Clock Genes in the Heart
Characterization and Attenuation With Hypertrophy

Martin E. Young, Peter Razeghi, Heinrich Taegtmeyer

Abstract—We investigated whether the heart, like other mammalian organs, possesses internal clocks, and, if so, whether pressure overload–induced hypertrophy alters the clock mechanism. Clock genes are intrinsically maintained, as shown by rhythmic changes even in single cells. Clocks are believed to confer a selective advantage by priming the cell for the expected environmental stimulus. In this way, clocks allow anticipation, thereby synchronizing responsiveness of the cell with the timing of the stimulus. We have found that in rat heart all mammalian homologues of known *Drosophila* clock genes (*bmal1*, *clock*, *cry1*, *cry2*, *per1*, *per2*, *per3*, *dbp*, *hlf*, and *tef*) show circadian patterns of expression and that the induction of clock output genes (the PAR [rich in proline and acidic amino acid residues] transcription factors *dbp*, *hlf*, and *tef*) is attenuated in the pressure-overloaded hypertrophied heart. The results expose a new dynamic regulatory system in the heart, which is partially lost with hypertrophy. Although the target genes of these PAR transcription factors are not known in the heart, the results provide evidence for a diminished ability of the hypertrophied heart to anticipate and subsequently adapt to physiological alterations during the day. **(*Circ Res*. 2001;88:1142-1150.)**

Key Words: anticipation ■ circadian rhythms ■ rat

All mammalian cells investigated to date seem to possess internal circadian clocks.[1] Such clocks are even present when isolated cells from peripheral tissues are cultured, suggesting that the basic clock mechanism is intrinsic and self-sustained, allowing the cell to anticipate and integrate changes in its normal environment.[1–4] As the culture is maintained, the amplitude of the clock diminishes (followed by gene expression markers), as does the timekeeping of the duration of each cycle.[3] However, reintroduction of serum to the culture normalizes the clock, suggesting that one or more humoral factors act as a zeitgeber (timekeeper).[5] There are three major components of biological clocks. These are (1) input signals, such as light in the case of the mammalian eye or humoral factors for peripheral tissues; (2) the clock mechanism itself; and (3) the output genes.

The heart responds to both acute and chronic changes in its environment. Acutely, it responds to alterations in workload, hormones, and fuel supply, through allosteric, covalent, and topographical alterations in preexisting proteins.[6–9] Chronically, the heart responds to sustained alterations in these same environmental factors, by the synthesis of new proteins, through both transcriptional and posttranscriptional mechanisms.[10,11] For example, the heart responds to sustained pressure overload by increasing the size of the cardiomyocytes (hypertrophy), a process requiring changes in both gene expression and protein synthesis.[12] How the responsiveness of the myocardium to physiological stimuli alters during the day has not previously been considered.

Intrinsic circadian rhythms have not been characterized in the heart at the transcriptional level. We therefore set out to investigate, in the rat heart, the level of gene expression of all of the major components involved in these rhythms over a 24-hour period. Because circadian rhythms are potentially involved in the daily adaptation of the heart, we hypothesized that the same factors might be involved in the development of hypertrophy (adaptation to sustained pressure overload). We therefore investigated whether circadian rhythms are altered in the hypertrophied heart and found that the rhythms of the major genes involved in the clock mechanism, with the exception of the period genes, were not affected by pressure overload–induced hypertrophy. In contrast, the output genes (and the period genes to a lesser extent) showed impairment in the level of induction during the light period. Pressure overload had no effect on the periodicity of the rhythms for any of the genes investigated. The results provide evidence for a diminished ability of the hypertrophied heart to anticipate and subsequently adapt to physiological alterations during the day.

Materials and Methods

Aortic Constriction

The care and use of animals followed the guidelines of the Animal Welfare Committee at the University of Texas–Houston Health Science Center. Cardiac hypertrophy was induced in male Wistar rats (160 to 210 g initial weight) by constriction of the ascending

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Primer and Probe Sequences Used in Real-Time Quantitative RT-PCR

