According to the Heart and Stroke Foundation of Canada, American Heart Association, and the British Heart Foundation, cardiovascular disease (CVD) is a leading cause of death worldwide (Heart and Stroke Foundation, 2014; Heran et al., 2011; Hunt et al., 2009; Rosamond et al., 2008). Although procedural interventions have markedly increased survival following acute adverse cardiac events, the incidence of chronic
diseases, such as heart failure, has more than doubled over the past 30 years (Go et al., 2013). Furthermore, mortality rates for heart failure remain high, with ~300,000 deaths per year and 5-year survival rates of ~50% (Go et al., 2013). CVDs, as with multiple common diseases, are the product of complex gene-environment interactions, wherein genetic information intrinsically influences the responsiveness of an individual to environmental stimuli and stresses. For example, genetic polymorphisms influence susceptibility of individuals to CVD risk factors, such as nutrients (quantity and quality), physical activity (duration and intensity), and sleep (duration and quality). It has long been appreciated that both cardiovascular physiology (e.g., heart rate, blood pressure) and pathology (e.g., onset of arrhythmia and myocardial infarction [MI]) exhibit time-of-day-dependent patterns (Carson et al., 2000; Degaute et al., 1994; Degaute et al., 1991; Delp et al., 1991; Muller et al., 1989; Richards et al., 1986). In recent years, investigation of neurohormonal rhythms, concurrent with the serendipitous discovery of molecular circadian clocks in cardiovascular-relevant cell types, has created new opportunities for the identification of novel mechanisms influencing cardiac processes. Such studies have revealed complex interactions between extra- and intracardiac mechanisms that affect function and dysfunction of the heart over the course of the day. Here, we provide an overview of circadian biology as it relates to cardiac function and discuss how to translate these findings to clinical cardiology as well as medicine in general.

TIME-OF-DAY-DEPENDENT OSCILLATIONS IN CARDIAC PROCESSES

Life on earth is subject to a 24-h day and night cycle. Organisms have therefore evolved mechanisms for the anticipation and adaptation to time-of-day-dependent fluctuations in the environment. The cardiovascular system is an excellent example. Marked time-of-day-dependent variations are observed in multiple cardiovascular parameters, in terms of both physiology (e.g., heart rate, QT interval, contractility) and pathophysiology (e.g., timing of onset of arrhythmias, MI, and sudden cardiac death) (Carson et al., 2000; Degaute et al., 1994; Degaute et al., 1991; Delp et al., 1991; Muller et al., 1989; Richards et al., 1986; Tofler et al., 1995). Many of these rhythms mirror behavioral fluctuations. That is, both heart rate and cardiac contractility are elevated during the wake period in humans, when physical activity is increased (Clarke et al., 1976; Hu et al., 2004; Scheer et al., 1999). Classically, many cardiovascular function rhythms have been attributed to fluctuations in neurohumoral rhythms, although an underlying circadian component appears to play an important role. For example, heart rate rhythms in humans correlate closely with the daily biases of autonomic nervous activity, which oscillate over 24-h periods, while sympathetic nervous activity is lowest and cardiac vagal markers are highest during sleep (Scheer et al., 2010). Healthy subjects also exhibit daily variations in left ventricular indices, consistent with day-night changes in sympathovagal tone (Karabag et al., 2011; Voutilainen et al., 1996). Somewhat surprisingly, time-of-day-dependent variations in cardiac function (e.g., both heart rate and contractility) observed in vivo persist when rodent hearts are perfused under controlled conditions in an ex vivo setting (Bray et al., 2008; Young et al., 2001a). Importantly, these findings suggest that not only do circadian variations in the neurohumoral axes acutely contribute to time-of-day-dependent fluctuations in cardiac function, but so too do oscillations in various intrinsic properties of the heart. These intrinsic properties are summarized in Figure 1 and detailed subsequently.

Time-of-Day-Dependent Rhythms in Cardiac Contractility and Electrophysiology

As highlighted above, time-of-day-dependent oscillations in heart rate and contractility persist when rodent hearts are allowed to reach a steady state in the ex vivo setting (Bray et al., 2008; Young et al., 2001a). In both cases, heart rate and contractility peak during the dark phase. Furthermore, when subjected to a workload challenge (increased afterload plus epinephrine), ex vivo perfused hearts exhibit greater cardiac output during the dark phase, revealing increased contractile reserve at this time (Bray et al., 2008). The latter is consistent with anticipation of increased workload demand (e.g., physical activity) when the organism is awake. Mechanistically, several possibilities exist with regard to how contractility is intrinsically increased during the active period, including time-of-day-dependent fluctuations in contractile proteins, ion homeostasis, metabolism (to adequately meet energetic demands), and signaling. The former two are the focus of this section.

When one is considering contractile proteins, myosin often comes to mind. Myosin is a complex of 2 heavy chains and 4 light chains; 2 major myosin heavy chain (MHC) isoforms are expressed in the heart, namely MHCα and MHCβ, which differ in their kinetics and force generation efficiency (Sieck and Regnier, 2001). Time-of-day-dependent oscillations have been reported in the rodent heart for both isoforms at the transcript level (Wang et al., 1999; Young et al., 2001b). The half-life of MHC proteins is
generally considered to be longer than 24 h, raising concerns that MHCα and MHCβ protein levels are unlikely to oscillate over the course of the day (although this has not been reported to date). However, myosin ATPase Ca^{2+}-sensitivity exhibits a marked daily variation in mouse hearts, peaking during the dark phase (Podobed et al., 2014a). This was associated with time-of-day-dependent rhythms in the protein levels of various myofilament components, including MyBP-C, desmin, tropomyosin, troponins I and T, and titin cap (Tcap) (Podobed et al., 2014a; Podobed et al., 2014b). For example, murine sarcomeric Tcap mRNA is circadian regulated, and the protein levels peak around wake time; this is consistent with the increased sensitivity of myosin ATPase to calcium at this time (Podobed et al., 2014a; Podobed et al., 2014b). Collectively, these data indicate that myofilament composition and function differ markedly, depending on the time of day.

In terms of ion homeostasis, Ca^{2+} plays an integral role in excitation-contraction coupling. Elegant studies by Collins and Rodrigo (2010) report variations in Ca^{2+} transients depending on the time of day at which adult rat cardiomyocytes were isolated. More specifically, both diastolic and systolic Ca^{2+} levels, as well as peak Ca^{2+} release and relaxation of the Ca^{2+} transient, are elevated in cardiomyocytes isolated during the light phase (Collins and Rodrigo, 2010). However, peak L-type Ca^{2+} current density is lower in cardiomyocytes during the light phase, despite increased gene and protein expression of the voltage-gated calcium channel subunit α1D (Collins and Rodrigo, 2010; Ko et al., 2011). Rhythms in K^{+} transients have also been reported in the heart. Following a screen of 14 different K^{+} channels in the rat heart, Yamashita et al. (2003) reported that Kv1.5 and Kv4.2 protein levels exhibit opposing daily rhythms, peaking during the dark and light periods, respectively. Importantly, oscillations in these channels are associated with predicted time-of-day-dependent differences in K^{+} transients (Yamashita et al., 2003). More recently, Jeyaraj et al. (2012) confirmed oscillations in Kv4.2 (the alpha subunit of the transient outward K^{+} current) and reported similar rhythms in KChIP2 (the

