Circadian regulation of renal function

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Urinary excretion of water and all major electrolytes exhibit robust circadian oscillations. The 24-hour periodicity has been well documented for several important determinants of urine formation, including renal blood flow (RBF), glomerular filtration (GFR), tubular reabsorption and tubular secretion. Disturbance of the renal circadian rhythms is increasingly recognized as a risk factor for hypertension, polyuria and other diseases and may contribute to renal fibrosis. The origin of these rhythms has been attributed to the reactive response of the kidney to circadian changes in volume and/or in the composition of extracellular fluids which are entrained by rest/activity and feeding/fasting cycles. However, numerous studies have shown that most of the renal excretory rhythms persist for long periods of time even in the absence of periodic environmental cues. These observations led to the hypothesis of the existence of a self-sustained mechanism enabling the kidney to anticipate various predictable circadian challenges to homeostasis. The molecular basis of this mechanism remained unknown until the recent discovery of the mammalian circadian clock made of a system of autoregulatory transcriptional/translational feedback loops which have been found in all tissues studied, including the kidney. Here, we present a review of the growing evidence demonstrating the involvement of the molecular clock in the generation of renal excretory rhythms.

Circadian rhythms in renal function have been studied since the middle of the 19th century. In 1861, Edward Smith, one of the pioneers in circadian physiology, published the first documented evidence for the existence of circadian oscillations in renal excretion of urea and water (1) (for an excellent historical review tracing the early stages in the development of the experimental chronobiology see (2)). Later studies showed that sodium, potassium, chloride and other major electrolytes also follow circadian excretory patterns. Because most of the excretory rhythms are maintained in kidney transplant patients (3), it was concluded that either humoral factors or yet unknown intrinsic renal mechanisms (or both) are involved in their generation. Analysis of circulating factors revealed that blood levels of vasopressin, aldosterone and many other hormones responsible for maintaining water and electrolyte balance exhibit circadian oscillations (4, 5). Until recently, it was thought that these hormonal rhythms are entrained principally by circadian changes in the volume and/or composition of extracellular fluids produced by the rest/activity and feeding/fasting cycles. The evidence for the existence of an intrinsic renal mechanism remained elusive because of the difficulty in dissociating this mechanism from the effects of circadian circulating factors. The discovery of the circadian timing system allowed major advance in the understanding of the origin of renal excretory rhythms. Several recent studies have clearly demonstrated that at least a part of the hormonal rhythms can be attributed to a self-sustained mechanism driven by the circadian clock at the site of synthesis and/or release of these hormones (see below). It was also shown that the kidney itself possesses an intrinsic circadian clock potentially involved in transcriptional/translational control of thousands of genes of the renal transcriptome.

CIRCADIAN CLOCK

The great majority of physiological processes run with a periodicity of ~ 24 hours. The ~ 24 hour period length gave rise to the name *circadian* which is composed of two latin words *circa*

(about) and *dies* (day). Functionally, circadian rhythms are thought to provide an important advantage by allowing the organism to anticipate the upcoming environmental changes. The molecular basis of circadian rhythms in mammals was uncovered at the end of the 20th century. It was shown that the mammalian circadian clock is a hierarchically organized system of individual cellular oscillators orchestrated by a self-sustained central pacemaker residing in the suprachiasmatic nucleus (SCN) of the hypothalamus (reviewed in (6)). The SCN pacemaker is synchronized with the external world primarily by the light/dark cycle. Its activity imposes the feeding pattern through the control of the rest/activity cycle. The feeding time is thought to be the dominant time cue for resetting circadian oscillators in peripheral tissues. However, the latter are capable of sustaining circadian rhythms for long periods of time in the absence of the SCN synchronization, thus demonstrating a high degree of autonomy. Central and peripheral oscillators share a similar core clock based on a system of autoregulatory transcriptional/translational feedback loops composed of the transcriptional activators Clock, BmalI and Npas2, and of the feedback repressors Cry1, Cry2, Per1 and Per2 (Figure 1). Circadian oscillations of the core clock entrain circadian rhythms in expression of output genes which are, in turn, translating these transcriptional oscillations into tissue-specific functional rhythms. Current estimates indicate that up to 10% of all genes are under the control of circadian transcriptional factors.

