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Molecular basis of circadian rhythmicity in renal physiology and pathophysiology

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Abstract

Accumulating evidence suggests that the molecular circadian clock acts as a master regulator of gene expression in the kidney. In this brief review, an overview of the molecular and physiological evidence for the kidney clock and the implications for the regulation of renal physiology and pathophysiology are presented. Accumulating evidence suggests that the molecular circadian clock acts as a master regulator of gene expression in the kidney. Global transcriptomic approaches have revealed the important finding that there are thousands of genes in the kidney subject to regulation by the molecular clock. Candidate gene approaches have also yielded information regarding regulation of renal sodium transport genes by the molecular clock. To date, the evidence linking the molecular kidney clock to rhythmic renal function provides strong support for the concept that circadian control of gene expression underlies rhythms in physiological function.

Our planet operates on an approximate 24 hour circadian cycle with alternating periods of light and dark. The molecular circadian clock evolved to provide organisms with the ability to adapt to these predictable changes in the external environment. In its simplest form, found in archaeobacteria, the clock consists of just three proteins that maintain circadian rhythmicity via transcriptional and posttranslational regulatory mechanisms (Ishiura *et al.*, 1998). In mammals, the clock is more complex, but the basic mechanism is similar: a core group of transcription factors coordinately regulate the expression of an ever-expanding list of target genes. In mammals, the proteins CLOCK and BMAL1 heterodimerize and interact with E-box promoter elements to drive transcription of clock target genes. In a regulatory loop, Cryptochrome and Period (CRY and PER) proteins feedback on CLOCK and BMAL1 to inhibit transcription (Partch *et al.*, 2014). The molecular clockwork has perhaps been best studied in the suprachiasmatic nucleus of the brain which is where the central clock is located and in the liver, an example of a peripheral clock. These two clocks coordinate rhythmic fluctuations in behavior and metabolism, for example. It is now well accepted that the molecular clock is present and functional in a variety of peripheral tissues, including the

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kidney. Indeed, oscillating expression of the core clock genes has been detected in the kidney (Figure 1)(data derived from CircaDB (Pizarro *et al.*, 2013)).

Urinary electrolyte excretion and glomerular filtration rate vary with the time of day and this is true in rodents, primates, and humans (for review (Gumz *et al.*, 2015; Gumz, 2016)). These laboratory and clinical observations were well established before the first clock gene, *per*, was discovered in *Drosophila*. Detection of a molecular clock in the kidney provides evidence for the mechanism underlying the known circadian fluctuations that have been detected in renal function.

Unbiased transcriptomic studies have shed significant light on the extent to which the molecular clock acts as a master regulator of gene expression in several organs, including the kidney. In the first study of this type, Firsov and colleagues characterized gene expression over a 24 hour period using microdissected cortical collecting duct (CCD) and distal convoluted tubule/ connecting tubule (DCT/CNT) nephron segments (Zuber *et al.*, 2009). They identified hundreds of clock target genes in this manner. In this same study, it was demonstrated that Clock KO mice exhibited lower blood pressure than WT control mice, but maintained the normal circadian rhythm of blood pressure. In a subsequent study by this same group, Nikolaeva *et al.* showed that time-dependent changes in plasma aldosterone were evident in wild type mice but this pattern was disrupted in Clock KO animals (Nikolaeva *et al.*, 2012). These authors also linked the reduced BP phenotype previously identified in these mice to decreased levels of 20-HETE in the kidney of Clock KO mice relative to control mice. Overall, both BP and 20-HETE were reduced in Clock KO versus control mice although the apparent circadian rhythms in these parameters were unaffected.

In a landmark study, Hogenesch and colleagues sought to describe circadian gene expression in multiple mouse tissues, including the kidney (Zhang *et al.*, 2014). Providing finer resolution of time points, these investigators harvested tissues every two hours over a 48 hour period and characterized circadian gene expression using a combination of RNA-Seq and microarray. In total, considering all of the tissues studied, nearly 50% of the mouse genome is subject to circadian regulation. Tissue ranking demonstrated that the kidney was second only to the liver in terms of the absolute number of oscillating transcripts. These data clearly support a role for the molecular kidney clock in the regulation of renal function.

