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RESEARCH ARTICLE

Rhythmic profiles of cell cycle and circadian clock gene transcripts in mice: a possible association between two periodic systems

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SUMMARY

The circadian system shapes the rhythms of most biological functions. The regulation of the cell cycle by a circadian clock was suggested to operate *via* stages S, G2 and G2/M. This study investigated a possible time link at stages G1 and G1/S as well. The daily expression profiles of cell cycle markers (*Ccnd1*, *Ccne1* and *Pcna*) and circadian clock genes (*Per2* and *Clock*) were monitored in liver and esophagus (low and high proliferation index, respectively) of BALB/c mice. Locomotor activity displayed a 24 h rhythm, establishing the circadian organization of the suprachiasmatic nucleus. In the liver, the mRNA level of *Per2* and *Clock* fitted the circadian rhythm with a 7.5 h shift. This temporal pattern suggests that the liver harbors a functional circadian clock. The rhythm of the analyzed cell cycle genes, however, was of low significance fitness and showed an opposite peak time between *Pcna* and *Clock*. These results indicate a weak regulatory role of the circadian clock. In the esophagus, the rhythms of *Clock* and *Per2* mRNA had a similar peak time and non-circadian periods. These results suggest either that the esophagus does not harbor a functional circadian apparatus or that the phenotypes stem from differences in phase and amplitude of the rhythms of its various cell types. The similarity in the rhythm parameters of *Clock*, *Ccne1* and *Pcna* transcripts questions the control of the circadian clock on the cell cycle along the G1 and G1/S stages. Yet the G1/S transition may play a role in modulating the local clock of proliferating tissues.

Key words: esophagus, liver, locomotor activity, transcript rhythm, time cue, SCN.

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INTRODUCTION

In mammals, the circadian time-keeping system shapes the rhythms of many physiological and behavioral functions. In rodents, a central circadian pacemaker located in the hypothalamic suprachiasmatic nucleus (SCN) is capable of sensing time cues from the external surroundings and internal body milieu. This major pacemaker is assumed to synchronize the peripheral secondary tissue's clocks (e.g. liver, heart, olfactory bulbs, pineal gland, etc.), utilizing neuronal and humoral pathways (Buijs and Kalsbeek, 2001; Gachon et al., 2004; Schibler and Sassone-Corsi, 2002). Consequently, physiology and behavior can be adjusted to the habitat conditions (Kalsbeek and Buijs, 2002). Virtually the same genes are involved in the molecular mechanism of the circadian clock in the SCN and peripheral tissues (Dibner et al., 2010; Mazzoccoli et al., 2012; Rana and Mahmood, 2010). Yet, the resulting phenotype of the clock tends to be tissue specific (Dibner et al., 2010; Rana and Mahmood, 2010; Yoo et al., 2004). The clock mechanisms are based on transcriptional–translational feedback loops of positive and negative effects. The CLOCK/BMAL1 protein complex constitutes the basic positive limb of the feedback loop, whereas the PER/CRY complex forms its negative limb. Other gene products were reported to shape the phenotype of the circadian clocks (Dardente and Cermakian, 2007; Dibner et al., 2010; Ko and Takahashi, 2006; Welsh et al., 2010; Yagita et al., 2001).

The cell division cycle is a time-dependent sequence of stages (G1, S, G2 and M) that are based on interlocking loops of positive and negative elements (resembling the circadian clock mechanism) (Alberts et al., 1994).

The overall rhythm pattern of the cell cycle is determined by the tissue's circadian clock and modified by developmental programming, illumination and metabolic (feeding) cycles (Borgs et al., 2009; Kondratov et al., 2007). In rodents, for example, regularly scheduled meal hours synchronize the timing of S-phase in the cornea, bone marrow, lymphoid system and intestine (Canaple et al., 2003; Li et al., 2009). The rhythm of the cell cycle stages in many tissues (bone marrow, lymphoid system, alimentary tract epithelium, skin, etc.) oscillates in a circadian but tissue-dependent manner (Abrahamsen et al., 1993; Bjarnason and Jordan, 2000; Canaple et al., 2003; Haus et al., 1983; Khapre et al., 2010; Kondratov et al., 2007; Matsuo et al., 2003; Potten et al., 2002; Smaaland et al., 2002). The different responsiveness of various cell types to ex-tissue signals further amplifies the diversity in the rhythmic pattern of the cell cycle between tissues (Edgar et al., 2001; Edmunds, 1992).