ROLE OF MOLECULAR CLOCK IN THE HOMEOSTATIC CONTROL OF WATER AND ELECTROLYTE BALANCE BY THE KIDNEY.

Water It is well established that the rate of urine formation by the kidney follows a well-defined circadian rhythm with a maximum excretion which takes place during the activity phase. This excretory pattern has been shown to persist for several days when activity/feeding cycles are either completely reversed or when water and meals are taken at regular intervals

throughout the 24-hour period (7). Upon water restriction the volume of excreted water is rapidly decreased and cycles disappear, thereby reflecting domination of the reactive mechanism of water conservation over anticipatory circadian functional rhythms. Urinary output of water depends on several parameters including circulating vasopressin levels, variations in the osmotic pressure along the cortico-medullary axis, the renal blood flow (RBF) and the glomerular filtration rate (GFR). Hence, self-sustained circadian oscillation of one of these factors or their combinations would be capable of entraining circadian rhythms in water diuresis. Circadian variations in both, RBF and GFR are well documented. Moreover, it has been demonstrated that both, RBF and GFR are oscillating in-phase with rhythms of urinary excretion of water and several major electrolytes (8). However, the self-sustained rhythmicity has only been shown for the GFR (9). Whether the cortico-medullary osmotic gradient is following a circadian profile remains unknown. Data concerning circadian variations in blood vasopressin concentration remain limited due, in part, to the low circulating levels of this hormone. A few available data indicate that maximal vasopressin levels are reached at the beginning of the activity phase (10). Vasopressin is synthesized in the paraventricular (PVN), supraoptic (SON) and suprachiasmatic (SCN) nuclei of hypothalamus. Significant circadian changes in vasopressin mRNA and protein abundance have only been detected in the SCN, in which about one-third of neurons synthesize this hormone (11, 12). The SCN-derived vasopressin is considered one of the major rhythmic outputs of the central pacemaker which is involved, among other functions, in the control of hypothalamo-pituitary axis. Hence, it was proposed that oscillations in the SCN-derived vasopressin might be involved in the circadian release of this hormone from the posterior pituitary (13). This interesting theory, however, requires further investigation.

The involvement of the molecular clock in renal water handling was recently tested in *Clock*-deficient mice. Zuber et al., have demonstrated that several key genes regulating water

reabsorption in the distal nephron and the collecting duct exhibit circadian patterns of mRNA expression (14). Expression levels of vasopressin type 2 receptor (V2R) and the aquaporin-2 (aqp-2) and aquaporin-4 (aqp-4) water channels have been shown to follow temporarily synchronized circadian oscillations with the maximal expression which takes place in the second half of the activity phase. The suppression of *Clock* leads to significant changes in the expression levels of these transcripts. The phenotype analysis of *Clock*-deficient mice revealed an impaired capacity of the kidney to concentrate urine, a condition called as a partial diabetes insipidus. Collectively, this study provided the first direct evidence of the role of circadian timing system in water homeostasis.

Sodium As mentioned above, urinary excretory rhythms of sodium, potassium, chloride and other major electrolytes parallel both, the RBF and the GFR oscillations. However, these rhythms differ significantly in their amplitudes. Indeed, both RBF and GFR rhythms show a low amplitude of ~ 20% of the daily mean (9), whereas the circadian amplitude of sodium excretion, for instance, is several-fold greater. This difference in amplitudes clearly indicates that the tubular component plays a dominant role. Doi et al., have recently shown that the circadian clock controls renal sodium reabsorption via a mechanism modulating aldosterone production by the adrenal glands (15). In this study, double knockout of the circadian repressors *Cry1* and *Cry2* has been used to demonstrate that the permanent activation of the circadian clock results in the significantly increased plasma aldosterone levels. The analysis of transcriptional profiles in adrenal glands of *Cry1/Cry2* knockout mice allowed the identification of a molecular mechanism underlying this increase in aldosterone production. It was shown that *Cry1/Cry2* knockout mice exhibit chronic overexpression of type VI 3β-hydroxyl-steroid dehydrogenase (Hsd3b6), one of the key enzymes in adrenal aldosterone biosynthesis in mice. *In vitro* promoter analysis has shown that expression of Hsd3b6 could be