Gene	Primers/Probe	Sequence
β-actin	Forward	5′-CCTCTGAACCCTAAGGCCAA-3′
	Reverse	5′-AGCCTGGATGGCTACGTACA-3′
	Probe	5′-FAM-TGACCCAGATCATGTTTGAGACCTTCAAC-TAMRA-3′
anf	Forward	5′-AGTGCGGTGTCCAACACAG-3′
	Reverse	5′-CTTCATCGGTCTGCTCGCT-3′
	Probe	5′-FAM-TCTGATGGATTTCAAGAACCTGCTAGACCAC-TAMRA-3′
bmal1	Forward	5′-TCCGATGACGAACTGAAACAC-3′
	Reverse	5′-CTCGGTCACATCCTACGACAA-3′
	Probe	5′-FAM-CAAAAATCCATCTGCTGCCCTGAGAAT-TAMRA-3′
clock	Forward	5′-GAACTTGGCGTTGAGGAGTCT-3′
	Reverse	5′-GTGATCGAACCTTTCCAGTGC-3′
	Probe	5′-FAM-AGACAGCTGCTGACAAAAGCCAAGATTCTG-TAMRA-3′
cry1	Forward	5′-CATCAACAGGTGGCGATTTT-3′
	Reverse	5′-CCCGAATCACAAACAGACGA-3′
	Probe	5′-FAM-TTTAATTTTCGTAGATTGGCATCAAGATCCTCAAGA-TAMRA-3′
cry2	Forward	5′-GTGTGAATGCAGGCAGCTG-3′
	Reverse	5′-ACAGGGCAGTAGCAGTGGAA-3′
	Probe	5′-FAM-ATGTGGCTGTCCTGCAGTGCTTTCTTC-TAMRA-3′
per1	Forward	5′-GGTTCAGGATCCCACGAAG-3′
	Reverse	5′-AAGAGTCGATGCTGCCAAAG-3′
	Probe	5′-FAM-AGCACCTCAGCCAGCATCACCC-TAMRA-3′
per2	Forward	5′-GCAGCCTTTCGATTATTCTTC-3′
	Reverse	5′-GCTCCACGGGTTGATGAAG-3′
	Probe	5′-FAM-ATTCGATTCCGCACACGCAACG-TAMRA-3′
per3	Forward	5′-CCAGGATGTGTGTTTCTTGAAGTAG-3′
	Reverse	5′-GGTGTATGGCAACCATCAGAG-3′
	Probe	5′-FAM-AGCAGTGCCTTTGCTGGGTTACCTACCT-TAMRA-3′
dbp	Forward	5′-AGGCAAGGAAAGTCCAGGTG-3′
	Reverse	5′-TCTTGCGTCTCTCGACCTCTT-3′
	Probe	5′-FAM-CCGAGGAACAGAAGGATGAGAAGTACTGGAG-TAMRA-3′
hlf	Forward	5′-CTCGCAAACGGAAGTTCTCC-3′
	Reverse	5′-TGGCCGCCATATTGTTCTT-3′
	Probe	5′-FAM-CCCAGTACTTGTCATCCTTCAAATCATCAGG-TAMRA-3′
tef	Forward	5′-ACCGTGTCCAGCACAGAATC-3′
	Reverse	5′-CATCAACCTCCACACAGTTGG-3′
	Probe	5′-FAM-TGGAAAAGGAGAGGGAGACACCAAGTCC-TAMRA-3′

aorta, as described previously.[13] The diameter of the lumen of the aorta after constriction was equivalent to that of an 18-gauge needle. In control animals, sham operations were performed without constriction of the aorta. As gene expression can be affected by multiple factors, including physical activity, hormone levels, and food intake, animals were allowed to recover from surgery for 7 weeks. During this time, the initial modest elevation in pressure on the heart using an 18-gauge needle for the banding procedure increased as the animals grew (310 to 430 g final weight). This more gradual increase in pressure on the heart with age is more akin to the situation in humans, as opposed to an acute, dramatic pressure overload. A total of 108 animals were studied, of which 55 were controls and 53 were banded.

One week before they were euthanized, rats were housed in a separate environment-controlled room, in which a strict 12-hour light/12-hour dark cycle regimen was enforced (lights on at 7 AM; zeitgeber time [ZT] 0). On the day of the experiment, rats were killed every 3 hours from 7 AM to 7 AM the following day. To ensure the reproducibility of the gene expression cycles, at least two control and two banded animals were killed at each time point on 3 separate days. After administration of pentobarbital (100 mg/kg IP), hearts were rapidly isolated, freeze-clamped in liquid nitrogen, and stored at −80°C before RNA extraction.

RNA Extraction and Quantitative Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR)

RNA extraction and quantitative RT-PCR of samples was performed using previously described methods.[10,14–16] Specific quantitative assays were designed from rat sequences available in GenBank (Table), except in the cases of *cry1*, *cry2*, *per1*, and *per3*, for whom the rat sequences were not available. In contrast, these genes have been cloned in the mouse. Multiple assays were then designed using