Cell proliferation timing is regulated *via* the expression of key cell cycle genes. Clock gene proteins (PER1/2 and CLOCK-BMAL complex) are assumed to regulate the cell proliferation timing through some cell cycle-related genes, such as *Wee1*, *c-Myc* and *Ccnd1* (Fu et al., 2002; Gery et al., 2006; Khapre et al., 2010; Rana and Mahmood, 2010; Sahar and Sassone-Corsi, 2007). For example, extensive translation of PER1/2 leads to inhibition and even arrest of the cell cycle in colon cancer cells (Khapre et al., 2010). In

contrast, inhibition of *Per2* transcription *in vivo* almost doubled the daily amplitude of the tumor growth rhythm in breast cancer patients (Yang et al., 2009).

In this study, mRNA patterns of two clock genes (*Clock* and *Per2*) and three cell cycle (G1 and S)-related genes (*Ccnd1*, *Ccne1* and *Pcna*) were monitored in two tissues of BALB/c inbred mice strain: esophagus, which is considered to have a relatively high proliferation index (Muskhelishvili et al., 2003; Scheving et al., 1979), and liver, with a relative quiescent status (Counts et al., 1996; Khapre et al., 2010; Michalopoulos and DeFrances, 1997). The choice of *Clock* and *Per2* was based on studies that include these genes as regulators in the gating of the cell cycle stages (G1 to S transition) (Johnson, 2010; Lahti et al., 2012). Locomotor activity was monitored in order to establish the circadian organization of the SCN's master pacemaker (Davis and Viswanathan, 1996; Earnest et al., 1999; Reebs and Maillet, 2003).

MATERIALS AND METHODS

Animals

BALB/c male mice, 2.5 months old, were housed in cages with food and water freely available. All mice were adapted to the experimental conditions for 3 weeks prior to the experiment. For the molecular studies, 16 mice were housed at a density of 3–4 per cage and for locomotor activity monitoring, a pair of mice was housed in a cage. The procedures followed the guidelines of Tel-Aviv University's Animal Care Committee.

Illumination regimen

Mice were exposed to a 12 h light:12 h dark cycle, with lights on at 06:00 h ('normal' conditions). Light intensity, at cage level, was 700 lx.

Tissue sampling

Every 6 h, starting at Zeitgeber time (number of hours and minutes past light onset) ZT 2 (08:00 h) and over a period of 24 h, 3–4 mice were killed by cervical dislocation. The esophagus and the right lobe of the liver were dissected from each mouse, rinsed with saline and immersed separately in RNAlater stabilization reagent (Ambion, Austin, TX, USA).

RNA extraction

Total RNA was isolated from both tissues with the PARIS kit (Ambion) following the manufacturer's instructions. DNA and protein residues were removed by applying the RNase-Free DNase Set (Qiagen, Valencia, CA, USA), followed by the RNeasy MiniElute kit (Qiagen) according to the manufacturer's instructions.

RNA concentration and quality

Optical density in each sample was measured at 260, 230 and 280 nm wavelengths using a GeneQuantPro spectrophotometer (Amersham BioSciences AB, Uppsala, Sweden), in order to assess the RNA concentration (A_{260}) and the levels of DNA and protein contamination (A_{260}/A_{230} and A_{260}/A_{280}, respectively).

cDNA library construction

The same amount of total RNA from each tissue of each mouse was reverse transcribed to single stranded cDNA using the Ommniscript kit with RNase inhibitor (Qiagen), according to the manufacturer's protocol. Briefly, the reaction (50 µl total volume) mix containing 10× RT buffer, deoxyribonucleotide triphosphate, random primers, $MgCl_2$, Omniscript RT enzyme, RNase inhibitor and RNA samples (including blanks) was incubated at 37°C for 60 min in a PTC-100 Thermal Cycler (MJ Research, Waltham, MA, USA).

Quantitative real time PCR (qRT-PCR)

In each of the tissues, mRNA levels of the target genes were assessed with specific TaqMan Assays-on-Demand Gene Expression (Applied Biosystems, Foster City, CA, USA) (Table 1). The transcript levels were determined by quantitative RT-PCR using ABI PRISM 7000 (Applied Biosystems). Table 1 details the primer sequences and amplicon length of each cDNA. Amplifications were performed in 20 µl volume reactions containing: 1 µg of cDNA, Universal TaqMan master mix (Applied Biosystems) and a primers/probe mix (Applied Biosystems) according to the manufacturer's instructions. The qRT-PCR reactions on each of the cDNA samples were run in triplicate. The $2^{-\Delta\Delta Ct}$ technique (Livak and Schmittgen, 2001) was applied in order to retrieve the relative mRNA level of the five genes. The expression level of each gene was normalized according to the reference gene *Gapdh* in the same animal along the 24 h time span.

