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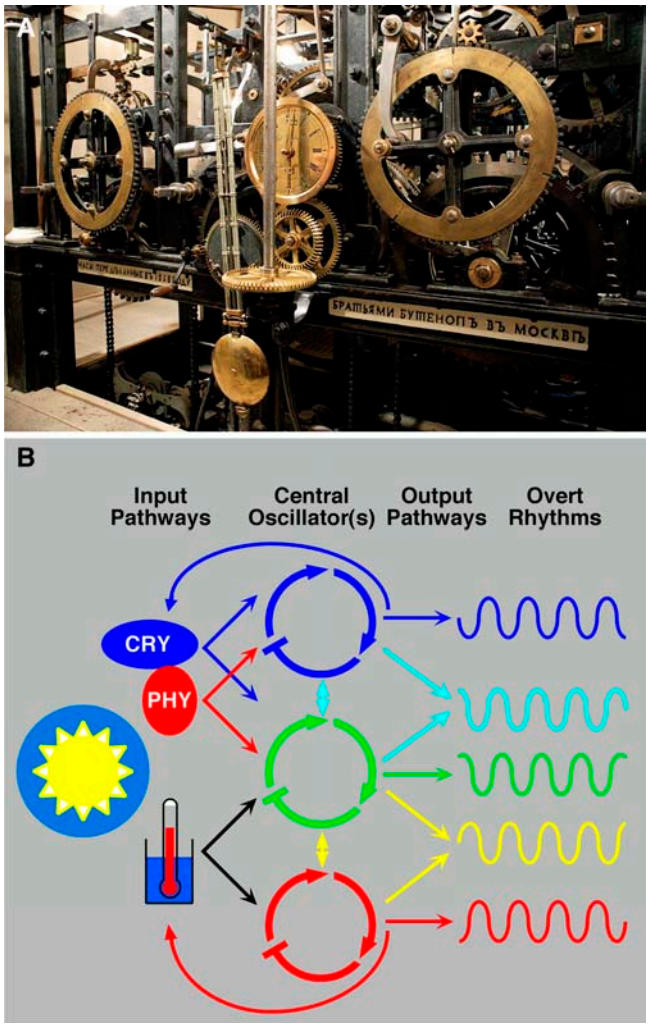
A Wheel within a Wheel: Temperature Compensation of the Circadian Clock

Circadian clocks influence numerous physiological and developmental functions in most if not all living organisms. Questions of how biological clocks operate have long intrigued biologists. How do circadian clocks keep accurate time in the midst of constantly changing internal and external conditions, such as seasonally varying light/dark cycles, large diurnal and seasonal temperature shifts, and a myriad of physiological and biochemical changes during development? Identification and analysis of core components of the clock in diverse organisms, including animals, plants, and fungi, have shown that the clock mechanism likely consists of interlocking negative and positive feedback loops. However, there is very little conservation among the core clock components between these groups, suggesting that numerous clocks have evolved independently and that circadian rhythmicity is an important adaptive feature of most organisms.

Defining characteristics of circadian rhythms are that they are free-running, maintaining a periodicity of ~24 h under constant external conditions; they can be entrained by external cues, principally light and temperature cycles; and they exhibit temperature compensation, maintaining relatively constant periodicity over a broad range of physiological temperatures. Most biochemical processes speed up as the temperature rises; this would wreak havoc with the timing of daily rhythms, and circadian clocks have evolved to avoid this problem. Circadian clocks are often modeled as consisting of three main components: input pathways, the central oscillator or core clock mechanism, and output pathways resulting in circadian rhythms. Although it has been useful to view circadian systems in this fashion, it has become clear that these are likely arbitrary groupings with substantial overlap and complex interconnections (McClung et al., 2002). Newer models of clocks may begin to resemble complex mechanical clocks with their multiple interconnecting wheels and wheels within wheels (see figure panel A). If we imagine additional inputs and outputs feeding in and out of each of these wheels, we may begin to approximate the workings of a biological clock (see figure panel B).



Clock Mechanisms.

(A) Internal mechanisms of St. Petersburg's cathedral tower clock. (©*Nikodim. Image from BigStockPhoto.com*.)

(B) Model of a simple circadian clock system consisting of a set of input (entrainment) pathways, multiple central oscillators, and sets of output pathways. (*From McClung et al. [2002], reproduced with author's permission*.)

