Running a little late: chloroplast Fe status and the circadian clock

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In plants, the circadian clock is known to regulate a wide variety of processes and it is clear that the clock-conferred ability of plants to anticipate daily changes in environmental conditions confers significant fitness advantages. Two new studies highlight complex reciprocal interactions between the clock and chloroplast iron status and provide significant new insight into the role of iron in control of circadian period length.

Iron (Fe) uptake and homeostasis are essential for plant growth and survival. These processes are carefully regulated to ensure adequate supplies of Fe to maintain metabolism while avoiding the negative effects of Fe over-accumulation. Indeed, it is clear that Fe uptake, efflux, long-distance transport and storage are controlled via transcriptional and post-transcriptional regulatory mechanisms and in recent years, a number of new components of the Fe-deficiency signalling

cascade have been identified (Walker and Connolly, 2008; Palmer and Gueriot, 2009).

In plants, the chloroplast represents a major sink for Fe; approximately 90% of the Fe found in leaves is contained within plastids, where it is associated with the photosystems, haem and chlorophyll. In addition, significant amounts of Fe are stored in chloroplasts in the form of ferritin-Fe. Chloroplast Fe homeostasis poses significant challenges owing, in part, to the fact that while Fe is essential for chloroplast function, excess Fe participates in the generation of hydroxyl radicals, which may exacerbate oxidative stress associated with normal functioning of photosynthetic electron transport reactions. It is not yet known how plants sense Fe but it is assumed that photosynthetic organisms possess a mechanism to gauge chloroplast Fe status.

Figure 1 The interaction of Fe with the circadian clock. (A) Circadian Fe responses likely require a putative chloroplast-derived Fe signal that is transduced to the nucleus. Circadian Fe responses also require functional plastids and phytochromes which relocate to the nucleus in response to light to regulate clock genes. Under Fe-sufficient conditions, the clock runs normally on a ~24 h cycle, as shown in blue. Regulated clock output genes include a set of genes involved in Fe homeostasis. (B) Under Fe limitation and in Fe homeostasis mutants, the free-running period is extended by up to 3 h, as shown in red. (C) In dark-grown (etiolated) seedlings and in phytochrome mutants, Fe sensing is uncoupled from the circadian clock. The FRP of the plant is unaffected by iron status.

Circadian rhythms are endogenous, ~24 h rhythms that are generated by the circadian clock. The clock controls expression of ~30% of nuclear genes in Arabidopsis; a significant subset of the clock-controlled transcriptome is involved in diurnal responses to changing light and temperature and it is clear that the proper functioning of the clock confers a significant fitness advantage (Dodd *et al*, 2005; Graf *et al*, 2010). Interestingly, recent work has illuminated interactions between the clock and both cell metabolism (Farré and Weise, 2012) and photosynthetically-generated ROS (Lai *et al*, 2012). In addition, Duc *et al* (2009) reported that the nuclear circadian clock component TIC (TIME FOR COFFEE) controls genes involved in the response to Fe overload in Arabidopsis. These studies suggest a likely interaction between the clock and chloroplast Fe levels. Two new reports in *The EMBO Journal* and *Plant Physiology* confirm this connection and shed considerable light on the molecular mechanisms that underlie the interaction between the clock and Fe status (Hong *et al*, 2012; Salomé *et al*, 2013).

Salomé *et al* (2013) report in *The EMBO Journal* that growth of plants under Fe-limiting conditions results in a circadian period lengthened by up to 3 h. The authors took advantage of promoter:luciferase (*Pro:LUC*) lines created using a variety of promoters from both clock and clock-controlled genes and monitored luciferase activity under free-running conditions to elegantly demonstrate a clear and robust response. They noted that modulation of the free-running period (FRP) by Fe occurs rapidly (within 24 h) and the FRP showed a linear relationship with the log of Fe concentration supplied in the medium. In addition, they measured the effects of changing Fe status on the FRP in two mutants with defects in the Fe-deficiency response: *irt1* lacks the ferrous iron transporter responsible for Fe uptake from the soil while *fit* lacks a bHLH transcription factor that is involved in induction of the Fe uptake machinery (including IRT1). Both mutants displayed a lengthened period under intermediate Fe concentrations that was rescued by growth with high (100 µM) Fe. Together these data indicate that the plant adjusts the period of the circadian clock based on physiological Fe status.

