Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

Circadian clocks regulate cardiac arrhythmia susceptibility, repolarization, and ion channels

Brian P Delisle[a], John L Stumpf[a], Jennifer L Wayland[a], Sidney R Johnson[a], Makoto Ono[a], Dalton Hall[a], Don E Burgess[a,b] and Elizabeth A Schroder[a,c]

Daily changes in the incidence of sudden cardiac death (SCD) reveal an interaction between environmental rhythms and internal circadian rhythms. Circadian rhythms are physiological rhythms that alter physiology to anticipate daily changes in the environment. They reflect coordinated activity of cellular circadian clocks that exist throughout the body. This review provides an overview of the state of the field by summarizing the results of several different transgenic mouse models that disrupt the function of circadian clocks throughout the body, in cardiomyocytes, or in adult cardiomyocytes. These studies identify important roles for circadian clocks in regulating heart rate, ventricular repolarization, arrhythmogenesis, and the functional expression of cardiac ion channels. They highlight a new dimension in the regulation of cardiac excitability and represent initial forays into understanding the complexities of how time impacts the functional regulation of ion channels, cardiac excitability, and time of day changes in the incidence of SCD.

Addresses
[a] Department of Physiology, University of Kentucky, 800 Rose Street, MS508, Lexington, KY 40536-0298, United States

[b] Department of Science and Health, Asbury University, One Macklem Drive, Wilmore, KY 40390, United States
[c] Department of Internal Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, University of Kentucky, 740 S. Limestone Street, L543, Lexington, KY 40536-0284, United States

Corresponding author: Schroder, Elizabeth A (eschr0@uky.edu)

Current Opinion in Pharmacology 2021, **57**:13–20

This review comes from a themed issue on **Cardiovascular and renal**

Edited by **Martin Young**

For complete overview about the section, refer Cardiovascular Renal (2021)

Available online 9th November 2020

https://doi.org/10.1016/j.coph.2020.09.015

Introduction

Half of all heart disease deaths are caused by sudden cardiac death (SCD), and most cases of SCD are triggered by cardiac arrhythmias [1,2]. Many different types of heart disease result in SCD, including acquired and congenital cardiovascular diseases such as coronary artery disease, heart failure, cardiomyopathies, and arrhythmogenic syndromes. Over the past 30 years, several studies identified time of day changes in the incidence of SCD [3–9]. Longitudinal analysis of the literature shows that these patterns have changed over time, perhaps reflecting changes in the clinical management and causes of SCD [10•,11,12••]. Moreover, studies focused on specific types of heart disease show disease-specific time of day patterns in the incidence of SCD [7,13,14]. Determining how and why patients with different types of heart disease die at certain times of day could lead to new breakthroughs in understanding the etiology and tailored therapeutic strategies.

Daily changes in the incidence of SCD reflect an interaction between external and internal day-night rhythms that resonate with the 24-hour period. Circadian rhythms are internal physiological rhythms that evolved to anticipate predictable changes in the environment [15]. Cardiovascular circadian rhythms include daily rhythms in heart rate (HR), ventricular repolarization, blood pressure, and cardiac metabolism [16–21]. These rhythms provide an advantage in healthy individuals but might precipitate deadly events in populations living with heart disease [4,16•,22–25]. For example, morning increases in sympathetic tone that are beneficial in healthy individuals could trigger arrhythmias or the reactivity of the heart in patients with disease [9,16•]. An additional layer of complexity is that behaviors or disease states which disrupt circadian rhythms (e.g. alignment with external environmental rhythms, rhythm amplitudes, or synchrony between circadian rhythms), might also impact arrhythmogenic triggers and/or heart reactivity to precipitate SCD.

Circadian rhythms

Circadian rhythms are generated by endogenous circadian clock mechanisms that temporally optimize cellular and biological functions with daily changes in behavior and the environment [26•,27–32]. The central circadian clock mechanism is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. In mammals, the SCN is synchronized to daily light-dark cycles through direct photic input from the retina [31]. The SCN sends neurohumoral signals that regulate organism behavior and aligns the phase of the circadian clocks in the peripheral tissues (peripheral circadian clocks) [15,33••,34,35,36•,37].

Behaviors that are important for aligning central and peripheral circadian clocks are eating and activity. Almost every cell, including cardiomyocytes, possesses an intrinsic circadian clock mechanism that performs a ubiquitous time keeping mechanism and the tissue specific expression of genes called clock output genes [15].

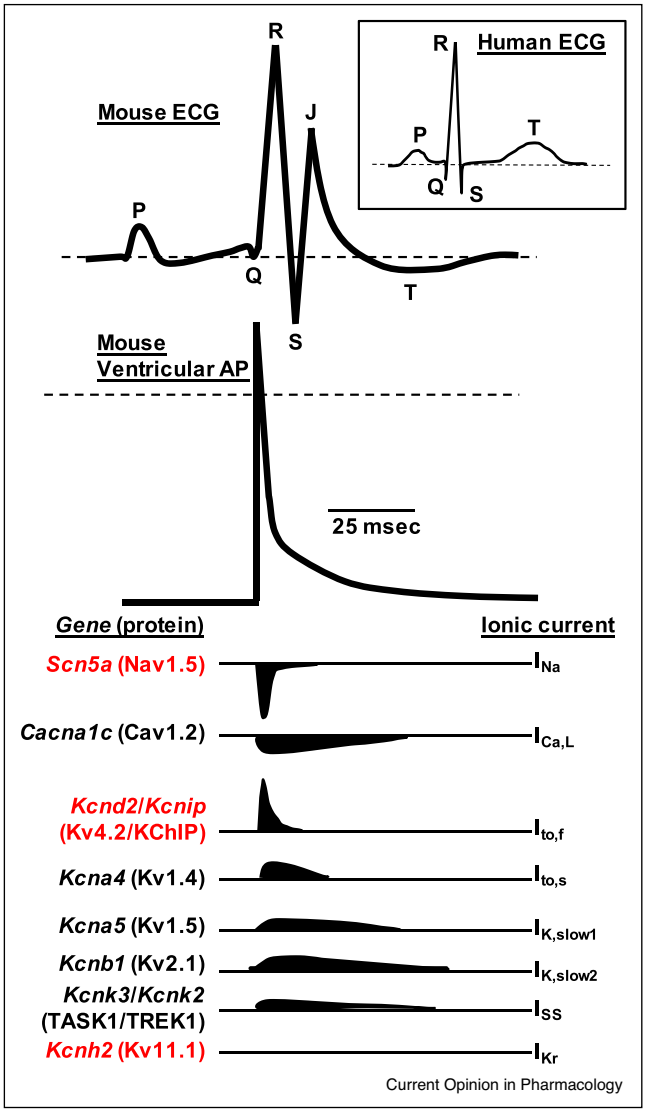
The circadian clock is a transcription-translation feedback loop composed of the basic helix-loop-helix transcription factors BMAL1 and CLOCK and the proteins that regulate their expression, including Period (PER) and Cryptochrome (CRY). BMAL1 and CLOCK hetero-dimerize to activate the transcription of *Per* and *Cry* genes, and PER and CRY proteins form multimers in the cytoplasm of the cell, that translocate to the nucleus, and act to inhibit BMAL1 and CLOCK function [31,32,38,39]. This periodicity of the loop takes approximately 24 hours to cycle and is the fundamental mechanism underlying circadian rhythms [15]. These components of the circadian clock can directly and indirectly regulate the tissue-specific expression of clock output genes [40,41].

