

# The Rhythm of the Light: How Light and the Clock Drive Cycling of Transcript Levels in Barley

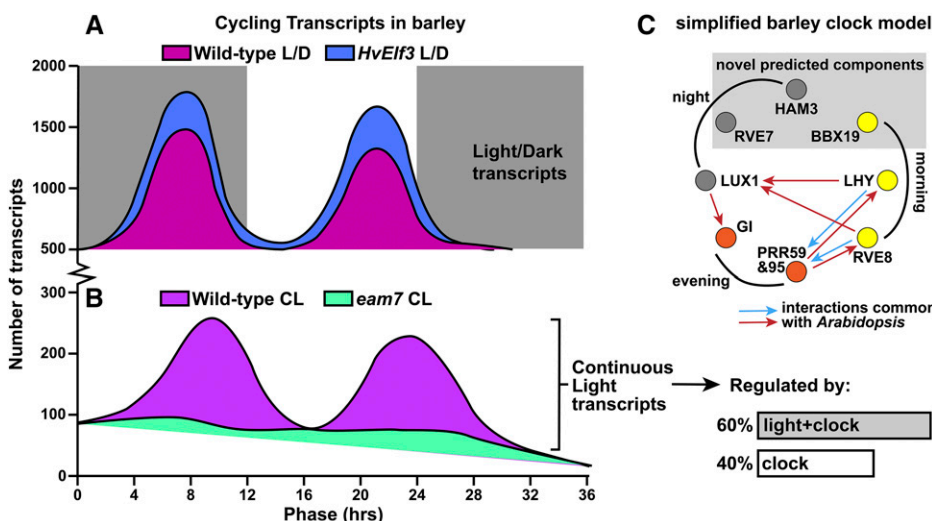
Circadian rhythms are ubiquitous among living things. A core set of so-called clock genes and their products assemble an oscillatory network that provides a rhythm to anticipate dawn and dusk (Hsu and Harmer, 2014). The circadian clock regulates the day/night rhythms of plants, and light itself has many ways of feeding information into the functioning of the clock (Oakenfull and Davis, 2017). If plants are transferred from normal day/night cycles into continuous light, these oscillations continue for a while, but the amplitude dampens out and the oscillations disappear after a few days.

Circadian rhythms affect almost all aspects of plant growth, from photosynthesis and flowering to responses to biotic and abiotic stress (Greenham and McClung, 2015). So perhaps not surprisingly, in crop plants domestication often selected for mutants in circadian genes. Clock gene mutations in various crop species alter flowering time and growth cycles (Bendix et al., 2015), and these alterations in many species help adapt plants native to equatorial regions to the long-day photoperiods and short growing seasons of more northern regions, such as in the case of tomato (*Solanum lycopersicum*; Müller et al., 2016) and barley (*Hordeum vulgare*; Faure et al., 2012; Campoli et al., 2013).

In this issue of *Plant Physiology*, Müller et al. (2020) show how transcriptomics, combined with the modeling of a gene regulatory network, can provide a flying start into unraveling the inner workings of the circadian clock in barley. On top of that, they are able to show the importance of light in driving circadian rhythms, compared with role of the clock gene network in doing so.

As a base data set, the authors performed an experiment to map cycling genes in day/night and continuous light conditions. RNA sequencing showed that, in wild-type cv Bowman, the expression of 84% of genes followed a rhythmic day/night cycle (Fig. 1A, wild type). During 36 h of continuous light, 20% of all gene expression still showed cycling behavior (Fig. 1B, wild type). Plants mutated in *HvELF3* and *HvLUX1* (orthologs of well-known clock genes from *Arabidopsis thaliana* *EARLY FLOWERING3* and *LUX ARRYTHMO*) did not exhibit any cycling in gene expression in continuous light. The cycling of these circadian regulated genes tends to follow a bimodal pattern: transcripts increase at the end of night and again at the end of day. The aforementioned 20% of genes that cycled in continuous light in cv Bowman showed this bimodal behavior. However, in *early maturity7* (*eam7*) mutants, this bimodal pattern was lost completely (Fig. 1B). Interestingly, in *Hvelf3* in normal day/night cycles, the amount of cycling transcripts had a higher peak at dawn and a higher peak at dusk compared with cv Bowman, showing a pronounced bimodal pattern (Fig. 1A). These data showed that the core clock genes in barley influence the bimodal phase of cycling genes and that the light/dark cues determine for the most part if genes cycle or not.

To be able to generate new hypotheses and novel clock genes from their RNA sequencing experiment, the authors created a computational model that showed the regulatory relationships of the barley clock without a priori knowledge of the network (Mombaerts et al., 2019). As an input for the model, they used genes that showed cycling behavior in continuous light and were



**Figure 1.** The phase of cycling transcripts in continuous light or light/dark cycles is disturbed by mutations in clock genes. A, Phase distribution of cycling transcripts from the wild type and *Hvelf3* in light/dark (L/D) cycles. B, Phase distribution of cycling transcripts from the wild type and *eam7* in continuous light (CL). Based on the model in C, the relative contribution of the clock and light to the cycling of transcripts in continuous light could be calculated. C, Simplified depiction of the barley core oscillator model. Based on Müller et al. (2020), figures 2A, 3B, and 4C.

common between cv Bowman and *eam7*. To avoid computational limitations, they filtered their gene set by searching for Arabidopsis orthologs that were classified by Gene Ontology as being circadian, with a high signal-to-noise ratio in the circadian cycle. In an ideal world, this network would have been centered around *HvLUX1* and *HvELF3*, since these genes are indispensable for continuous light cycling transcripts; however, only *HvLUX1* could be used, because the signal-to-noise ratio for *HvELF3* transcripts was too low.

By aligning the genetic and expression data from Arabidopsis with those of barley, they generated a model that predicts the degree of commonality to the Arabidopsis clock. Some components are missing, but the model nonetheless predicts a number of novel genes involved in oscillatory regulation. *HvBBX19*, *HvRVE7*, and *HvHAM3* were, as of yet, unknown as clock components; however, these genes can now be tested to investigate their function in governing the cycling rhythms of genes in barley (Fig. 1C).

With the help of the authors' models, it was also possible to investigate the proportion of transcripts cycling in continuous light that were regulated primarily by the circadian clock compared with the proportion regulated primarily by the light in the normal day/night cycle. It turns out that about 40% reveal a phase dominated by the circadian clock, and the remaining 60% of transcripts are under the control of light signaling pathways or coregulated by light signaling and clock (Fig. 1B). Basically, this means that the clock helps transcripts cycle but the light sets the time!

In conclusion, this work by Müller et al. (2020) gives plant scientists new tools to investigate circadian rhythms of barley. This is also important for plant breeding and agronomy, since many cultivars of other (grain) crops like barley have mutations in genes

considered as part of the core clock (Bendix et al., 2015). Therefore, knowledge of the clock can help adapt species to new growing environments.

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