Locomotor activity

Locomotor activity was monitored for the last week of the adaptation period. Activity data were collected every 15 s and recorded in 15 min time bins using electromagnetic sensors. In order to minimize the effects of external interruptions (e.g. water exchange, food supply and injections), the data recorded during the first 2 h following each known interruption were removed.

Daily rhythm parameters and statistical analysis

We use Two-dimensional Table Curve (TableCurve 2D, Jandel Scientific, San Diego, CA, USA) to assess the presence of periods equal to or longer than 12 h. The software derives the 'basic' characteristics for each of the rhythm components: period, acrophase (waveform-approximated peak time) and amplitude (half of the waveform-approximated extent of variation) with their standard errors (Weigl et al., 2004; Weigl et al., 2010). We refer in this study to periods of three domains: 24±4 h (circadian), and 18±2 and 13±3 h (semidian).

Statistics

The fit of the expression patterns to a rhythmic function was evaluated by two statistical parameters that were obtained using TableCurve 2D: *P*-value of the rhythm and degrees of freedom (d.f.)-adjusted r^2 (d.f. r^2). Two-sided unpaired *t*-tests with Welch correction were utilized to assess significant differences between

Table 1. Primer and probe sequences used for qRT-PCR

Gene transcript	Assay no.	Amplicon length (bp)	Sense primer
Clock	Mm00455950_m1	81	5′-GAGACAGCTGCTGACAAAAGCCAAG-3′
Per2	Mm00478113_m1	73	5′-TCCAACATGCAACGAGCCCTCAGAC-3′
Ccnd1	Mm00432367_m1	63	5′-GAGGATAGCAGTCAGCCCTGGGATG-3′
Ccne1	Mm00432359_m1	58	5′-TCTGTGCCACAGATGTGAAGTTCAT-3′
Gapdh	Mm99999915_g1	107	5′-TGAACGGATTTGGCCGTATTGGGCG-3′

Assay no. refers to the TaqMan Assay-on-Demand number (see Materials and methods).

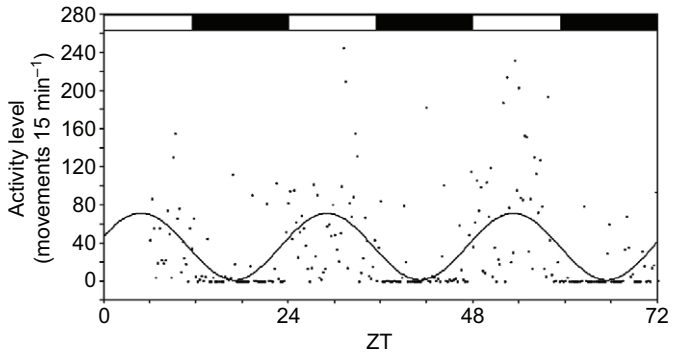

Fig. 1. Rhythmic pattern of locomotor activity of male BALB/c mice during the last 3 days of monitoring. Each point represents the level of locomotor activity in a 15 min time bin. The best-fitted rhythm function, including its statistical fitness, and the period and acrophase of each rhythm component were as follows: period 24:15 h, acrophase ZT 05:22 (ZT is Zeitgeber time, where ZT 0 indicates light onset and ZT 12 indicates dark onset), degrees of freedom-adjusted r^2 (d.f. r^2)=0.23, $P<10^{-5}$. The black and white bars indicate the 12 h dark and light period, respectively.