Mouse models for the study of circadian clocks, cardiac impacts

Mice are a useful animal model for understanding the impact of circadian clocks on physiology. Although they are nocturnal, many circadian rhythms and circadian clock mechanisms are conserved [42]. Mice can be genetically engineered to determine how germline, tissue-specific, and inducible (at select time points over lifespan) disruptions of circadian clock mechanisms impact their phenotype. Mice are also amenable to studies that determine how external time-keeping cues, including light and behavior, impact the entrainment and synchrony between central and peripheral circadian clocks [43–45].

This review summarizes how transgenic mouse models of circadian clock disruption impact mouse cardiac electrophysiology, with an emphasis on the electrocardiogram (ECG), repolarization, and the regulation of ion channels important for repolarization in mice and humans. A challenge to using mice is there are species-specific differences in cardiac electrophysiology that limits direct extrapolation to humans [46]. Mouse hearts beat about 10 times faster than human hearts, and although their ECG waveforms bear several similarities to humans, there are notable differences (Figure 1; modified from Ref. [46]). Human and mouse ECG waveforms have distinct P, QRS, and T waves. The P wave is generated by the depolarization of the atrium; the QRS waves are generated by the depolarization of the ventricles, and the T wave reflects ventricular repolarization. The RR interval is often used to measure HR and the QT interval measures the time it takes the heart to repolarize. Abnormally slow or fast HR, or short or long QT intervals are risk factors for arrhythmias. There is a direct relation between HR and the QT interval, such that the QT interval increases or decreases with the preceding RR interval [47]. In order to measure the QT interval at different HR, formulas are used to calculate the HR corrected QT (QTc) interval. The QTc interval is a clinically valuable biomarker that identifies abnormalities in ventricular repolarization [48]. Surprisingly, humans and mice share similar QTc interval correction formulas, but measuring the end of the T wave in mouse ECGs is

Figure 1



Cardiac electrophysiology in the mouse.
Shown is a *cartoon* of a mouse electrocardiogram (ECG) and a human ECG (inset). Unlike the human ECG, the mouse ECG shows a prominent J wave and an inverted T wave. Below the mouse ECG is a schematic showing a mouse ventricular action potential (AP) and several cardiac ion channel genes (proteins) and their corresponding ionic currents that shape the ventricular AP. Several groups show the expression of the gene transcripts in red and/or their corresponding ionic currents are reduced by circadian clock disruption in cardiomyocytes.

challenging [47,49]. The reason is mouse hearts initially repolarize very quickly and generate a predominant J wave after the QRS complex and the T-waves are small and inverted (Figure 1) [47,50,51]. Another difference between human and mouse hearts is the size. Mouse hearts are smaller, and although they can sustain arrhythmias, the underlying mechanisms for these arrhythmias are potentially different [52]. Nonetheless, mouse models are widely employed by cardiac electrophysiologists because they are practical for determining how genetic, pharmacological, and/or environmental manipulations impact arrhythmogenic triggers and/or pro-arrhythmic changes in the cardiac substrate [53,54•].

Circadian clocks regulate heart rate and arrhythmia susceptibility

Bmal1 knockout mice (*Bmal1*$^{-/-}$) suffer from a number of systemic abnormalities, including dilated cardiomyopathy and premature death [55,56]. Similar to humans, wild type (WT) mice have a 24-hour rhythm in HR, but studies have shown that this rhythm is lost in *Bmal1*$^{-/-}$ mice [57]. Specifically in WT mice, HR increases during the active (dark) phase. The *Bmal1*$^{-/-}$ mice did not show an increase in HR during the active phase. A dampening in the daily rhythm of HR was also seen in mice engineered to express a dominant negative Clock mutation (ClockΔ19) that disrupts the circadian clock mechanism throughout the body [57]. Similar to *Bmal1*$^{-/-}$ mice, these mice showed a diminished daily rhythm of HR by reducing the HR increase during the active phase. To study the impact of the cardiomyocyte circadian clock on heart function, Durgan *et al.* engineered mice to selectively express ClockΔ19 in the heart driven by the α-myosin heavy chain promoter [58]. Compared to WT mice, mice that express ClockΔ19 only in cardiomyocytes had a slower HR and a damped daily rhythm. Studies that investigated mice engineered for the cardiomyocyte specific deletion of *Bmal1* (H-*Bmal1*$^{-/-}$) showed these animals developed progressive cardiomyopathy with age and had a decreased lifespan. However, HR was unchanged in these mice [59]. An inducible transgenic model that allows for the cardiomyocyte specific deletion of *Bmal1* in adult cardiomyocytes (iCSΔ*Bmal1*$^{-/-}$) did not develop dilated cardiomyopathy but had a slower HR compared to control animals [60]. Taken together, the data suggest that systemic disruption of the circadian clock mechanism abrogates the daily rhythm in HR by slowing HR during the active phase. Cardiomyocyte-specific disruption of the circadian clock by expressing ClockΔ19, but not deletion of *Bmal1*, slows HR. Selectively deleting *Bmal1* from adult cardiomyocytes slows HR, but unlike *Bmal1*$^{-/-}$ or *H-Bmal1*$^{-/-}$ mice, these mice do not develop cardiomyopathy or suffer pre-mature mortality.