directly regulated by circadian transcriptional factors. Under a standard salt diet the *Cry1/Cry* deficient mice exhibit normal blood pressure. However, already on the second day of the high salt intake there was a significant increase in arterial pressure, suggesting salt-sensitive hypertension. Analysis of human genome revealed Hsd3b1 as a functional counterpart of mouse Hsd3b6, thereby identifying this gene as a new candidate for salt-sensitive hypertension in humans. Interestingly, Zuber et al, have shown that mice with the whole-body deletion of the circadian transcriptional activator *Clock* exhibit decreased expression of the α subunit of the epithelial sodium channel (αENaC), a modified rhythm of urinary sodium excretion and a significantly reduced blood pressure (14). Collectively, these two studies have demonstrated that the activation level of the circadian clock results either in salt-sensitive hypertension, when the molecular clock is permanently active, or in decreased blood pressure, when the molecular clock is downregulated (Figure 2). Recently, Gumz et al., proposed that the circadian repressor Per1 can generate the sodium excretory rhythms via a direct control of αENaC promoter (16). However, the molecular mechanism of this control remains unclear.

The predictive circadian regulation of aldosterone production has a clear physiological meaning. The plasma aldosterone levels start to rise several hours before the beginning of the activity phase and remain elevated for ~ 12 hours. Aldosterone is a steroid hormone which requires a delay of several hours before its genomic effects become apparent. Thus, the rhythm of the aldosterone's effect on sodium reabsorption in the kidney could be responsible for the synchronous circadian variations in arterial blood pressure. It should be noted, however, that the sodium excretion rhythms persists in adrenalectomized rats, thereby indicating that others as yet uncharacterized factors might be involved (17, 18).

Potassium The urinary excretion of potassium is characterized by the highly stable circadian oscillations that have been shown to persist for more than a week when food intake,

posture and activity are evenly distributed throughout day and night (19). Upon fasting, however, these rhythms demonstrate a major difference from water and sodium excretory oscillations. Indeed, potassium excretory rhythms are maintained for at least 24 hours upon fasting and the amount of potassium excreted in the urine exceeds that in the extracellular fluid and in the gut. This suggests that (i) potassium flux between the intracellular and extracellular compartments is controlled by a circadian mechanism and (ii) circadian timing system could play an important role in the overall potassium balance. Moore-Ede et al., have shown that renal capacity to excrete potassium is significantly lower during the inactivity phase (20). Steele et al., have demonstrated that the circadian pattern of urinary potassium excretion is mostly determined by circadian changes in the intratubular potassium concentration in the cortical collecting duct (CCD) and to a significantly lesser extent by variations in the urine flow rate (21). These observations indicate that potassium secretion in the distal nephron and the collecting duct follows a circadian pattern. Experiments with adrenalectomized rats receiving or not receiving aldosterone and/or dexamethasone replacement have shown that circadian rhythms of urinary potassium excretion remain unchanged (19). The existence of other cyclic circulating factors controlling potassium secretion or participation of the intrinsic renal clock in generation of these rhythms remains to be determined.

Calcium Circadian variations of plasma calcium, urinary calcium and of regulators of calcium homeostasis such as calcitonine, parathormone (PTH) or vitamin D have been described in humans and other species (22, 23). Similarly, circadian rhythms have been described for bone turnover markers (hydroxyproline, C-terminal telopeptide of type I collagen, and osteocalcin) (24-26). However, the physiological relevance of calcium cycling remains elusive. Alterations of circadian rhythms may increase renal stone formation (27-30). Intestinal calcium absorption could differ depending on the time food is provided (31). But the

most spectacular effects of circadian rhythms on mineral metabolism have been described for the bone (32) and have been attributed to the circadian rhythm of the parathormone (PTH). PTH has a very short half-life; it is secreted in a pulsatile manner at a basal rate and regulates plasma calcium concentrations. In normal humans, PTH has a circadian rhythm with a peak between 01:00 and 03:00am and a trough occurring at approximately 10:00-11:00am and which has been shown to be independent of sleep/wake or light/dark cycles, meals or posture (22, 23). How circadian variations of PTH levels affect bone turnover is not yet well understood. Experiments performed on mice perfused continuously with PTH have shown a decrease in bone mass, while mice stimulated daily by pulses of PTH presented a gain in their bone mass (33). Similar observations have been made in humans. On one hand, high concentrations of PTH and loss of circadian rhythm is associated with decreased bone mass in primary hyperparathyroidism (34). On the other hand, recombinant 1-34 amino terminal fragment of PTH (teriparatide) is an effective treatment for osteoporosis when injected once a day (35, 36). Overall, circadian variations have been described for all the different players in calcium metabolism, but the underlying specific molecular mechanisms remain largely unknown.