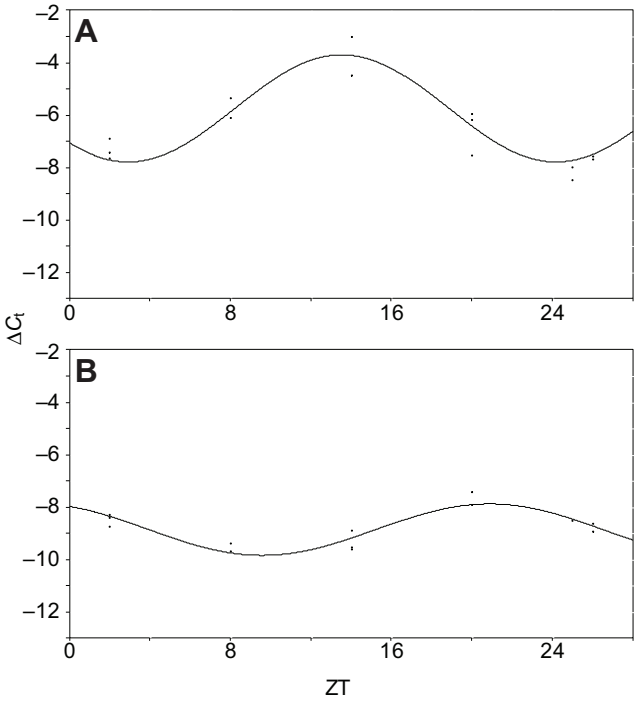

Fig. 2. Rhythmic patterns of two clock gene transcripts in the liver of male BALB/c mice: *Per2* (A) and *Clock* (B). For each of the mice, the mRNA level of the gene of interest was normalized to that of the reference gene *Gapdh*. The best-fitted rhythm function and its statistical fitness were as follows: (A) period 21:14 h, acrophase ZT 13:34, amplitude 2.04 cycles, d.f. r^2=0.79, $P=1.7\times10^{-4}$; (B) period 22:31 h, acrophase ZT 20:52, amplitude 0.98 cycles, d.f. r^2=0.74, $P=8.5\times10^{-4}$. The lower the negative ΔC_t, the more abundant the target gene transcript. ZT indicates time (h:min) after light onset.

the parameters of the different curves. Differences between amplitudes of <20% and between acrophases and periods of <2 h were not considered biologically meaningful. Differences of <10% in the actual rhythm fitness index (d.f. r^2) was also regarded as biologically non-significant.

RESULTS

The rhythms of mRNA levels of two clock genes (*Per2* and *Clock*) were monitored in the liver and esophagus and compared to that of locomotor activity, a behavioral marker of the SCN circadian pacemaker's temporal organization. In addition, this study assayed the daily transcript levels patterns of three cell cycle genes (*Ccnd1*, *Ccne1* and *Pcna*) in these two peripheral tissues.

Locomotor activity

The parameters of the 3 day activity pattern of BALB/c mice are presented in Fig. 1. The circadian period best fitted the activity data with an acrophase at ZT 05:15.

Pattern of mRNA level in the liver

The profiles of *Per2* and *Clock* transcripts exhibited significant circadian rhythms (Table 2; Figs 2, 3). Yet, there were phenotypic differences: the amplitude of *Per2* mRNA rhythm was 2-fold more robust than that of *Clock* mRNA (P=0.006), and ~7 h phase difference was found between the acrophases of these two transcripts. Similar temporal and amplitude relationships between these gene transcripts were documented previously (Liu et al., 2007).

In the case of the cell cycle genes, the rhythm of *Pcna* also demonstrated a significant circadian rhythm of 25:17 h. However, the *Ccnd1* transcript level was of a shorter period (18:19 h). The acrophases of *Pcna* and *Ccnd1* rhythms were shifted by ≥4:24 h ($P\leq0.003$). No significant rhythm could be detected for *Ccne1* mRNA.

Pattern of mRNA level in the esophagus

In the esophagus, mRNA levels of all examined genes monitored over 24 h showed significant rhythms (Table 3; Figs 4, 5). The oscillations of *Clock*, *Ccne1* and *Pcna* were similar in their temporal pattern: they all had ~17 h period and two acrophases around

Table 2. The rhythmic profiles of selected circadian clock- and cell cycle-related gene transcripts in the livers of male BALB/c mice

Rhythm parameter/ gene transcript	d.f. r^2 (P-value)	Period (h:min)	Acrophase (ZT)	Amplitude (cycles)
Per2	0.79 ($P=1.7\times10^{-4}$)	21:14±1:34	13:34±0:29	2.04±0.3
Clock	0.74 ($P=8.5\times10^{-4}$)	22:31±1:01	20:52±0:31	0.98±0.15
Ccnd1	0.395 (P=0.028)	18:19±1:28	04:24/22:43±0:55	0.5±0.14
Ccne1	n.s. rhythm*	–	–	–
Pcna	0.579 (P=0.0033)	25:17±2:02	08:35±0:55	0.8±0.16

d.f. r^2, modified r^2 value according to the number of degrees of freedom; ZT, zeitgeber time (ZT0 indicates light onset, ZT12 indicates dark onset).
Values (±s.e.m.) of period, acrophase and amplitude are given.
Amplitude level is given in cycles (1 cycle is equivalent to a 2-fold difference in the transcript level).
*The transcript pattern does not fit to any rhythm with period(s) between 10 and 28 h (P>0.05).

