Biochemical and Biophysical Research Communications 451 (2014) 408–414

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

The circadian rhythm controls telomeres and telomerase activity

Wei-Dar Chen [a,1], Ming-Shien Wen [a,*,1], Shian-Sen Shie [b], Yu-Lun Lo [c], Hung-Ta Wo [a], Chun-Chieh Wang [a], I-Chang Hsieh [a], Tsong-Hai Lee [d], Chao-Yung Wang [a,*]

[a] Department of Cardiology, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taiwan
[b] Department of Infectious Diseases, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taiwan
[c] Department of Thoracic Medicine, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taiwan
[d] Stroke Center and Department of Neurology, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taiwan

ARTICLE INFO

Article history:
Received 27 July 2014
Available online 7 August 2014

Keywords:
Circadian rhythm
Telomerase activity
Telomere
Aging

ABSTRACT

Circadian clocks are fundamental machinery in organisms ranging from archaea to humans. Disruption of the circadian system is associated with premature aging in mice, but the molecular basis underlying this phenomenon is still unclear. In this study, we found that telomerase activity exhibits endogenous circadian rhythmicity in humans and mice. Human and mouse *TERT* mRNA expression oscillates with circadian rhythms and are under the control of CLOCK–BMAL1 heterodimers. CLOCK deficiency in mice causes loss of rhythmic telomerase activities, *TERT* mRNA oscillation, and shortened telomere length. Physicians with regular work schedules have circadian oscillation of telomerase activity while emergency physicians working in shifts lose the circadian rhythms of telomerase activity. These findings identify the circadian rhythm as a mechanism underlying telomere and telomerase activity control that serve as interconnections between circadian systems and aging.

1. Introduction

Circadian rhythms exist in organisms from archaea to humans [1]. In mammals, the central clock of the hypothalamic suprachiasmatic nucleus and peripheral clocks in tissues coordinate multiple aspects of behavior and physiology [2]. The oscillatory rhythms regulate cell growth, hormonal homeostasis, electrolyte balance, energy metabolism, cardiovascular physiology, and sleep, with external environmental changes synchronizing with the rotation of the earth [3]. Disturbance of circadian rhythms often results in abnormal energy metabolism, increased carcinogenesis, cardiovascular diseases, sleep disturbances, and early aging [4]. Recent advances have supported evidence for a link between circadian rhythms and the physiology changes in aging. Flies with disturbed light/dark cycles or mutations in core circadian genes have reduced life span [5]. Mice deficient in BMAL1, CLOCK, or PER1/2 proteins develop early aging phenotypes [6]. In human, disturbances in circadian timing in shift workers is associated with an increased risk of coronary artery diseases and cancer [7].

Telomeres are DNA–protein complexes that protect chromosome ends and maintain genomic integrity. Telomeres shorten with cell division and impose a replicative limit on the growth of primary cells in culture. Telomere shortening is mainly compensated by the enzyme telomerase, which adds back telomeric DNA [8]. The regulation of telomerase activity requires integration at multiple levels and includes TERT expression control, enzyme phosphorylation, telomere complex subunit assembly, and transport [10].

The aging process in humans is associated with changes in circadian rhythm patterns [11]. Aged mice have decreased sensitivity to the effects of light entrainment and show reduced amplitude of circadian gene expression [4]. Cellular senescence impairs circadian rhythmicity, and introduction of telomerase rescues clock genes expression that has decreased due to senescence [12]. Cancer cells also have oscillatory rhythms in DNA synthesis and telomerase activity [13]. These findings suggest potentially important links between circadian rhythms and telomeres or telomerase. However, the exact mechanisms and potential interactions between circadian rhythms and telomere are still unclear.

2. Materials and methods

2.1. Cell culture and antibody

Multiple and independent mouse embryonic fibroblasts (MEFs) from wild-type and *Clock*$^{-/-}$ knockout mice were derived and assayed. To assess the oscillation of gene expression, cells were

* Corresponding authors. Address: 5 Fu-Hsing Street, Taoyuan 333, Taiwan. Fax: +886 3 3289134.
E-mail address: cwang@ocean.ag (C.-Y. Wang).
[1] W.D.C. and M.S.W. contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.07.138
0006-291X/

synchronized using serum shock. Antibodies against mTERT (Santa Cruz), Per2 (Millipore), Rev-Erbα (Cell Signaling), and Lamin A/C (Abcam) were used for Western blot.

2.2. Animals

Clock$^{-/-}$ mice on a C57BL/6J background were obtained from Dr. Reppert and Dr. Weaver (University of Massachusetts Medical School, Worcester, MA) [14]. The animal experiments were in accordance with the guidelines of Chang Gung University and Chang Gung Memorial Hospital Institutional Animal Care and Use Committee.

2.3. Telomerase assay

Telomerase extracts were prepared with CHAPS lysis buffer. The telomerase repeat amplification protocol (TRAP) assay was performed with 0.1/0.4 μg protein from the mouse liver/lung or 0.01 μg protein from human peripheral blood leukocytes. After PCR amplification and electrophoresis, gels were stained with SYBR Green nucleic acid gel stain (Molecular Probes).

2.4. Transcriptional assay

hTERT promoter regions of various lengths in pGL3-basic plasmids (Promega) were gifts from Dr. Hiroyuki Kugoh (Tottori University, Yonago, Japan). pcDNA3.1 constructs containing *mCry1* and *mCry2* were gifts from Dr. Steven M. Reppert (University of Massachusetts Medical School, MA, USA). The cells were lysed 48 h after transfection and analyzed with a dual-luciferase reporter assay system (Promega).

2.5. Chromatin immunoprecipitation (ChIP)

ChIP was performed with livers as previously described [15]. The cross-linked nuclei were immunoprecipitated using an antibody against CLOCK (Abcam) or BMAL1 (Millipore) or c-Myc (N-262, Santa Cruz). Primers for regions downstream of the TERT promoter were used as negative control. The result was normalized to input control without immunoprecipitation and reported as percent input.