Our own studies have employed a candidate gene approach. The renal epithelial sodium channel (ENaC) is a critical determinant of sodium balance and blood volume thus it is important for blood pressure regulation. Following the novel finding that the clock gene *Per1* is an early aldosterone response gene (Gumz *et al.*, 2003), we tested the hypothesis that *Per1* mediated downstream aldosterone action on the alpha subunit of ENaC (α ENaC)(Gumz *et al.*, 2009). *In vitro* methods using several different renal collecting duct cell lines demonstrated that knockdown of *Per1* resulted in decreased α ENaC expression. *In vivo*, *Per1* KO mice on the salt-sensitive 129/sv genetic background, exhibited reduced α ENaC mRNA levels in the renal medulla and this affect was associated with increased levels of sodium in the urine. Consistent with a role for *Per1* in the regulation of ENaC, we later found that *Per1* KO mice on the salt-sensitive, hypertensive 129/sv background maintained

normal circadian rhythms but exhibited much lower blood pressure compared to wild type control mice (Stow *et al.*, 2012).

Given the large difference in blood pressure between WT and Per1 KO mice, we investigated additional renal sodium transport genes. We identified the peptide hormone endothelin-1 (ET-1), a negative regulator of ENaC, as a Per1 target gene in collecting duct cells and *in vivo* in the renal cortex (Stow *et al.*, 2012; Richards *et al.*, 2014b). Using genetic knockdown and pharmacological inhibition in a cell model of the distal convoluted tubule, we showed that the Na-Cl cotransporter NCC and its regulatory kinase WNK1 were also Per1 target genes (Richards *et al.*, 2014a). A similar approach in HK2 human kidney proximal tubule cells revealed that the Na⁺/H⁺ exchanger NHE3 and the Na-glucose cotransporter SGLT1 were Per1 target genes as well (Solocinski *et al.*, 2015). Together, these data suggest a role for Per1 in the coordinate, positive regulation of sodium reabsorption in the kidney, consistent with the established blood pressure phenotype in Per1 KO mice on the 129/sv background strain.

Taken together, the transcriptomic and candidate gene data support a role for the molecular clock as a master regulator of gene expression in the kidney. If this is the case, knockout of clock genes in the kidney should result in alteration of renal function. Indeed, we have demonstrated that 129/sv mice with reduced expression of Per1 exhibit a renal sodium wasting phenotype (Richards *et al.*, 2013a). In the first study to knockout a clock gene specifically in the kidney, Tokonami *et al.* showed that loss of Bmal1 in renin-producing cells of the juxtaglomerular apparatus (JGA) was associated with altered electrolyte handling by the kidney and reduced blood pressure (Tokonami *et al.*, 2014). These mice also exhibited altered glomerular filtration rate. In a new and exciting report, Nikolaeva *et al.* demonstrated that inducible KO of Bmal1 in the renal tubules resulted in a variety of metabolic disturbances (Nikolaeva *et al.*, 2016). Plasma urea was elevated in tubule-specific Bmal1 KO mice compared to control mice and this effect was associated with upregulation of arginase 2 in the proximal straight tubules of the KO mice. Arginase 2 catalyzes the last step in the urea cycle, converting L-arginine to L-ornithine and urea. Importantly, the tubular Bmal1 KO mice exhibited defects in drug metabolism pathways; the KO mice had a reduced ability to excrete the loop diuretic furosemide. These findings have implications for our understanding of how the kidney clock contributes to pharmacokinetics and also shed light on how chronotherapy may benefit certain individuals.

To date, the evidence linking the molecular kidney clock to rhythmic renal function provides strong support for the concept that circadian control of gene expression underlies rhythms in physiological function. However, several questions remain to be answered. What makes the kidney clock tick? Light is accepted as the dominant zeitgeber to entrain the central clock which is thought to then signal to the peripheral clocks through neuronal and humoral pathways to synchronize their functions. Available evidence suggests that water intake may not be a potent cue (Mistlberger & Rechtschaffen, 1985). Food, however, is a critical zeitgeber and some peripheral clocks may be more sensitive to this entrainment cue. Wu *et al.* showed that clock gene expression in metabolically active tissues including the kidney, liver, and heart, could be dramatically altered by uncoupling the feeding cycle from the mouse's normal day/night rest/activity cycle (Wu *et al.*, 2008; Wu *et al.*, 2010). Does the

clock function in a similar manner among the myriad of cell types in the kidney? The differences in the phenotypes of two unique kidney specific (renin vs. Pax8 driven Cre) Bmal1 KO mice suggest that the clock mechanism may differ between distinct cell types (Tokonami *et al.*, 2014) (Nikolaeva *et al.*, 2016). New studies using a variety of cell type clock gene KO and also evaluation of the tubular versus vascular cell types in the kidney are needed. What role do circulating hormones play? Cortisol has been proposed as a synchronizing signal yet adrenalectomized animals still exhibit strong circadian fluctuations in physiological function (Moore-Ede, 1986). Our identification of Per1 as an aldosterone target gene has been validated by the findings of several other groups that aldosterone induces Per1 expression in the kidney and cardiomyoblasts (Tanaka *et al.*, 2007; Le Billan *et al.*, 2015). Per1 appears to be unique among the clock genes in being induced by aldosterone and cortisol. Evidence in other systems suggests that clock proteins and nuclear hormone receptors interact to affect circadian functions in metabolism (Lamia *et al.*, 2011) and the same is likely true for kidney function as well. In line with a connection between hormone signaling and the molecular clock, we found that Per1 and MR both interact with a region of the α ENaC promoter containing an aldosterone response element and an E-box sequence in close proximity to one another (Richards *et al.*, 2013b). Chromatin immunoprecipitation studies in mouse cortical collecting duct cells demonstrated that the signals for MR and Per1 both increased in response to aldosterone treatment *in vitro*. *In vivo* studies exploring the connection between the kidney clock and blood pressure-regulating hormones are needed.