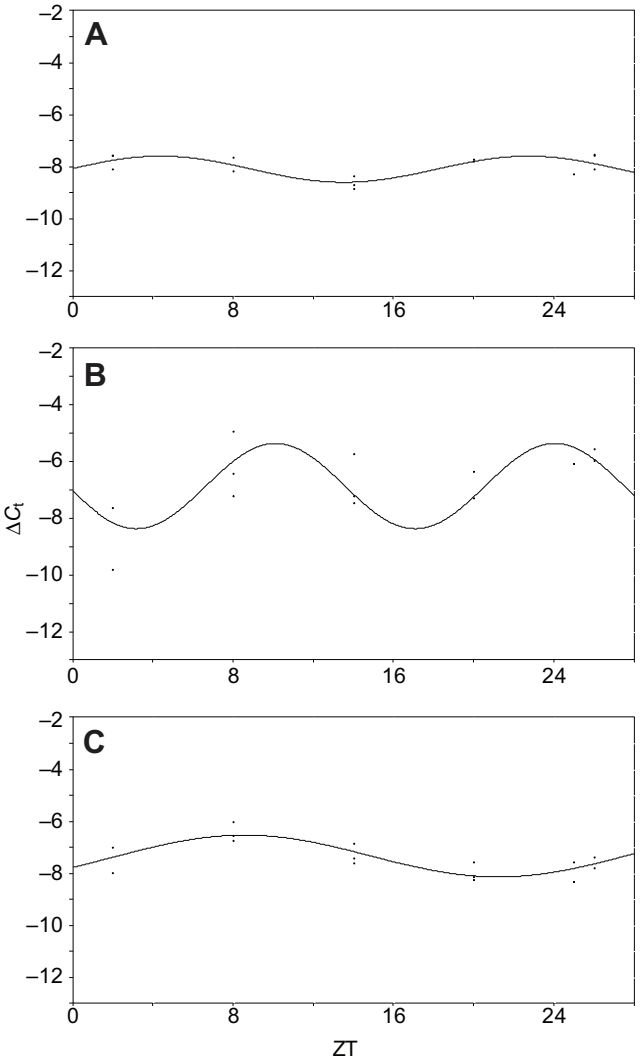

Fig. 3. The best-fitted rhythmic patterns of selected cell cycle-related gene transcripts in the liver of male BALB/c mice. (A) *Ccnd1* (G1 stage), (B) *Ccne1* transcript (G1/S transition) and (C) *Pcna* (marker of cell proliferation, S stage). For each of the mice, the mRNA level of the gene of interest was normalized to that of the reference gene *Gapdh*. The best-fitted rhythm function and its statistical fitness were as follows: (A) period 18:19 h, acrophase ZT 04:24/22:43, amplitude 0.5 cycles, d.f. r^2=0.39, P=0.028; (B) non-significant rhythm (P>0.05); (C) period 25:17 h, acrophase ZT 08:35, amplitude 0.8 cycles, d.f. r^2=0.58, P=3.3×10^{-3}. The lower the negative ΔC_t, the more abundant the target gene transcript. ZT indicates time (h:min) after light onset.

ZT 05:30 and ZT 22:30. *Per2* mRNA showed a clear semi-dian period and the pattern of *Ccnd1* was the only one to fit a circadian rhythm (21:17±0:28 h). The differences in the periods of the clock gene transcripts were highly significant (P<10^{-4}).

The acrophase (around ZT 05:30) was common to the cell cycle- and clock-related transcripts rhythms (Table 3) and there were also amplitude differences: the largest fluctuation was displayed by *Per2* transcript and the lowest by *Pcna* transcript (P=0.03).

DISCUSSION

Various studies have suggested that the circadian clock paces the timing of the cell cycle by synchronizing the expression of G1/S- and G2/M-related genes (Borgs et al., 2009; Khapre et al., 2010; Rana and Mahmood, 2010). At the same time, Oikonomou and Cross argued that the phases of the cell cycle stages could act as a time cue for the peripheral clock in those tissues (phase locking model) (Oikonomou and Cross, 2010). This study compared a possible interaction between the circadian clock and the passage from G1 to S stage in two peripheral tissues of different proliferation index, liver (low) and esophagus (high).