Figure 1. Time-of-day-dependent oscillations in the intrinsic properties of the rodent heart. Multiple cardiac processes oscillate in the heart, ranging from contractility, metabolism, and signaling to gene and protein expression.
Cardiac metabolism and contractile function are inextricably linked. During periods of increased workload (e.g., exercise), cardiac metabolism (primarily glucose utilization) increases to fulfil the increased energetic demand on the heart (Allard et al., 1994; Goodwin et al., 1998). Conversely, impairment in cardiac energetics negatively affects contractility (as exemplified by mitochondrial dysfunction during heart failure) (Neubauer, 2007). Consistent with a need for continuous contraction throughout the lifespan of the organism, the heart is a metabolic omnivore, capable of using a host of substrates as energy sources (Taegtmeyer, 2000). Cardiac metabolism is notably sensitive to substrate availability, allowing the heart to switch between metabolic fuels depending upon circulating levels. Given marked fluctuations in energetic demand and substrate availability associated with sleep-wake and fasting-feeding cycles, it is therefore not surprising that time-of-day-dependent oscillations have been reported for numerous cardiac metabolism parameters (Figure 2). Next we briefly review current knowledge regarding daily variations in primary energy sources for the heart, namely glucose and fatty acids. The discussion focuses on studies directly investigating metabolic fluxes, as opposed to indirect markers of metabolism, such as gene and protein expression of metabolic enzymes (as these measures do not necessarily correlate with metabolic flux). For a more complete account of time-of-day-dependent variations in cardiac metabolism, including amino acid and protein metabolism, the reader is directed to recently published reviews focusing on this topic (Chatham and Young, 2012; Tsai and Young, 2009).

To date, the majority of studies investigating daily variations in cardiac metabolism have been performed in rodent models. Of these, time-of-day-dependent oscillations in oxidative and nonoxidative metabolism of glucose and fatty acids have been described with the greatest level of detail, through use of ex vivo working rat and mouse heart perfusions. An advantage of this ex vivo system is that acute neurohumoral and workload influences that vary over the course of the day in the in vivo setting can be maintained constant during metabolic measures ex vivo. Such studies have revealed elevated rates of glucose oxidation during the dark phase in both ex vivo perfused rat and mouse hearts, which are also associated with increased rates of nonoxidative glucose metabolism (i.e., glycolysis and glycogen synthesis) at this time (Durgan et al., 2007; Durgan et al., 2011a; Young et al., 2001a). Glycogen content peaks in the rodent heart at the dark-to-light phase transition, consistent with elevated rates of glycogen synthesis during the awake-dark period (Durgan et al., 2007). It has been hypothesized that increased glucose uptake during the dark phase would facilitate ATP generation at a time of increased energetic demand as well as enable efficient storage of excess glucose as glycogen in anticipation of the upcoming period of fasting during the inactive-sleep-light phase (Durgan et al., 2011a). Unlike glucose utilization, oleate (fatty acid) oxidation does not exhibit a significant time-of-day-dependent oscillation in the ex vivo perfused rat or mouse (FVB background) heart (although a slight oscillation is observed in C57B6 mouse hearts; Young, unpublished observations), consistent with fatty acids serving primarily as a foundation for the energetic demands of the heart, as opposed to a reserve of ATP during periods of increased energetic demand (Tsai et al., 2010; Young et al., 2001a). However, triglyceride synthesis does oscillate in a time-of-day-dependent manner in the mouse heart, peaking near the end of the dark phase (consistent with peak myocardial triglyceride content at the dark-to-light phase transition) (Tsai et al., 2010). Far less is known regarding time-of-day-dependent oscillations in protein and amino acid metabolism and mitochondrial function in the heart or whether rhythms in glucose and fatty acid utilization observed in ex vivo perfused hearts are similar in the in vivo setting (in the presence of neurohumoral oscillations).
Collectively, these observations suggest that the intrinsic metabolic properties of the myocardium vary markedly over the course of the day.

**Time-of-Day-Dependent Rhythms in Signal Transduction**

Multiple extracellular influences known to affect cardiac processes oscillate over the course of the day. It is therefore not surprising that numerous studies have reported time-of-day-dependent oscillations in the activation status of signaling components in the heart. For example, consistent with increased energetic demand during the active period, both the phosphorylation and activity of AMPK-activated protein kinase are elevated in the mouse heart at this time; this would likely promote substrate utilization, thus helping to balance ATP demand with generation (Tsai et al., 2010). Studies by Sachan et al. (2011) suggest that the activity of the Ca\(^{2+}\)-activated phosphatase calcineurin exhibits a daily variation in the heart, with lowest activity at the sleep-to-wake transition. It has been hypothesized that increased β-adrenergic signaling in the heart at this time promotes PKA-mediated phospholamban phosphorylation and subsequently increased SERCA2 activity, thereby diminishing Ca\(^{2+}\) availability for calcineurin activation (Sachan et al., 2011). The phosphorylation status of various additional kinases has also been reported to exhibit marked time-of-day-dependent variations in rodent hearts, including AKT, GSK3\(β\), and ERK (Durgan et al., 2010; Ko et al., 2011). These kinases play important roles in transducing an array of extracellular stimuli and stresses, including mechanical (e.g., stretch) and humoral (e.g., insulin) processes (Abel, 2004; Baba et al., 2003). It is important to note that not only have oscillations in extracellular stimuli been observed, but so too have rhythms in the responsiveness of the heart to these factors. For example, the responsiveness of the heart to β-adrenergic stimulation exhibits a daily variation in multiple parameters, including calcium transients, heart rate, and arrhythmia induction (Bray et al., 2008; Collins and Rodrigo, 2010). As discussed later in the review, this potentially reflects differences in expression levels of critical signaling components in the heart over the course of the day.

**EXTRACELLULAR VERSUS INTRACELLULAR MODULATORS OF CARDIAC FUNCTION**

As evidenced by the preceding section, functional properties of the heart display clear rhythmic differences dependent upon the time of day. Questions therefore arise with regard to the mechanisms mediating these rhythms. Classically, time-of-day-dependent oscillations in cardiovascular physiology (e.g., heart rate, cardiac output) and pathology (e.g., the onset of adverse cardiac events, such as MI or arrhythmia) have been attributed to fluctuations in various neurohumoral factors. These include, but are not limited to, sympathetic-autonomic-adrenergic stimulation, endocrine factors (e.g., cortisol, growth hormone, insulin, and various adipokines), nutrients (e.g., glucose and lipids), pro- and antithrombolytic factors (e.g., plasminogen activator inhibitor 1), and vascular resistance (for reviews, see Gamble et al., 2014; Young, 2006). However, evidence has begun to emerge suggesting that in addition to these extracellular influences, distinct cardiac processes are directly regulated by intracellular mechanisms in a time-of-day-dependent manner.

Dissecting the relative contributions of extrinsic versus intrinsic influences on a biological process in the in vivo setting is a challenging undertaking. This is particularly true when studying time-of-day-dependent rhythms in humans; differentiating between the contributions of behaviors such as sleep-wake and fasting-feeding cycles versus an intrinsic mechanism presents significant experimental hurdles. However, a number of groups have designed appropriate experimental protocols in order to achieve this goal. For example, Scheer and Shea have developed desynchrony protocols (DPs), wherein healthy volunteers are subjected to contiguous 20- or 28-h days, in terms of periodicity of enforced sleep, exercise, and feeding schedules. These elegant studies have revealed persistent 24-h rhythms for a number of important cardiovascular-relevant parameters (including heart rate, blood pressure, and platelet activation) during the DP (Scheer et al., 2010; Scheer et al., 2011; Shea et al., 2011). In other words, time-of-day-dependent oscillations in these parameters do not appear to be driven simply by daily behaviors but are instead mediated by intrinsic circadian mechanisms.

