Circadian biology: **Clocks for the real world**

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The circadian system of *Neurospora crassa* includes a molecular feedback loop that is entrainable by light. A recent study has shown that a second, elusive oscillator interacts with the feedback loop to drive output rhythms.

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In her best-selling book *Longitude*, Dava Sobel [1] describes John Harrison's invention of a clock that kept perfect time, regardless of the changes in climate inevitable on a long sea voyage. The accurate timing allowed eighteenth-century sailors to pinpoint their position on the globe for the first time. Clocks are vitally important to us all, not only those consciously-consulted devices that mark out our days but also the biological timers that synchronise our physiology with the external, day–night cycle.

Biological clocks may be ubiquitous across taxa, as the systems that have been defined so far in eukaryotes and prokaryotes that display rhythms share a number of important properties. Their period, τ, is approximately twenty-four hours under constant environmental conditions, which has led to their being known as 'circadian'. Although they are endogenous, circadian clocks are set, or 'entrained', by external signals such as light and temperature, which are collectively called 'Zeitgebers' (meaning time-givers); the problem of resetting can be appreciated by anyone who has suffered jet lag after changing time zones.

Like Harrison's nautical clocks, the circadian system is compensated against changes in the environment, keeping time equally well at both high and low temperatures and regardless of nutritional supplementation or metabolic activity. More recently, molecular studies on the circadian clocks of a variety of organisms have revealed a further common property: they include a molecular feedback loop through which a few, critical proteins regulate their own rhythmic synthesis [2]. The shared properties of circadian oscillators listed above serve to distinguish them from the mass of feedback regulation that is quite widespread in both signalling and metabolic pathways.

Figure 1

(a) Three-component model of the circadian system. **(b)** The frq–Frq–Wc feedback loop and its putative interactions with the Frq-less oscillator in the control of rhythmic conidiation in *Neurospora*.

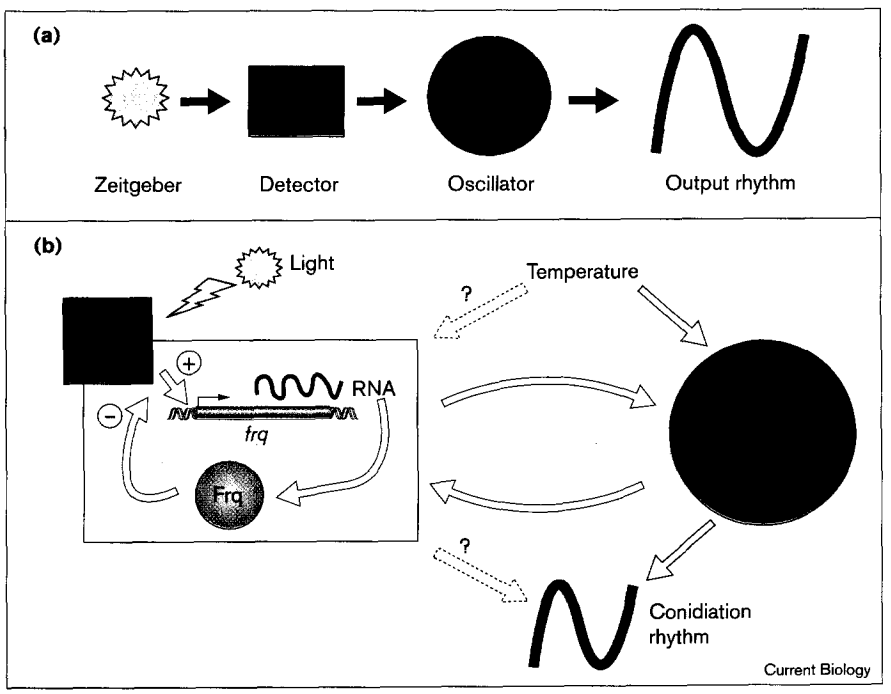
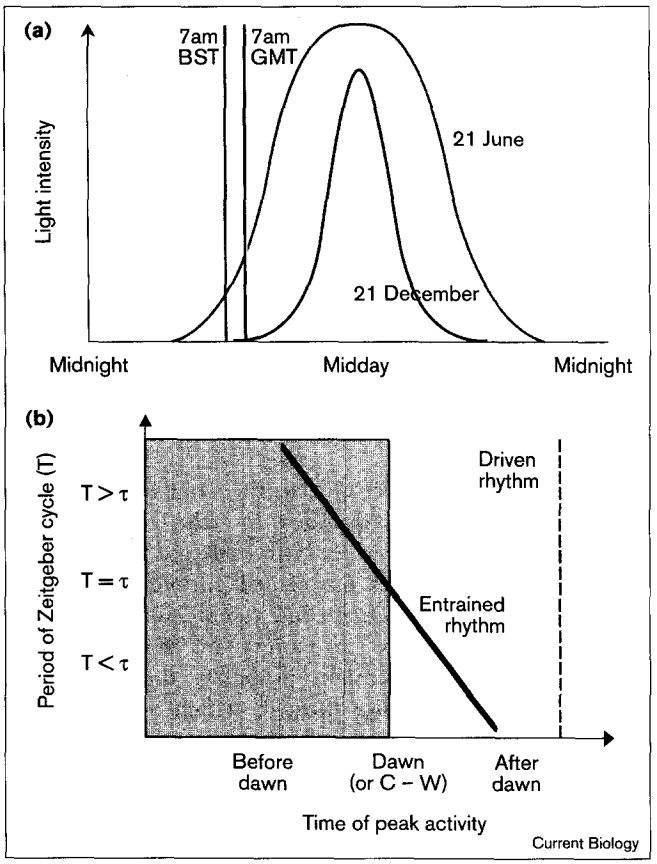