Another remaining question pertains to how the kidney clock functions in pathophysiological states. There is limited evidence using models of hypertension in clock gene knockout models but two such studies provide additional evidence for an important connection between the renin angiotensin aldosterone system (RAAS) and the molecular clock. A recent study investigated the effects of angiotensin II administration in mice lacking all three Period genes (Per1, 2, and 3; triple KO (TKO))(Pati *et al.*, 2016). AngII treatment on a normal diet resulted in loss of blood pressure dipping in TKO mice under constant darkness conditions, whereas a normal dipping profile was maintained in control mice. This effect was associated with worsened vascular hypertrophy in TKO mice compared to controls. Interestingly, placement of these mice on a low salt diet resulted in over-activation of the RAAS in TKO mice including higher levels of plasma and kidney renin. A 2010 report from Okamura and colleagues also linked the circadian clock to the RAAS. High salt diet administration in Cry1/Cry2 KO mice resulted in salt-sensitive hypertension that was ameliorated following treatment with the mineralocorticoid receptor (MR) antagonist spironolactone (Doi *et al.*, 2010). Cry1/Cry2 KO mice exhibited much higher plasma aldosterone levels compared to control mice and became hypertensive in response to a high salt diet. The salt-sensitive hypertension observed in these mice was ameliorated following treatment with the mineralocorticoid receptor (MR) antagonist spironolactone. These investigators looked further into gene expression using a transcriptomics approach in the adrenal gland and identified a candidate gene that was highly overexpressed: Hsd3b6. This gene encodes the enzyme 3 β -HSD, a hydroxysteroid dehydrogenase which catalyzes a reaction early in the steroidogenesis pathway. A direct role for the kidney clock in these models of hypertension has not yet been tested. It will be interesting to probe the role of the

clock in the end organ damage that occurs in vascular tissues as well as the kidney in chronic hypertension.

Given the accumulating evidence supporting a role for the kidney clock as a master regulator of gene expression and consequently renal function, it seems likely that disruption of this clock in disease states such as chronic kidney disease would exacerbate renal pathology. Indeed, the *tau* mutant hamster, a model of circadian misalignment and clock dysfunction, exhibits a dramatic cardiorenal syndrome resulting in early death (Martino *et al.*, 2008). Correction of the circadian phenotype early in life restores longevity and prevents renal and cardiac fibrosis in these hamsters. It is tempting to speculate that humans with circadian disruption resulting from shift work or sleep/wake disorders may be at increased risk for kidney disease. It is well established that non-dipping blood pressure in humans is associated with decreased cardiovascular survival (Stow & Gumz, 2011). A small study in humans linked disturbed circadian rhythms in the RAAS to renal damage and nocturnal hypertension (Isobe *et al.*, 2015). Additional preclinical and clinical studies are clearly needed in order to ascertain the physiological and pathophysiological links between the circadian clock and renal function.

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New Findings

- a. **What is the topic of this review?** This brief symposium report is focused on the molecular and physiological evidence that supports a key role for the circadian clock in the regulation of kidney function.
- b. **What advances are highlighted?** Progress in understanding the molecular mechanism of the kidney clock is reviewed here, including new results from global “omics” studies and candidate gene approaches. The molecular kidney clock is a master regulator of gene expression affecting renal electrolyte and drug handling and also blood pressure.

Figure 1. Core Clock Gene Expression Oscillates in the Kidney

Data were derived from CircaDB (Pizarro *et al.*, 2013). Expression of mRNA for the core circadian genes Bmal1, Clock, Per1, and Cry2 exhibits significant circadian rhythm oscillation. The y-axis represents relative mRNA levels and the x-axis represents circadian time where CT0 means “lights on” and CT12 “lights off.” Shaded areas correspond to the dark/active phase for mice whereas the white areas correspond to light/rest phase. Figure adapted from Solocinski and Gumz (Solocinski & Gumz, 2015).