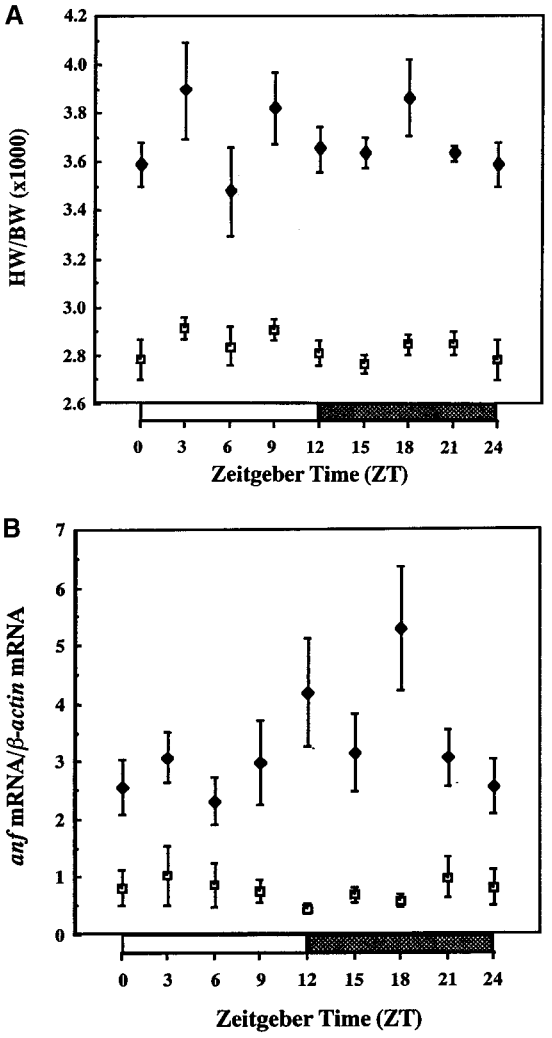

Figure 1. Effects of 7 weeks of aortic constriction on HW/BW ratio (A) and *anf* expression (B) during a 24-hour period. Values are mean±SEM for 6 to 8 observations. All gene expression values are normalized against the housekeeping gene product *β-actin*. $P<0.05$ for all comparisons between control (□) and banded (◆) animals at the same zeitgeber time.

the mouse sequence for these genes and subsequently tested for compatibility in the rat. Primers and probes were designed from nonconserved sequences of the genes (allowing for isoform specificity), spanning sites where two exons join (splice sites) when such sites are known (preventing recognition of the assay to any potential contaminating genomic DNA). Standard RNA was made for all assays by the T7 polymerase method (Ambion), using total RNA isolated from the rat heart. The correlation between the C_t (the number of PCR cycles required for the fluorescent signal to reach a detection threshold) and the amount of standard was linear over at least a 5-log range of RNA for all assays (data not shown). The level of transcripts for the constitutive housekeeping gene product *β-actin* was quantitatively measured in each sample, to control for sample-to-sample differences in RNA concentration. PCR data are reported as the number of transcripts per number of *β-actin* molecules.

Statistical Analysis

Data are presented as the mean±SEM for between six and eight hearts in each group. Statistically significant differences between groups were calculated by the Student *t* test between control and hypertrophied hearts at the same zeitgeber time. A value of $P<0.05$ was considered significant.

Results

Aortic Constriction Induces Both Trophic and Gene Expression Markers of Hypertrophy

Seven weeks after the initial surgery, the heart weight–to–body weight (HW/BW) ratio was increased by 30% (Figure 1A). This increase was due to increased heart weight as opposed to fluctuations in body weight (data not shown). The HW/BW ratio did not undergo obvious rhythmic changes during the day (Figure 1A).

Pressure overload is known to cause the reexpression of several fetal genes in the heart, including atrial natriuretic factor (*anf*). This genetic marker of hypertrophy was elevated at all time points investigated in pressure-overloaded hearts (Figure 1B). The average level of *anf* expression throughout the 24-hour period was 4.6-fold greater in the pressure-overloaded hearts. There was a trend for a steady increase in *anf* expression during the dark phase in control animals, with a subsequent decrease during the light phase. This trend was not observed in the pressure-overloaded hearts.

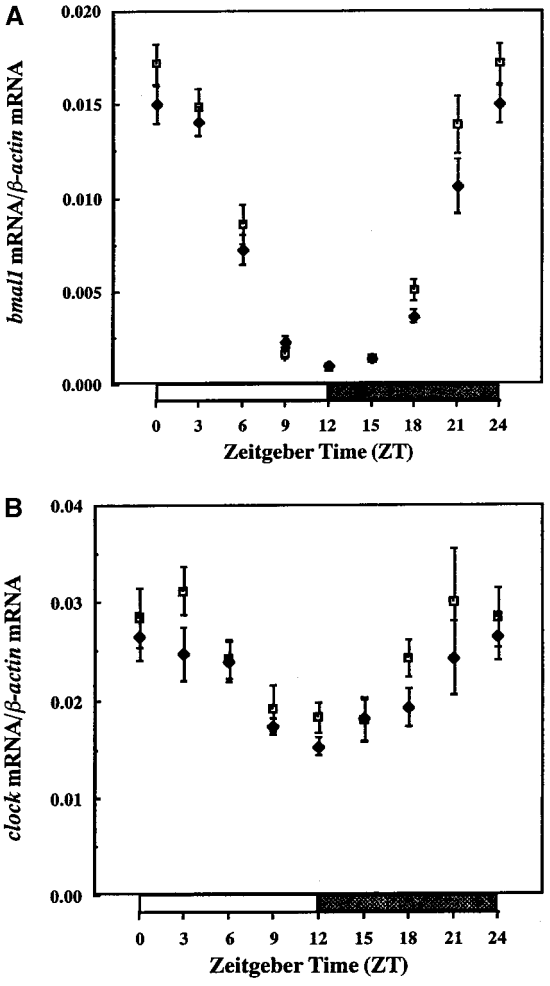

Figure 2. Circadian expression of *bmal1* (A) and *clock* (B) in control and hypertrophied hearts. Values are mean±SEM for 6 to 8 observations. All expression values are normalized against the housekeeping gene product *β-actin*. There were no statistically significant differences for comparisons between control (□) and banded (◆) animals at the same zeitgeber time.