Despite the absence of cardiomyopathy and pre-mature mortality, iCSΔ*Bmal1*$^{-/-}$ mice did suffer arrhythmias [60]. Compared to control mice, iCSΔ*Bmal1*$^{-/-}$ mice had an increased number of premature ventricular contractions and long sinus pauses. The increase in arrhythmia susceptibility was intrinsic to iCSΔ*Bmal1*$^{-/-}$ hearts because electromechanical stimulation of isolated iCSΔ*Bmal1*$^{-/-}$ heart preparations showed they were more sensitive to conduction block. These data demonstrate a protective role for the cardiomyocyte circadian clock against arrhythmias in adult hearts.

A common mechanism that underlies susceptibility to ventricular arrhythmias and SCD is abnormalities in ventricular repolarization [48,61,62]. Abnormally short or long QT intervals increase the risk for deadly arrhythmias that cause SCD in humans. Although there are many possible molecular mechanisms that underlie the cardiac arrhythmias in iCSΔ*Bmal1*$^{-/-}$ mice, one clear difference in these animals was the QTc interval. ECG telemetry studies showed that inducing the deletion of *Bmal1* in adult cardiomyocytes prolonged the QTc interval [63]. Similar to humans, the daily variation in the mouse QTc interval is normally very small; however, in iCSΔ*Bmal1*$^{-/-}$ mice the QTc interval was longer during the inactive (light) phase compared to the active phase [19,63]. This suggests that the cardiac circadian clock mechanism buffers against daily increases in the QTc interval by limiting how long it takes the ventricles to repolarize at slow HRs.

Cardiac circadian clock regulates ion channels

The observation that the iCSΔ*Bmal1*$^{-/-}$ mice show arrhythmias and a prolonged QTc interval in the absence of cardiomyopathy suggests the cardiac circadian clock mechanism regulates the functional expression of cardiac ion channel genes important for cardiac repolarization. Mouse ventricular APs have a rapid upstroke, a large transient repolarization phase, and a gradual repolarization phase to the resting membrane potential (mouse ventricular APs lack a distinct plateau phase; Figure 1) [46,64,65]. The rapid upstroke is caused by an influx of Na^+ current (I_{Na}) through *Scn5a*-encoded Nav1.5 channels. The rapid and gradual ventricular repolarization is caused by several different K^+ currents ($I_{to,f}$, $I_{to,s}$, I_{Kslow1}, I_{Kslow2}, I_{SS}) (Figure 1). $I_{to,f}$ is conducted by the *Kcnd2*-encoded Kv4.2 channels; $I_{to,s}$ is conducted by the *Kcna4*-encoded Kv1.4 channels; I_{Kslow1} is conducted by *Kcna5*-encoded Kv1.5 channels; I_{Kslow2} is conducted by *Kcnb1*-encoded Kv2.1 channels, and I_{SS} is conducted through *Kcnk3*-encoded and *Kcnk2*-encoded TASK1 and TREK1 channels. Mouse ventricular myocytes also express the rapidly activating delayed rectifier K^+ current (I_{Kr}) conducted by the *Kcnh2*-encoded Kv11.1 channel, but I_{Kr} is not expected to play a primary role in normal ventricular repolarization [66].

Several different investigators show that the expression of *Scn5a*, *Kcnd2*, and *Kcnh2* mRNA transcript levels in the

mouse ventricle is circadian, because they oscillate with a period of ~24 hours in constant darkness [60,63,67–69]. The circadian oscillation in *Scn5a* and *Kcnh2* transcript levels, but not *Kcnd2*, were lost in the hearts of iCSΔ*Bmal1*$^{-/-}$ mice. Voltage-clamping ventricular myocytes isolated from iCSΔ*Bmal1*$^{-/-}$ hearts showed that the peak I_{Na} and I_{Kr} are 30% and 50% smaller, respectively, compared to control myocytes [60,63].

I_{Kr} does not play a major role in the cardiac repolarization in mice [65,70,71], and as such, the loss of I_{Kr} is unlikely responsible for the abnormal prolongation of the QTc interval in iCSΔ*Bmal1*$^{-/-}$ mice. Therefore, the cardiomyocyte circadian clock mechanism likely regulates other cardiac K^+ channel transcripts important for mouse repolarization. Jeyaraj *et al.* [68•] showed that the transcripts for *Kcnip2*, which encodes an auxiliary K^+ channel subunit protein needed to generate native $I_{to,f}$, was also circadian in mouse hearts. They found that BMAL1 directly regulates the circadian expression of the transcription factor gene, *Krüppel-like factor 15* gene (*Klf15*), and used several transgenic mice to demonstrate that KLF15 drove the circadian expression of *Kcnip2* [68•]. They also demonstrated that, compared to control hearts, *Bmal1*$^{-/-}$ hearts had reduced *Kcnip2* transcript levels; most ventricular myocytes isolated from *Bmal1*$^{-/-}$ hearts lacked $I_{to,f}$; and *Bmal1*$^{-/-}$ ventricular APs were longer than in control myocytes [68•]. Consistent with these data, iCSΔ*Bmal1*$^{-/-}$ hearts also showed a reduction in overall *Kcnip2* mRNA levels, as well as several other K^+ channel transcripts important for mouse ventricular repolarization, including *Kcna5* and *Kcnb1* [21]. Taken together the data suggest the circadian clock in the heart might directly or indirectly regulate the functional expression of several cardiac K^+ channel genes important for repolarization.

There are discrepancies in studies between which cardiac channel transcripts show a circadian pattern of expression. This is likely the result of multiple factors including but not limited to: the absolute amplitude of the circadian component for many cardiac channel transcripts is small [67]; the number of animals used to measure mRNA at a specific time point; the frequency with which the transcript levels were measured (e.g. every 2, 4, or 6 hours); and the duration of the experimental collection timeframe (1 or more days). Regardless of the root cause of the discrepancies, these transgenic studies clearly demonstrate the importance of the cardiac circadian clock in regulating the functional expression for the predominant cardiac Na^+ channel and several K^+ channels.