Magnesium Several studies have shown circadian changes in the urinary excretion of magnesium (37-39), but to our knowledge, the molecular events involved in the changes in the expression of the magnesium transporters still need to be unravelled.

Phosphate Variation in the urinary excretion of phosphate is expected in relation to food intake. However, even in constant conditions (food intake taken as hourly snacks, constant light and rest) important variations in phosphate excretion have been observed (37, 40). This data suggests that phosphate excretion is controlled by an endogenous mechanism independent

of food intake or other systemic cues. Bielesz and colleagues have explored the putative regulation of one of the main phosphate-sodium co-transporters NaPi-IIa by such a mechanism in the rat kidney and have shown that the abundance of NaPi-IIa was unchanged and that the brush border NaPi-IIa activity was only slightly changed over 24 hours (41). However, whether the molecular clock is involved in the control of the endogenous circadian rhythm of phosphate excretion is unknown so far.

Acid-base Acid-base effectors and regulators are highly dependent on circadian oscillators. In humans, urine pH is lower during the night with a trough around 4am (42). Several acid-base transporters have been shown to have circadian variations of the expression levels and might be involved in urinary pH changes. The sodium/proton exchanger 3 (NHE3/SLC9A3) displays a strong 24-hour oscillation of its renal mRNA expression and its promoter contains an E-Box that can be regulated by the Bmal1-Clock complex (43). Oscillating expression of the V-ATPase might also contribute to the circadian variations of urinary pH, in comparison to what has been found in the vas deferens of the moth (44). However, the contributing role of the molecular clock to this phenomenon and its physiological function remain to be established.

Erythropoietin (EPO) Erythropoietin levels show a robust circadian rhythm (45). More than 10 times variation in amplitude has been observed in constant darkness and normoxia for mice kidney EPO mRNA over 24 hours and an E-Box has been identified in the promoter region of the EPO gene and has been shown to regulate EPO expression (46). However, the significance of the 24h variation of EPO levels is not known. It is noteworthy that EPO administration in normal and dialysed patients could be influenced by the circadian rhythm (47) and deserves further chronopharmacological studies.

CLINICAL RELEVANCE OF RENAL EXCRETORY RHYTHMS

The dysfunction of the circadian clock or its misalignment with behavioural cycles has been implicated in pathogenesis of many diseases. For instance, long-term night work is associated with a significant increase in the risk of breast cancer (48), metabolic syndrome (49) and ischemic heart disease (50). In the individuals with essential hypertension, abnormal rhythm of sodium reabsorption by the kidney has been associated with a blunted decrease in nighttime blood pressure, a condition characterized by a significantly increased risk of end organ damage (51). The abnormal rhythm of natriuresis is also associated with nocturnal polyuria in chronic kidney disease and in the elderly and in children with enuresis (52). In hamsters, it has been shown that a point mutation in the circadian regulatory gene, casein kinase-1ε, leads to a disorganization of the circadian clock accompanied by cardiomyopathy, extensive cardiac and renal fibrosis and renal tubular dilation (53). As discussed above, disturbance in renal rhythms may influence calciuria, phosphaturia, natriuria, urinary pH and diuresis, but also other risk factors for stone formation, including citrate and oxalate urinary excretion (28, 29).

PERSPECTIVES

To what extent the intrinsic renal clock is contributing to the generation of renal rhythms still remains to be evaluated. Efforts will be needed to addressing this quest in two ways. First, mice models carrying kidney-specific deletion of the different elements of the molecular clock will allow the direct exploration of the role of the clock function in the cells of the whole kidney or in specific segments. These experiments might be coupled with the silencing of systemic cues by using adrenalectomized or parathyroidectomized mice whenever possible. Second, new genetic tools will contribute to the evaluation of the clock system in the renal function, especially in humans, provided that a thorough circadian phenotyping has been

performed. Undoubtedly, renal circadian predictive physiology and renal pathophysiology are entering a new promising era.

