
RESEARCH NEWS**How does a fungus know the time of day?**

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The timing of sunrise and sunset has a remarkable influence on the lives of plants and animals. Diurnal animals, like most birds, wake up at sunrise and

begin their activities as if they know that an early bird gets the worm. So do most of the mammals including humans, except perhaps students in the hostel!

Conversely, nocturnal animals wake up at sunset and go to sleep at dawn¹. Nowhere can this be witnessed more spectacularly than in the caves of Madurai

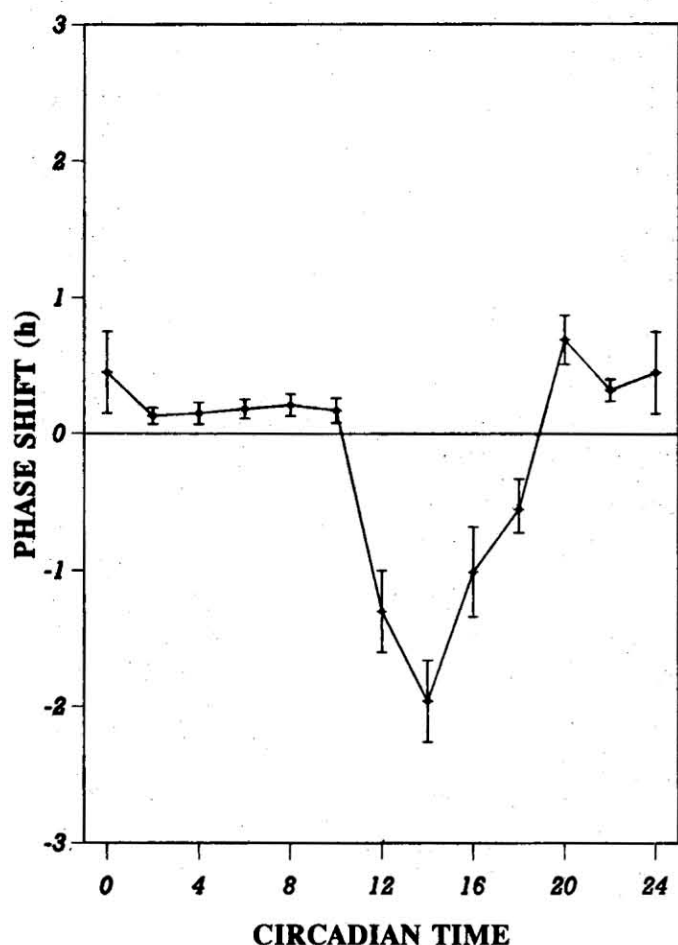


Figure 1. A Phase Response Curve (PRC) depicts the sensitivity of the clock to perturbations at various phases. Any point in a 24-hour cycle is a phase and is measured in circadian time (CT). The time at which a diurnal animal wakes up and a nocturnal animal goes to sleep (dawn) for example, is CT0. Every hour after CT0 is expressed as CT1, 2 and so on. An animal free-running in constant conditions (for e.g. DD) starts its activity every day at constant time intervals. Looking at the times of onset of activity for a few days, it is possible to predict the expected time of onset of subsequent days. If such a free-running organism is exposed to some perturbation (for example, a brief light pulse), the onset of activity can get advanced or delayed or remain unchanged depending on the time of perturbation. The time difference between the expected time of onset and the observed time of onset of activity is called 'phase shift'. A plot of the phase shifts against the circadian phase at which the perturbations were administered gives a PRC. Conventionally, the delay phase shifts are denoted as negative values and plotted below 0 and advance shifts are positive values plotted above 0. The mid point 0 is when no phase shifts occurred. Given here is the PRC for the field mouse *Mus booduga* constructed for fluorescent light pulses of 1000 lx intensity for 15 minutes duration⁶. In a PRC, the phases 0–12 are called the subjective day and phases 12–24 (0) are called subjective night. Note that maximum delay phase shifts occurred at CT 14 which is at the early subjective night and maximum advance phase shifts occurred at CT 20 which is at late subjective night. A light pulse in subjective day evoked minimum response. What is the significance of such phase shifts? *Mus booduga* is a nocturnal animal and it starts its activity at CT 12. A pulse at early subjective night evokes a delay response. It may probably be so because the animal 'thinks' that it has woken up early that day and that is the reason for 'seeing' light at that time of the day. So, it should delay its time of wake-up the next day, and hence is the resultant delay phase shift. Alternatively, a light pulse at the late subjective night evokes advance responses. In this case, the animal might think that it overslept on the day of the pulse and hence should advance its onset of activity the next day. At subjective day, the animal expects to see light, any way, and hence the minimum response. The PRC reveals the ecological advantage that circadian clocks confer on organisms. The clock is well tuned to escape predator pressure and increase survival value.

where one can see tens of thousands of bats emerge almost simultaneously at dusk, forming a cloud of bats that cannot be easily forgotten².