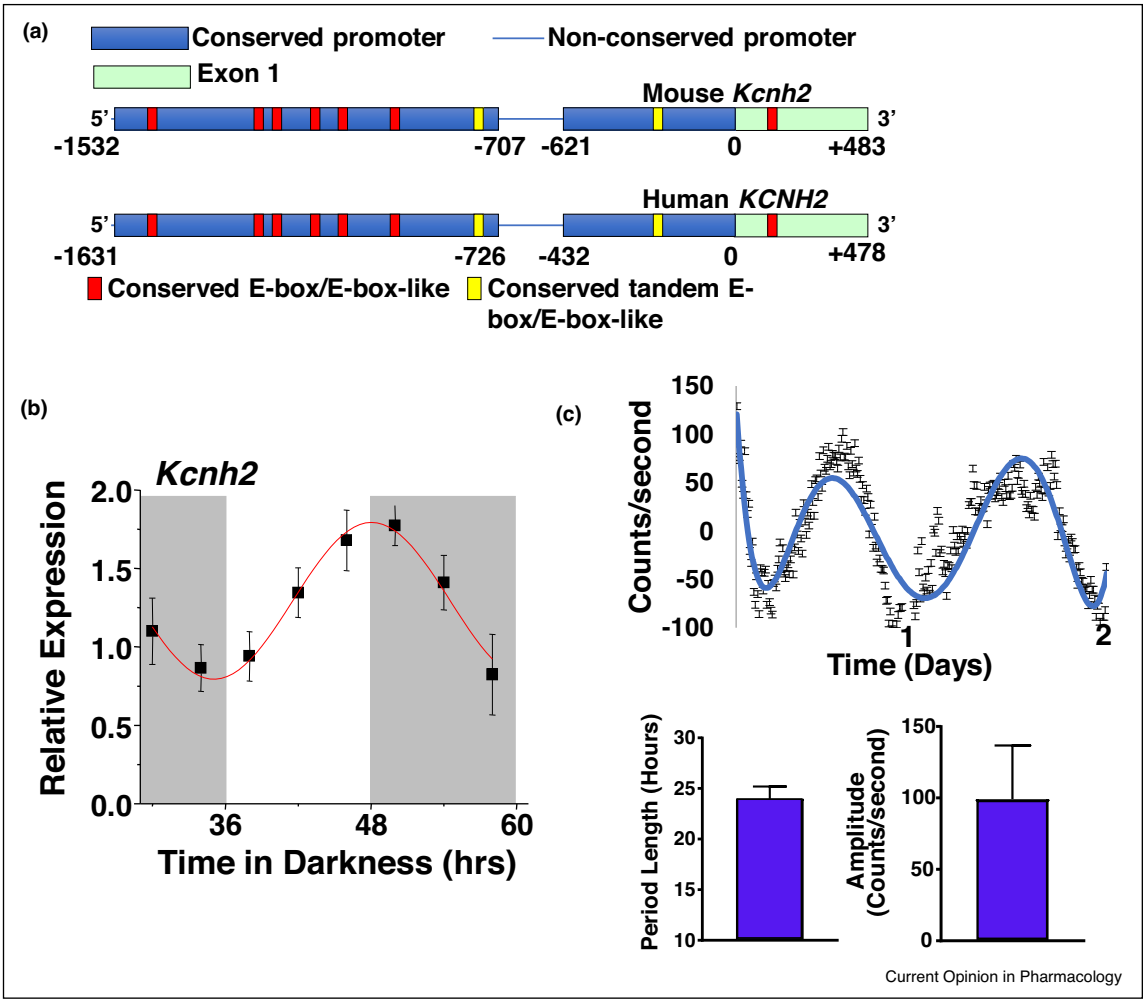
Most studies agree that *Kcnh2* transcripts show a robust circadian oscillation in the mouse heart (Figure 2b) [63,67,69]. A circadian oscillation pattern of *Kcnh2* mRNA was also detected in rat hearts, suggesting a conserved mechanism. This raises the intriguing possibility that human *KCNH2* expression might be regulated by the cardiomyocyte circadian clock. This would be an important finding because, although *Kcnh2*-encoded Kv11.1 channel proteins are not critical for normal cardiac repolarization in mice, I_{Kr} is critical for normal cardiac repolarization in humans. Loss of I_{Kr} is linked to life-threatening drug induced and inherited arrhythmias [71–74].

Circadian clock transcription factors directly regulate gene expression by binding to canonical and non-canonical E-box elements [75–77]. Sequence alignment of the mouse, rat, and human *Kcnh2/KCNH2* promoters that encode the full-length Kv11.1 channel protein showed two homologous regions of conservation upstream of exon 1. Sequence analysis of the conserved regions revealed several conserved tandem E-box motifs (Figure 2a) [69,77].

The Lumicycle allows the measurement of real time promoter activity every 10 min over the course of several days in cultured cells expressing a promoter driving luciferase expression [78–82]. Luciferase is a good reporter for measuring circadian promoter activity because it has a short protein half-life (~4 hours). This enables researchers to quantify circadian oscillations in luciferase activity as a read out of promoter activity *in vitro*. Lumicycle data for human inducible pluripotent stem cell derived cardiomyocytes expressing the cloned human *KCNH2* promoter driving luciferase expression showed the cloned promoter activity oscillates with a periodicity of ~24 hours (Figure 2c). The data suggest that the circadian clock regulation of mouse and human *Kcnh2/KCNH2* is conserved. More research is needed to understand how the circadian clock in the human heart impacts *KCNH2*, I_{Kr}, and susceptibility to arrhythmias, but similar to mice, these data provide a link between the cardiac clock mechanism and cardiac repolarization in humans.

Does the observation that several different cardiac ion channel mRNA transcripts oscillate suggests that this causes circadian changes in channel protein levels? Ionic currents and cardiac excitability? Circadian rhythms in HR or even the incidence of SCD? The answer is not straightforward. What is clear is that disruption of the cardiac circadian clock regulates the transcript levels for several cardiac ion channels, key macroscopic currents important for depolarization and repolarization, and cardiac excitability. However, connecting changes in transcript levels to protein levels and overall function is not direct. Generally speaking, the amplitude of rhythmic mRNA in tissues is much larger than that for their corresponding proteins. This is because proteins are typically more stable and as such have small circadian oscillations [83,84]. The circadian expression of transcripts encoding proteins with longer half-lives is expected to contribute to their overall accumulation and steady-state protein levels. For cardiac ion channels