Circadian clock phenotype

Under 'normal' conditions, the hierarchical model of the circadian time-keeping system anticipates that all peripheral clocks will be synchronized to the SCN circadian pacemaker and according to a ~24 h period (Dibner et al., 2010; Kowalska and Brown, 2007; Lowrey and Takahashi, 2011; Mohawk et al., 2012). The expression patterns of the positive and negative limb of the basic feedback loop (*Clock* and *Per2* in our case) should be close to opposite in their phases in order for the clock to oscillate. The circadian profile of the locomotor activity predicts that the SCN pacemaker is responsible for the management of the circadian oscillations. The best-fitted liver mRNA rhythms of *Per2* and *Clock* resemble the locomotor's activity circadian period. The circadian acrophases of *Per2* and *Clock* transcripts were shifted by about 7.5 h; a similar timing alignment was reported in other tissues with circadian clocks, such as the lung (Liu et al., 2007).

In the esophagus, in contrast, *Clock* and *Per2* transcripts exhibited non-circadian rhythms of diverse periods and amplitudes, and one similar acrophase (ZT 05:24, ZT 06:02). Several explanations can be proposed for the non-circadian phenotype: (i) the esophagus harbors neither a self-sustaining nor a SCN-paced circadian time device; (ii) the circadian phenotypes of other variables in the esophagus (but not the analyzed clock genes) are directly governed by the SCN and not by the tissue's clock; and (iii) genes other than *Per2* (*Per1* for example) function as the principal factors in the negative limb of the esophagus rhythm (Johnson, 2010). However, the relatively large amplitude of the *Per2* mRNA rhythm does not favor this alternative. In addition, the non-circadian phenotype could also be the outcome of the differences in phase and amplitude between the various cell types in that tissue (epithelial, connective, muscular and nervous) (DeNardi and Riddell, 1991; Henrikson et al., 1997; Scheving and Gardner, 1998).

Degree of proliferation in the examined peripheral tissues

In the liver, the lack of significant oscillations in the transcript level of *Ccne1* and the relatively low amplitude of the *Pcna* proliferation marker rhythm (0.8 cycles) seem to support the quiescent status of most of the hepatocytes (Counts et al., 1996; Khapre et al., 2010; Michalopoulos and DeFrances, 1997).

In the esophagus, however, the significant amplitude of the rhythm of both *Ccne1* and *Pcna* transcripts affirms the relatively higher proliferation level of this tissue (Muskhelishvili et al., 2003; Scheving et al., 1979). According to Scheving and colleagues, most of the replicating cells are located in the sub-mucosal epidermis (Scheving et al., 1998).

Timing control of the cell cycle stages

At the protein level it was shown that the joint activity of cyclins D1 and E1 (in complexes with the cyclin-dependent kinases, CDKs) promotes the transcription of *Pcna* (Kondo et al., 2006). Several studies found the patterns of these complexes to be shaped by the cyclins' rhythms (Bjarnason and Jordan, 2000; Bjarnason et al., 1999; Morgan, 1995; Murray, 1992; Rensing et al., 2001). Thus, the timing of S stage is assumed to be determined by the G1 cyclins.

In the present study, the rhythm of *Ccnd1* differed significantly from that of *Pcna* in both tissues. Thus, it seems probable that the

Table 3. The rhythmic profiles of selected circadian clock- and cell cycle-related gene transcripts in the esophagus of male BALB/c mice

Rhythm parameter/ gene transcript	d.f. r^2 (P-value)	Period (h:min)	Acrophase (ZT)	Amplitude (cycles)
Per2	0.969 ($P<10^{-5}$)	12:01±0:09	06:02/18:03±0:11	3.78±0.6
Clock	0.852 ($P=3\times10^{-5}$)	17:39±0:31	05:24/23:02±0:23	2.76±0.3
Ccnd1	0.959 ($P<10^{-5}$)	21:17±0:28	06:16±0:14	2.98±0.17
Ccne1	0.559 ($P=9.2\times10^{-3}$)	17±1:02	05:31/22:31±0:40	2.14±0.46
Pcna	0.795 ($P=6.8\times10^{-4}$)	17:02±0:41	06:20/23:22±0:22	2.15±0.3

d.f. r^2, modified r^2 value according to the number of degrees of freedom; ZT, zeitgeber time (ZT0 indicates light onset, ZT12 indicates dark onset).
Values (±s.e.m.) of period, acrophase and amplitude are given.
Amplitude level is given in cycles (1 cycle is equivalent to a 2-fold difference in the transcript level).