2.6. Telomere Q-FISH

First-passage MEFs were incubated with 0.1 μg/mL colcemide (Gibco) overnight and then fixed in 3.7% formaldehyde in Tris-buffered saline (TBS) for metaphase spreads. Quantitative fluorescent *in situ* hybridization (Q-FISH) was performed with the telomere peptide nucleic acid (PNA) FISH kit/FITC (DakoCytomation) according to the manufacturer's protocol.

2.7. Human subjects

Participants received the Munich Chronotype Questionnaire and Pittsburgh Sleep Quality Index evaluation to exclude sleep disorders and mid-sleep on free days (MSF; the half-way point time between sleep-onset and sleep-end) earlier than 1:00 AM or later than 8:00 AM. Non-emergency physicians were from the departments of pathology, nuclear medicine, radiation oncology, and otolaryngology. Blood tests were performed at the ends of the night shift at 10:00 AM and 5:00 PM later. The clinical research studies were conducted according to the principals by the *Declaration of Helsinki*. Written informed consent was obtained from all study participants prior to enrolment. The protocol was approved by the institutional review board of Chang Gung Memorial Hospital.

2.8. Statistical analysis

Physiological and molecular parameters were analyzed with a cosinor model to determine whether there is a 24 h rhythm. A mixed model analysis of variance was used with standard least-square regression and the restricted maximum likelihood method with SPSS (IBM) and ClockLab software (Actimetrics) to determine the amplitude and phase of the sinusoidal function and to determine whether there were significant relationships. Values were expressed as mean ± standard deviation. Data were compared using Student's *t*-tests or analysis of variance (ANOVA) or Lomb–Scargle analysis, where appropriate. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Circadian oscillation of telomerase activity

To determine whether telomerase activity exhibits circadian rhythms, we assessed telomerase activity at 4-h intervals in the livers and lungs of wild-type mice. The mice were analyzed on the first day under constant darkness (DD) after entrainment for 14 d under a 12 h light/12 h dark (LD) cycle. Under these conditions, telomerase activity was rhythmic and exhibited significant circadian rhythmicity (Fig. 1A and B). The peak telomerase activity was noted at circadian time (CT) 17 and the trough at CT 1, with a peak-to-trough ratio of 2.1. Transcriptional control of telomerase reverse transcriptase (TERT) plays a crucial role in the regulation of telomerase activity [16]. It is possible that mouse *TERT* mRNA and protein also exhibit a circadian rhythm to drive the oscillation of telomerase activity. To test this possibility, we first searched the circadian expression profiles data base (CircaDB) [17]. In CircaDB, *mTERT* transcripts from mouse livers showed significant oscillations (Supplementary Fig. 1). We then examined the *mTERT*, *mPer2*, and *mRev-Erbα* mRNA expression levels in the same livers that were analyzed for telomerase activity. *mTERT* transcripts in livers and lungs showed circadian oscillation with a peak-to-trough ratio of 1.7. *mPer2* and *mRev-Erbα* transcripts have higher amplitude circadian oscillations with peak-to-trough ratios of 10.7 and 14.4, respectively. *mTERT* transcripts peaked at CT 9, *mPer2* at CT 17, and *mRev-Erbα* at CT 5 (Fig. 1C). These results indicate that telomerase activity and *mTERT* mRNA level oscillate with an endogenous circadian rhythm.

3.2. CLOCK deficiency results in decreased telomerase activity and telomere shortening

Given the existence of a circadian rhythm in both telomerase activity and *mTERT* transcription, circadian rhythm disruption may have direct effects on telomerase activity or *mTERT* transcription. We studied the oscillation of telomerase activity in CLOCK-deficient mice. CLOCK-deficient mice have reduced life span but less severe phenotypes than BMAL1-deficient mice [18]. In CLOCK-deficient mice, behavioral rhythms are preserved, while peripheral tissue rhythms are only systemically driven [19]. We analyzed telomerase activity in CLOCK-deficient and wild-type mice on the first and second days, under constant darkness (DD) after entrainment for 14 d under a 12 h light/12 h dark (LD) cycle. The telomerase activity in the livers of CLOCK-deficient mice did not show significant circadian oscillation as compared to that for the wild-type mice (Fig. 2A). The average telomerase activity was also significantly decreased in the livers of CLOCK-deficient mice (Fig. 2A). The balance between telomerase activity and incomplete DNA replication during cell division determines telomere length [16]. The fact that CLOCK-deficient mice have no telomerase