Figure 2



Circadian regulation of *Kcnh2/KCNH2.*

(a) Promoter schematic for mouse and human *Kcnh2/KCNH2*. There are two regions of homology ~1.6 kB upstream of exon 1. **(b)** The circadian mRNA expression profile of *Kcnh2* is shown. Dark and light bars represent subjective night and day extrapolated from their prior light:dark (L:D) cycle before release in constant darkness (DD). (n = 4/time point) This Figure is modified from Ref. [63]. **(c)** The human *KCNH2* promoter was cloned into the pGL3-Basic vector upstream of luciferase to generate the human *Kcnh2* promoter:reporter construct (h*KCNH2*:Luc). We transiently transfected human inducible pluripotent stem cell derived cardiomyocytes with h*KCNH2*:Luc. The cells were synchronized using serum shock [86]. Data shown are Cosine fit mean lumicycle data ± SEM showing the circadian expression pattern of luciferase activity. Data were collected at 10 min intervals (n = 5; inset shows the JTK_cycle [67,87] calculated period and amplitude).

with long half-lives, long-term disruptions in cardiac circadian clock regulation (like the transgenic models described above) would be needed to reduce steady-state ion channel protein levels. Whether or not reductions in channel protein levels result in a decrease in functional current depends on a number of additional steps that are known to regulate the functional expression of cardiac ion channels. This includes but is not limited to post-transcriptional regulation (splicing, mRNA degradation, etc.), coordinate regulation of transcripts in microtranslatomes [85••], co-translational assembly with other pore-forming or auxiliary ion channel subunits, native protein folding, channel transport (trafficking) on and off the cell surface membrane, second messenger regulation, and channel degradation. Future studies that incorporate a temporal dimension in the regulation of ion channel function are needed to understand how the circadian system regulates ion channel function and cardiac excitability. These studies are expected to help clinician scientists understand why certain types of cardiomyopathies and arrhythmia syndromes show an increase (or decrease) in the incidence of SCD at certain times of day.

Conclusion

Clinician scientists have long recognized that certain patients with certain types of heart disease show a time of day change in risk for SCD. Daily changes in the incidence of SCD likely reflect an interaction between external and internal circadian rhythms. Many aspects of the cardiovascular system show strong circadian rhythms. These rhythms are generated by central and peripheral circadian clock mechanisms. Transgenic mouse models designed to disrupt these clocks systemically or solely in the heart show clear effects on heart rate and pathology, including cardiomyopathy, arrhythmia susceptibility, and ventricular repolarization. Inducible models that disrupt cardiac circadian clock signaling identified a key role for regulating ion channel transcript levels that are important for ventricular depolarization and repolarization in mice and humans. Future studies are needed to determine how disruption in clock signaling impact other aspects of ion channels function, including protein levels, cell surface expression and second messenger regulation. Additional important questions directly relevant to human health are: How do time keeping cues that impact central and cardiac circadian clocks affect cardiac excitability and arrhythmia susceptibility in healthy or diseased hearts? Could lifestyles known to disrupt, desynchronize, or misalign circadian clocks and rhythms represent a modifiable risk factor for SCD in humans by modifying cardiac ion channel function and excitability?

Conflict of interest statement

Nothing declared.

References and Recommended reading

Papers of particular interest, published within a period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

CRediT authorship contribution statement

Brian P Delisle: Conceptualization, Writing - original draft, Writing - review & editing. **John L Stumpf:** Investigation, Data curation, Formal analysis, Writing - review & editing. **Jennifer L Wayland:** Writing - original draft, Writing - review & editing. **Sidney R Johnson:** Writing - original draft, Writing - review & editing. **Makoto Ono:** Writing - original draft, Writing - review & editing. **Dalton Hall:** Writing - original draft, Writing - review & editing. **Don E Burgess:** Data curation, Formal analysis, Writing - review & editing. **Elizabeth A Schroder:** Conceptualization, Writing - original draft, Writing - review & editing.

Acknowledgements

This work was supported by the National Heart Lung and Blood award 1R01HL141343-01A1. The authors would like to thank Alexander Alimov and John McCarthy (Department of Physiology, University of Kentucky) for technical assistance.

References

1. Shah M, Akar FG, Tomaselli GF: **Molecular basis of arrhythmias**. *Circulation* 2005, **112**:2517-2529.

2. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR *et al.*: **Heart disease and stroke statistics-2019 update: a report from the American Heart Association**. *Circulation* 2019, **139**:e56-e528.

3. Willich SN, Levy D, Rocco MB, Tofler GH, Stone PH, Muller JE: **Circadian variation in the incidence of sudden cardiac death in the Framingham heart study population**. *Am J Cardiol* 1987, **60**:801-806.

4. Muller JE, Tofler GH, Willich SN, Stone PH: **Circadian variation of cardiovascular disease and sympathetic activity**. *J Cardiovasc Pharmacol* 1987, **10(Suppl. 2)**:S104-S109 discussion S110-101.

5. Muller JE, Ludmer PL, Willich SN, Tofler GH, Aylmer G, Klangos I, Stone PH: **Circadian variation in the frequency of sudden cardiac death**. *Circulation* 1987, **75**:131-138.

6. Matsuo K, Kurita T, Inagaki M, Kakishita M, Aihara N, Shimizu W, Taguchi A, Suyama K, Kamakura S, Shimomura K: **The circadian pattern of the development of ventricular fibrillation in patients with Brugada syndrome**. *Eur Heart J* 1999, **20**:465-470.

7. Takigawa M, Kawamura M, Noda T, Yamada Y, Miyamoto K, Okamura H, Satomi K, Aiba T, Kamakura S, Sakaguchi T *et al.*: **Seasonal and circadian distributions of cardiac events in genotyped patients with congenital long QT syndrome**. *Circ J* 2012, **76**:2112-2118.

8. Black N, D'Souza A, Wang Y, Piggins H, Dobrzynski H, Morris G, Boyett MR: **Circadian rhythm of cardiac electrophysiology, arrhythmogenesis, and the underlying mechanisms**. *Heart Rhythm* 2019, **16**:298-307.

9. Willich SN, Goldberg RJ, Maclure M, Perriello L, Muller JE: **Increased onset of sudden cardiac death in the first three hours after awakening**. *Am J Cardiol* 1992, **70**:65-68.

10. • Wong CX, Brown A, Lau DH, Chugh SS, Albert CM, Kalman JM, Sanders P: **Epidemiology of sudden cardiac death: global and regional perspectives**. *Heart Lung Circ* 2019, **28**:6-14.

This paper provides a relevant review of the global and regional epidemiological data available on SCD. It underscores the need to improve our understanding of risk factors and underlying conditions, as well as, how these vary across regional populations and ethnicities.