Insight into the molecular mechanisms mediating this response came first through the analysis of the *hy6* mutant line, which has a defect in the chloroplast haem oxygenase HO1; HO1 functions in the biosynthesis of the phytochrome chromophore. Analysis of *TOC1:LUC* activity in the *hy6* background revealed that HY6 is required for the circadian Fe response (i.e., the FRP in *hy6* did not vary with Fe supply but rather was short regardless of Fe supply). These data, combined with other data showing that Fe-dependent changes in the FRP do not appear to rely on haem, implicated phytochrome chromophore synthesis in the Fe circadian response. Consistent with this idea, the authors showed that inhibition of plastid translation abrogates the lengthening of period under Fe deficiency.

To assess the importance of light for the circadian Fe response, Salomé *et al* (2013) examined *TOC1:LUC* activity in etiolated (dark-grown) seedlings that were previously entrained using thermocycles. They showed that despite proper functioning of the clock, Fe supply does not affect the FRP in etiolated seedlings. These data are consistent with

the notion that the circadian Fe response depends on light. Given the importance of both *hy6* (which lacks active phytochromes) and light in the change in circadian period in response to Fe status, the authors predicted that proper phytochrome function is required for the circadian Fe response. Indeed, loss of PHYA and PHYB resulted in loss of period adjustment in response to low Fe. Further studies implicated HEMERA in the circadian Fe response; HEMERA is known to participate in translocation of phytochromes to the nucleus and thus is important for photomorphogenesis.

In a separate report, Hong *et al* (2012) confirm an Fe-deficiency-dependent lengthening of the period of the circadian clock. In addition, these authors used *PRO:LUC* lines to profile expression of genes with demonstrated roles in Fe homeostasis. They reported circadian control of promoter activity for *IRT1*, *FER1* (encoding Fe storage protein Ferritin 1) and *bHLH39* (encoding a transcription factor involved in the Fe-deficiency response). Together these data confirm the findings of Salomé *et al* (2013) and additionally show that while Fe status regulates the circadian period, the clock reciprocally regulates expression of Fe homeostasis genes.

Hong *et al* (2012) went on to investigate the possible involvement of various clock components in regulation of clock periodicity by Fe status. Interestingly, the authors provide clear evidence for involvement of ZTL (an F-box protein that controls turnover of clock components TOC1 and PRR5) as well as CCA1 and LHY (myb transcription factors and central clock components) in the circadian Fe response. These results are not in agreement with those of Salomé *et al* (2013) who did not find evidence to support the involvement of ZTL, CCA1 and LHY in the process. These differences could be owing to growth conditions and/or the specific *PRO:LUC* constructs used in the two studies. Additional work will be required to clarify the differences. Despite this, the two studies are largely consistent and serve to highlight the robust interaction between Fe and the circadian clock (see Figure 1). It is important to note that these studies have important implications for experimental design of future studies focused on Fe homeostasis in plants. Moreover, these reports have set the stage for future studies aimed at further elucidation of the molecular mechanism(s) involved in both sensing of chloroplast Fe status and integration of light, circadian and Fe signals to control Fe homeostasis.

Note added in proof: While this manuscript was in review, complementary work showing that Fe deficiency results in a lengthened circadian period was published (Chen, Y-Y, Wang Y, Shin L-J, Wu J-F, Shanmugam V, Tsednee M, Lo J-C, Chen C-C, Wu S-H, Yeh K-C. Iron is involved in maintenance of circadian period length in Arabidopsis. *Plant Physiology*, doi: <http://dx.doi.org/10.1104/pp.112.212068>).

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Conflict of interest

The authors declare that they have no conflict of interest.

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