The DP studies in humans clearly highlight the importance of intrinsic mechanisms in modulating crucial parameters important for healthy cardiovascular function. However, these studies are not able to dissociate the relative contributions of neurohumoral rhythms that persist under these conditions (i.e., extracellular factors) versus a mechanism that is intrinsic to the heart (i.e., intracellular influences). To address this level of dissection, animal- and cell-based studies have been used. For example, investigation of metabolism and contractility in ex vivo perfused hearts at different times of the day, after a steady state is achieved (e.g., over a 30-min time period), reduces acute neurohumoral influences. Cardiomyocytes have also been freshly isolated from rodent hearts at distinct times of the day, revealing marked differences in parameters such as calcium...
homeostasis and responsiveness to fatty acids (Collins and Rodrigo, 2010; Durgan et al., 2006). Although these studies highlight that the intrinsic properties of the heart and cardiomyocyte change as a function of time of day, such studies are unable to account for the chronic mechanisms by which neurohumoral influences may persist ex vivo (e.g., changes in protein expression).

CONTRIBUTION OF THE CARDIOMYOCYTE CIRCADIAN CLOCK

Evidence discussed in the preceding section suggests not only that cardiovascular processes oscillate in a time-of-day-dependent manner when behavioral rhythms are controlled but that the intrinsic properties of the heart and cardiomyocyte fluctuate over the course of the day. This leads to the question of whether an intrinsic mechanism within the cardiomyocyte modulates cardiac processes in a time-of-day-dependent manner. One such candidate mechanism is the cell autonomous circadian clock.

Circadian Clocks

The mammalian circadian clock can be defined as a set of proteins that generate transcriptionally based positive and negative feedback loops with a free-running period of approximately 24 h (Edery, 2000; Takahashi et al., 2008). This cell autonomous molecular mechanism provides the selective advantage of anticipation, allowing a cell, organ, or organism to prepare for a stimulus or stress prior to its onset. The clock mechanism is ubiquitous in nature, having been identified in virtually all mammalian cells investigated to date. In general terms, 3 important factors should be considered: (1) the clock mechanism; (2) factors that synchronize (entrain) the clock (known as zeitgebers); and (3) those genes and processes that are directly regulated by the clock. Critical to the mammalian clock mechanism are 2 transcription factors, CLOCK and BMAL1. The CLOCK and BMAL1 heterodimer binds to E-boxes located in the promoter region of various genes, resulting in induction (Gekakis et al., 1998; Hogenesch et al., 1998). These genes include multiple PER and CRY isoforms, which, upon accumulation of the translation products, translocate into the nucleus and inhibit CLOCK/BMAL1 transcriptional activity (Kume et al., 1999; Shearman et al., 2000). A second negative feedback loop involves REV-ERBa. Briefly, the CLOCK and BMAL1 heterodimer induces expression of REV-ERBa, which in turn represses ROR-mediated expression of BMAL1 (Yin and Lazar, 2005). Numerous posttranslational modifications (PTMs) are also essential for the normal functioning of the mammalian clock mechanism, including protein phosphorylation, ubiquitination, sumoylation, ADP-ribosylation, and O-GlcNAcylation (Cardone et al., 2005; Durgan et al., 2011a; Hardin and Yu, 2006; Katada and Sassone-Corsi, 2010; Tamaru et al., 2009).

The Cardiomyocyte Circadian Clock

Various circadian clock components and output genes oscillate in a time-of-day-dependent manner in both the vasculature and the heart (Durgan et al., 2005; McNamara et al., 2001; Rudic et al., 2005; Young et al., 2001b). Consistent with the autonomous nature of the circadian clock, findings by Davidson et al. (2005) indicated persistent 24-h oscillations in bioluminescence for ex vivo vascular and cardiac explants from transgenic rats in which luciferase expression is driven by the clock component Per1. Transcript and protein levels of clock components have also been reported to oscillate in all in vitro cultured cell types present in the intact heart (following serum-induced synchronization), including vascular smooth muscle cells, endothelial cells, fibroblasts, and cardiomyocytes (Balsalobre et al., 1998; Durgan et al., 2005; McNamara et al., 2001; Takeda et al., 2007). In the latter case, both clock components (Bmal1, Per2, Rev-erBa) and output genes (Dbp) oscillate with a periodicity of approximately 24 h for at least 3 days (Durgan et al., 2005). Furthermore, phases of these gene expression oscillations in vitro are essentially identical to those observed in the intact heart, wherein Bmal1 is antiphase to Per2 and Dbp (Durgan et al., 2005). Collectively, these data are consistent with the presence of an autonomous circadian clock within cardiomyocytes. Given that all cell types in the intact heart possess circadian clocks, it is possible that cardiac processes are influenced by clocks within any of these cells types. For example, the vascular smooth muscle clock may modulate coronary flow in a time-of-day-dependent manner, thereby affecting cardiac metabolism through substrate delivery. For the sake of simplicity, the current review focuses solely on the cardiomyocyte circadian clock.

Investigating the Cardiomyocyte Circadian Clock

Circadian clocks influence a host of biological processes ranging from behaviors to endocrine factor secretion, neural output, and metabolic homeostasis (Takahashi et al., 2008). Given that cardiovascular function is known to be modulated by all of these parameters, it is not surprising that blood pressure, heart rate, and cardiac contractility are altered...
in various mouse models in which circadian clock components are genetically disrupted in a germline fashion. For example, germline BMAL1 null mice exhibit bradycardia (attenuated increase in heart rate during the dark phase) and an age-dependent depression of systolic function (Curtis et al., 2007; Lefta et al., 2012). These mice also exhibit alterations in the expression of various cytoskeletal proteins in the heart (although only one time of the day was investigated) (Lefta et al., 2012). Similarly, DBP/HLF/TEF triple knockout mice exhibit hypertrophic cardiomyopathy (Wang et al., 2010). Collectively, these observations are consistent with the concept that circadian clock function and output are essential for normal cardiac contractility.

In an attempt to dissociate the role of the cardiomyocyte circadian clock from extracellular factors that are altered in germline knockout/mutant rodents, 2 primary tactics have been taken. These include investigation of isolated cardiomyocytes in culture and (to a greater extent) generation of cardiomyocyte-specific, circadian clock–disrupted mouse models. For the in vivo murine models, 2 circadian clock components have been targeted in a cardiomyocyte-specific manner to date: CLOCK and BMAL1. Due to apparent overlapping functions of CLOCK and NPAS2 (suggesting a level of redundancy), a dominant negative transgenic approach has been taken for CLOCK. The CLOCK<sup>−/−</sup> mutant previously described by Vitaterna et al. (1994), which lacks exon 19 encoding for the transactivation domain of CLOCK, was selectively expressed in cardiomyocytes through use of the MHCα promoter (termed cardiomyocyte-specific CLOCK mutant; CCM) (Durgan et al., 2006). BMAL1 has been ablated from cardiomyocytes, in both constitutive (termed cardiomyocyte-specific BMAL1 knockout; CBK) and inducible (termed inducible cardiomyocyte-specific deletion of BMAL1; iCSΔbmal1) manners (Durgan et al., 2011b; Schroder et al., 2013). In all models, clock output gene oscillations are depressed in the heart, whereas clock function is normal in other peripheral tissues investigated. In addition, in the cases of the CCM and CBK models, behaviors and neurohumoral factors have been reported to be normal (relative to control littermate mice), consistent with cardiac specificity (Durgan et al., 2011b; Young et al., 2014). The subsequent sections highlight how these models have been used to identify novel roles for the cardiomyocyte circadian clock.