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In *Arabidopsis*, the first clock proteins to be identified include the Myb domain transcription factors CIRCADIAN CLOCK ASSOCIATED1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) and the pseudoresponse regulator protein TIMING OF CAB EXPRESSION1 (TOC1). TOC1 promotes transcription of *CCA1* and *LHY* by an unknown mechanism, and CCA1 and LHY feedback to inhibit *TOC1* transcription by binding to the *TOC1* promoter (Alabadí et al., 2001). The core wheels of the clock supplement this loop with interlocked negative feedback loops that affect not only the transcription but also the stability and activity of these core proteins, resulting in circadian oscillations within the clock and affecting output pathways (McClung, 2006).

Investigations into temperature compensation of the circadian clock are beginning to uncover the nature of more of the wheels within wheels, or interlocking loops, that constitute the core clock mechanism and what controls the rate at which they turn. In this issue of *The Plant Cell*, **Gould et al. (pages 1177–1187)** show that a component of temperature compensation of the circadian clock in *Arabidopsis* is a function of balancing expression levels between *GIGANTEA* (*GI*) and the core clock components *LHY* and *CCA1*. In addition to providing insight into temperature compensation of the circadian clock, this work enhances our understanding of the multiple loops that surround the core clock mechanism and offers support for the idea that GI has a fundamental function in one of the core loops.

It was previously shown by Edwards et al. (2005) that there is considerable variation in temperature compensation parameters among *Arabidopsis* accessions, and *GI* was identified as a candidate for one of the principle quantitative trait loci (QTL) influencing temperature compensation by mapping of near-isogenic lines. GI is a novel nuclear-localized protein (Huq et al., 2000) that was originally characterized for its role in the induction of flowering by long daylength (Araki and Komeda, 1993; Koornneef et al., 1998) and has also been found to function in the inhibition of hypocotyl elongation by red light (Huq et al., 2000; Tseng et al., 2004) and in regulation of the circadian clock (Fowler et al., 1999; Park et al., 1999; Huq et al., 2000). Based on characterization of double loss-of-function *lhy cca1* alleles, Locke et al. (2005) suggested a mathematical model wherein GI functions in a second, interlocking feedback loop with *TOC1* that is required to maintain circadian amplitude and proper period length, in addition to mediating part of the light input to the clock.

Gould et al. investigated the role of *GI* in temperature compensation of the clock, first by analyzing the circadian rhythms of leaf movements in the wild type and a *gi* null mutant over a range of physiological temperatures. There was no difference in the period of leaf movement between the mutant and wild type at 17°C, but a significant period shortening was observed in the mutant relative to the wild type at both higher and lower temperatures, consistent with a role for *GI* in temperature compensation (i.e., extending the temperature range at which accurate leaf movement rhythms can be maintained). Similar observations were recorded in the response of transgenic wild type and *gi* mutant plants expressing a *CAB* promoter:luciferase reporter gene.

The authors next conducted gene expression analyses of *GI* and core clock components *TOC1*, *LHY*, and *CCA1* over a range of temperatures in the wild type and *gi* mutants and also simulated expression profiles for these genes under these conditions using the mathematical interlocking loop model described by Locke et al. (2005). The experimental data suggested that increasing temperature affects *GI* and *LHY* expression in an opposing and counterbalanced manner, and the simulations confirmed that this balance would contribute toward temperature compensation. As temperature increases, *LHY* expression decreases, and this is counterbalanced by increases in *GI* (and *TOC1*). The authors also uncovered distinct roles for *LHY* and *CCA1* in temperature compensation. *CCA1* was not found to play a significant role at higher temperatures, but at lower temperatures, it appeared to play a greater role than and largely substitute for *LHY* in counterbalancing *GI*.

