Metaclocks

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We live in a '24-hour' culture in which transatlantic air travel and shift-work are part of normal life for many people. These types of desynchronization—being awake when the body expects to be asleep, as with jet-lag—disrupt our daily physiological cycles and are increasingly being linked to diseases such as diabetes, obesity and cancer. Daily rhythms are also impaired in the elderly, as well as in patients with common neurodegenerative conditions such as Alzheimer disease and fronto-temporal dementia. As a result, so-called circadian-clock disruption is widespread in modern societies (Reddy *et al*, 2010).

Circadian clocks have been studied in many experimental organisms, as well as humans. In each case, molecular models have been developed that converge on a common mechanistic logic: transcriptional-translational feedback loops. Such models exhibit varying degrees of complexity, but the central idea is that timed expression of clock genes facilitates delayed negative feedback, whereby the encoded clock proteins eventually repress their own cognate promoter sequences. As these proteins are subsequently degraded, the cycle begins again. This process takes approximately 24 hours, hence the term circadian (in Latin: *circa*, about; *diem*, day).

Although these models have been able to account successfully for a large body of experimental evidence, there have been several inconsistencies, suggesting gaps in our knowledge. This has led us and others to hypothesize that, although transcriptional regulation is clearly relevant to the temporal coordination of organismal physiology, and life in general, the actual timekeeping mechanism might be biochemical in nature (Merrow & Roenneberg, 2001; O'Neill & Reddy, 2011; Roenneberg & Merrow, 1999).

Testing this hypothesis using traditional approaches has proven difficult, because these methods generally rely on transcriptional reports of the clock. Moreover, drugs that inhibit gene expression for more than 24 hours tend to be highly cytotoxic, so using a pharmacological approach has proven impossible. To overcome these obstacles, we first had to identify a post-translational biomarker for cellular rhythms—that is, one that did not rely on transcription. It transpired that we had already done much of the hard work in a proteomics screen we performed on mouse liver, which identified the oxidation of peroxiredoxin (PRX) proteins as a potential marker of the clockwork (Reddy *et al*, 2006). Peroxiredoxin proteins are highly conserved anti-oxidant proteins that scavenge cellular reactive oxygen species (ROS), most notably hydrogen peroxide (Woo *et al*, 2003).

We optimized a new platform for assaying cellular rhythms in the absence of transcription using erythrocytes (red blood cells). Mature human erythrocytes naturally have no nucleus or other organelles, and therefore no DNA. They are readily purified and express PRX proteins at high levels—approximately 0.5% of total cellular protein—as a defence against ROS generated by haemoglobin auto-oxidation. We took purified erythrocytes and cultured them at constant temperature (37 °C), sampling every 4 hours for up to three days. Western blot analyses of the time-courses revealed clear circadian rhythms in PRX oxidation. Furthermore, these rhythms could be entrained by temperature cycles, and were temperature-compensated (displaying approximately the same period at 32 °C and 37 °C). As such, the oscillations in red blood cells met the classic criteria for circadian rhythms.

We then extended our observations to include erythrocyte redox status and ATP levels, which also seem to show some circadian regulation. Thus, we showed that in the absence of transcription—on which all previous models of the clockwork in higher organisms are based—circadian rhythms in basic biochemical reactions are still observed, signifying the presence of an endogenous clock within the cells (O'Neill & Reddy, 2011).

Having established that human cells can sustain circadian rhythms in the absence of gene expression, we investigated the cross-talk between the new, non-transcriptional oscillations and the transcriptional clock mechanisms previously identified in nucleated cells. To do this, we examined rhythms in cells from circadian-mutant (cryptochrome-deficient) mice. Although cells from these mutants were thought to be arrhythmic in conventional clock-gene assays, we still observed PRX rhythms. They were, however, not completely normal, implying that although purely biochemical mechanisms are able to sustain 24-hour rhythms, they must normally reciprocally interact with gene-expression cycles.

These findings were echoed in a parallel study of PRX oscillations in the alga *Ostreococcus tauri*, thus establishing identical 24-hour oscillations in an organism separated from humans by 1,000 million years of evolution (O'Neill *et al*, 2011).

This work suggests a new paradigm for understanding cellular timekeeping and the way in which the cellular clock might keep time using the rhythms of metabolism (a 'metaclock'). Perhaps the most surprising outcome is that PRX oscillations are indicative of an evolutionarily ancient biochemical timekeeping mechanism that is conserved in disparate eukaryotes. Indeed, seeing just how far back this conservation goes is an active and exciting challenge for clock biologists.

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