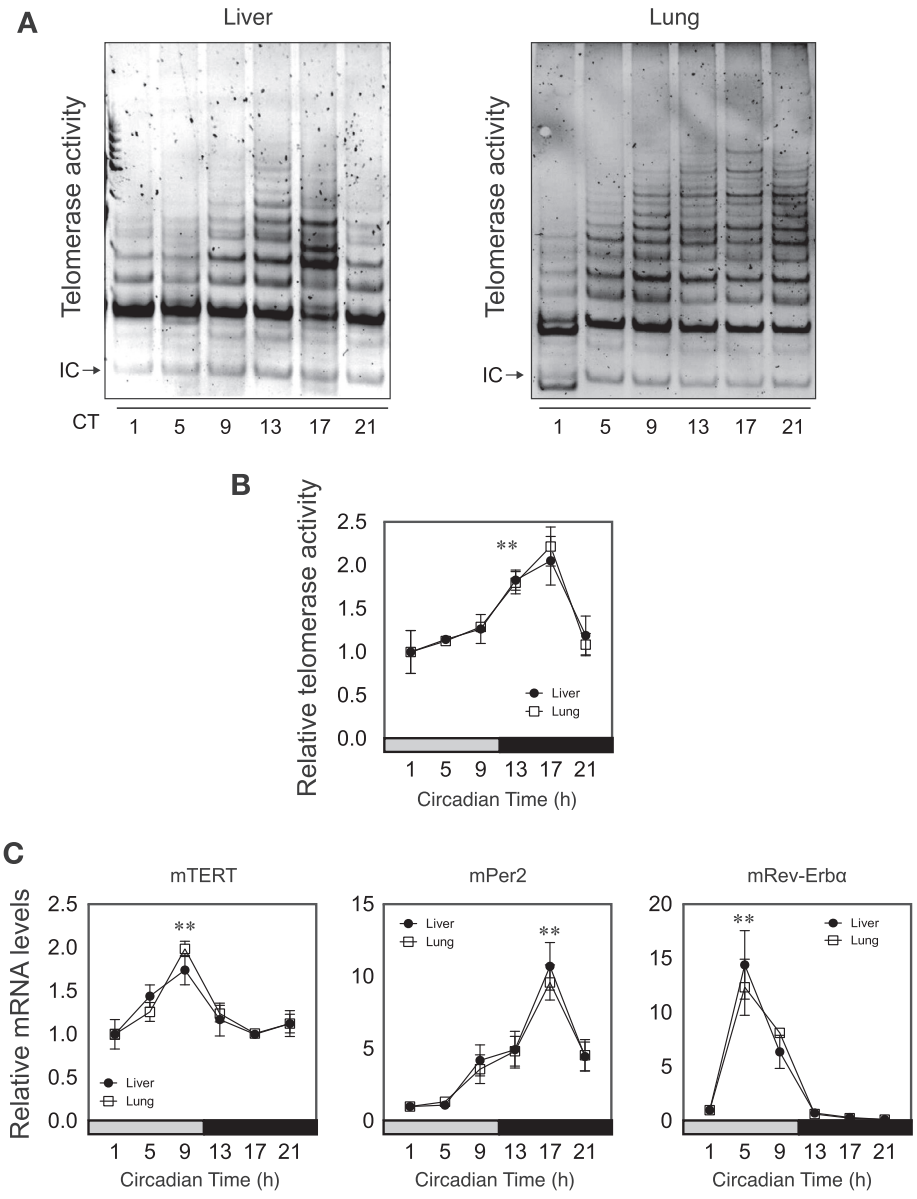

Fig. 1. Circadian oscillation of telomerase activities in mouse liver and lung. Mouse livers and lungs were sampled every 4 h under constant darkness and analyzed with a telomerase repeat amplification protocol (TRAP) assay. (A) Representative gel images from mice liver and lung. (B) Quantification by densitometry. Values were normalized to the internal control first and to CT 1 (mean ± SD, $n = 6$ mice per CT). (C) Liver and lung mRNAs were analyzed and normalized to levels of CT1 (mean ± SD, $n = 6$ mice per CT). $^{**}P < 0.01$ by one-way ANOVA (effect of time).

activity oscillation prompted us to test whether CLOCK deficiency would affect telomere length. First-passage MEFs were isolated from CLOCK-deficient and wild-type mice and subjected to quantitative fluorescent *in situ* hybridization (Q-FISH) analysis with a telomere-specific peptide nucleic acid (PNA) probe. We determined the average telomere fluorescence for each MEF and the corresponding frequency distribution. CLOCK-deficient MEFs showed a significant decrease in average telomere length compared to wild-type MEFs (Fig. 2B). This result indicated that CLOCK deficiency in mice has a critical impact on telomerase activity and telomere length.

3.3. TERT transcripts oscillation require CLOCK

We then asked whether CLOCK is required for oscillation of the *mTERT* transcript. We analyzed mRNA accumulation profiles for 72 h following the serum shock in wild-type and CLOCK-deficient MEFs. As reported previously, *mPer2* transcripts were rhythmic in wild-type MEFs but had a much smaller or arrhythmic amplitude in CLOCK-deficient MEFs [14]. The *mTERT* transcripts showed a rhythm antiphasic to that of *mPer2* transcripts in wild-type MEFs but were arrhythmic in CLOCK-deficient MEFs (Supplementary Fig. 2A). To confirm the oscillation of *mTERT* transcripts *in vivo*, we analyzed mRNA from the same wild-type and CLOCK-deficient livers previously tested for telomerase activity. *mPer2* transcripts in CLOCK-deficient livers showed similar rhythms when compared to wild-type livers while *mRev-Erbα* transcripts had reduced rhythm amplitudes in CLOCK-deficient livers [20]. Indeed, *mTERT* transcripts in CLOCK-deficient mice were arrhythmic compared to transcripts from wild-type mice (Supplementary Fig. 2B).

3.4. Regulation of hTERT gene transcription by CLOCK/BMAL1

Human and mouse *TERT* (*hTERT* and *mTERT*) genes have CLOCK–BMAL1 E-box sites near the 5'end of the gene (Supplementary Fig. 3A), similar to other clock-regulated genes [9,21]. To