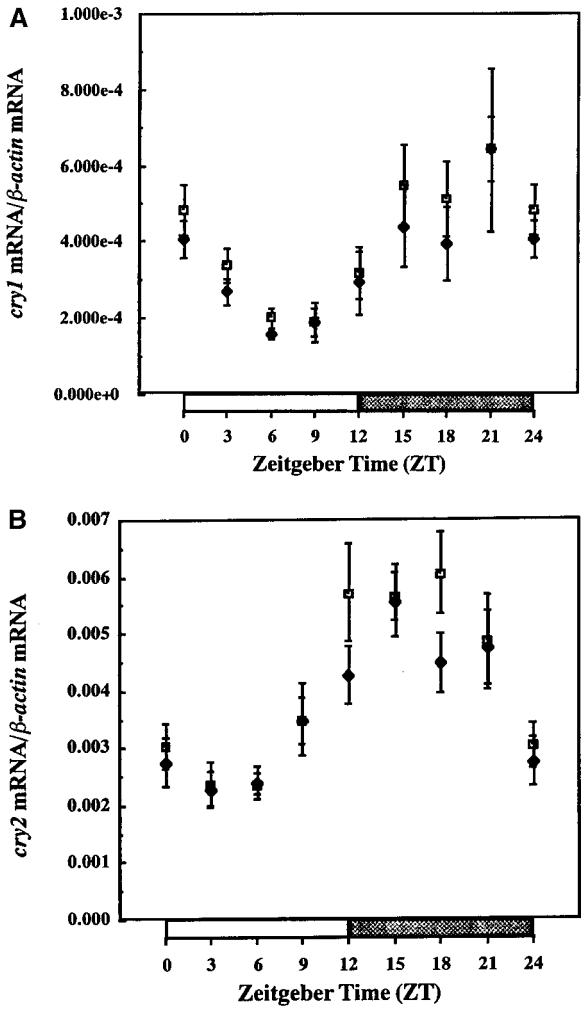

Figure 3. Expression levels of *cry1* (A) and *cry2* (B) in control and hypertrophied hearts during the day. Values are mean±SEM for 6 to 8 observations. All expression values are normalized against the housekeeping gene product *β-actin*. There were no statistically significant differences for comparisons between control (□) and banded (◆) animals at the same zeitgeber time.

Expression of Clock Genes in Normal and Hypertrophied Hearts

Mammalian homologues of known *Drosophila* clock components include CLOCK, BMAL1 (brain and muscle ARNT-like protein 1), PER (periods 1, 2, and 3), and CRY (cryptochromes 1 and 2).[1,2,17–22] Using real-time quantitative RT-PCR, we compared the rhythms of the mRNA encoding for these proteins in control and hypertrophied hearts. *bmal1*, a basic helix-loop-helix/PER-ARNT-SIM (bHLH/PAS) transcription factor, undergoes striking circadian rhythms in the heart (Figure 2A). There was a 19-fold induction of *bmal1* during the day (from peak [ZT0] to trough [ZT12]). There were no differences in the level of *bmal1* expression between control and hypertrophied hearts, when compared at the same time points. BMAL1 heterodimerizes with a second bHLH/PAS transcription factor, CLOCK. *clock* expression, like that of *bmal1*, undergoes a circadian rhythm in the heart, albeit less dramatically (2-fold from peak [ZT0] to trough [ZT12]; Figure 2B). No significant difference in the level of *clock* expression was observed between control and hypertrophied hearts.

We investigated the expression rhythms of *cry* (1 and 2) and *per* (1, 2, and 3) mRNA in control and hypertrophied hearts. Unlike *bmal1* and *clock*, *cry1* and *cry2* appear to have slightly different rhythms compared with one another (Fig-

