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Molecular Clocks and the Human Condition:

Approaching their characterization in human physiology and disease

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Abstract

Molecular clockworks knit together diverse biological networks and compelling evidence from model systems infers their importance in metabolism, immunological and cardiovascular function. Despite this and the diurnal variation in many aspects of human physiology and the phenotypic expression of disease, our understanding of the role and importance of clock function and dysfunction in humans is modest. There are tantalizing hints of connection across the translational divide and some correlative evidence of gene variation and human disease but most of what we know derives from forced desynchrony protocols in controlled environments. We now have the ability to monitor quantitatively ex vivo or in vivo the genome, metabolome, proteome and microbiome of humans in the wild. Combining this capability, with the power of mobile telephony and the evolution of remote sensing affords a new opportunity for deep phenotyping, including the characterization of diurnal behavior and the assessment of the impact of the clock on approved drug function.

Keywords

Aging; Human physiology; Molecular clocks; Remote sensing

Introduction

Molecular clocks play a fundamental role in integrating tissue function. The complex regulatory features of the network, its high degree of redundancy and conservation all speak to its likely importance; studies in rodents suggest that up to half the transcriptome is subject to circadian oscillation [1,2]. Our understanding of clockworks in the molecular era began in plants, moved to flies, zebrafish and mice and is now ripe for elucidation in the model system of fundamental relevance, the human. There has long been interest in and some suggestion of a role for clockworks in the human condition. Many physiological variables, such as temperature and blood pressure, together with levels of many hormones, oscillate in a diurnal fashion. Furthermore, we have some evidence consistent with a role for clock dysfunction in human disability and disease. For example, hormonal rhythms lose time and

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CONFLICT OF INTEREST

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amplitude as they finally taper off with increasing age. Oscillatory function is dampened in patients with neurodegenerative disease. Clinical phenotypes of many human diseases, including myocardial infarction and stroke, asthmatic exacerbations, depression and attempted suicides oscillate in their incidence as a function of time of day. Intriguingly, many of our physiological habits – what and how much we eat and drink, when we choose to exercise, our ability to consolidate and retrieve complex memories also vary with time of day, at the extremes resulting in so called night owls or morning larks. All of these connections are, however, mere suggestions, rather than established manifestations of our human clocks. Still, in the blink of an evolutionary eye, our lifestyles have changed in ways that might be expected to disrupt our circadian rhythmicity. From a time of clear distinction of light from darkness, striking thermal contrasts and a need to eat, swiftly, what we had killed, we have electric light, air conditioning and the continuous drip feed of fast food and the distraction by our personalized electronics, available to us at all times of the day. We flit around the globe and up to a third of us indulge in shift work. Given the integrative role of clock function in model systems, a breakdown would be expected to be manifest in metabolic and immunological dysfunction [1] and indeed we see an increased incidence of such disorders in shift workers [3] and speculate how disordered clock function may relate to the increasing incidence of the metabolic syndrome in the developed world.

Translational hints of linkage

While there are scant data implicating directly the clock in human physiology and disease – the association of a *Per-2* variant (which renders it susceptible to phosphorylation and accelerated degradation) with a human sleep disorder and its recapitulation with knock in of the variant in the mouse being the most persuasive [4] – there are some hints across the translational divide. Disruption of the endothelial clock undermines the diurnal variability in susceptibility to thrombogenesis in mice [5]. Forced desynchrony protocols – in which subjects are asked to sleep on short or long cycles to which the circadian system cannot entrain - have been used in humans to segregate circadian from diurnal rhythms. Such studies suggest that oscillation in the prothrombotic plasminogen activator -1 is under circadian control in humans [6]. Disruption of the clock in mice predisposes to accelerated aging [7] and indeed more specifically, deletion of forward limb components, such as *Bmal-1*, or *Npas-2* together with *Clock*, predisposes to neurodegeneration, gliosis, loss of inter-regional connectivity and increased oxidative stress in the brain of mice [8]. Ambulant 24 hour rhythms of glucose, as do many endocrine rhythms as mentioned above, mark chronological age in humans. More provocatively, comparisons amongst middle aged, apparently healthy individuals also segregate glucose rhythms in those predisposed to longevity (more than two first order nonagenarian relatives) from the rest of us [9]. We have found recently that postnatal deletion of *Bmal-1*, a core clock component attenuates some, but not all features of the accelerated aging phenotype observed in the conventional knock outs. Strikingly, lifespan, dramatically shortened when *Bmal-1* is absent during development, is normal when deletion occurs post-natally. We do not know how functional is the clock during development; this may be an off target effect of *Bmal-1*. However, it does remind us of the Barker hypothesis which first proposed the relevance of prenatal experience to lifespan and the development of diseases of adulthood, specifically the