11. Arias MA, Pachon M, Akerstrom F, Puchol A, Rodriguez-Padial L: **Circadian variations of ventricular arrhythmias and sleep-disordered breathing in HF patients**. *J Am Coll Cardiol* 2014, **64**:1968.

12. •• Ni YM, Rusinaru C, Reinier K, Uy-Evanado A, Chugh H, Stecker EC, Jui J, Chugh SS: **Unexpected shift in circadian and septadian variation of sudden cardiac arrest: the Oregon sudden unexpected death study**. *Heart Rhythm* 2019, **16**:411-415.

This paper is an observational study with significant public health implications. This prospective study examined incidence of sudden cardiac arrest (SCA) in 1535 individuals in Portland, Oregon. They found that SCA in their population did not follow the typical morning or Monday incidence of SCA. These data may provide evidence for impacts of new societal demands on the incidence of SCA and warrants further research.

13. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, Denjoy I, Guicheney P, Breithardt G, Keating MT *et al.*: **Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias**. *Circulation* 2001, **103**:89-95.

14. Stramba-Badiale M, Priori SG, Napolitano C, Locati EH, Vinolas X, Haverkamp W, Schulze-Bahr E, Goulene K, Schwartz PJ: **Gene-specific differences in the circadian variation of ventricular repolarization in the long QT syndrome: a key to sudden death during sleep?** *Ital Heart J* 2000, **1**:323-328.

15. Mohawk JA, Green CB, Takahashi JS: **Central and peripheral circadian clocks in mammals**. *Annu Rev Neurosci* 2012, **35**:445-462.

16. • Thosar SS, Butler MP, Shea SA: **Role of the circadian system in cardiovascular disease**. *J Clin Invest* 2018, **128**:2157-2167.
This review examines the role of circadian rhythms and the impact of environmental factors on cardiovascular health with a focus on vascular components. They also discuss clinical implications of both the development of cardiac pathology and treatment in the context of circadian rhythms.

17. Bray MS, Young ME: **The role of cell-specific circadian clocks in metabolism and disease**. *Obes Rev* 2009, **10(Suppl. 2)**:6-13.

18. Bray MS, Young ME: **Diurnal variations in myocardial metabolism**. *Cardiovasc Res* 2008, **79**:228-237.

19. Molnar J, Zhang F, Weiss J, Ehlert FA, Rosenthal JE: **Diurnal pattern of QTc interval: how long is prolonged? Possible relation to circadian triggers of cardiovascular events**. *J Am Coll Cardiol* 1996, **27**:76-83.

20. Okutucu S, Karakulak UN, Sahiner L, Aytemir K, Demiri E, Evranos B, Fatihoglu SG, Kaya EB, Kabakci G, Tokgozoglu L *et al.*: **The relationship between circadian blood pressure pattern and ventricular repolarization dynamics assessed by QT dynamicity**. *Blood Press Monit* 2012, **17**:14-19.

21. Xie Z, Su W, Liu S, Zhao G, Esser K, Schroder EA, Lefta M, Stauss HM, Guo Z, Gong MC: **Smooth-muscle BMAL1 participates in blood pressure circadian rhythm regulation**. *J Clin Invest* 2015, **125**:324-336.

22. Elliott WJ: **Circadian variation in the timing of stroke onset: a meta-analysis**. *Stroke* 1998, **29**:992-996.

23. Manfredini R, Boari B, Smolensky MH, Salmi R, la Cecilia O, Maria Malagoni A, Haus E, Manfredini F: **Circadian variation in stroke onset: identical temporal pattern in ischemic and hemorrhagic events**. *Chronobiol Int* 2005, **22**:417-453.

24. Goldberg RJ, Brady P, Muller JE, Chen ZY, de Groot M, Zonneveld P, Dalen JE: **Time of onset of symptoms of acute myocardial infarction**. *Am J Cardiol* 1990, **66**:140-144.

25. Twidale N, Taylor S, Heddle WF, Ayres BF, Tonkin AM: **Morning increase in the time of onset of sustained ventricular tachycardia**. *Am J Cardiol* 1989, **64**:1204-1206.

26. • Takahashi JS: **Molecular components of the circadian clock in mammals**. *Diabetes Obes Metab* 2015, **17(Suppl. 1)**:6-11.

27. Tei H, Okamura H, Shigeyoshi Y, Fukuhara C, Ozawa R, Hirose M, Sakaki Y: **Circadian oscillation of a mammalian homologue of the Drosophila period gene**. *Nature* 1997, **389**:512-516.

28. van der Horst GT, Muijtjens M, Kobayashi K, Takano R, Kanno S, Takao M, de Wit J, Verkerk A, Eker AP, van Leenen D *et al.*: **Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms**. *Nature* 1999, **398**:627-630.

29. Kume K, Zylka MJ, Sriram S, Shearman LP, Weaver DR, Jin X, Maywood ES, Hastings MH, Reppert SM: **mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop**. *Cell* 1999, **98**:193-205.

30. Konopka RJ, Benzer S: **Clock mutants of *Drosophila melanogaster***. *Proc Natl Acad Sci U S A* 1971, **68**:2112-2116.

31. Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacner LD, King DP, Takahashi JS, Weitz CJ: **Role of the CLOCK protein in the mammalian circadian mechanism**. *Science* 1998, **280**:1564-1569.

32. Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD, Dove WF, Pinto LH, Turek FW, Takahashi JS: **Mutagenesis and mapping of a mouse gene, clock, essential for circadian behavior**. *Science* 1994, **264**:719-725.

33. •• Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U: **Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus**. *Genes Dev* 2000, **14**:2950-2961.
Although not a recent paper, this paper is important as it was the first to describe a role for the environment (feeding) in uncoupling the central and peripheral circadian clocks in the body. It has important implications; in that lifestyle factors may lead to circadian dyssynchrony with impacts on the heart.

34. Balsalobre Aurélio, Damiola Francesca, Schibler Ueli: **A serum shock induces circadian gene expression in mammalian tissue culture cells**. *Cell* 1998, **93**:929-937.

35. Yamazaki S, Numano R, Abe M, Hida A, Takahashi R, Ueda M, Block GD, Sakaki Y, Menaker M, Tei H: **Resetting central and peripheral circadian oscillators in transgenic rats**. *Science* 2000, **288**:682-685.