Such periodic behaviour is not restricted to sleep and wakefulness alone. Our body temperature, for example, shows a rhythmic rise and fall with the highest value at about noon and the lowest value when we are in deep sleep. Even plants obey the sun – their leaves droop during night and open at dawn³. Mice kept in cages exercise on their running wheels at night (they are nocturnal) and rest during the day. It turns out that this rhythmic behaviour does not merely mimic the movement of the sun. Living organisms have an internal clock that allows them to oscillate their activities with fixed periodicities. The most commonly observed periodicity is *circadian*, meaning 'about 24 hours'. When the same individuals are kept in continuous light (LL) or continuous darkness (DD) they still show a periodicity of about 24 hours. For example, the internal clock that drove the temperature cycle of one of us when denied information about the day–night cycle, showed a periodicity of 24 hours and 28 minutes^{4,5}. If continuously denied access to external cues the internal clock will gradually drift out of phase with the day–night cycle as small changes will add up every day. This state is called *free-run*. In nature, organisms do not free-run because the biological clock is fine-tuned every day in relation to the time of sunrise and sunset, much as we might reset our watches periodically. Such fine tuning is called *entrainment*. Biological clocks will also entrain to light–dark (LD) cycles, other than what is found in nature, if provided appropriate artificial LD cycles in the laboratory. For instance, when the field mouse *Mus booduga* was kept under conditions of 22 hours of light and only 2 hours of darkness, it remained active only for about 2 hours and 28 minutes and rested for the remaining 21 hours 32 minutes⁶! The adaptive significance of timing activities in relation to the day–night cycle is quite obvious – a diurnal animal would be quite lost and vulnerable at night as would a nocturnal animal during the day.

One of the lowest organisms to have a well-developed biological clock is the fungus *Neurospora crassa*, a favourite model system in molecular genetics. This fungus produces asexual spores called conidiospores and the release of

these spores is rhythmic and occurs once in about 24 hours. This can be observed by bands called conidial bands and the intervals at which these bands are produced mark the period of the rhythms. The conidation cycle entrains for example to LD 12:12 hours, so that banding occurs once in 24 hours. As expected, the conidation rhythm free runs with a periodicity of about (but not exactly) 24 hours when the cultures are grown under LL or DD. *Neurospora crassa* has become one of the genetically best studied organisms⁷, thanks to Beadle and Tatum who made it famous with their one-gene-one-enzyme hypothesis⁸. Little wonder then that *N. crassa* is now being used along with *Drosophila melanogaster*, to unravel the genetic basis of the biological clock^{9,10}.

Feldman and Hoyle¹¹ first isolated mutants of *N. crassa* which showed drastic alterations in period length. These mutants repeatedly mapped on one locus, the *frq*, which is therefore, clearly responsible for clock functions. This is reminiscent of the similar *per* locus of *D. melanogaster* which is known to be the canonical clock gene in that system. The *frq* gene has since been cloned and sequenced¹². The *frq* of *N. crassa* shows 44% identity and 23% conservative substitution with the *per* gene of *Drosophila*, suggesting the evolutionary relatedness of *frq* and *per* in spite of their being located in rather distantly-related organisms. As in the case of *Drosophila per* gene, the intracellular concentrations of *frq* mRNA as well as FRQ protein also vary rhythmically, with a periodicity mirroring the conidation rhythm, both in the wild type as well as in the short period, long period and arrhythmic mutants. Thus, the *frq* mRNA and protein levels are elevated during the day and depressed during night. These patterns appear to be brought about by the ability of the FRQ protein to negatively autoregulate *frq* gene effectively at night, but not during the day¹³. Cultures free-running with different periodicities can be brought to the same phase and made synchronous by inducing a burst of FRQ protein synthesis showing that FRQ protein is the 'state variable' of the clock of *Neurospora* – a state variable is a central clock component whose

rhythmic expression or activity, not mere presence in the cell, is essential for the normal operation of the clock¹⁴.

But establishing FRQ protein as the state variable of the clock is not enough. The more important question concerns how entrainment to the external 24 hour cycle occurs in nature. The Phase Response Curve (PRC) (Figure 1) is a convenient tool to study the mechanism of entrainment. Since a brief pulse of light instantaneously resets most biological clocks, Pittendrigh argued that light, when received by a photoreceptor, effects an instantaneous change in a state variable which triggers various changes leading to overt expression of rhythms^{15,16}. If *frq* encodes a state variable, Pittendrigh's model would predict that a light pulse should bring about an immediate increase in *frq* mRNA production. Crosthwaite *et al.*^{17,18} have now confirmed this prediction in a series of elegant experiments. This means that transcription of the *frq* gene is the first step in light-mediated resetting of the *Neurospora* circadian clock. Even as this is a major advance in our understanding of the molecular machinery constituting the biological clock, it opens up a new paradox. If the presence of light induces the transcription of the *frq* gene, how does *Neurospora* express a fire-running rhythm under LL? Measurement of levels of *frq* mRNA under LL showed that although there is probably an underlying low amplitude oscillation, there is a generally elevated level of *frq* mRNA under continuous light. If this answers one question it opens up another – whatever happened to the autoregulation of *frq* – why did it not bring down *frq* mRNA levels under LL? It turns out that continuous light abolishes the autoregulatory capacity which is however witnessed the moment cultures are transferred from light to dark. These experiments have thus demonstrated that rapid induction of *frq* mRNA is the first step in response to light and that this, with the aid of its autoregulatory property, allows the *Neurospora* clock to entrain to external LD cycles as well as to appropriately reset in response to a light pulse given at any phase of the cycle. The free-running of the endogenous clock under LL or DD, however, must depend on intra-cellular cues other than rapid in

duction of *frq* mRNA and its subsequent autoregulation. This latter aspect remains poorly understood. Nevertheless, it is fair to say that the mystery surrounding the circadian clock has begun to melt under the power of molecular technology!

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