Figure 2



(a) Daylengths are longer in summer than in winter, yet people wake up at the same time throughout the year, experiencing phase disturbances only when clocks are put forward or back, giving one twenty-five or twenty-three hour day. BST, British summer time; GMT, Greenwich mean time. **(b)** Entrainment of a biological clock causes the phase relationship between an observed rhythm and the Zeitgeber to vary, depending on the period of the Zeitgeber (T) and of the clock (τ). In contrast, an activity that is driven by the light or temperature step occurs at a fixed time relative to dawn (or the cold–warm transition of a temperature cycle, C–W), independently of T. The shaded box indicates darkness or cold temperature.

A recent study by Merrow *et al.* [3] has added to our knowledge of circadian systems by demonstrating a means to assay a hitherto elusive additional oscillator in the fungus *Neurospora crassa*, and showing that this second oscillator interacts with a feedback loop long considered to be the heart of the circadian clock in this species.

Clock models

On a very simple level, the circadian clock can be visualised as a three-component system: detectors to provide input from the Zeitgebers, a central oscillator to generate a rhythm, and an output through which the rhythm is expressed (Figure 1a). This model, though accepted as oversimplified, has helped provide a successful experimental paradigm for the identification of mutants with abnormal rhythms and subsequent studies of the relevant genes.

One productive system for such experiments is *Neurospora*, which produces a band of spores (conidia) once every twenty-two hours. The conidiation rhythm persists in darkness, but can be entrained to light or temperature cycles. Many rhythm mutants in *Neurospora* have been mapped to the *frequency* (*frq*) gene, and the allele of *frq* present determines both the period of the conidiation rhythm and its temperature and nutritional compensation. The *frq* gene and its products are linked through the 'white collar' (Wc) proteins, which act as transcriptional activators of *frq* [4]. The Frq proteins inhibit *frq* activation, making a negative feedback loop that cycles once per circadian day, driving a rhythm in *frq* RNA levels [2] (Figure 1b).

The rapid activation of *frq* transcription by light, mediated by the Wc proteins, can account for the entraining effects of light signals on the *Neurospora* circadian clock. Null mutants of *frq* that produce no Frq protein are, at first sight at least, arhythmic under constant conditions. These and other results make the *frq*–Frq–Wc feedback loop look very much like a part of a circadian clock that controls the conidiation cycle.

Setting the clock

For a circadian clock to be of use it must, like our watches, run on local time. This occurs through entrainment to an environmental signal. The most obvious Zeitgeber is the cycle of day and night. Even an 'eyeless' rodent, the blind mole rat *Spalax ehrenbergi*, has retained diminutive, subcutaneous eyes, apparently for the sole purpose of detecting light for entrainment [5]. In a similar way, we use the light–dark cycle as a primary Zeitgeber, but in addition we can be driven voluntarily by our clocks and watches (see Figure 2a): daylight-saving time in the winter provides these timers with just one hour's adjustment to the changing photoperiod, twice each year.