Cardiomyocyte Clock Control of the Cardiac Transcriptome

The circadian clock mechanism is transcriptional in nature. Multiple laboratories have therefore rationalized that identification of clock-controlled output genes would reveal potential processes that are regulated by this molecular mechanism. The first large-scale network approach to examine circadian gene expression in the murine heart was performed using high-density oligonucleotide microarrays, revealing that ~9% of cardiac genes are rhythmic under circadian (constant darkness) conditions (Storch et al., 2002). Since mammals, including humans, live in a 24-h day-night environment, Martino et al. (2004) investigated murine heart gene expression rhythms under light-dark conditions, demonstrating that ~13% of genes are rhythmic. It is worthwhile to note that circadian microarray data from heart tissue (as well as additional organs) are readily available through the open access interactive website created by the Hogenesch laboratory, the Circadian Expression Profiles Data Base (CircaDB; http://bioinf.itmat.upenn.edu/circa/) (de Lichtenberg et al., 2005; Glynn et al., 2006; Hughes et al., 2010; Pizarro et al., 2013). These global microarray approaches have identified a wide variety of genes that are important for key biological pathways, including growth and renewal, metabolism, transcription and translation, and molecular signal pathways (Martino et al., 2004; Martino et al., 2007a). Accordingly, both hypothesis-testing and hypothesis-generating approaches have been used to meticulously identify specific cardiomyocyte circadian clock–regulated genes.

The first cardiomyocyte circadian clock–controlled genes were reported fewer than 10 years ago. Here, expression of the fatty acid–responsive genes <i>pdk4</i> and <i>ucp3</i> oscillated in serum-synchronized cardiomyocytes in vitro in a manner comparable to the intact rodent heart in vivo (Durgan et al., 2005). Furthermore, the transcriptional responsiveness of both isolated cardiomyocytes and intact rodent hearts to fatty acids is dependent not only on time of day but also on the cardiomyocyte circadian clock (Durgan et al., 2006; Stavinoha et al., 2004). Additional candidate approaches have revealed transcriptional regulation of metabolic (e.g., <i>dgat2</i>) and ion homeostasis (e.g., <i>scn5a</i>) genes (Schroder et al., 2013; Tsai et al., 2010). However, most recently, through the use of microarray analyses in both CCM and CBK hearts, the extent of cardiomyocyte circadian clock control of the cardiac transcriptome has been revealed. Genetic disruption of CLOCK (CCM) and BMAL1 (CBK) influences as much as 10% of the cardiac transcriptome, including genes affecting signal transduction, protein turnover, and metabolism (Bray et al., 2008; Young et al., 2014).

The circadian clock has the potential of influencing gene expression in a number of ways. The clock mechanism itself is composed of multiple transcriptional modulators, all of which have the potential to
Martino and colleagues reported that only ~50% of protein abundance is controlled in a daily manner. The clock also directly regulates the expression of a host of transcription factors, including the PAR family (DBP, HLF, and TEF), which in turn influence gene expression (Falvey et al., 1995; Fonjallaz et al., 1996; Ripperger et al., 2000). It is worthy to note that concern always exists with regard to indirect effects on gene expression in animal models of genetic manipulation (both constitutive and inducible). For these reasons, a combined microarray and in silico analysis involving both CCM and CBK mice was recently applied to identify novel direct CLOCK and BMAL1 target genes in the heart. This stringent analysis highlighted 19 genes as being direct CLOCK and BMAL1 target genes, as they (1) are differentially expressed in a similar manner in both CCM and CBK hearts (relative to littermate controls); (2) possess conserved putative E-boxes in the promoter region; and (3) are identified in a BMAL1 chromatin immunoprecipitation coupled with deep sequencing analysis in mouse liver. Identified genes are involved in clock function (e.g., rev-erba), metabolism (e.g., nampt), and signaling (e.g., pik3r1) (Young et al., 2014). Clearly, additional studies are required to elucidate fully the mechanisms by which the cardiomyocyte circadian clock regulates the cardiac transcriptome.

Cardiomyocyte Clock Control of the Cardiac Proteome

To improve our understanding of the extent to which the cardiomyocyte clock influences heart function, it is critical to appreciate the influence that this mechanism has on the cardiac proteome, given that proteins underlie fundamental biochemical processes in cells including cardiomyocytes. Using large-scale proteomic techniques, including 2-dimensional difference in gel electrophoresis (2D-DIGE) and mass spectrometry (MS), investigators discovered that the abundance of up to ~8% of the soluble cardiac proteome changes over the course of the day (Podobed et al., 2014a). Importantly, a role for the cardiomyocyte circadian clock in protein abundance was demonstrated in CCM hearts using 2D-DIGE/MS and Western blot analyses. For example, proteins with increased abundance in CCM versus wild-type hearts include PDHE1α, GOT2, and DLST, while decreased abundance is observed for ALDH2, LDHB, ECHS1, and BDH1 (Podobed et al., 2014a). Many of these are rate-limiting enzymes that play crucial roles in vital metabolic pathways that provide substrate for the TCA cycle.

There is considerable speculation about how protein abundance is controlled in a daily manner. Martino and colleagues reported that only ~50% of the fluctuations in the cardiac proteome are concurrent with underlying day-night gene expression patterns (Podobed et al., 2014a), an observation that is strikingly similar to that demonstrated on a global scale by Reddy et al. (2006) for the hepatic proteome. A recent study by Luck and colleagues (2014) used a statistical model to demonstrate that rhythmic production and rhythmic degradation (half-life) of mRNAs, as well as half-life of proteins, play an important role in timing of peak abundances. The circadian clock also regulates a number of other factors that contribute to daily oscillations in the proteome, including a large number of E3 ligases, proteasome components, regulators of translation initiation and elongation, and numerous mediators of PTMs in multiple tissues (Duffield et al., 2002; Reddy et al., 2006). This is likely the case for the cardiomyocyte circadian clock.

