Gene 548 (2014) 101–111

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Evidence for species-specific clock gene expression patterns in hamster peripheral tissues

Vivian Meyer, Alexander Lerchl *

MoLife Research Center, Jacobs University Bremen, Campus Ring 1, D-28759 Bremen, Germany

ARTICLE INFO

Article history:
Received 8 April 2014
Received in revised form 12 June 2014
Accepted 8 July 2014
Available online 10 July 2014

Keywords:
Circadian rhythm
Clock gene function
Peripheral oscillators
Testis
Cricetidae

ABSTRACT

Rhythmic oscillations that repeat every 24 h can be found in numerous behavioral and physiological functions. Beside the endogenous master clock in the suprachiasmatic nucleus (SCN), peripheral oscillators exist that can disengage from the master clock rhythm by different mechanisms. The fact that core clock genes in peripheral tissues do not always have the same characteristics as in the SCN suggests that their function may vary in different organs. Additionally, suggestions about species-specific variation in expression peak and nadir times, especially in the testis, led to the need for systematical investigations on clock gene expression patterns in different organs and species under standardized methodological conditions. Therefore, daily gene expression patterns of the clock genes *Bmal1*, *Period1*, *Period2*, *Clock*, *Cryptochrome1* and *Cryptochrome2* were recorded at each of eight time points during a 24 hour period in the testis, kidney, liver, spleen and heart of three hamster species (*Phodopus sungorus*, *Phodopus roborovskii* and *Cricetulus griseus*; family: Cricetidae). Clock gene expression was found to be rhythmic in all investigated organs, however with inconsistent results in the testis. Complex cosinor analysis revealed species differences in temporal gene expression patterns regarding their orthophase, number of peaks, and amplitude for all genes and organs with most pronounced differences in the testis. The results of this study strongly indicate that clock gene expression in peripheral tissues is species-specific and that their functions might be at least partly connected to clock-unrelated traits that vary between the investigated species. Further studies should aim at clarifying the specific roles of clock genes in the testis.

1. Introduction

Behavioral and physiological rhythms that repeat every 24 h can be found in various organisms, from unicellular prokaryotes over plants, insects, and vertebrate animals to humans (Allada et al., 2001; Kondo and Ishiura, 2000; McWatters and Devlin, 2011; Pöggeler et al., 1991; Roenneberg et al., 2013). Chronobiological studies of the past decades suggest that almost every physiological parameter shows daily variation (Steinlechner, 2012). Circadian cycles are endogenously maintained through a core oscillator that receives input signals, e.g. the photoperiod, and generates output signals that can lead to altered physiology and behavior. The core oscillator consists of self-sustaining delayed transcriptional/translational feedback loops of clock genes, producing regular circadian fluctuations of their positive and negative elements in antiphase. In the example of the sleep–wake cycle in mammals, the core oscillator is located in the suprachiasmatic nucleus (SCN) and composed of clock gene expression feedback loops. The so-called "central pacemaker" in the SCN can receive input from other body regions, for instance the intergeniculate leaflet in the brain or directly from the retina to synchronize RNA and protein oscillations to environmental cues like the light–dark cycle as an external zeitgeber (Dibner et al., 2010). Consequently, neuronal and humoral signals are generated to alter downstream processes in the brain and peripheral organs. One well-known output pathway of the SCN is the nightly innervation of the pineal gland leading to melatonin production. The "darkness hormone" melatonin is distributed all over the body and binds to specific receptors at relevant brain areas and peripheral organs, signaling the time of day; therefore, under 24-hour light–dark cycles, beside other direct mechanisms, G-protein coupled melatonin receptors at the SCN are part of the process that leads to the entrainment of the circadian rhythms to become daily rhythms (Reiter, 1993).

It is generally accepted that other self-sustaining oscillators than the light-entrainable oscillator in the SCN exist and are governed by the SCN master clock. One example is the food-entrainable oscillator (FEO) which was first described in SCN-lesioned rats that showed periodically increased locomotion, body temperature, and adrenal hormone

Abbreviations: SCN, suprachiasmatic nucleus; BMAL1/ARNTL, aryl hydrocarbon receptor nuclear translocator-like; PER1/2, period circadian clock 1/2; CLOCK, circadian locomotor output cycles kaput; CRY1/2, cryptochrome 1/2; FEO, food-entrainable oscillator; NPY, neuropeptide Y; RORα/β, retinoid-related orphan receptor α/β; DD, constant darkness; CT, circadian time; LD, light–dark cycle; 16L8D, light–dark cycle with 16 hour light and 8 hour darkness; NCBI, National Center for Biotechnology Information; no RT, no reverse transcription control; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FDR, False Discovery Rate.