metabolic syndrome [10]. Epidemiological data have strengthened belief in the Barker hypothesis and this has largely been attributed to epigenetic perturbation by environmental exposures of the mother during pregnancy. A provocative consideration is that while this may be so, the anticipatory role of the clock might condition the impact of such insults, attenuating them at certain times of day. Integration of clock function and environmental influences is nicely illustrated in the mouse. Thus, disruption of clock genes variably influence the diurnal oscillation of blood pressure. However, while the blood pressure response to a stress paradigm is conditioned by clock time, as might be expected, disabling the clock by deletion of *Bmal-1* abrogates the stress induced response, irrespective of clock time [11]. The influence of the clock on physiological responses is likely to be as a modulator of environmental influences increasingly as we ascend the evolutionary tree, rather than a direct determinant of life and death.

Clock disruption influences the inflammatory response in mice. Lethality in response to an injection of bacterial lipopolysaccharide (LPS) varies with clock time of administration in mice and this diurnal variability is abolished by deletion of *Bmal-1* in myeloid cells [12]. The inflammatory response to LPS injection also varies as a function of clock time in humans [13], suggesting the potential relevance to clock dysfunction to the development of inflammatory diseases, such as atherosclerosis. Studies in mice suggest that this might be an interesting biology to pursue. For example, atherogenesis is accelerated by global deletion of *Bmal-1* or mutation of *Clock* to undermine its partnership with *Bmal-1*, consistent with the anti-inflammatory action, at least of *Bmal-1*. However, we have found that myeloid cell deletion of *Bmal-1* restrains atherogenesis, suggesting that in this lineage it may act to promote inflammation.

Finally, we found more than a decade ago that the discrete gene cassettes that oscillated in diurnal fashion in aorta (and perivascular fat) recapitulated the metabolic syndrome – genes relevant to vascular integrity, cholesterol and glucose metabolism and adipocyte maturation [14] – and that disruption of the clock in various ways predisposed to fasting hypoglycemia [15]. Since then, this phenotype has been recapitulated by gene deletion in liver [16] while its deletion in pancreas, skeletal muscle and the adipocyte predisposes to diabetes, increased oxidative capacity and obesity respectively [17–19]. Perhaps the aspect of metabolic function that has attracted most attention to the clock recently has been the demonstration that in mice and flies, time restricted feeding (TRF) has proven protective against diet induced obesity and diabetes and against aging dependent decline in cardiac function respectively [20,21]. Despite the absence of any actual human data, these observations have been recounted in the popular press and provoked much interest in a similar strategy for overweight humans. It remains to be seen, in properly controlled experiments, whether it will work and more importantly whether TRF can be sustained by humans, given that in mice there appears to be no “legacy effect” when they depart from the regime.