Cardiomyocyte Clock Control of Cardiac Metabolism

Through the use of both CCM and CBK mouse models, metabolism has emerged not only as an important clock output but also as an integral clock component. Evidence has emerged suggesting that the metabolism of carbohydrate, fatty acid, amino acid, ketone body, and even NAD+ is regulated by the cardiomyocyte circadian clock (Bray et al., 2008; Durgan et al., 2011a; Tsai et al., 2010; Young et al., 2014). For example, time-of-day-dependent oscillations in both oxidative and nonoxidative (glycolysis and net glycogen synthesis) glucose metabolism observed in contracting intact wild-type hearts ex vivo are abolished in CCM hearts (due to an inability to increase metabolic fluxes during the dark phase) (Durgan et al., 2011a). Similarly, CBK hearts exhibit depressed glucose utilization during the dark phase (only this time of day has been investigated to date) (Young et al., 2014). Impaired glucose utilization following disruption of the cardiomyocyte clock is associated with depressed rates of glucose uptake (Durgan et al., 2011a). Conversely, submaximal rates of fatty acid oxidation are elevated in both CCM and CBK hearts (relative to wild-type littermates), suggesting an important role for the cardiomyocyte circadian clock in substrate selection (as opposed to a general depression of oxidative metabolism) (Bray et al., 2008; Young et al., 2014). In terms of nonoxidative fatty acid utilization, the cardiomyocyte circadian clock regulates rates of myocardial triglyceride turnover; time-of-day-dependent oscillations in net triglyceride synthesis (and total triglyceride levels) are severely attenuated in CCM hearts (which correlates with attenuated rhythms in dgat2 expression, a direct CLOCK and BMAL1 target gene encoding for the
committed step in triglyceride synthesis) (Tsai et al., 2010; Young et al., 2014). Ketone body oxidation has also recently been found to be decreased in both CCM and CBK hearts, consistent with decreased gene and protein expression, as well as activity, of β-hydroxybutyrate dehydrogenase 1 (BDH1; a direct CLOCK and BMAL1 target gene) (Young et al., 2014).

Metabolism has emerged as an integral component in the circadian clock mechanism (Bass and Takahashi, 2010; Gamble and Young, 2013). Two recent examples include glucose and NAD⁺ metabolism. In the latter case, studies in extracardiac tissues (e.g., liver) have revealed that cellular NAD⁺ levels are modulated by cell autonomous clocks through direct regulation of NAMPT (a critical component in the NAD⁺ salvage pathway) (Peek et al., 2013; Ramsey et al., 2009). NAD⁺ is an obligate substrate in multiple posttranslational modifications, including ADP-ribosylation and deacetylation. NAD⁺ therefore serves as a feedback loop in the clock, through promotion of BMAL1 deacetylation in a time-of-day-dependent manner (Nakahata et al., 2009). Several lines of evidence suggest that this mechanism operates in the heart, including the following: (1) NAD⁺ levels oscillate in the heart in a time-of-day-dependent manner (Powanda and Wannemacher, 1970); (2) Namp7 has been identified as a direct CLOCK/BMAL1 target gene in the heart, consistent with decreased expression (gene and protein) in both CCM and CBK hearts (Young et al., 2014); and (3) NAD⁺ levels are decreased in CCM hearts (Durgan et al., 2011a). More recently, Durgan et al. (2011a) highlighted that protein O-GlcNAcylation is an important metabolism-based PTM that influences the cardiomyocyte circadian clock. Protein O-GlcNAcylation is a reversible covalent modification akin to phosphorylation, wherein an O-GlcNAc moiety (derived from glucose via the hexosamine biosynthetic pathway; HBP) is added or removed from serine residues on target proteins, thereby affecting protein activity, locality, and/or stability (Hart et al., 2011). The cardiomyocyte circadian clock appears to promote protein O-GlcNAcylation during the dark phase through increased glucose uptake and regulation of HBP enzymes (which oscillate in wild-type, but not CCM, hearts) (Durgan et al., 2011a). Importantly, several clock components become O-GlcNAc modified (including BMAL1), which appears to affect the timing of the circadian clock (Durgan et al., 2011a; Li et al., 2013; Ma et al., 2013).

Cardiomyocyte Clock Control of Signal Transduction

An important role of circadian clocks is anticipation of stimuli and stresses prior to their onset. In this regard, a plethora of neurohumoral factors exhibit time-of-day-dependent oscillations, in association with sleep-wake and fasting-feeding cycles. It is therefore not surprising that evidence has emerged suggesting that circadian clocks influence various signaling pathways that transduce extracellular stimuli and stresses to appropriate cellular responses. When one considers oscillations in physical activity and feeding status over the course of the day, 2 humoral factors that readily come to mind include epinephrine and insulin. Evidence exists in support of the concept that the cardiomyocyte circadian clock modulates sensitivity of the heart to both these extracellular factors.

In the ad libitum fed mouse, the phosphorylation status of numerous insulin signaling cascade components exhibits time-of-day-dependent oscillations, including AKT, GSK3β, and ERK (Durgan et al., 2010; Ko et al., 2011). Importantly, the cardiomyocyte circadian clock may modulate capacity through this signaling pathway, as these daily oscillations were markedly attenuated in CCM hearts (Durgan et al., 2010; Ko et al., 2011). Consistent with this concept, Pik3r1, encoding for the p85α regulatory subunit of phosphatidylinositol 3-kinase (PI3K; a critical component in the insulin signaling pathway), was recently reported to be a direct CLOCK:BMAL1 target gene in the heart (Young et al., 2014). Such observations have led to the hypothesis that the cardiomyocyte clock regulates myocardial insulin sensitivity in anticipation of feeding-fasting cycles. Although this is an attractive hypothesis, additional studies are required to elucidate fully the extent to which this mechanism modulates insulin signaling. It is noteworthy that evidence exists in support of the concept that extracardiac circadian clocks affect whole body insulin sensitivity (Rudic et al., 2004; Shi et al., 2013).

Time-of-day-dependent oscillations in cardiac sensitivity to β-adrenergic stimulation have been shown at multiple levels. For example, epinephrine stimulation of heart rate peaks during the dark phase (murine wake period) in wild-type murine hearts, a time-of-day-dependency that is absent in CCM hearts (Bray et al., 2008). Limited information is available regarding clock control of β-adrenergic signaling components, although circumstantial evidence exists. β-Adrenergic receptor density exhibits a daily variation in rat hearts, although whether this is mediated by the cardiomyocyte circadian clock has not been investigated (Witte et al., 1995). Microarray analyses have identified numerous β-adrenergic signaling cascade components as being differentially expressed in CCM and CBK hearts relative to littermate controls (e.g., prkar1a, encoding for the regulatory domain of protein kinase A); however, whether this is due to direct circadian clock regulation has yet to be determined (Bray et al., 2008; Young et al., 2014).
Cardiomyocyte Clock Control of Cardiac Contractility and Electrophysiology

Several lines of evidence support the concept that circadian clocks affect cardiac contractile function over the course of the day. Light increases heart rate in a dose-dependent manner in humans, and this effect of light on heart rate is dependent on the time of day of testing (Scheer et al., 1999). Daily heart rate rhythms are abolished in rats following bilateral lesions in the hypothalamic suprachiasmatic nucleus (SCN), the location of the central circadian clock (Saleh and Winget, 1977). Wake-time heart rate and amplitude of oscillations are reduced in both germ-line ClockK19/Δ19 and BMAL1 null mice (Curtis et al., 2007; Sei et al., 2008). Similarly, PER2 mutant mice lack the daily variations in heart function observed in wild-type mice (as assessed by echocardiography analyses) (Wu et al., 2011). A distinct role for the cardiomyocyte circadian clock in mediating time-of-day-dependent variations in cardiac contractility was highlighted through the use of CCM mice. These mice exhibit diminished light-dark variations in heart rate both in vivo and ex vivo as well as abolished time-of-day-dependent rhythms in contractile reserve (i.e., cardiac output at elevated workload) (Bray et al., 2008). Moreover, purified cardiac myofilaments from CCM hearts do not exhibit a rhythm in calcium sensitivity across the light-dark cycle (unlike wild-type littermate hearts) (Podobed et al., 2014a). More recently, Titin-cap (Tcap), a key component of the myocardial sarcomere structure that underlies cardiac contraction (Candasamy et al., 2014; Gregorio et al., 1998; Linke and Hamdani, 2014; Valle et al., 1997; Zou et al., 2006), was shown to be transcriptionally targeted by the core circadian mechanism proteins CLOCK and BMAL1, leading to its rhythmic expression at both the mRNA and protein levels in the heart (Podobed et al., 2014b). Taken together, these studies strongly suggest that the cardiomyocyte circadian clock directly regulates heart rate and contractility in a time-of-day-dependent manner.

