

Cellular clocks: circadian rhythms in primary human fibroblasts

Almost 300 years ago, a French astronomer made the observation that daily leaf movement continues even when a plant is kept in constant darkness (De Mairan 1729). These so-called circadian rhythms exist at all levels of biology, ranging from gene expression to complex behaviours. They are controlled by a cellular clock that has been observed in organisms from all phyla. The underlying molecular mechanism has been described using genetic strategies that identified a set of clock genes that function in a transcriptional regulatory loop (Young and Kay 2001). Mutations in any of these genes can cause disruption in some facet of circadian timing, and micro-array studies suggest that – in complex organisms – most cells are capable of generating circadian oscillations (Panda *et al* 2002). Thus, circadian clocks are cell-based.

Circadian clocks are entrained to exactly 24 h in nature, where organisms use various cues from the environment (zeitgebers) that cycle reliably and thus precisely represent the rotation of the Earth. The best understood and perhaps the strongest zeitgeber is light, which changes systematically in intensity and in spectral quality over the course of each day, in addition to the changing ratio of light and darkness over the course of the year. Similar to physical oscillators, biological clocks will entrain differently depending on their period and amplitude. A relationship between external (entraining) and internal (circadian) period has been noted in animals (Hoffmann 1963; Pittendrigh and Daan 1976) and – at least in young adults – in humans (Duffy and Czeisler 2002). In general, a long free running circadian period entrains late in the day relative to a shorter one. Spore formation in fungal clock mutants with a short period occurs earlier in the night compared to wild type strains, and hamsters are active earlier or later in the 24 h light-dark cycle according to their free running period (Pittendrigh and Daan 1976; Merrow *et al* 1999). Importantly, the palette of clock-regulated physiologies also reflects the phase of entrainment. The implications of physiological chronotype are substantial, ranging from optimizing medical treatment to quality of life and shift work. To understand chronotype we need to first understand free running rhythms, and then what happens when they are entrained.

Obviously, in humans, circadian rhythms are typically observed in the entrained state. Pioneering experiments did study free running rhythms in humans, showing an approximate 25 h period in many parameters, including sleep/wake cycles and core body temperature. The complexity of a circadian system was apparent in this early work when activity and temperature rhythms dissociated, resulting in two free-running rhythms in a single individual (Aschoff 1965). More recently, human rhythms are studied in forced desynchrony protocols, that estimate the period to be closer to 24 h (Czeisler *et al* 1999). What is apparent to chronobiology researchers is the cumbersome nature of this sort of human experimentation, requiring sequestration of subjects for days, weeks or months in temporal isolation. This is at the least expensive and it can also be psychologically challenging for subjects. Thus, more efficient tools to study human rhythms are overdue.

A recent publication in *PLoS* describes such a tool, namely a luminescent reporter that is used to follow cellular oscillations (Brown *et al* 2005). The authors built on their previous work showing that fibroblast cell lines in tissue culture could be synchronized such that they display coordinated circadian regulation of gene expression (Balsalobre *et al* 1998). Here (Brown *et al* 2005), primary human cells were transformed with a lentiviral vector to insert a fusion of the clock gene *BMAL1* promoter and the coding region of firefly luciferase. The recipient cells were derived either from skin biopsies (fibroblasts), hair roots (keratinocytes) or peripheral blood (monocytes).

In all cases, circa-24 h oscillations in BMAL1-driven luminescence were observed from the cultures, but the fibroblast system is – at least at this stage – the most robust. The authors compared different fibroblasts cultures from the same individual and from different individuals, showing that intra-individual differences are smaller than inter-individual differences. Thus, the individual cells appear to represent the characteristics of the donor's clock.

The critical question is: can this tool replace some aspect of the temporal isolation experiments on humans? All indications are that this will indeed be the case. Importantly, a distribution of cellular free running periods was observed amongst fibroblasts tested from 19 individuals. The range was, however, larger than would be expected according to the before mentioned isolation experiments, with periods running from 23 h to over 26 h. To put this into perspective, the authors investigated fibroblast rhythms in mice and compared them to behavioural rhythms. Interestingly, while in all cases a correlation between cellular and behavioural periods was seen, the cell phenotype was more extreme. For instance, a mutant mouse with a short, 23.4 h period in running wheel activity showed a very short 20 h oscillation in gene expression. This is not entirely surprising because it is apparent that the circadian rhythm of a tissue is more robust and consolidated than at the level of its dissociated cells (Welsh *et al* 1995; Vansteensel *et al* 2003).

The circadian system is a collection of oscillators – within the body, within organs and tissues, and even perhaps at the molecular level within individual cells. The tools described by Brown *et al* (2005) open up exciting new possibilities for investigations into the molecular biology and genetics of clocks at the level of the cell as well as the system. Any number of other clock gene- or clock-regulated promoters can be employed to explore molecular clock regulation. Non-invasive sources of cells will surely be developed. Cells from different tissues can be investigated, to determine developmental and epigenetic effects on the clock genotype or even one day to use the circadian biology as a tool for detecting pathologies at the tissue level. For basic research on human chronobiology, the new tools will significantly accelerate our understanding of how clock genotype and cell-based rhythms shape the chronotype distribution in human behaviour.

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