36. • Tong M, Watanabe E, Yamamoto N, Nagahata-Ishiguro M, Maemura K, Takeda N, Nagai R, Ozaki Y: **Circadian expressions of cardiac ion channel genes in mouse might be associated with the central clock in the SCN but not the peripheral clock in the heart**. *Biol Rhythm Res* 2013, **44**:519-530.
This paper is important because it demonstrated a role for the central circadian clock in the regulation of ion channel expression in the heart employing SCN ablation and adrenergic blockade. It suggests the complexity of the circadian system and the critical need to parse out the relative contribution of the central and peripheral clocks to the regulation of functional ion channels in the heart in order to better understand arrhythmogenesis.

37. Partch CL, Green CB, Takahashi JS: **Molecular architecture of the mammalian circadian clock**. *Trends Cell Biol* 2014, **24**:90-99.

38. Jin X, Shearman LP, Weaver DR, Zylka MJ, De Vries GJ, Reppert SM: **A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock**. *Cell* 1999, **96**:57-68.

39. Darlington TK, Wager-Smith K, Ceriani MF, Staknis D, Gekakis N, Steeves TDL, Weitz CJ, Takahashi JS, Kay SA: **Closing the circadian loop: CLOCK-induced transcription of its own inhibitors per and tim**. *Science* 1998, **280**:1599-1603.

40. Ripperger JA, Schibler U: **Rhythmic CLOCK-BMAL1 binding to multiple E-box motifs drives circadian Dbp transcription and chromatin transitions**. *Nat Genet* 2006, **38**:369-374.

41. Stratmann M, Suter DM, Molina N, Naef F, Schibler U: **Circadian Dbp transcription relies on highly dynamic BMAL1-CLOCK interaction with E boxes and requires the proteasome**. *Mol Cell* 2012, **48**:277-287.

42. Florez JC, Takahashi JS: **The circadian clock: from molecules to behaviour**. *Ann Med* 1995, **27**:481-490.

43. Valentinuzzi VS, Buxton OM, Chang AM, Scarbrough K, Ferrari EA, Takahashi JS, Turek FW: **Locomotor response to an open field during C57BL/6J active and inactive phases: differences dependent on conditions of illumination**. *Physiol Behav* 2000, **69**:269-275.

44. Challet E, van Reeth O, Turek FW: **Altered circadian responses to light in streptozotocin-induced diabetic mice**. *Am J Physiol* 1999, **277**:E232-E237.

45. Aschoff J: **Exogenous and endogenous components in circadian rhythms**. *Cold Spring Harb Symp Quant Biol* 1960, **25**:11-28.

46. Nerbonne JM: **Mouse models of arrhythmogenic cardiovascular disease: challenges and opportunities**. *Curr Opin Pharmacol* 2014, **15**:107-114.

47. Mitchell GF, Jeron A, Koren G: **Measurement of heart rate and Q-T interval in the conscious mouse**. *Am J Physiol* 1998, **274**: H747-H751.

48. Jervell A, Lange-Nielsen F: **Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval and sudden death**. *Am Heart J* 1957, **54**:59-68.

49. Bazett HC: **An analysis of the time-relations of electrocardiograms**. *Heart* 1920, **7**:353-370.

50. Kaese S, Verheule S: **Cardiac electrophysiology in mice: a matter of size**. *Front Physiol* 2012, **3**:345.

51. Boukens BJ, Rivaud MR, Rentschler S, Coronel R: **Misinterpretation of the mouse ECG: 'musing the waves of Mus musculus'**. *J Physiol* 2014, **592**:4613-4626.

52. Killeen MJ, Sabir IN, Grace AA, Huang CL: **Dispersions of repolarization and ventricular arrhythmogenesis: lessons from animal models**. *Prog Biophys Mol Biol* 2008, **98**:219-229.

53. Dobrev D, Wehrens XHT: **Mouse models of cardiac arrhythmias**. *Circ Res* 2018, **123**:332-334.

54. • Clauss S, Bleyer C, Schüttler D, Tomsits P, Renner S, Klymiuk N, Wakili R, Massberg S, Wolf E, Kääb S: **Animal models of arrhythmia: classic electrophysiology to genetically modified large animals**. *Nat Rev Cardiol* 2019, **16**:457-475.
This review provides a broad overview of animal models employed in the study of arrhythmias. They suggest employing multiple models to address hypotheses and provide a comprehensive overview of different models and currently used species for arrhythmia research, as well as, providing extensive pros and cons of each model.

55. Lefta M, Campbell KS, Feng HZ, Jin JP, Esser KA: **Development of dilated cardiomyopathy in Bmal1-deficient mice**. *Am J Physiol Heart Circ Physiol* 2012, **303**:H475-H485.

56. Kondratov RV, Kondratova AA, Gorbacheva VY, Vykhovanets OV, Antoch MP: **Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock**. *Genes Dev* 2006, **20**:1868-1873.

57. Curtis AM, Cheng Y, Kapoor S, Reilly D, Price TS, Fitzgerald GA: **Circadian variation of blood pressure and the vascular response to asynchronous stress**. *Proc Natl Acad Sci U S A* 2007, **104**:3450-3455.

58. Durgan DJ, Trexler NA, Egbejimi O, McElfresh TA, Suk HY, Petterson LE, Shaw CA, Hardin PE, Bray MS, Chandler MP *et al.*: **The circadian clock within the cardiomyocyte is essential for responsiveness of the heart to fatty acids**. *J Biol Chem* 2006, **281**:24254-24269.

59. Kohsaka A, Das P, Hashimoto I, Nakao T, Deguchi Y, Gouraud SS, Waki H, Muragaki Y, Maeda M: **The circadian clock maintains cardiac function by regulating mitochondrial metabolism in mice**. *PLoS One* 2014, **9**:e112811.

60. Schroder EA, Lefta M, Zhang X, Bartos DC, Feng HZ, Zhao Y, Patwardhan A, Jin JP, Esser KA, Delisle BP: **The cardiomyocyte molecular clock, regulation of Scn5a, and arrhythmia susceptibility**. *Am J Physiol Cell Physiol* 2013, **304**:C954-C965.

61. Romano C, Gemme G, Pongiglione R: **Rare cardiac arrythmias of the pediatric age. Ii. Syncopal attacks due to paroxysmal ventricular fibrillation. (Presentation of 1st Case in Italian Pediatric Literature)**. *Clin Pediatr (Bologna)* 1963, **45**:656-683.