Evidence has also emerged in support of the concept that the cardiomyocyte circadian clock directly influences cardiac electrophysiology. For example, Schroder et al. (2013) highlighted direct regulation of Scn5a (encoding for the voltage-gated Na+ channel NaV1.5) by the cardiomyocyte circadian clock. Concomitant with attenuated rhythms in Scn5a expression in iCSABm1a1 hearts, these mice exhibit bradycardia and decreased Na+ current in isolated ventricular myocytes (although only a single time of the day was investigated) (Schroder et al., 2013). Although several Ca2+ and K+ channels have been identified as being differentially expressed in CCM and CBK hearts (relative to littermate controls) (Bray et al., 2008; Young et al., 2014), to date no studies have reported Ca2+ and/or K+ transients in models of cardiomyocyte-specific circadian clock disruption. It is likely that once performed, such studies will reveal the extent to which the cardiomyocyte circadian clock affects cardiac ion homeostasis.

DISRUPTION OF NORMAL CIRCADIAN RHYTHMS AS A CAUSE OF HEART DISEASE

Jurgen Aschoff, widely considered to be a cofounder of the field of chronobiology, wrote in his 1965 Science publication, “Circadian Rhythms in Man,” that daily rhythmicity is a key component of our physiology. Furthermore, he provided insightful examples of applications for night shift workers, jet air travel, space crews, and military use and thoughts on manipulation of artificial zeitgebers to influence the circadian oscillator (Aschoff, 1965). While these examples were only theoretical or preliminary experiments at the time, they formed a basis for many fundamental studies in contemporary chronocardiology today, as described in more detail below (Figure 3).

Timing Considerations for the Onset and Severity of Adverse Cardiovascular Events

Adverse cardiovascular events are the leading cause of mortality in the Western world (Heart and Stroke Foundation 2014; Heran et al., 2011; Hunt et al., 2009; Rosamond et al., 2008), and a number of recent reports indicate that these events do not occur randomly throughout the day. For example, MI (Cohen et al., 1997; Goldberg et al., 1990; Kanth et al., 2013; Muller et al., 1985; Toffler et al., 1992; Toffler et al., 1990; Willich et al., 1991), stroke (Marler et al., 1989; Willich et al., 1989), angina (Cannon et al., 1997), ventricular tachyarrhythmia (Behrens et al., 1995; Eksik et al., 2007; Toffler et al., 1995) (as well as defibrillation energy requirements [Venditti et al., 1996] and ventricular refractoriness [Kong et al., 1995]), dissection or rupture of aortic aneurysms (Manfredini et al., 2004; Mehta et al., 2002; Sumiyoshi et al., 2002), and sudden cardiac death (Muller et al., 1987; Willich et al., 1992; Willich et al., 1987) exhibit a daily pattern, with the highest incidence in the early morning hours between ~0600 and 1200 h; the nadir occurs during sleep time between ~0300 and 0600 h. Moreover, there is a time-of-day-dependence on infarct size in patients with ST-segment elevation (i.e., MI), with worse outcomes around wake time, as compared with later in the day (Reiter et al., 2012; Suarez-Barrientos et al., 2011). It is postulated that sympathetic balance plays a causal role for the onset of a
number of these pathologies, as patients with autonomic nervous system dysfunction do not exhibit daily variations in the timing of MI onset (Fava et al., 1995; Kuniyoshi et al., 2008; Zarich et al., 1994). Hu and colleagues (2004) used a fractal analysis of heartbeat dynamics to support the notion that there is an endogenous circadian rhythm that influences the timing of adverse cardiac events. Similarly, the DP studies described earlier have highlighted circadian control of several neurohumoral factors known to increase cardiovascular risk, including plasminogen activator inhibitor-1 (PAI-1), a critical regulator of fibrinolysis (Scheer et al., 2010; Scheer et al., 2011; Scheer and Shea, 2014). Consistent with the concept that the onset of adverse cardiac events may be driven by endogenous circadian mechanisms, Couch (1990) reported that the incidence of sudden cardiac death was higher in the afternoon among visitors travelling to Kauai, compared with the normal early morning peak among Hawaiian residents. Thus, collectively these studies reveal striking time-of-day rhythms in the onset and severity of clinically relevant adverse cardiovascular events.

Circadian and Sleep Disturbances Cause Heart Disease in Humans

An important new frontier for circadian biology is its intersection with the field of sleep; the circadian clock may have a major influence on sleep. This is supported by the finding that mutations in genes encoding for circadian clock components are associated with human sleep syndromes, such as advanced phase sleep disorder, a condition in which patients go to sleep early in the evening and wake up extremely early in the morning (Jones et al., 1999; Toh et al., 2001; Xu et al., 2005). Although a direct link between the clock mechanism, sleep, and heart disease has not yet been investigated, the general notion of circadian-coupled factors influenced by disturbing sleep and mechanistically contributing to heart disease has been proposed. Sleep disturbance adversely affects our daily physiology, and this undoubtedly has detrimental consequences on the cardiovascular system, as described below.

A classic example is the common sleep-related breathing disorder obstructive sleep apnea (OSA), which can disturb the normal wake time and sleep time variation in sympathetic nervous activity, blood pressure, and cardiac vagal tone, leading to increased...
risk of heart disease and worse outcomes (Bradley and Floras, 2003, 2009; Floras, 2005; Marin et al., 2005; Martino and Sole, 2009). Conversely, maintaining daily physiology, such as with nocturnal treatment with continuous positive airway pressure (CPAP), reverses the disturbance and reduces the cardiovascular consequences (Gilman et al., 2008; Kaneko et al., 2003; Mansfield et al., 2004; Usui et al., 2005). At a molecular circadian level, CPAP also normalizes the impaired circadian clock gene expression profiles, as observed in blood cells of OSA patients (Burioka et al., 2008).

Several additional lines of evidence (in addition to OSA) support the notion that disturbing circadian rhythms and sleep has adverse effects on the cardiovascular system: (1) insomnia alone is associated with a moderate increase in MI risk (Laugsand et al., 2011); (2) insufficient sleep of short or long duration is associated with an increased all-cause mortality risk (Kripke et al., 1979; Wingard and Berkman, 1983), including risk of heart disease (Ayas et al., 2003); (3) evening chronotypes are more at risk for some cardiovascular changes in heart rate and blood pressure compared with morning chronotypes (Merikanto et al., 2013); and (4) later chronotypes also tend toward social jet lag, which refers to the misalignment of social and biological clocks, which could contribute to increased cardiovascular risk (Rutters et al., 2014). Interestingly, it has been shown that time-of-day-dependent gene expression oscillations in the murine heart change following fewer than 12 h of sleep deprivation (Anafi et al., 2013).

