopsis. Plant J 2002; 31:639-47.
17. Laby RJ, Kincaid MS, Kim D, Gibson SI. The Arabidopsis sugar-insensitive mutants sis4 and sis5 are defective in abscisic acid synthesis and response. Plant J 2001; 23:587-96.
18. Pego JV, Kortstee AJ, Huijser C, Smeekens SC. Photosynthesis, sugars and the regulation of gene expression. J Exp Bot 2000; 51:407-16.
19. Rolland F, Baena-Gonzalez E, Sheen J. Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu Rev Plant Biol 2006; 57:675-709.
20. Kubasek WL, Shirley BW, McKillop A, Goodman HM, Briggs WB, Ausubel FM. Regulation of flavonoid biosynthetic genes in germinating Arabidopsis seedlings. Plant Cell 1992; 4:1229-36.
21. Loreti E, Povero G, Novi G, Solfanelli C, Alpi A, Perata P. Gibberellins, jasmonate and abscisic acid modulate the sucrose-induced expression of anthocyanin biosynthetic genes in Arabidopsis. New Phytol 2008; 179:1004-16.
22. Dijkwel PP, Huijser C, Weisbeek PJ, Chua NH, Smeekens SC. Sucrose control of phytochrome A signaling in Arabidopsis. Plant Cell 1997; 9:583-95.
23. Falk KL, Behal RH, Xiang C, Oliver DJ. Metabolic bypass of the tricarboxylic acid cycle during lipid mobilization in germinating oilseeds. Regulation of NAD^+-dependent isocitrate dehydrogenase versus fumarase. Plant Physiol 1998; 117:473-81.

24. To JP, Reiter WD, Gibson SI. Mobilization of seed storage lipid by Arabidopsis seedlings is retarded in the presence of exogenous sugars. BMC Plant Biol 2002; 2:4.

25. Oelmüller R, Levitan I, Bergfeld R, Rajasekhar VK, Mohr H. Expression of nuclear genres as affected by treatments acting on plastids. Planta 1986; 168:482-92.

26. Gray JC, Sornarajah R, Zabron AA, Ducket CM, Khan MS. Chloroplast control of nuclear gene expression. In: Mathis P, ed. Photosynthesis, from Light to Biosphere. Kluwer, Dordrecht 1995:543-50.