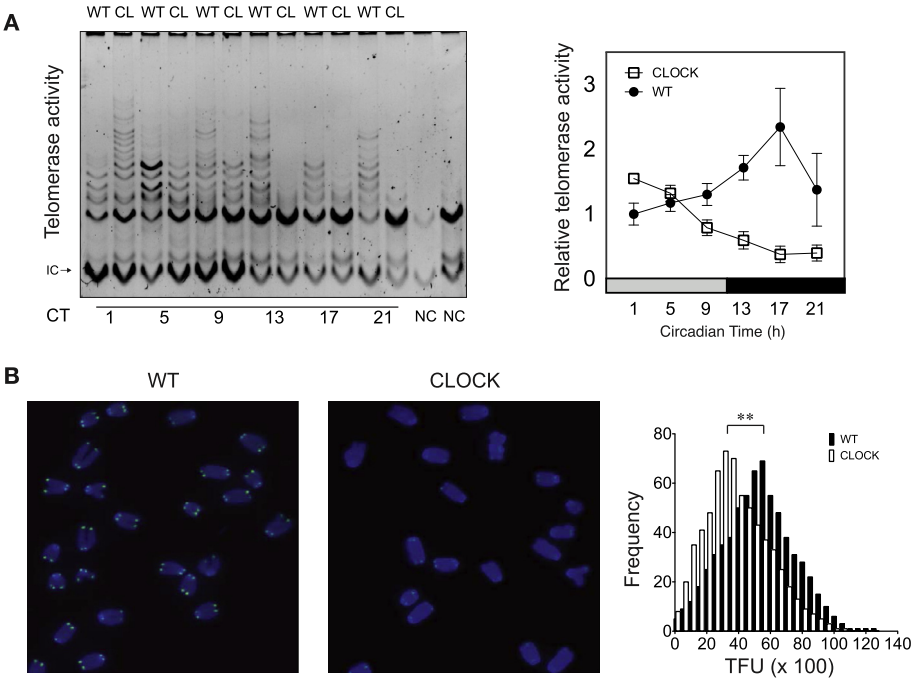

Fig. 2. Telomerase activity and telomere length in wild-type and CLOCK-deficient mice. (A) Telomerase activities were analyzed in livers from wild-type and CLOCK-deficient mice every 4 h for 24 h. Quantifications were done by densitometry and shown in the right panel. Values were normalized to the internal control first and to CT1 of the first day (mean ± SD, $n = 3$ mice per CT). (B) Quantitative telomere-FISH of metaphase spreads of MEFs from wild-type and CLOCK-deficient mice. Left panel: representative images of wild-type and CLOCK-deficient MEFs. Right panel: images were captured and analyzed with TFL-Telo program. Mean telomere fluorescence units (TFUs) were calculated, as well as the frequency of chromosome ends sampled. $^{**}P < 0.01$. WT, wild-type; CL, CLOCK-deficient; NC, negative control.

investigate whether CLOCK–BMAL1 regulates *hTERT* or *mTERT* mRNA expression through the modulation of *hTERT* or *mTERT* promoter activity, we first tested the effects of CLOCK–BMAL1 on the promoter activity of a 1.4-kb fragment of the *hTERT* promoter (hTERT1412). A luciferase activity assay showed that CLOCK or BMAL1 alone could increase the *hTERT* promoter activity by 1.75 or 2.25-fold, respectively. Cotransfection of both CLOCK and BMAL1 could increase *hTERT* promoter activity by 3.55-fold (Fig. 3A). Moreover, CRY1 and CRY2 could inhibit the CLOCK–BMAL1 effects on *hTERT* promoter activity. We tested the transcriptional activity of CLOCK–BMAL1 in 1.4-kb, 1.0-kb, and 233-bp fragments of the *hTERT* promoter (hTERT 1412, hTERT 1047, and hTERT 233, respectively). The 233-bp fragment, which contains 2 E-box sites, had similar transcriptional activity when comparing to the 1412-bp or 1047-bp fragments of *hTERT* promoters, indicating that this 233-bp *hTERT* fragment is responsible for CLOCK–BMAL1 transcriptional activity (Fig. 3B). Mutation analysis showed that the E-box1 located at −165 was responsible for most transcriptional activity in this 233-bp fragment (Fig. 3C). The *mTERT* promoter also contains 2 E-boxes at the −808 and +1 sites (Supplementary Fig. 3A). Similar to the results for *hTERT*, CLOCK–BMAL1 could increase *mTERT* promoter activity by 3-fold (Supplementary Fig. 3B). Fragment analysis of the *mTERT* promoter showed that the 1.9-kb and 824-bp fragments had similar transcriptional responses to CLOCK–BMAL1, whereas 356-bp fragment exhibited no response to CLOCK–BMAL1 (Supplementary Fig. 3C). This result implied that E-box −808 was responsible for the CLOCK–BMAL1 transcriptional activity in the *mTERT* promoter. Mutation analysis of E-box −808 and E-box +1 in the *mTERT* promoter further confirmed this finding (Supplementary Fig. 3D). We then investigated if CLOCK–BMAL1 could bind to this fragment *in vivo*. We performed chromatin immunoprecipitation (ChIP) assays with nuclear extracts obtained from mouse livers at different circadian times. As a positive control, we showed rhythmic binding of CLOCK to the *mPer1* and *mPer2* promoters, as described previously (Fig. 3D) [22,23]. The binding of CLOCK to the *mTERT* promoter was robust and exhibited significant circadian variation with the highest peak at CT 5 (Fig. 3E). Previous studies have shown that c-Myc forms heterodimers with Max and binds directly to the *hTERT* promoter E-boxes [24]. c-Myc induces *hTERT* expression and regulates telomerase activity [25]. *c-myc* is also a clock-controlled gene and its expression oscillates with the circadian rhythm [26,27]. It is possible that the regulation of *hTERT* by the circadian rhythm also occurs through indirect clock control of *c-myc*. To investigate this possibility, we performed c-Myc ChIP for mouse livers. However, the binding of c-Myc to the *hTERT* promoter did not vary over time (Fig. 3F) ($P > 0.05$, one-way analysis of variance). Since CLOCK–BMAL1 is a heterodimer, the CLOCK ChIP results should be able to be replicated by BMAL1 ChIP. We performed BMAL1 ChIP in wild-type and CLOCK-deficient mice. Indeed, the bindings of BMAL1 to the *mPer1* and *mPer2* promoter in wild-type mice were rhythmic but with lower amplitudes in CLOCK-deficient mice (Fig. 3G). The binding of BMAL1 to *mTERT* promoter also exhibited a circadian rhythm with peak at CT 5 in wild-type mice. However, in CLOCK-deficient livers, the binding of BMAL1 to *mTERT* promoters showed no rhythm with significantly lower amplitude (Fig. 3H).