Shift Work and Heart Disease

The World Health Organization (2002) defines shift work leading to circadian disruption as a risk factor for human disease. This notion extends to heart disease and is supported by clinical studies (reviewed in Akerstedt and Knutsson, 1997; Akerstedt et al., 1984; Boggild and Knutsson, 1999; Knutsson and Boggild, 2000). For example, shift work is associated with an increased incidence of MI, ischemic stroke, and all coronary events, as based on a meta-analysis of 34 studies in 2,011,935 individuals (Vyas et al., 2012). Furthermore, an increased risk of coronary heart disease was found among 79,109 nurses with 6 or more years of rotating night shiftwork, compared with those who had never performed shift work (Kawachi et al., 1995). Similarly, an occupational health report in The Lancet revealed an association between shift work and ischemic heart disease, and this could be detected within the first 2 decades of shift work (Knutsson et al., 1986). Shift work is associated with physiologic consequences on the heart, including changes in cardiac sympathetic and vagal autonomic control (Furlan et al., 2000), perturbation of 24-h blood pressure profiles (Chau et al., 1989), and altered metabolic profiles (Karlsson et al., 2001). Experimentally, circadian misalignment in healthy humans can alter autonomic, endocrine, and metabolic predictors of cardiovascular risk (Scheer et al., 2009). Thus, given all of these findings, it is tempting to speculate that these disturbances contribute to the higher incidences of cardiovascular disease reported in shift workers.

Whole Body Light-Dark Misalignment and Animal Models of Heart Disease

The biological effects of light on the internal circadian clock mechanism profoundly influence cardiac physiology, and disturbing the light-dark cycle adversely affects the heart. For example, hearts isolated from mice following light-dark cycle manipulations no longer exhibit time-of-day-dependent variations in contractile performance ex vivo on a Langendorff apparatus, compared with mice housed on normal 24-h light and dark cycles (Podobed et al., 2014a). Moreover, deleterious effects on survival are seen in cardiomyopathic hamsters subjected to chronic shifts in the light-dark cycle, compared with littermate controls (Penev et al., 1998). Furthermore, altering the light-dark cycle worsens cardiac remodeling in the murine pressure-overload-induced cardiac hypertrophy model; the exacerbated remodeling ceases only once the animals are returned to their normal 24-h light-dark environment (Martino et al., 2007a). Thus, disturbing light-dark cycles affects cardiac function and worsens heart disease in murine models.

Disturbing Day-Night Rhythms Adversely Affects Remodeling after Myocardial Infarction

Alibhai et al. (2014) reported that short-term disruption of the daily light and dark environment worsens outcome in the murine MI model induced by left anterior descending coronary artery ligation (heart attack model). More specifically, altering the light and dark period for just the first 5 days post-MI impairs the healing process by altering innate inflammatory recruitment crucial for scar formation and ventricular remodeling (Alibhai et al., 2014). Even though the animals are returned to the normal light and dark cycle after just 5 days, the short-term disruption leads to a worse outcome 8 weeks after MI (as exemplified by infarct expansion, declining cardiac function, and altered myofilament profiles) (Alibhai et al., 2014). These observations have profound
clinical significance; environmental disruptions in intensive care units may impair recovery of patients (Buxton et al., 2012; Drouot et al., 2008; Patel et al., 2008). Reconsidering how we practice medicine by maintaining daily light and dark cycles and patients’ biohythms holds promise as a nonpharmacological approach to improve outcomes in acute care settings (Sharma and Canty, 2014; Stecker, 2014).

**Abnormal Entrainment in +/-tau Hamsters Causes Cardiomyopathy**

Entrainment to the light and dark cycle is also important for healthy cardiovascular physiology, and abnormal entrainment has profound adverse effects on the heart. This is well exemplified by the Tau Syrian hamsters, which were the first characterized mammalian genetic model of circadian clock function. The animals carry a naturally occurring mutation in the autosomal locus termed tau, which encodes for casein kinase I epsilon (CK1epsilon), a core component of the circadian clock mechanism (Lowrey et al., 2000). Indeed, the Tau hamsters originally helped define the SCN as the master circadian pacemaker (Ralph and Menaker, 1988). An important role in the maintenance of health and longevity has also been indicated in studies using these animals (Hurd and Ralph, 1998). That is, the +/-tau heterozygotes exhibit periods of ~22 h with abnormal entrainment and reduced longevity, compared with the wild-types that have free-running periods of ~24 h and normal entrainment and lifespan (Hurd and Ralph, 1998). This discordance between the external 24-h environment and the internal 22-h circadian period of the +/-tau hamsters causes dilated cardiomyopathy (Martino et al., 2008). Notably, only if +/-tau hamsters are housed on 22-h light-dark cycles consistent with their internal clock mechanism is the heart disease prevented (Martino et al., 2008). These data highlight that a failure to entrain properly, thereby preventing synchrony of the intrinsic circadian mechanism with the external light-dark environment, causes organ pathology, including cardiac disease.

**Genetic Models of Circadian Disruption and Heart Disease**

Although the circadian system has long been known to play a role in regulating major neural and endocrine pathways, it is only recently that circadian clock components have been identified along with sufficient technologies for murine genetic manipulation, thus enabling investigation of the direct consequences of disruption of this mechanism on cardiac physiology and pathophysiology. Experimental studies demonstrating that the circadian clock mechanism plays a causal role in cardiovascular disease are described in more detail below.

**Germline Circadian Clock Manipulation Leads to Heart Disease in Mice**

A role for the circadian mechanism in a variety of clinically relevant cardiovascular conditions has been studied in mice, using transgenic, mutant, and knockout models. For example, mice lacking Bmal1 (Bmal1–/-) develop dilated cardiomyopathy, with reduced cardiac contractility, and sarcomeric disorganization at the cellular level (Lefta et al., 2012). In addition, simultaneous knockout of the clock output transcription factors DBP, HEF, and TEF results in hypertrophic cardiomyopathy (Wang et al., 2010). A role for the circadian mechanism on modulation of acquired heart disease has also been suggested. Virag et al. (2013, 2010) reported that functional deletion of the gene Period 2 in mice attenuated cardiac injury following both experimentally induced MI (i.e., permanent ligation of the left anterior descending coronary artery) and ischemia-reperfusion (I/R), implicating both reduced inflammation and preservation of mitochondrial function in cardioprotection. In contrast, Eckel et al. (2012) reported reduced tolerance of Period 2 null mice to I/R, highlighting a need for future studies to dissect the functional contribution of this clock component toward cardioprotection. Studies investigating not only disruption of Period 2 but also CLOCK mutant (ClockΔ19/Δ19) and Bmal1−/− mice have associated circadian clock disruption with metabolic pathologies (Carvas et al., 2012; Lefta et al., 2012; Marcheva et al., 2010). These later studies are also of particular clinical interest given that metabolic disorders, such as diabetes (Kannel and McGee, 1979), obesity (Eckel and Krauss, 1998), and metabolic syndrome (Lakka et al., 2002), are risk factors for heart disease.