* Corresponding author at: Campus Ring 6, D-28759 Bremen, Germany.
E-mail address: a.lerchl@jacobs-university.de (A. Lerchl).

http://dx.doi.org/10.1016/j.gene.2014.07.019
0378-1119/

secretion in anticipation of food (food anticipatory activity; Krieger et al., 1977; Stephan, 1984). Food entrainment can lead to altered clock gene expression phases in peripheral organs like the liver (Damiola et al., 2000). Blum et al. (2012) reviewed different mechanisms by which oscillators like the FEO may disengage from SCN control when entrainment signals like food are presented regularly outside of the light-entrained schedule. These mechanisms include for example the decreased activation of SCN cells receiving retinal input. Photic and non-photic signals are processed in the intergeniculate leaflet which leads to neuropeptide Y (NPY) secretion in the SCN (Harrington et al., 1987). Activated NPY receptors in the SCN can lead to decreased melatonin secretion in the pineal gland (Gamble et al., 2006) which finally results in a reduction of activated SCN cells that also integrate direct photic information from the retina.

In the case of the SCN, main genetic mechanisms of the core oscillator have been identified (Reppert and Weaver, 2001; Toh, 2008). Except from *Clock* all other clock genes exhibit more or less pronounced daily oscillations in gene expression. The protein CLOCK forms a heterodimer with BMAL1 protein when this is abundant in sufficient amount in the cytoplasm. Subsequently, BMAL1 is acetylated and the complex relocates into the nucleus where it binds to a transcription factor binding site (E-Box) in the promoter region of the target genes *Period* (*Per1*, *Per2*, *Per3*), *Cryptochrome* (*Cry1*, *Cry2*), *Retinoid-related Orphan Receptor* (*Rorα*, *Rorβ*, *Rorγ*) and *Rev-Erb* (*Rev-Erbα*, *Rev-Erbβ*) to enhance their transcription. Since *Bmal1* has one expression peak during a 24-hour cycle, this regulation appears only during distinct hours of the day and leads to similar but antiphase expression patterns of the target genes. This means that *Per* and *Cry* peak approximately 12 h later than *Bmal1*. Accordingly, PER and CRY proteins form heterodimers and after phosphorylation by casein kinase 1δ or casein kinase 1ε they also relocate into the nucleus where CRY suppresses CLOCK:BMAL1-induced transcription of *Per* and *Cry*. In contrast to the former, this leads to decreased gene expression, describing a negative feedback loop. *Bmal1* oscillations are mediated by ROR and REV-ERB which compete for binding to another transcription factor binding site in the promoter region of *Bmal1*, and have inhibiting (ROR) and activating (REV-ERB) function, respectively (Okamura et al., 2002; Toh, 2008).

Oscillating clock gene expression is suggested to occur in most mammalian tissues and variable cell types (Balsalobre, 2002). A large number of clock genes were found to show similar expression patterns in the SCN and peripheral organs of mice (Yamamoto et al., 2004). Nevertheless, there are some differences. For example, peak expression time can be shifted by several hours in the different tissues. Whereas the expression of *Clock* does not oscillate in the SCN, it does in many peripheral organs (Yamamoto et al., 2004). It was suggested that tissues, which are primarily composed of immature and differentiating cells, like testis and thymus, show only dampened or no clock gene expression cycles at all (Alvarez and Sehgal, 2005; Morse et al., 2003). However, these observations are controversial since other research groups indeed found daily (24 h) and ultradian (12 h; 8 h) rhythmic components in temporal expression patterns of *Per1*, *Per3*, *Bmal1*, *Cry1*, and *Cry2* in the murine testis (Bebas et al., 2009; Liu et al., 2007; Zylka et al., 1998).