DISCLOSURE

The authors declared no competing interests.

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Figure legends:

Figure 1. Schematic presentation of circadian molecular clock.

Figure 2. Transcriptional activity of circadian clock controls arterial blood pressure in mice: its constitutive activation leads to salt-sensitive hypertension whereas its suppression results in decreased blood pressure.

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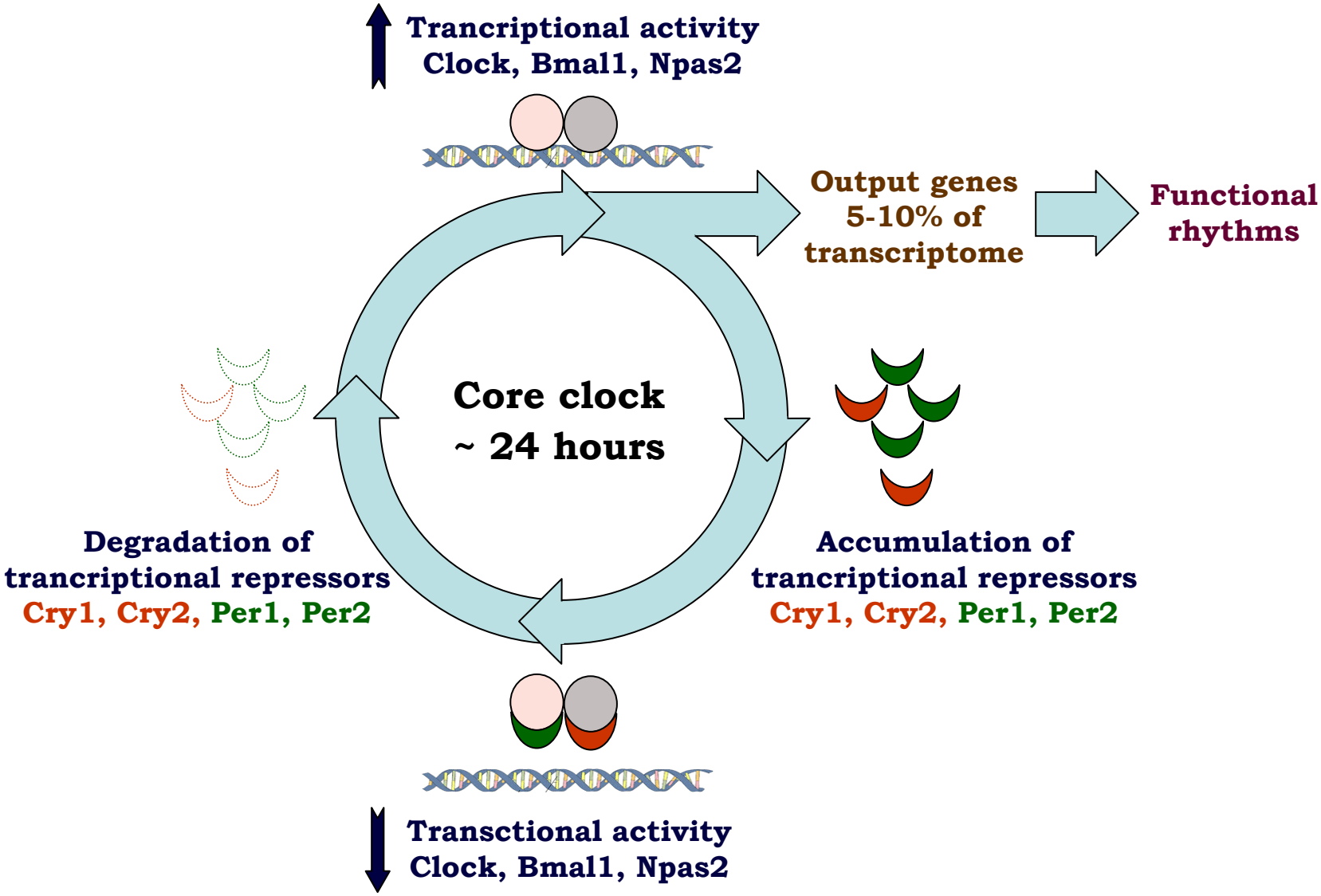
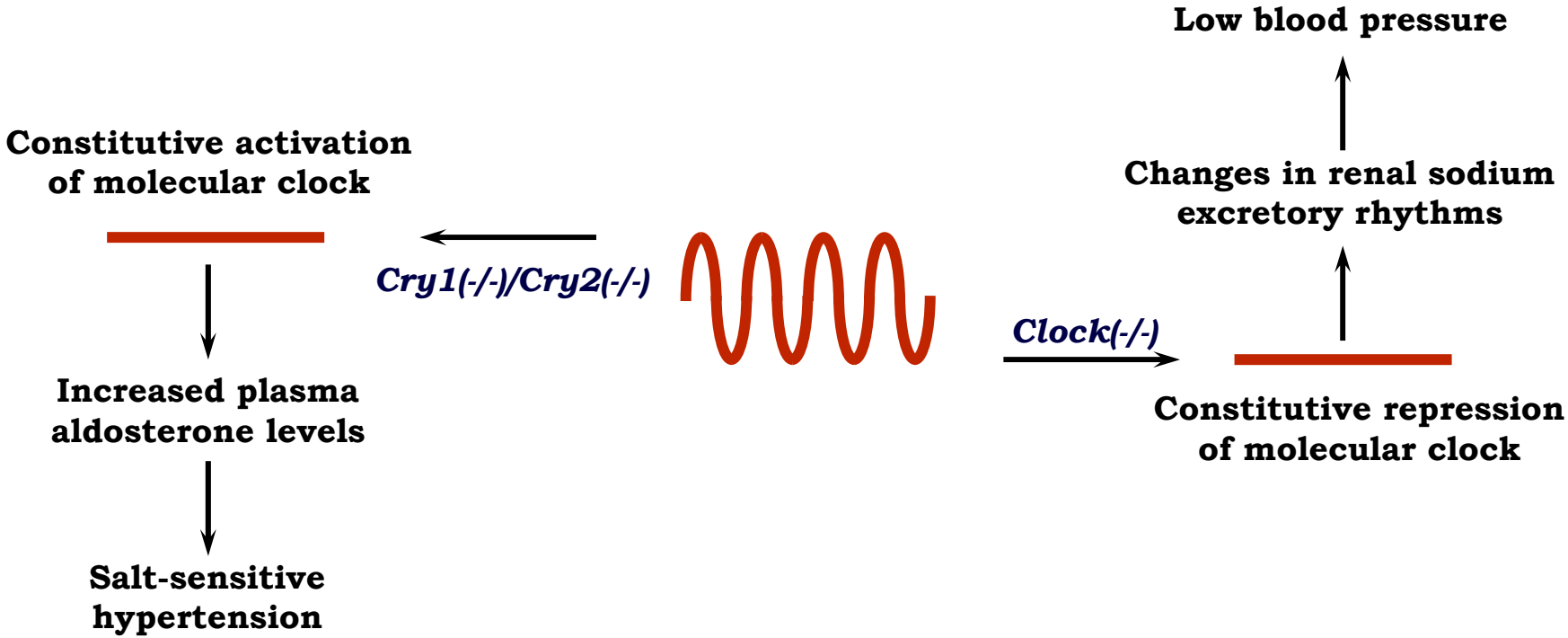

Trancriptional activity
Clock, Bmal1, Npas2
Output genes
5-10% of
transcriptome
Functional
rhythms
Core clock
~ 24 hours
Degradation of
trancriptional repressors
Cry1, Cry2, Per1, Per2
Accumulation of
trancriptional repressors
Cry1, Cry2, Per1, Per2
Transctional activity
Clock, Bmal1, Npas2


Low blood pressure
Changes in renal sodium excretory rhythms
Constitutive activation of molecular clock
Cry1(-/-)/Cry2(-/-)
Clock(-/-)
Increased plasma aldosterone levels
Constitutive repression of molecular clock
Salt-sensitive hypertension