**Cardiomyocyte-Specific Circadian Clock Disruption and Heart Disease**

As highlighted in previous sections, delineating the causal role of the circadian mechanism on the heart has been approached using sophisticated genetic manipulations in a tissue-specific manner. Through the use of both CCM and CBK mouse models, important roles for the cardiomyocyte circadian clock in cardiac pathology have been revealed. For example, CBK mice develop an age-onset dilated cardiomyopathy and decreased lifespan (Young et al., 2014), comparable to the phenotype observed in germline BMAL1 null mice (Lefta et al., 2012) and
consistent with the concept that BMAL1 plays critical roles in cardiac form and function. CCM mice, like CBK mice, are predisposed to development of cardiac hypertrophy, although they do not transition to cardiac dilatation or systolic dysfunction (Durgan et al., 2011b). Both models also exhibit increased cardiac expression of fetal genes, such as mhcβ and anf, reminiscent of remodeling (Durgan et al., 2011b; Young et al., 2014). In contrast, the substrate reliance pattern typically observed in the fetal and remodeled heart (i.e., increased glucose reliance concomitant with decreased fatty acid reliance) is not recapitulated in CCM and CBK hearts, suggesting that the cardiomyocyte circadian clock differentially affects “fetal versus adult” processes (Bray et al., 2008; Young et al., 2014). However, evidence exists in support of the concept that the cardiomyocyte circadian clock modulates responsiveness of the heart to adverse stresses, in terms of remodeling and fetal gene expression. For example, wild-type mice exhibit a time-of-day-dependent variation in isoproterenol-induced cardiac growth (i.e., hypertrophy) and anf induction that is dependent on the cardiomyocyte circadian clock (i.e., lack of oscillation in CCM mice) (Durgan et al., 2011b). CCM mice have also been used to investigate oscillations in I/R tolerance. Infarct (or scar) size is dependent on the time of day at which I/R occurs in wild-type mice; hearts with I/R at the sleep-to-wake transition have larger infarcts and worse outcomes (Durgan et al., 2010). In contrast, daily variations in I/R tolerance are attenuated in CCM mice (Durgan et al., 2010). Collectively, these observations suggest that the cardiomyocyte circadian clock influences responsiveness of the heart to pathological stresses.

**FUTURE DIRECTIONS**

In light of the aforementioned discoveries, what questions remain unanswered? In terms of the cardiomyocyte circadian clock, the full extent by which this mechanism influences cardiac physiology and pathophysiology remains incomplete. Use of murine models of genetic manipulation, including whole-body, cell-specific, and inducible transgenics and knockouts, will undoubtedly provide considerable insight with regard to not only the cardiac processes governed by the cardiomyocyte circadian clock but also the mechanisms by which this occurs. Specific questions might include these: (1) To what extent does the cardiomyocyte circadian clock influence protein turnover in the heart? (2) What extracellular stimuli does the heart anticipate on a daily basis? (3) By what mechanisms does cardiomyocyte circadian clock disruption (either genetically or environmentally induced) lead to heart disease?

(4) Does the cardiomyocyte circadian clock contribute to increased arrhythmias at the beginning of the active phase? (5) How does the cardiomyocyte circadian clock modulate responsiveness of the heart to adverse stresses, such as pressure overload or ischemic events? (6) How is the cardiomyocyte circadian clock regulated (e.g., what zeitgebers entrain the cardiomyocyte circadian clock)? (7) In what manner is the cardiomyocyte circadian clock altered during disease states? With regard to modulation of the cardiomyocyte circadian clock, relatively little is known. To date, both electrical stimulation and dexamethasone have been shown to influence function of the clock in isolated cardiomyocytes, while clock gene expression oscillations have been reported to be altered in the heart in animal models of restricted feeding, diabetes, MI, and aging (Bray et al., 2013; Durgan et al., 2011b; Kung et al., 2007; Qi and Boateng, 2006; Young et al., 2002; Young and Bray, 2007). Whether alterations in clock function contribute to the etiology of cardiac disease remains unknown.

Significant opportunity also exists to integrate circadian-cardiovascular biology with other major physiologic systems. For example, experiments investigating circadian regulation of the cardiorespiratory axes are virtually unknown. These would be of particular interest to the study of heart and lung disease, given the profound association between circadian and sleep disruption (e.g., obstructive sleep apnea) and heart failure (Bradley and Floras, 2003, 2009; Floras, 2005; Marin et al., 2005; Martino and Sole, 2009; Sole and Martino, 2010). Integration with the renal system could also be interesting in light of the well-documented clinical significance of daily rhythms on hypertension (Ohkubo et al., 2002; Verdecchia et al., 1993). Also, kidney disease is considered an independent risk factor for cardiovascular disease (Chobanian et al., 2003; Sarnak et al., 2003). An important role for the circadian clock mechanism in the structure and function of the renal system has been demonstrated (Gumz et al., 2009; Richards et al., 2014).

In terms of clinical translation, the opportunity exists to design de novo biomarker tests based on the discoveries from microarray, proteomics, mass spectrometry, and other types of high-throughput data generated over a 24-h period. Indeed, proof-of-concept studies have already revealed the potential for novel day and night differences in gene and protein biomarkers of heart disease (Martino et al., 2007b; Tsimakouridze et al., 2012). Measuring body-time-of-day will significantly facilitate the translation of functional “omics” to personalized medicine (Kasukawa et al., 2012; Minami et al., 2009; Ueda et al., 2004). Minor modifications of these preclinical studies will also readily increase feasibility for routine clinical assessments in humans: for example, by sampling blood instead of tissue, and through the design
of simple point-of-care genetic and/or protein- or ELISA-based tests that can be used in clinicians’ offices or by patients at home. Extension to investigate the cardiac metabolome, lipidome, or changes in the breathalome could also be interesting and warrants future investigation. Implementation could well be served by mission statements in academia, product development by the biotechnology sector, and medically perhaps through chronobiology clinics.

Finally, with regard to new therapeutic approaches, several studies have demonstrated that timing of therapy (chronotherapy or chronopharmacology) offers significant promise. Examples include the following: (1) Martino et al. (2011) showed that administration of the short-acting angiotensin-converting enzyme inhibitor (ACEi) captopril at sleep time has direct beneficial effects on the heart. That is, administration at the beginning of the light period (murine rest time) significantly reduced adverse cardiac remodeling in mice subjected to pressure overload-induced cardiac hypertrophy. Conversely, ACEi given at wake time is not different compared with placebo (Martino et al., 2011). (2) As mentioned above, CPAP therapy benefits patients with OSA and heart disease, and this is administered at night (e.g., Arias et al., 2005; Kaneko et al., 2003; Tkacova et al., 1998; Usui et al., 2005. (3) In another example, hemodialysis patients with renal disease have improved heart function when treated at night compared with conventional daytime therapy (Chan et al., 2002; Culleton et al., 2007). (4) Bonten et al. (2014) reported that administration of low-dose aspirin at night reduces the circadian rhythm of platelet reactivity the following morning, which may coincide with a reduced risk of acute MI during the early morning hours. (5) Several investigators have demonstrated that administration of blood pressure medications at night restores nocturnal blood pressure profiles, and this is associated with reduced cardiovascular risk (e.g., reviewed in Hermida et al., 2013a; Hermida et al., 2014; Hermida et al., 2013b; Smolensky et al., 2010). It is important to note that many pharmacological agents, including World Health Organization essential medicines, have short half-lives and therefore will potentially benefit from chronotherapeutic application (Zhang et al., 2014). Thus, therapeutic consideration of physiologic and molecular rhythms offers significant promise for cardiovascular (and other) disease treatments in clinical settings.

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