Since hamsters are established animal models when studying the effect of different photoperiods on various physiological functions, they are common experimental animals in the field of chronobiology. However, till now, there are only few studies on clock gene expression in peripheral organs of hamsters. Researchers found that the photoperiod has varying influence on clock gene expression in central and peripheral tissues (Carr et al., 2003) and that SCN and heart samples of torpid animals show different clock gene expression patterns than those of normothermic animals (Crawford et al., 2007). Tong et al. (2004) presented temporal expression patterns of *Bmal1* and *Per1* in the kidney, heart, liver, spleen, muscle and testis of Syrian hamsters (*Mesocricetus auratus*) maintained in DD (constant darkness). Following the transcriptional/translational feedback model of the core oscillator in the SCN, it was expected that both gene products cycle in antiphase. However, in the testis, both of them had an expression peak at circadian time 21 (CT21). Therefore, the authors expanded their study and showed that *Bmal1* mRNA was homogenously distributed among seminiferous tubules while *Per1* mRNA was confined to only some tubules and was less abundant at CT3 than at CT9, CT15, or CT21 (Tong et al., 2004). Another study with Djungarian hamsters (*Phodopus sungorus*) kept at 16L:8D (16 hour light, 8 hour darkness) confirmed the strong oscillations of *Per1* expression in the testis (Klose et al., 2011). The difference in peak (CT21 vs. zeitgeber time 3 [ZT3]) and nadir time (CT3 vs. ZT12) between both studies may be due to species differences and/or to the different light conditions. In contrast, *Bmal1* showed only weak oscillations in the testis which is in accordance with previous findings of hamsters kept under DD for only 3–5 days after 14L:10D conditions (Klose et al., 2011; Tong et al., 2004). These and other findings led to discussions about possible variations in clock gene function and interaction in different organs and species (Dibner et al., 2010). In order to clarify these issues, further systematical investigations on clock gene expression patterns in different organs and species under standardized methodological conditions are needed.

We therefore hypothesized that daily clock gene expression shows species-specific patterns in the testis and possibly also in other peripheral tissues. Furthermore, differences between the investigated organs were expected which would promote the hypothesis of potential clock–unrelated clock gene functions. With the aim to address these issues, daily clock gene expression patterns of *Bmal1*, *Per1*, *Per2*, *Clock*, *Cry1*, and *Cry2* were investigated in the testis, kidney, liver, spleen and heart of three different hamster species, the Djungarian, Roborovski, and Chinese hamsters. The Djungarian hamster is widely used in chronobiological studies due to its strong sensitivity to photoperiod in both circadian and seasonal rhythms. Additionally we were interested in the Chinese hamster, since it has remarkably big visible male reproductive organs together with a remarkably long duration of spermatogenesis when compared to other rodent and mammalian species (Oud and De Rooij, 1977). Hence, we proposed to also find differences in their testicular gene expression when compared to the Djungarian hamster. Finally, the Roborovski hamster was chosen because it belongs to the same genus as the Djungarian hamster and might therefore have similar clock gene expression patterns.

2. Methods

2.1. Animals

Djungarian hamsters (*P. sungorus*), Roborovski hamsters (*Phodopus roborovskii*) and Chinese hamsters (*Cricetulus griseus*) were born and raised in the animal facility of Jacobs University Bremen. They were kept under long-day photoperiod (16L:8D) in a room which was equipped with a 150 cm Polylux XL fluorescent lamp (58 W, warm white; Osram, München, Germany). Illumination level during the light phase, measured with a Light Meter (72-6693, Tenma, Tokyo, Japan), was 250 lx. Animals were kept in polycarbonate cages on softwood bedding and a handful of hay in groups of two or three animals and were separated to individual cages one week before the experiments. Five male hamsters per species (age: 4-8 months) were sacrificed every 3 h using terminal CO_2 anesthesia at each of eight time points during a 24-hour period. Tissue samples of the testis, kidney, liver, spleen, and heart were collected and frozen immediately in liquid nitrogen. Animal experiments were performed according to the legal requirements of Germany, and the Bremen State Commission for Animal Welfare approved the experiments following §8a of the German Animal Welfare Law (522-27-11/02-00 [114]).

2.2. Quantitative RT-PCR

Total RNA was extracted from all 600 tissue samples using the RNeasy Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's