The conclusions of Gould et al. are in agreement with the antagonistic balance model of temperature compensation of the circadian clock proposed by Ruoff (1992) and Ruoff and Rensing (1996) and the interlocking loop model of Locke et al. (2005). The antagonistic balance model is based on a much earlier idea described by Hastings and Sweeney (1957), who proposed that temperature change similarly affects two reactions that have opposing effects on the period of the oscillation, resulting in temperature compensation, a principle that is also observed in mechanical temperature-compensated pendulum clocks and in electronic crystal oscillators (Ruoff and Rensing, 2004). The interlocked loop model of Locke et al. (2005) predicts one loop wherein *GI* is part of an unknown component (called *Y*) that positively regulates *TOC1* expression and is itself negatively regulated by TOC1, interlocked (through *TOC1*) with the core clock loop wherein TOC1 positively regulates *LHY* and *CCA1*, the products of which feed back to negatively regulate *TOC1* expression. The work of Gould et al. provides general support for this model and further postulates a counterbalancing mechanism between *GI* and *LHY* at high temperature and *GI* and *CCA1* at lower temperatures that results in temperature compensation.

However, it is apparent that the model is incomplete, and the work of Gould et al. raises additional questions and suggests avenues for further investigation. It was shown that high temperature causes an increase in *GI* expression, which positively influences *TOC1* expression, and TOC1 positively influences *LHY/CCA1*, but high temperature also causes a decrease in *LHY* expression. Conversely, low temperature causes a decrease in *GI* and *TOC1* but an increase in *CCA1* and to a lesser extent *LHY*. Gould et al. suggest that *GI* exerts temperature-dependent regulation on *TOC1*, thereby maintaining the amplitude of CCA1 and LHY to sustain accurate and robust clock function, but this does not fully explain the temperature effects on CCA1 and LHY. How do temperature changes feed in to both of these loops to cause opposing effects, and how are the effects on corresponding gene activities balanced?

It is clear that there are other factors affecting temperature compensation and

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the core clock mechanism. For example, if the component *Y* of the interlocked loop model (Locke et al., 2005) consisted solely of *GI*, then *gi* mutants would always be arrhythmic, which does not fit with experimental observations. However, Gould et al. showed that *gi* mutants are arrhythmic under some conditions, suggesting that *GI* is a component of *Y*, but *Y* likely includes others factors in addition to *GI*. Other genes likely influence temperature compensation independently of putative *Y* function, such as the MADS box gene *FLC*, recently shown by Edwards et al. (2006) to influence temperature compensation of the clock at high temperatures, and the clock-related gene *ZEITLUPE* (*ZTL*), which Edwards et al. (2005) identified in addition to the *GI* locus as a candidate for another one of the principal QTL affecting temperature compensations.

One of the important missing wheels in models of the clock mechanism is what controls turnover of LHY and CCA1, and furthermore, how this might be linked to temperature compensation of the clock. Ruoff et al. (2005) have shown that factors affecting stability of the *Neurospora* core clock protein FRQ determine the period length as well as temperature compensation of the circadian clock. Certainly an important avenue for further study is the possible link between temperature compensation of the clock and function of *ZTL*, which encodes an F-box protein that targets TOC1 for proteasomal degradation, thereby playing a critical role in one of the principle loops of the clock (Más et al., 2003; Han et al., 2004). In addition, it contains a PAS/LOV domain similar to that of phototropins, suggesting that it may have a photoreceptor function, and KELCH repeats, which are implicated in protein–protein interactions. ZTL has been shown to interact with the PHYB and CRY1 photoreceptors in a yeast two-hybrid system (Jarillo et al., 2001; see also Kevei et al., 2006), so it may function in light entrainment of the clock. Since Edwards et al. (2005) identified *ZTL* and *GI* as candidates for one of the major temperature compensation QTL in *Arabidopsis*, it may be of interest to investigate whether ZTL interacts with GI and/or LHY/CCA1 independently of its effect on TOC1.

Another question is what is the nature of the functional difference between LHY and CCA1 with respect to temperature compensation? Previous data had suggested largely redundant function, and Locke et al. (2005) had lumped them together as a single component of their model—clearly their effects must now be teased apart. Gould et al. suggest that the switch in importance of LHY versus CCA1 at higher versus lower temperatures might reflect differences in temperature-dependent biochemical properties of the two proteins, but this remains to be tested.

Despite the many remaining questions, the work of Gould et al. provides solid support for the interlocked loop model of the circadian clock in higher plants and shows that antagonistic balance between interlocked loops containing *GI* and *LHY/CCA1* can account for a major component of temperature compensation of the clock in *Arabidopsis*. In addition, it provides evidence that *LHY* and *CCA1* play distinct roles in temperature compensation. It is likely that further investigation will reveal additional wheels within wheels that influence light and temperature signal inputs as well as stability and turnover of the core components.

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