The circadian body clock: a piece of biology crying out for formal analysis?

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1 Circadian rhythms

Circadian (*circa-* approximately *-dies* one day) rhythms are those daily biological cycles that persist when an individual is held in experimental isolation, deprived of time cues. Our most obvious circadian rhythm is the daily cycle of sleep and wakefulness, although this is accompanied by equally pronounced changes in most critical physiological and metabolic processes (e.g. heart rate and blood pressure, hormone secretion, liver and kidney function). Circadian rhythms are therefore a central property of human life, setting the tempo for wider society. Disruption of circadian co-ordiantion arising from jet-lag, rotational shift-work and neurodegenerative disease imposes a heavy burden for cardiovascular, metabolic and mental health (Hastings et al., 2003).

2 Circadian clocks - biological oscillators

Circadian rhythms are not simply a human phenomenon, however, and can be observed in all major taxa, from cyanobacteria upwards (Edgar et al., 2012). The persistence of such rhythms in isolation reveals the operation of underlying biological timers or circadian clocks. Under normal conditions these internal clocks are synchronised by environmental cues, most notably the cycle of light and darkness, so that internal biological time becomes predictive of solar time. This is the principal evolutionary adaptive value of circadian clocks because they allow organisms to anticipate, and therefore prepare for, the opportunities and challenges of day and night. By mapping the ability of individual pulses of light (a proxy for dawn and dusk) to phase-shift overt biological rhythms an exhaustive library of phase response curves has been constructed (Johnson, 1992). This has confirmed that across all biological groups tested, circadian clocks are pivoted around a true oscillator, most frequently modelled as a limit-cycle oscillator (Winfree, 1970).

3 The brain's circadian clock: the SCN

The principal circadian clock in mammals is a cluster of ca. 10,000 nerve cells deep in the brain sitting at the base of a structure called the hypothalamus and connected to the optic nerve (the source of entraining photic cues). The suprachiasmatic nucleus (SCN) is a remarkable structure, with a capacity to define ca. 24 hour cycles even when isolated in a culture dish. Being nervous tissue, the most accessible intrinsic circadian rhythm is the daily cycle of spontaneous electrical activity recorded in culture - SCN neurons are electrically active during circadian (i.e. projected) daytime, action potentials being fired at ca. 10 Hz, whilst firing rate falls to below 1 Hz in projected night (Atkinson et al., 2011). It is presumed that this intrinsic rhythm of electrical activity is the means by which the SCN can signal circadian time to the rest of the brain and thus the organism.

4 Circadian clocks genes and a delayed negative feedback loop

The past decade has witnessed a revolution in our understanding of the biological mechanisms of circadian timekeeping, with the identification of circadian "clock genes", first in fruit flies and fungus, and soon afterwards in mice and humans. Extensive genetic and biochemical analysis has led to the development of a canonical model for circadian pacemaking with a common principle of delayed negative feedback across all biological groups. In mammals, the feedback loop is pivoted around *Period* and *Cryptochrome* genes, which are activated at the start of circadian daytime, leading to the progressive accumulation of Period (Per) and Cryptochrome (Cry) proteins in SCN neurons (Welsh et al., 2010). These protein associate into complexes that suppress the activation of their cognate genes. With gene expression thereby silenced at the beginning of circadian night time, the existing Per/Cry complexes are degraded by dedicated cellular pathways and no longer replenished. By the end of circadian night, therefore, negative regulation is relieved and the process is due to start again with a new circadian dawn defined by renewed gene expression.

5 Imaging the ticking clockwork

In order to monitor these molecular events in the clock, we make extensive use of SCN tissue from genetically modified mice in which it is possible to monitor the activation of *Period* and *Cryptochrome* genes by means of enzyme luciferase, the enzyme employed by fireflies to catalyse luciferin and thereby emit bioluminescence. Light emission from SCN tissue in a sealed culture dish can be recorded for several weeks by photomultiplier tubes or by CCD camera (Hastings et al., 2005). As the feedback loop progresses through day and night phases, then so the activation of *Per* and *Cry* genes increase and decrease, leading to sinusoidal rhythm of bioluminescence. In contrast to the sloppiness of most biochemical measure, these molecular oscillations are beautifully stable and precise. The period and waveform of the bioluminescence rhythms are sensitive to changes in the stability of the Per and Cry proteins - genetic or pharmacological manipulations can generate SCN with periods ranging between 20 and 30 hours (Maywood et al., 2007, Meng et al., 2010).

6 Cellular synchrony across the SCN circuit

CCD imaging of bioluminescence rhythms from SCN tissue reveals the molecular cycle within the individual neurons that together form a neural circuit within the SCN. Gene activation of individual neurons is synchronised across the circuit but progresses as a wave from neuron to neuron in a stereotypical direction and speed (Maywood et al., 2006). The mechanism and meaning of this wave are unclear, although in genetically modified SCN lacking a particular neurotransmitter, the wave and synchrony break down and individual SCN neurons continue to oscillate but with reduced overall amplitude and with no phase coherence. The circadian behaviour of mice carrying this mutation is poorly defined and their daily physiology disorganised.

7 Conclusion

As a non-mathematician, I am acutely aware of the enormous potential for formal analysis of the circadian system, in particular the properties of the SCN as a molecular pacemaker. At the level of the delayed negative feedback loop that constitutes the core oscillation, the almost crystalline quality of the data from bioluminescence recordings should allow us to unravel the competing dynamics of gene activation and protein degradation. At the level of the SCN tissue,

the wave of gene expression has yet to be formally described, analysed and understood. Finally, the question of how cell-to-cell signalling augments the cell-autonomous timing properties of SCN neurons remains unanswered.

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