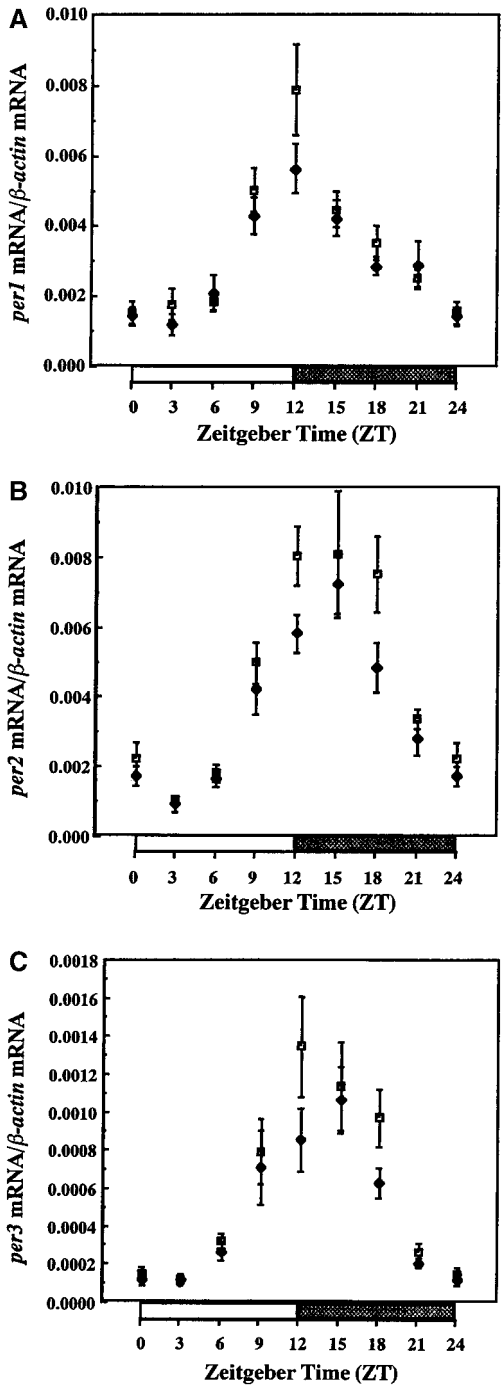

Figure 4. Comparison of *per1* (A), *per2* (B), and *per3* (C) expression between control and hypertrophied hearts during a 24-hour period. Values are mean±SEM for 6 to 8 observations. All expression values are normalized against the housekeeping gene product *β-actin*. $P<0.05$ at ZT12 and ZT18 for *per2*, and $P<0.05$ at ZT18 for *per3*, for comparisons between control (□) and banded (◆) animals at the same zeitgeber time.

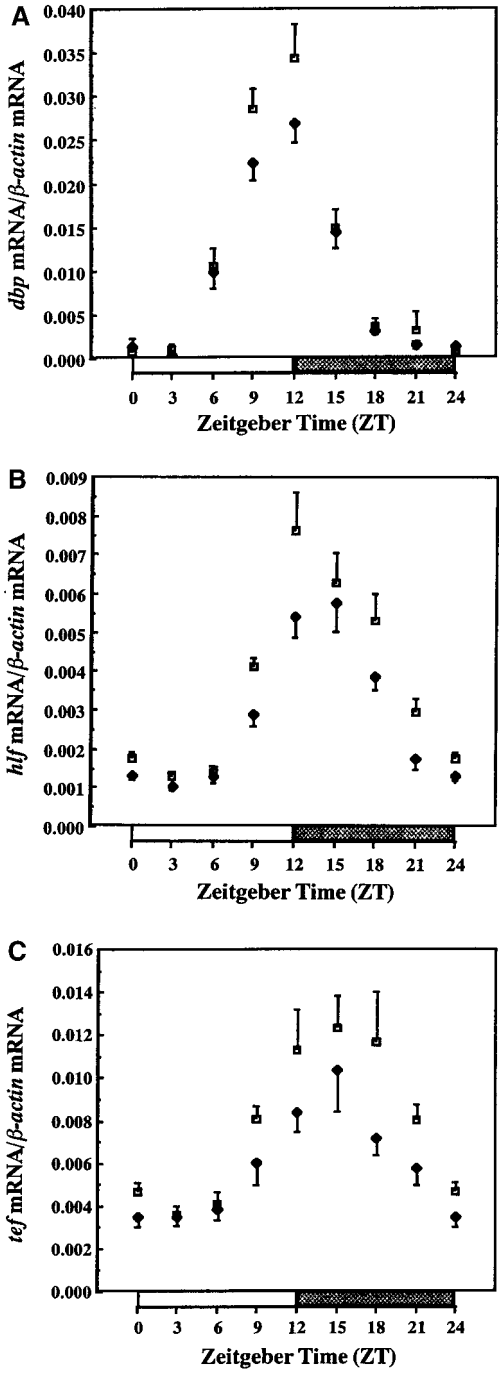

Figure 5. Impaired induction of *dbp* (A), *hlf* (B), and *tef* (C) PAR transcription factors in the hypertrophied heart during the day. Values are mean±SEM for 6 to 8 observations. All expression values are normalized against the housekeeping gene product *β-actin*. $P<0.05$ at ZT9 for *dbp*; at ZT9, ZT12, ZT18, and ZT21 for *hlf*; and at ZT21 for *tef*, for comparisons between control (□) and banded (◆) animals at the same zeitgeber time.

ures 3A and 3B). *cry1* expression has a peak at ZT21 and a trough at ZT9, whereas *cry2* has an earlier peak at ZT15 and a trough at ZT3. The induction of both *cry1* and *cry2* was 3-fold. No significant differences were observed between control and hypertrophied hearts. The circadian rhythms of the 3 *per* gene isoforms were very similar to one another, with peaks at ZT12/15 and troughs at ZT0/3 (Figures 4A through 4C). The levels of induction of *per1*, *per2*, and *per3* were 5-, 8-, and 10-fold, respectively. In all three cases, there was a trend for decreased induction of the *per* genes in the hypertrophied heart, with no differences in expression at the basal (trough) levels.

Impaired Induction of Clock Output Genes in the Hypertrophied Heart

Clock output genes include the PAR (rich in proline and acidic amino acid residues) transcription factors *dbp* (albumin D-element binding protein), *hlf* (hepatic leukocyte factor), and *tef* (thyrotrophic embryonic factor).[23–25] The circadian rhythms of the genes for these three PAR factors are very similar, with peaks at ZT12/15 and troughs at ZT0/3 (*tef* appears to have a slightly later peak compared with *dbp* and *hlf*; Figures 5A through 5C). The levels of induction of *dbp*, *hlf*, and *tef* were 41-, 6-, and 3- fold, respectively. There was an impairment in the level of induction of these transcription factors in the hypertrophied heart compared with the control hearts. No differences were observed in the basal levels of expression.