phases of G1 and S stages are independently controlled. In the esophagus, for example, the highly significant circadian period of *Ccnd1* might imply a synchrony in the G1 stage between the tissue cells. In the case of the short S stage, however, the shorter and less significant period of the *Pcna* transcript rhythm could point to a degree of desynchronization in the timing of this cell cycle stage. In addition, the fact that this study addressed the transcript patterns rather than the protein levels could also contribute to the different findings obtained here and previously (Kondo et al., 2006).

Possible interaction between the cell cycle and circadian clock genes

The coupling of the cell cycle stages to the tissue's clock device predicts that functions of the cell cycle stage will oscillate according to the local clock (Gérard and Goldbeter, 2012).

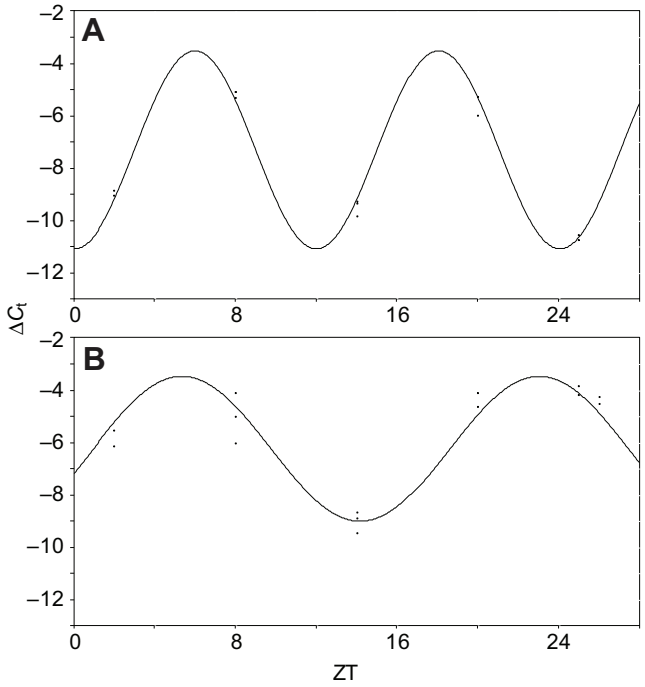

Fig. 4. Rhythmic patterns of two clock gene transcripts in the esophagus of male BALB/c mice: *Per2* (A) and *Clock* (B). For each of the mice, the mRNA level of the gene of interest was normalized to that of the reference gene *Gapdh*. The best-fitted rhythm function and its statistical fitness were as follows: (A) period 12:01 h, acrophase ZT 06:02/18:03, amplitude 3.78 cycles, d.f. r^2=0.97, $P<10^{-5}$; (B) period 17:39 h, acrophase ZT 05:23/23:02, amplitude 2.76 cycles, d.f. r^2=0.85, $P=3\times10^{-5}$. The lower the negative ΔC_t, the more abundant the target gene transcript. ZT indicates time (h:min) after light onset.

In the esophagus, the similarity of the rhythmic profiles of *Ccne*1 and *Pcna* transcripts (G1 to S-related genes) and the absence of