3.5. Circadian rhythm of telomerase activity in emergency physicians and non-emergency physicians

To determine whether a circadian rhythm exists for telomerase activity and *hTERT* mRNA in humans, we investigated telomerase activity and *hTERT* mRNA profiles in leukocytes from 10 healthy volunteers. Telomerase activity in leukocytes exhibited a significant circadian rhythm with the highest peak at ZT 5 (12:00 PM) and the lowest at ZT 9 (4:00 PM). Unlike the oscillation pattern in the mice, there was a second peak at CT13 (8:00 PM) (Fig. 4A and B). *hTERT* mRNA in leukocytes also had circadian oscillation but with a much lower amplitude when compared to *mTERT* (Fig. 4C). These data support that circadian rhythm of telomerase

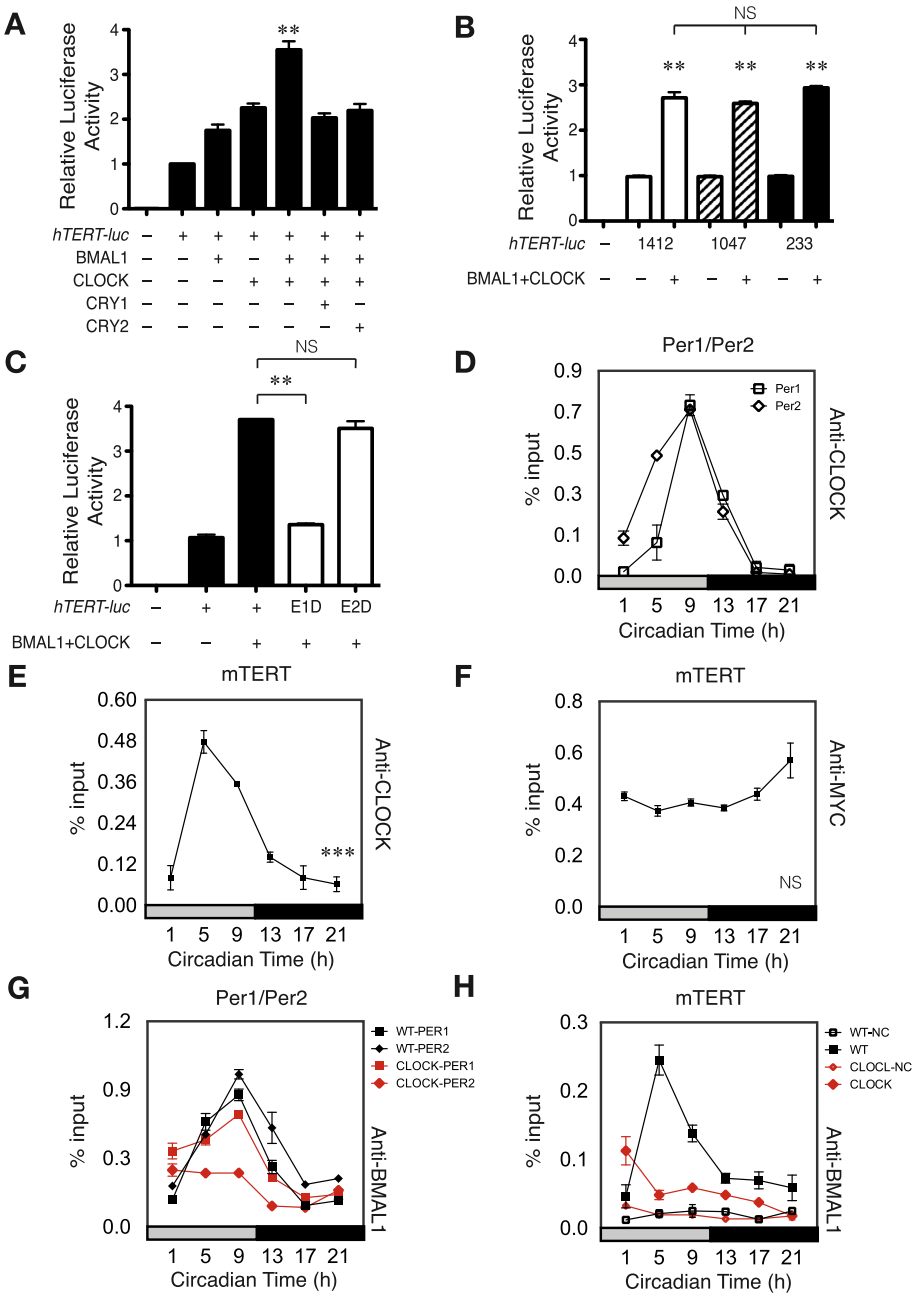

Fig. 3. Transactivation of *mTERT* and *hTERT* promoters by CLOCK–BMAL1 heterodimers to the E-boxes. (A) Effects of CLOCK, BMAL1, Cry1, and Cry2 on the transactivation of 1412 bp *hTERT* promoter in 293T cells or U2OS cells (mean ± SD, $n = 3$). (B) Effects of CLOCK–BMAL1 heterodimers on the transactivation of 1412 bp, 1047 bp, and 233 bp *hTERT* promoters (mean ± SD, $n = 3$). (C) Effects of CLOCK–BMAL1 heterodimers on the transactivation of 233 bp *hTERT* promoters with E-box 1 mutation (E1D) or E-box 2 (E2D) mutation (mean ± SD, $n = 3$). (D) Binding of CLOCK to the *Per1* and *Per2* promoters in mouse livers analyzed by ChIP (mean ± SD, $n = 3$). (E) Binding of CLOCK to *mTERT* promoters in mouse livers analyzed by ChIP (mean ± SD, $n = 3$). (F) c-Myc ChIP analysis on *mTERT* promoters in mouse livers (mean ± SD, $n = 3$). (G) Binding of BMAL1 to the *Per1* and *Per2* promoters in wild-type and CLOCK-deficient livers analyzed by ChIP (mean ± SD, $n = 3$). (H) Binding of BMAL1 to *mTERT* promoters in wild-type and CLOCK-deficient livers analyzed by ChIP (mean ± SD, $n = 3$). $^{**}P < 0.01$, $^{***}P < 0.001$; NS, not significant; NC, qPCR of downstream region of the mTERT promoter as negative control.