Chrono-pharmacology and the human condition

Although it has been known, at least since the 1970s, that time of administration may influence aspects of drug action, this has had minimal influence on clinical practice. Recently, it has been noted that the targets of roughly 2/3 of the top 100 selling drugs

oscillate in a diurnal fashion and that most of these drugs have half-lives of 6 hours or less. Irrespective of target oscillation [2], it is known that drug metabolizing enzymes and transporters are high amplitude oscillators in the liver and that pharmacokinetics is subject to variability as a function of time of drug dosing [22]. What is lacking are any data integrating time of drug administration, target oscillation, drug exposure and drug disposition. A nice place to consider this might be in hypertension, given its potential relevance to the phenomenon of nocturnal hypertension or “non-dipping” which complicates high blood pressure in roughly 40% of cases. Indeed, Rudic and colleagues have modeled this condition by infusing angiotensin II into Per-2 knock out mice. Interestingly, controlled, but not rapid release melatonin administered in the morning to patients attenuates nocturnal hypertension in humans [23].

An example of the functional relevance of target oscillation attained in mice has been that of mTOR [24]. Here the survival rate of mice with xenograft tumors is conditioned by the time of administration of everolimus [25]. Similarly, we have found that while administration of indomethacin influences the microbiome, there is a reciprocal impact on indomethacin disposition, gut bacteria mediating its deglucuronidation. Finally, Hermedia and colleagues have reported a time dependent hypotensive effect of low dose aspirin in a variety of randomized controlled trials in different hypertensive patient populations [26]. Although this has not been observed by some others [27] we have recently recapitulated this phenomenon in mice and demonstrated its dependence on oscillatory expression of mir-22 which negatively regulates prostaglandin G/H synthase -1 (*PTGHS-1*), colloquially known as cyclooxygenase -1, the target of low dose aspirin, in the kidney.

The emergence of novel compounds from chemical screens that disrupt the clock in various ways offers a tempting opportunity for preclinical characterization. However, given the history of targeting nuclear receptors and the integrative role of the clock in physiological function, one wonders how safely it might be tampered with directly to attain clinical benefit. Most of the preclinical science to make such decisions with respect to drug development remains to be done.

Because we can

The development of remote sensing technologies, exemplified by those based on smart phones, makes this a particularly appealing time to perform detailed human phenotyping relevant to the clock. We can access oscillatory gene expression in circulating cells and hair follicles *ex vivo* [28], the oscillatory metabolome in exhaled breath, urine and plasma [29, 30], the oscillatory microbiome which, at least in mice, is subject to clock control [31] and phone based apps can record such variables as our communications, exercise, sleep, mood, pain, dietary content and frequency, grazing radius, temperature, blood glucose and cytokines.

To date, the most careful studies have been performed under very controlled conditions, exemplified by those using forced desynchrony protocols that permit segregation of circadian and diurnal variability. In this regard, a particularly provocative finding related to blood pressure where the circadian and diurnal rhythms peak in opposite phases. The

biological implications of this finding remain to be elucidated. Along with such studies we need to collect data “in the field” to decide whether we can discriminate signals from noise and then define the factors that contribute to variability between individuals. Provocatively, the “ineffectiveness” of social behaviors [32], such as obesity and smoking habit can also be recorded. Encouragingly, we do have some positive controls – factors like temperature, salivary melatonin and indirect readouts like blood pressure – against which to interpret our findings with other quantitative outputs. For now, gathering robust human physiological data is a necessary baseline on which we can then design studies to probe more directly circadian function in “free running” humans. Then we can begin to determine how the clock influences our response to environmental perturbations, contributes to variability in drug response and as it unwinds, may influence our propensity to aging and disease.

“The only reason we have time is that everything doesn’t happen at once”.

Albert Einstein.

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CALLOUTS

- Studies in mice infer the importance of clockworks in human physiology and disease
- Presently our understanding of clockworks in humans derives largely from forced desynchrony protocols
- There are some correlative data suggesting linkage across the translational divide
- The development of quantitative omics and remote sensing technologies afford the opportunity to deeply phenotype diurnal oscillation of human biology, a prerequisite to understanding the relevance of clocks to parsing drug action.