Unlike our watch-driven behaviour, the mechanisms of entrainment and the dynamics of the circadian system ensure that the circadian clock continuously adapts to the changing proportions of light and dark during the year. And like any entrainable oscillator (circadian or not), the phase relationship between the observed rhythm and the entraining cycle varies systematically as the period length of the light–dark cycle changes (Figure 2b) [6]. Such patterns of 'daylight saving' adjustment are a hallmark of an entrainable clock.

There are, however, observations that indicate there is more to conidiation rhythms than light and *frq*. For example, conidiation becomes arhythmic in constant light, even at the level of moonlight. Furthermore, the *frq* null mutant first described by Loros and Feldman [7]

shows residual rhythmicity under certain conditions [7,8], demonstrating the presence of a Frq-less oscillator, which might also be known as the Feldman–Loros oscillator. This variable periodicity is observed after a latency of several days and is neither nutritionally nor temperature compensated [7,9]. In part because of their variability and intractability, such rhythms had been rather neglected until the recent series of experiments by Merrow *et al.* [3], which used temperature cycles as an alternative Zeitgeber to light.

Temperature

Merrow *et al.* [3] examined rhythmicity in *frq* mutants, using warm–cold temperature cycles of varying length as a Zeitgeber. Both experimental data and theoretical predictions show that, if an entrainable oscillator with a period of τ hours is entrained to an external rhythm of T hours, then the timing (phase) of the endogenous rhythm relative to the external rhythm depends on the difference between τ and T (see Figure 2b). This means that a change in the length (T) of the temperature cycle will cause an entrained conidiation rhythm to peak at a different time relative to the warm–cold transition. A response that is not entrained but driven directly by the Zeitgeber, however, will occur at a fixed phase regardless of T (see Figure 2b).

Merrow *et al.* [3] were able to show that the conidiation rhythm in a variety of *frq* mutants, including the null strain frq^9, could indeed be entrained by temperature, as was known to be the case for the intact circadian system [10]. The capacity of the frq^9 null mutant, in particular, to be entrained shows that *Neurospora* contains an entrainable oscillator that persists in the absence of Frq and can control conidiation, long considered a hand of the circadian clock. As the relationship between Zeitgeber period and conidiation phase behaved according to oscillator theory, and followed a similar trend in both Frq-containing and Frq-less strains, the Frq protein must make only a limited contribution to the mechanism of the Frq-less oscillator. Similar protocols using light–dark cycles, however, have indicated that *Neurospora* requires Frq for its response to light.

Multiple clocks

So where do these results leave the *frq*–Frq–Wc feedback loop? Despite these new findings, *frq* would still appear to be intimately involved in the *Neurospora* circadian clock, not least because it is *frq* which grants most of the properties that clockwatchers consider to be circadian essentials: a self-sustained period of around twenty-four hours, compensated for temperature and metabolic state, and responsive to light signals. All this would seem to make *frq* more than simply an input component. If the principle of Occam's razor is invoked, the *frq* and *wc* genes and their products still look and act like a circadian oscillator under the conditions used for the mutant identification and molecular studies.

Merrow *et al.* [3] have reminded us that the Frq-less oscillator exists in addition to the *frq*–Frq–Wc feedback loop, have made it more experimentally tractable and have revealed its influence on the intact circadian system. Their evidence documents feedback between the two oscillators (Figure 1b); changing the allele of *frq* changed the period length of the oscillatory system and thereby the phase of conidiation in temperature cycles of the same period. Feedback between the Frq-less oscillator and the Frq protein also determined the phase of *frq* RNA rhythms under temperature cycles.

This situation may be reminiscent of the 'A' and 'B' coupled oscillators that were proposed by Pittendrigh [6] to explain the responses to light and temperature of the *Drosophila* pupal eclosion rhythm. He suggested that this system is governed by two oscillators, the first of which, A, is entrainable by light and drives the second, B. The B oscillator generates the output rhythm and can, in addition, be entrained by temperature. If this model applies to the current *Neurospora* data, the A oscillator could represent the *frq*–Frq—Wc feedback loop, and the B oscillator the Frq-less oscillator.

And where next? The experimental tools provided by the new data should open up the Frq-less oscillator to molecular and genetic investigation. By re-interpreting formal models [6,11] in biochemical terms, the field of circadian biology is arriving at a new level of sophistication. Merrow *et al.* [3] have also shown the limitations of experiments that test only one of several potential Zeitgebers, whereas light and temperature are both important and often inseparable temporal cues in nature.

References

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