activity and *hTERT* mRNA exist in human leukocytes. We then asked if shift work could affect the telomerase rhythm in leukocytes. We recruited 14 physicians working in the emergency department and 13 physicians working in the non-emergency department without night duties under the OCEAN registry (Obesity and Clock for Elegant AgiNg registry). Working schedules for 1 month were reviewed before recruitment to confirm the shift work patterns. The blood samples were tested at 10:00 AM and 5:00 PM the day after night shifts for emergency physicians. Similar to the healthy volunteers, non-emergency physicians had high telomerase activity at 10:00 AM and low telomerase activity at 5:00 PM. Emergency physicians had similar low telomerase activity at 10:00 AM and 5:00 PM without rhythm after their shifts (Fig. 4D), indicating a significant effect of shift work on the circadian rhythm of telomerase activity in leukocytes.

4. Discussion

Both human and mice *TERT* promoters have 2 classic CLOCK–BMAL1 binding E-boxes with CACGTG sequences [28]. Our data suggest that CLOCK–BMAL1 does not bind to the E-box near the transcription start site, which is more evolutionarily conserved,

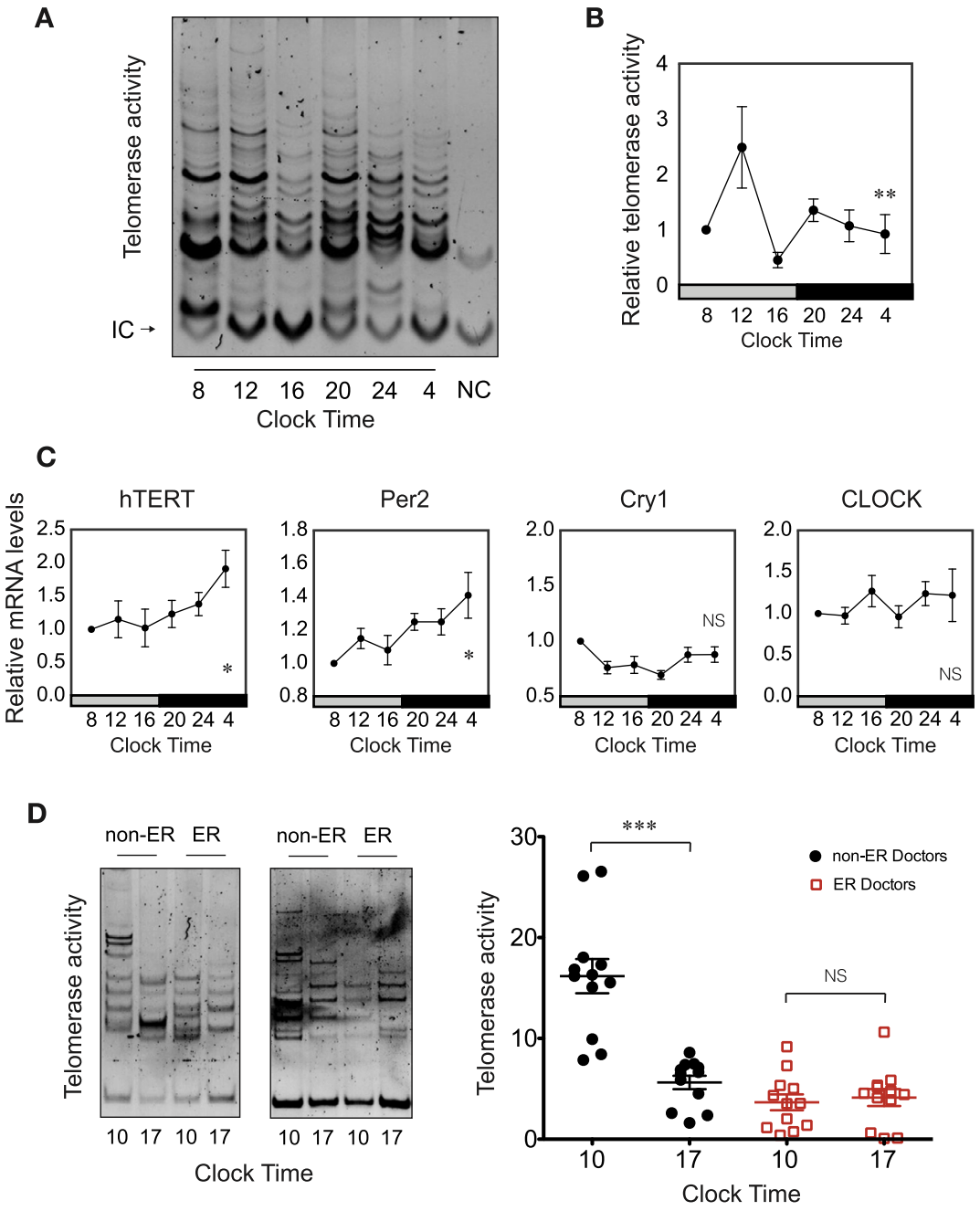

Fig. 4. Circadian oscillation of telomerase activity in humans. (A) Representative gel image of telomerase activity in peripheral blood leukocytes. Peripheral blood leukocytes were isolated and sampled every 4 h starting at 8:00 AM. (B) Quantification of leukocytes telomerase activity (mean ± SD, $n = 10$ per each time point) (C) *hTERT*, *Per2*, *Cry1*, and *CLOCK* mRNA profiles in leukocytes (mean ± SD, $n = 10$ per each time point, Lomb–Scargle analysis). (D) Representative telomerase activity gels and leukocyte telomerase activity in emergency physicians (ER doctors) and non-emergency physicians (non-ER doctors). Leukocytes were sampled at 10:00 AM and 5:00 PM the day after night shifts for emergency physicians (mean ± SD, $n = 14$ in ER doctors and $n = 13$ in non-ER doctors). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; NS, not significant.