Discussion

The present study exposes a new dynamic system of transcriptional regulation in the heart, which is summarized in Figure 6A. In a normal heart, the level of gene expression changes dramatically within a 24-hour period. Many of the genes we examined are transcription factors, with the potential to alter the expression of a large number of downstream target proteins. These transcription factors include BMAL1, CLOCK, DBP, HLF, and TEF. The identity of the target gene promoters to which these *trans*-acting factors bind remains relatively unknown, particularly in the case of the latter three PAR factors. In the heart, this family of transcription factors has not been investigated previously.

In addition to full characterization of the primary factors known to be involved in cellular clocks, we were interested in how such components, which are involved in the daily adaptation of the heart, might be altered in a chronically adapted heart, in the face of pressure overload. We found that the components of the clock mechanism itself were little affected in the hypertrophied heart. In contrast, known clock output genes (ie, the PAR transcription factors investigated) showed a significant impairment of induction during the 24-hour period. Whether this impairment of induction is amplified further in the target genes of these transcription factors, and whether these targets are directly involved in the development of hypertrophy, is unknown. Thus, in pressure overload–induced hypertrophy, the heart has partially lost its ability to anticipate environmental changes.

Cardiac Biological Clocks

Two key transcription factors involved in circadian rhythms, BMAL1 and CLOCK, both undergo rhythms during the day in the heart (Figure 2). In the case of BMAL1, the present results are consistent with previous studies.[26] However, whether the *clock* gene undergoes circadian rhythms in peripheral tissues is less clear.[1] Using real-time quantitative

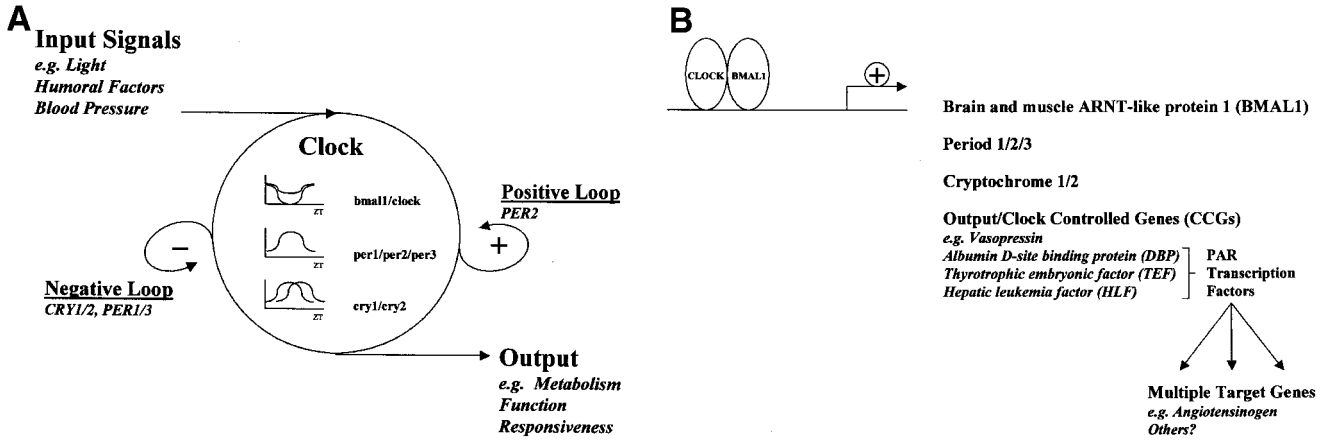

Figure 6. Intracellular biological clocks. A, Major components of biological clocks described in text. The self-sustained clock mechanism itself consists of interacting positive and negative loops, resulting in rhythmic expression of clock genes. The positive loop involves translocation of PER2 into the nucleus, which promotes BMAL1/CLOCK transcriptional activity. The latter increases expression of components of the negative loop (PER1/3 and CRY1/2), thereby reducing transcriptional activity of this heterodimer. Intracellular clocks are reset by external cues, allowing environmental communication. These input signals (zeitgebers) include light (eyes), humoral factors (hormones and fuels), and blood pressure. Output from the clock can affect metabolism, function, and responsiveness of the cell. B, Summary of the genes that are under transcriptional control of the BMAL/CLOCK heterodimer. See text for discussion.

RT-PCR, a highly sensitive technique, we have found that *clock* expression in the heart does indeed show a rhythmic pattern, in the same phase as *bmal1*, albeit at lower amplitude. Furthermore, the levels of expression of these two bHLH/PAS transcription factors are similar at their maximal levels (ZT0). These two factors form a transcriptionally active heterodimer.[19] The near equal level of expression of these two factors at ZT0 will promote effective dimerization. However, as the cycle progresses, the level of *bmal1* expression diminishes to a greater extent than that of *clock* and therefore becomes limiting.