62. Ward OC: **A new familial cardiac syndrome in children**. *J Ir Med Assoc* 1964, **54**:103-106.

63. Schroder EA, Burgess DE, Zhang X, Lefta M, Smith JL, Patwardhan A, Bartos DC, Elayi CS, Esser KA, Delisle BP: **The cardiomyocyte molecular clock regulates the circadian expression of Kcnh2 and contributes to ventricular repolarization**. *Heart Rhythm* 2015, **12**:1306-1314.

64. London B: **Cardiac arrhythmias: from (transgenic) mice to men**. *J Cardiovasc Electrophysiol* 2001, **12**:1089-1091.

65. Nerbonne JM, Kass RS: **Molecular physiology of cardiac repolarization**. *Physiol Rev* 2005, **85**:1205-1253.

66. Pond AL, Scheve BK, Benedict AT, Petrecca K, Van Wagoner DR, Shrier A, Nerbonne JM: **Expression of distinct ERG proteins in rat, mouse, and human heart. Relation to functional I(Kr) channels**. *J Biol Chem* 2000, **275**:5997-6006.

67. Pizarro A, Hayer K, Lahens NF, Hogenesch JB: **CircaDB: a database of mammalian circadian gene expression profiles**. *Nucleic Acids Res* 2013, **41**:D1009-D1013.

68. • Jeyaraj D, Haldar SM, Wan X, McCauley MD, Ripperger JA, Hu K, Lu Y, Eapen BL, Sharma N, Ficker E *et al.*: **Circadian rhythms govern cardiac repolarization and arrhythmogenesis**. *Nature* 2012, **483**:96-99.
This important paper was the first to demonstrate a role for circadian rhythms in the regulation of cardiac repolarization. In addition, it identified circadian transcription of ion channels as a mechanism for cardiac arrhythmogenesis.

69. Podobed PS, Alibhai FJ, Chow CW, Martino TA: **Circadian regulation of myocardial sarcomeric titin-cap (Tcap, telethonin): identification of cardiac clock-controlled genes using open access bioinformatics data**. *PLoS One* 2014, **9**: e104907.

70. Sanguinetti MC, Jiang C, Curran ME, Keating MT: **A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel**. *Cell* 1995, **81**:299-307.

71. Trudeau MC, Warmke JW, Ganetzky B, Robertson GA: **HERG, a human inward rectifier in the voltage-gated potassium channel family**. *Science* 1995, **269**:92-95.

72. Vandenberg JI, Perry MD, Perrin MJ, Mann SA, Ke Y, Hill AP: **hERG K(+) channels: structure, function, and clinical significance**. *Physiol Rev* 2012, **92**:1393-1478.

73. Crotti L, Celano G, Dagradi F, Schwartz PJ: **Congenital long QT syndrome**. *Orphanet J Rare Dis* 2008, **3**:18.

74. Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT: **A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome**. *Cell* 1995, **80**:795-803.

75. Rey G, Cesbron F, Rougemont J, Reinke H, Brunner M, Naef F: **Genome-wide and phase-specific DNA-binding rhythms of BMAL1 control circadian output functions in mouse liver**. *PLoS Biol* 2011, **9**:e1000595.

76. Yoshitane H, Ozaki H, Terajima H, Du NH, Suzuki Y, Fujimori T, Kosaka N, Shimba S, Sugano S, Takagi T *et al.*: **CLOCK-controlled polyphonic regulation of circadian rhythms through canonical and noncanonical E-boxes**. *Mol Cell Biol* 2014, **34**:1776-1787.

77. Nakahata Y, Yoshida M, Takano A, Soma H, Yamamoto T, Yasuda A, Nakatsu T, Takumi T: **A direct repeat of E-box-like elements is required for cell-autonomous circadian rhythm of clock genes**. *BMC Mol Biol* 2008, **9**:1.

78. Zhang X, Patel SP, McCarthy JJ, Rabchevsky AG, Goldhamer DJ, Esser KA: **A non-canonical E-box within the MyoD core enhancer is necessary for circadian expression in skeletal muscle**. *Nucleic Acids Res* 2012, **40**:3419-3430.

79. Ballesta A, Dulong S, Abbara C, Cohen B, Okyar A, Clairambault J, Levi F: **A combined experimental and mathematical approach for molecular-based optimization of irinotecan circadian delivery**. *PLoS Comput Biol* 2011, **7**:e1002143.

80. Tong X, Muchnik M, Chen Z, Patel M, Wu N, Joshi S, Rui L, Lazar MA, Yin L: **Transcriptional repressor E4-binding protein 4 (E4BP4) regulates metabolic hormone fibroblast growth factor 21 (FGF21) during circadian cycles and feeding**. *J Biol Chem* 2010, **285**:36401-36409.

81. Tamanini F: **Manipulation of mammalian cell lines for circadian studies**. *Methods Mol Biol* 2007, **362**:443-453.

82. Yamazaki S, Takahashi JS: **Real-time luminescence reporting of circadian gene expression in mammals**. *Methods Enzymol* 2005, **393**:288-301.

83. Lee C, Etchegaray JP, Cagampang FR, Loudon AS, Reppert SM: **Posttranslational mechanisms regulate the mammalian circadian clock**. *Cell* 2001, **107**:855-867.

84. Mauvoisin D, Wang J, Jouffe C, Martin E, Atger F, Waridel P, Quadroni M, Gachon F, Naef F: **Circadian clock-dependent and -independent rhythmic proteomes implement distinct diurnal functions in mouse liver**. *Proc Natl Acad Sci U S A* 2014, **111**:167-172.

85. •• Eichel CA, Rios-Perez EB, Liu F, Jameson MB, Jones DK, Knickelbine JJ, Robertson GA: **A microtranslatome coordinately regulates sodium and potassium currents in the human heart**. *eLife* 2019, **8**.
This paper employed multiple methodologies to demonstrate coregulation of transcripts encoding ion channels that regulate excitability in cardiomyocytes, specifically *Kcnh2* and *Scn5a*. They described these 'microtranslatomes' and put forth a new paradigm for the regulation of the functional expression of both channels at the membrane, and ultimately, control of electrical activity in heart.

86. Balsalobre A, Damiola F, Schibler U: **A serum shock induces circadian gene expression in mammalian tissue culture cells**. *Cell* 1998, **93**:929-937.

87. Hughes ME, Hogenesch JB, Kornacker K: **JTK_CYCLE: an efficient nonparametric algorithm for detecting rhythmic components in genome-scale data sets**. *J Biol Rhythms* 2010, **25**:372-380.