but binds to the E-box at −165 bp in humans or −808 bp in mice upstream. We believe that this observation suggests that despite humans and mice utilizing similar circadian machinery for telomerase control, there are still some differences between humans and mice that warrant future investigations. We initially thought it was a reasonable explanation that c-Myc binding to the *TERT* promoter was responsible for the rhythm of *TERT* mRNA levels based on the following evidence: c-Myc is a critical regulator of telomerase expression [16,29], E-boxes with CACGTG sequences are also recognized by c-Myc-Max heterodimers [30], and *c-Myc* mRNA levels oscillate with circadian rhythm [26,27]. However, our ChIP results suggest that despite c-Myc abundance oscillation with circadian rhythm, the binding of c-Myc to these E-boxes has no circadian rhythm. Our studies in CLOCK-deficient mice support that direct binding of the CLOCK–BMAL1 is the mechanism responsible for the oscillation of *TERT* mRNA levels and telomerase activity. Therefore, the regulation of *TERT* transcription is multilayered with CLOCK–BMAL1 and c-Myc. Further circadian studies with c-Myc conditional knockout mice will be interesting to dissect the complex interaction between c-Myc and CLOCK–BMAL1.

Our results showed that CLOCK-deficiency leads to profound changes in telomere lengths by Q-FISH. This difference exceeded what is seen in first generation telomerase KO mice. It is possible that circadian deficiency also affects telomerase length through

other signaling pathways. We have observed increased phosphorylation of histone H2A variant H2AX, a marker of DNA damage, in CLOCK-deficient MEFs after stimulation of staurosporine (Supplementary Fig. 4). We believe future studies dissecting circadian deficiency, DNA damage foci at telomere ends, and other evidence of telomere dysfunction will answer this question.

The circadian rhythm of telomerase activity has several important clinical impacts. First, telomerase expression is essential for most cancer cells but inactive in the majority of normal tissues [31]. Theoretically, it is an important target for cancer treatment. Several therapeutic agents targeting telomerase, such as imetelstat, GV100, and telomelysin, are in clinical trials for essential thrombocythemia, breast cancer or lung cancer [9]. It will be critical to take into account the daily oscillation of telomerase activity when administering these drugs or evaluating the effects of telomerase inhibition. Second, shift workers have a higher incidence of breast cancer [7], metabolic syndrome [1], and impaired immune function [32], a syndrome cluster similar to telomere syndromes in human or short telomeres phenotypes in mice [33]. Our findings support a possible link between circadian dyssynchrony and telomere dysfunction. It will be of great interest to study whether therapeutic measures for circadian rhythm can help maintain telomeres. A potentially important aspect of this work related to the link between circadian dyssynchrony and aging. Regulation of telomeres and telomerase by the circadian machinery provides direct evidence of interactions between aging and circadian regulation.

Acknowledgments

C.Y.W. received support from the National Health Research Institute (NHRI-EX100-9925SC), National Science Council (101-2314-B-182-100-MY3, 101-2314-B-182A-009), and Chang Gung Memorial Hospital (CMRPG3B1642, CMRPG3D1001, CMRPG3D0581, and CMRPG3C1761). M.S.W. received support from National Science Council and Chang Gung Memorial Hospital (CMRPG3A1073). S.S.S. received support from Chang Gung Memorial Hospital (CMRPG3C0721 and CMRPG3C0722).

We appreciate the technical assistance from Mei-Hsiu Lin, Hui-Chiu Chen, Yu-Jung Hu, and Hui-Ting Su.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.138.