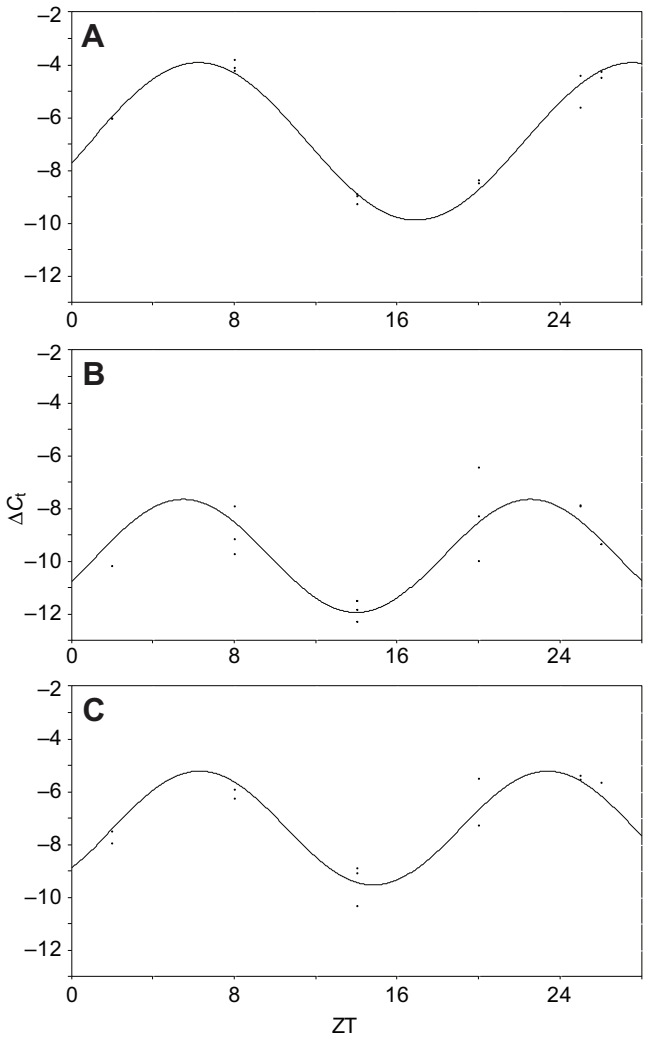

Fig. 5. The best-fitted rhythmic patterns of selected cell cycle-related gene transcripts in the esophagus of male BALB/c mice. (A) *Ccnd1* (G1 stage), (B) *Ccne1* transcript (G1/S transition) and (C) *Pcna* (marker of cell proliferation, S stage). For each of the mice, the mRNA level of the gene of interest was normalized to that of the reference gene *Gapdh*. The best-fitted rhythm function and its statistical fitness were as follows: (A) period 21:17 h, acrophase ZT 06:16, amplitude 2.98 cycles, d.f. r^2=0.96, $P<10^{-5}$; (B) period 17 h, acrophase ZT 05:31/22:31, amplitude 2.14 cycles, d.f. r^2=0.56, $P=9.2\times10^{-3}$; (C) period 17:02 h, acrophase ZT 06:20/23:22, amplitude 2.15 cycles, d.f. r^2=0.79, $P=6.8\times10^{-4}$. The lower the negative ΔC_t, the more abundant the target gene transcript. ZT indicates time (h:min) after light onset.

circadian rhythm of the clock genes oppose the possibility that the cell cycle rhythm in this tissue is paced by a local time device. The synchrony of the mRNA rhythms of *Ccne1*, *Pcna* and *Clock* (a similar period and a common acrophase, ZT 05:00–06:00), suggests that the G1/S transition operates as a time cue of the tissue clock apparatus, through regulating the expression rhythm of the *Clock* gene. It should be noted that the temporal alignment on the protein level could differ from the current results, which are based on the transcript level.

The 5 h shift between the second acrophases of *Per2* (ZT 18:03) and *Clock* (ZT 23:02) mRNA rhythms possibly implies a close relationship to the local clock device (esophagus). Thus, it seems that the rhythmic phenotypes of *Per2* and *Clock* transcripts reflect a combination of the G1/S transition effect and the output of the local clock apparatus.

In tissues of low proliferation index, the rhythm of clock gene expression is not expected to be modified by the cell cycle's signals. The circadian oscillations of *Per2* and *Clock* transcripts in the hepatic cells (locomotor-like period) suggest a SCN role in that tissue pace.

The expression rhythms of *Pcna* and *Clock* genes were similar in their circadian period and amplitude with about 12 h phase shift. Such a relationship questions whether the liver's circadian clock indirectly restrains the proliferation of the hepatocytes through pacing their timing pattern (Gérard and Goldbeter, 2012).

CONCLUSIONS

In conclusion, this study suggests the existence of two routes of interaction between the cell cycle and the clock time device in the esophagus and liver. The phase and amplitude of the G1 stage are governed by the circadian clock (either through *Per2* or *Clock*). Yet, in dividing cells, the G1/S checkpoint seems to act as a time cue that can modulate the circadian clock output of the same tissue. The extent of this effect might be influenced by the proliferation index of that tissue and could explain the non-circadian oscillations of the clock genes' phenotypes.

AUTHOR CONTRIBUTIONS

This research was conducted as part of Y.W.'s PhD thesis at Tel Aviv University. I.E.A. and L.P. guided and supervised the study. Y.W. and L.P. wrote and revised the manuscript.

COMPETING INTERESTS

No competing interests declared.

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