The BMAL1-CLOCK heterodimer binds to *cis*-acting elements (E-boxes) in the promoter region of various target genes, including *per*, *cry*, and *bmal1* itself (Figure 6B).[19,27] Once synthesized, PER and CRY isoforms translocate into the nucleus and generally act as repressors of BMAL1-CLOCK heterodimer function.[28] Such repression might be achieved by direct binding to the heterodimer components, binding to the E-box sequence, or binding to as-yet-unidentified cofactors. In the case of *Drosophila*, a protein named timeless (TIM) has been identified that binds to the PERs and may be involved in the translocation and/or repression mechanism mentioned above.[29,30] A mammalian TIM homologue was believed to be found, which has been shown to be permanently located in the nucleus.[28,31] However, recent reports suggest that this gene is not the true mammalian homologue to *tim*, and therefore it was not investigated in the present study.[32] The *per* and *cry* gene products therefore form a negative feedback loop, resulting in the downregulation of their own expression (Figure 6A). With the exception of *cry1*, the phases of these transcriptional modulators are very similar to one another and are opposite to those of both *bmal1* and *clock*. Thus, when the expression of *bmal1* and *clock* is at a maximum, the expression levels of *per1*, *per2*, *per3*, and *cry2* are at their lowest (Figure 6A).

Cryptochromes form a second class of photoreceptor found in the mammalian eye (the other being rhodopsin).[33] These proteins are putative vitamin B_2–based photopigments that contain a pterin as a chromophore; cryptochromes have been hypothesized to be involved in photoentrainment.[33] Indeed, mutation of *cry1* and *cry2* in mice results in a complete loss of circadian rhythmicity in wheel-running behavior, in addition to elevated arrhythmic levels of *per1* and *per2*.[34,35] However, the circadian rhythms of these mutant mice still have light sensitivity, suggesting that cryptochromes are not the only protein involved in photoentrainment.[35] The heart of the semitransparent zebrafish responds directly to light, through cryptochromes.[4] Despite the fact that mammals are opaque and peripheral tissues are not normally exposed to light, cryptochromes have been shown to be expressed in multiple internal organs, including the heart (although their circadian oscillations have not been investigated previously in this tissue).[33] Period gene products are believed to bind to and result in the translocation of the cryptochromes into the nucleus, where the latter inhibit the transcriptional activity of BMAL1-CLOCK (see above). The nature of the signal for cryptochrome activation in peripheral tissues is unknown. Evidence suggests that phosphorylation and/or degradation of PER proteins might be involved in clock modulation.[1,2] Phosphorylation of PER is catalyzed by casein kinase I ϵ (CKIϵ), the mammalian homologue to *Drosophila* DOUBLE-TIME, the mutation of which results in arrhythmia.[36] We speculate that factors (zeitgebers) affecting the activity of CKIϵ are involved in peripheral clock resetting.

The separation in the phases of cardiac *cry1* and *cry2* expression by 6 hours gives rise to several exciting hypotheses. For example, whether CRY1 and CRY2 differentially suppress the binding of the BMAL1-CLOCK heterodimer to different promoter regions is one possibility. As *cry2* expression is 10-fold greater than that of *cry1* in the heart, and given that the periodicity of *cry2* expression is in rhythm with the *per* genes, and opposite to *bmal1* and *clock*, it is likely that CRY2 is involved more in the negative feedback loop mechanism in the heart than is CRY1. Whether CRY1 is

involved in the repression of other genes or differentially modulates the PER isoforms is unknown. For example, PER2 is believed to promote *bmal1* expression, as part of the *bmal1* reexpression stage of the clock (positive loop; Figure 6A).[32] It is possible that CRY1 preferentially binds to PER2, resulting in subsequent translocation to the nucleus later than PER1 and PER3 and allowing for delayed *bmal1* induction. Alternatively, CRY1 and CRY2 may compete for PERs, and the later induction of CRY1 may displace CRY2 from its PER partner, altering its function at a specific moment in the cycle.

The BMAL1-CLOCK heterodimer can also activate the transcription of a number of clock-controlled genes (or output genes; Figure 6B). Clock-controlled genes identified to date include vasopressin and the family of PAR transcription factors *dbp*, *hlf*, and *tef*.[23–25,31] The latter three showed an expression pattern similar to that of the *per* genes and *cry2*. The maximal levels of expression of *hlf* and *tef* were similar to one another and were approximately one third that of *dbp*. Very little is known concerning the identity of the target genes of this family of PAR transcription factors. In extracardiac tissues, *dbp* has been shown to regulate angiotensinogen, steroid 15α-hydroxylase, and coumarin 7-hydroxylase expression.[37,38] No targets have been identified in the heart as yet. Clearly these transcription factors, of which the level of expression can change up to 40-fold within 1 day, must have physiologically relevant targets in cardiomyocytes.