References

[1] J. Bass, J.S. Takahashi, Circadian integration of metabolism and energetics, Science 330 (2010) 1349–1354, http://dx.doi.org/10.1126/science.1195027.
[2] J.P. DeBruyne, D.R. Weaver, S.M. Reppert, Peripheral circadian oscillators require CLOCK, Curr. Biol. 17 (2007) R538–R539, http://dx.doi.org/10.1371/journal.pbio.0050034.st001.
[3] E.E. Zhang, S.A. Kay, Clocks not winding down: unravelling circadian networks, Nat. Rev. Mol. Cell Biol. 11 (2010) 764–776, http://dx.doi.org/10.1038/nrm2995.
[4] S.A. Brown, K. Schmitt, A. Eckert, Aging and circadian disruption: causes and effects, Aging (Albany NY) 3 (2011) 813–817.
[5] S. Kumar, A. Mohan, V.K. Sharma, Circadian dysfunction reduces lifespan in *Drosophila melanogaster*, Chronobiol. Int. 22 (2005) 641–653, http://dx.doi.org/10.1080/07420520500179423.
[6] R.V. Kondratov, A role of the circadian system and circadian proteins in aging, Ageing Res. Rev. 6 (2007) 12–27, http://dx.doi.org/10.1016/j.arr.2007.02.003.
[7] K.L. Gamble, A.A. Motsinger-Reif, A. Hida, H.M. Borsetti, S.V. Servick, C.M. Ciarleglio, et al., Shift work in nurses: contribution of phenotypes and genotypes to adaptation, PLoS One 6 (2011) e18395, http://dx.doi.org/10.1371/journal.pone.0018395.t004.
[8] E. Sahin, R.A. Depinho, Linking functional decline of telomeres, mitochondria and stem cells during ageing, Nature 464 (2010) 520–528, http://dx.doi.org/10.1038/nature08982.
[9] P. Martinez, M.A. Blasco, Telomeric and extra-telomeric roles for telomerase and the telomere-binding proteins, Nat. Rev. Cancer 11 (2011) 161–176, http://dx.doi.org/10.1038/nrc3025.
[10] A. Wojtyla, M. Gladych, B. Rubis, Human telomerase activity regulation, Mol. Biol. Rep. 38 (2011) 3339–3349, http://dx.doi.org/10.1007/s11033-010-0439-x.
[11] S. Yamazaki, M. Straume, H. Tei, Y. Sakaki, M. Menaker, G.D. Block, Effects of aging on central and peripheral mammalian clocks, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 10801–10806, http://dx.doi.org/10.1073/pnas.152318499.
[12] T. Kunieda, T. Minamino, T. Katsuno, K. Tateno, J.-I. Nishi, H. Miyauchi, et al., Cellular senescence impairs circadian expression of clock genes in vitro and in vivo, Circ. Res. 98 (2006) 532–539, http://dx.doi.org/10.1161/01.RES.0000204504.25798.a8.
[13] Y. Qu, Z. Wang, X. Huang, C. Wan, C.-L. Yang, B. Liu, et al., Circadian telomerase activity and DNA synthesis for timing peptide administration, Peptides 24 (2003) 363–369, http://dx.doi.org/10.1016/S0196-9781(03)00050-0.
[14] J.P. DeBruyne, E. Noton, C.M. Lambert, E.S. Maywood, D.R. Weaver, S.M. Reppert, A clock shock: mouse CLOCK is not required for circadian oscillator function, Neuron 50 (2006) 465–477, http://dx.doi.org/10.1016/j.neuron.2006.03.041.
[15] J.P. Etchegaray, X. Yang, J.P. DeBruyne, A.H.F.M. Peters, D.R. Weaver, T. Jenuwein, et al., The polycomb group protein EZH2 is required for mammalian circadian clock function, J. Biol. Chem. 281 (2006) 21209–21215.
[16] E.H. Blackburn, C.W. Greider, J.W. Szostak, Telomeres and telomerase: the path from maize, *Tetrahymena* and yeast to human cancer and aging, Nat. Med. 12 (2006) 1133–1138, http://dx.doi.org/10.1038/nm1006-1133.
[17] A. Pizarro, K. Hayer, N.F. Lahens, J.B. Hogenesch, CircaDB: a database of mammalian circadian gene expression profiles, Nucleic Acids Res. 41 (2013) D1009–13, http://dx.doi.org/10.1093/nar/gks1161.
[18] Y.V. Dubrovsky, W.E. Samsa, R.V. Kondratov, Deficiency of circadian protein CLOCK reduces lifespan and increases age-related cataract development in mice, Aging (Albany NY) 2 (2010) 936–944.
[19] J.P. DeBruyne, D.R. Weaver, S.M. Reppert, CLOCK and NPAS2 have overlapping roles in the suprachiasmatic circadian clock, Nat. Neurosci. 10 (2007) 543–545, http://dx.doi.org/10.1038/nn1884.
[20] B. Kornmann, O. Schaad, H. Bujard, J.S. Takahashi, U. Schibler, System-driven and oscillator-dependent circadian transcription in mice with a conditionally active liver clock, PLoS Biol. 5 (2007) e34, http://dx.doi.org/10.1371/journal.pbio.0050034.
[21] E. Muñoz, R. Baler, The circadian E-Box: when perfect is not good enough, Chronobiol. Int. 20 (2003) 371–388, http://dx.doi.org/10.1081/CBI-120022525.
[22] N. Koike, S.-H. Yoo, H.-C. Huang, V. Kumar, C. Lee, T.-K. Kim, et al., Transcriptional architecture and chromatin landscape of the core circadian clock in mammals, Science 338 (2012) 349–354, http://dx.doi.org/10.1126/science.1226339.
[23] G. Rey, F. Cesbron, J. Rougemont, H. Reinke, M. Brunner, F. Naef, Genome-wide and phase-specific DNA-binding rhythms of BMAL1 control circadian output functions in mouse liver, PLoS Biol. 9 (2011) e1000595, http://dx.doi.org/10.1371/journal.pbio.1000595.
[24] Ç. Günes, S. Lichtsteiner, A.P. Vasserot, C. Englert, Expression of the hTERT gene is regulated at the level of transcriptional initiation and repressed by Mad1, Cancer Res. 60 (2000) 2116–2121.
[25] E.H. Blackburn, Switching and signaling at the telomere, Cell 106 (2001) 661–673.
[26] A. Repouskou, T.G. Sourlingas, K.E. Sekeri-Pataryas, A. Prombona, The circadian expression of c-MYC is modulated by the histone deacetylase inhibitor trichostatin A in synchronized murine neuroblastoma cells, Chronobiol. Int. 27 (2010) 722–741, http://dx.doi.org/10.3109/07420521003786800.
[27] L. Fu, H. Pelicano, J. Liu, P. Huang, C. Lee, The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo, Cell 111 (2002) 41–50.
[28] J.A. Ripperger, U. Schibler, Rhythmic CLOCK–BMAL1 binding to multiple E-box motifs drives circadian Dbp transcription and chromatin transitions, Nat. Genet. 38 (2006) 369–374, http://dx.doi.org/10.1038/ng1738.
[29] M.J. McEachern, A. Krauskopf, E.H. Blackburn, Telomeres and their control, Annu. Rev. Genet. 34 (2000) 331–358, http://dx.doi.org/10.1146/annurev.genet.34.1.331.
[30] T.K. Blackwell, L. Kretzner, E.M. Blackwood, R.N. Eisenman, H. Weintraub, Sequence-specific DNA binding by the c-Myc protein, Science 250 (1990) 1149–1151.
[31] J. van Tuyn, P.D. Adams, Signalling the end of the line, Nat. Cell Biol. 14 (2012) 339–341, http://dx.doi.org/10.1038/ncb2476.
[32] O.A. Habbal, A.A. Al-Jabri, Circadian rhythm and the immune response: a review, Int. Rev. Immunol. 28 (2009) 93–108, http://dx.doi.org/10.1080/08830180802645050.
[33] M. Armanios, E.H. Blackburn, The telomere syndromes, Nat. Rev. Genet. 13 (2012) 693–704, http://dx.doi.org/10.1038/nrg3246.