Adaptation of the Heart

The mechanisms by which the heart adapts to various stimuli has been an intense area of research. Factors known to affect the myocardium include humoral factors (such as substrate availability and hormones), neurohormones, and mechanical stress. All of these factors change during a single day. Food intake, hormones, sympathetic tone, blood pressure, and physical activity are all known to be altered at different times in the day. Despite this, very little is known concerning the adaptation or responsiveness of the heart during the 24-hour period to this assortment of physiological stimuli. It can be hypothesized that the normal heart adapts to pressure overload every day, but only chronic exposure to these conditions causes a full phenotypic response and the ensuing long-term complications. For example, blood pressure undergoes a circadian rhythm, with a trough during the day and a peak during the night (in the case of the rodent).[39] The phase of increased blood pressure may initiate alterations in the heart (biochemical and/or transcriptional, such as *anf* induction [Figure 1B]), similar to those observed in the hypertrophied heart, but the full development of hypertrophy does not occur because of the reduction of pressure later in the day. However, in response to pressure overload, these normal mechanisms are chronically activated (for example, induction of *anf*; Figure 1B).

Cardiomyocytes are not different from other cells. Its internal clock mechanism is important for the anticipation of the cell to changes in the environment within which it finds itself, thereby synchronizing responsiveness with the stimulus. These clocks are intrinsic within each cell and can continue even when the cells are in isolation.[3] However, external factors are required for the resetting of these clocks, forming a communication between the cell and its environment.[4,5] In the case of the suprachiasmatic nucleus of the brain (the central body clock), light sensing by the eyes appears to be the signal for the resetting of the clock (a process termed photoentrainment).[40] In contrast, peripheral tissues are not always in direct contact with external light and rely on other factors to modulate the clock mechanism. No single factor has been ascribed to the zeitgeber of peripheral tissues. It is possible that more than one factor is involved in the regulation of peripheral clocks in mammals, allowing for multilevel adaptation. Additional factors might include hormones, substrate availability, and blood pressure.

Impaired Rhythms in the Hypertrophied Heart

We compared the expression patterns of components of the biological clocks in normal and pressure overload–induced hypertrophied hearts for the following reasons. First, as blood pressure undergoes circadian rhythms during the day, it is possible that pressure acts as a zeitgeber for peripheral clocks. Thus, chronic pressure overload occurring locally on the heart would be expected to modulate the clock. Second, we wanted to know whether the mechanisms involved in circadian rhythms were also involved in the development of hypertrophy. These studies would also aid in the understanding of how the hypertrophied heart anticipates and adapts to additional environmental changes in the face of pressure overload. If the hypertrophied heart is less flexible in terms of adaptation to additional environmental factors, this "rigidity" may play a role in the subsequent development of cardiac dysfunction and failure.

In the rat, blood pressure, heart rate, and cardiac output are greatest during the dark phase, when the animal is most active and forages for food.[39] In the light phase, these parameters decrease in the more sedentary animal.[39] Aortic constriction increases the local pressure on the heart, which subsequently maintains cardiac output through the development of hypertrophy.[13] Mechanical stress on cardiomyocytes, caused by increased blood pressure, for example, induces a cascade of intracellular signaling events resulting in alterations in gene expression.[12] It could therefore be hypothesized that blood pressure acts as a zeitgeber for circadian rhythms in the heart through overlapping mechanisms compared with the mechanical stress–induced hypertrophic response. If this hypothesis were true, then aortic constriction should diminish the fluctuation of genes in the light phase, when pressure is normally decreased. Genes encoding for the PERs and the family of the PAR transcription factors are induced in the light. In the hypertrophied heart, the induction of these genes is blunted (with no effect on basal expression), suggesting that pressure may play a role in modulation of rhythms in the normal heart. However, as the amplitude of the rhythms is only partially attenuated, and no effect is seen on periodicity, it is likely that other factors in addition to pressure act as zeitgebers in the heart. This conclusion is consistent with other studies, both in vitro and in vivo.[5,41]

Assuming that internal clocks are important for the anticipation of cells to environmental stimuli, the present results

suggest that the hypertrophied heart is less able to adapt to changes in physiological factors during the day. Previous studies have shown that the hypertrophied heart can maladapt (fail) either with sustained pressure overload over longer periods of time or when an additional stress is placed on the heart.[42,43] For example, both pressure overload–induced and streptozotocin-induced diabetes mellitus result in specific programs of cardiac adaptation, allowing for maintenance of contractile function within their respective environments.[11,13] However, when both stresses are placed on the heart simultaneously, the myocardium fails.[43] Thus, the adapted heart appears less able to respond to additional stimuli. This is consistent with the present study in which the hypertrophied heart is unable to induce the expression of specific clock output genes (the PAR transcription factors). We have also found this to be true for the circadian rhythms of metabolic genes (authors' unpublished observation, 2001), suggesting that the hypertrophied heart anticipates and adapts less well to fluctuations in substrate/fuel availability, hormones, and energy demand/workload. Whether the impairment of the hypertrophied heart to adapt worsens with prolonged or more severe pressure overload is presently unknown.

Conclusions

The present study has characterized fully the major components of the intracellular clock in the heart, of which the level of gene expression changes dramatically during a 24-hour period. Many of the components of this system are transcription factors that have the potential to alter the expression of a host of as-yet-unidentified genes in the heart. In the case of the hypertrophied heart, the circadian rhythms of the PAR transcription factors are attenuated. Whether these circadian clocks are important for diurnal variation in cardiac function or whether attenuation of this mechanism contributes to the development of contractile dysfunction is currently unknown.

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Clock Genes in the Heart: Characterization and Attenuation With Hypertrophy

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