Published in final edited form as:

J Biol Rhythms. 2008 February ; 23(1): 95–98.

Mammalian Peripheral Circadian Oscillators Are Temperature Compensated

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Variations in ambient temperature present a unique obstacle to the timekeeping function of circadian clocks. Most biological reactions proceed with a temperature coefficient (Q_{10}) ~ 2 or 3, so that with every 10 °C increase in temperature, the reaction rate approximately doubles or triples. Therefore, a temperature-dependent clock would run faster at high temperatures than at low temperatures, and would not be a reliable predictor of time of day. So it is not surprising that mechanisms have evolved to ensure that the length of the circadian period remains relatively constant over a wide range of temperatures, a phenomenon known as temperature compensation.

In mammals, self-sustaining rhythms have been measured in the master circadian clock, located in the SCN, and in peripheral tissues in vitro (Yamazaki et al., 2000; Yoo et al., 2004). When separated from the entrainment of the SCN, each peripheral tissue expresses tissue-specific differences in circadian period and phase (Yoo et al., 2004). While previous studies have shown that the circadian period in the SCN, retina, and in fibroblast cell lines remains relatively constant across a range of temperatures, it is unknown whether mammalian peripheral clocks are temperature compensated (Tosini and Menaker, 1998; Ruby et al., 1999; Izumo et al., 2003; Tsuchiya et al., 2003).

To examine the effect of temperature on circadian oscillations, we measured PERIOD2::LUCIFERASE (PER2::LUC) rhythms in explants of central and peripheral tissues from *mPer2^{Luc}* mice at 29, 31, 33, 35, and 37 °C. At temperatures ranging from 31 to 37 °C, robust rhythms of PER2::LUC were measured in the SCN, pituitary gland, cornea, adrenal gland, and lung. In the liver, the rhythm of PER2::LUC was robust at 37 °C, but damped within 2 cycles at all other temperatures examined. At 29 °C, only the pituitary consistently maintained a robust PER2::LUC rhythm.

To determine if circadian clocks in mammalian central and peripheral tissues were temperature compensated, we calculated the average period of each tissue at various temperatures. The Q_{10} of the SCN, pituitary gland, cornea, adrenal gland, and lung were variable, but all were close to 1, suggesting that these tissues were temperature compensated (Fig. 1). Because the PER2::LUC rhythm of liver explants was not robust at temperatures below 37 °C, the Q_{10} of the liver could not be calculated.

Each peripheral tissue had a different Q_{10} , ranging from 0.89 to 0.96. Recent studies have reported tissue-specific expression patterns of circadian genes and mutating these genes results in variable, tissuespecific effects on the expression of other clock components (Lowrey and Takahashi, 2004; Sato et al., 2004; Akashi and Takumi, 2005; Guillaumond et al., 2005; Noshiro et al., 2005; Ko and Takahashi, 2006). Therefore, while the molecular mechanisms controlling temperature compensation are unknown, it is possible that tissue-dependent

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differences in the Q_{10} of the mammalian periphery results from variations in the expression and regulation of circadian genes.

Since *Per2* participates in the transcriptional/translational feedback loops that regulate the expression of other circadian genes, temperature-induced changes in the bioluminescent waveform of PER2::LUC expression could reflect variable processing of the components of the feedback loops. However, we found no differences in the waveforms of PER2::LUC expression between tissues cultured at 31 and 37 °C (Fig. 2). It is possible that the effects of temperature on the molecular mechanism of the clock are not reflected in rhythmic PER2::LUC expression and other circadian genes should also be assessed. Alternatively, our method may not be sensitive enough to detect temperature-induced changes in waveform.

Temperature-dependent variations in the molecular timekeeping mechanism could also be reflected in the phase of PER2::LUC expression. When we compared tissues cultured at 31 or 37 °C, we found that the phase of the PER2::LUC rhythm was temperature dependent in SCN slices, but not in the pituitary, lung, or cornea (Fig. 2). Upon further analysis, we found that the circadian phase of PER2::LUC expression was gradually delayed with decreasing temperature (Fig. 3). The phase shift induced by changing the ambient temperature in our experiments is consistent with a previous study that demonstrated that the SCN entrains to temperature cycles (Herzog and Huckfeldt, 2003).

In summary, our results demonstrate that the periods of mammalian peripheral tissues are temperature compensated over the range of 29 to 37 °C. We find tissuespecific differences in the Q_{10} and in temperature-induced shifts of circadian phase.

ACKNOWLEDGMENTS

This research was supported by the NIH (NS051278 to S.Y.) and by the Vanderbilt University Summer Research Program (to B.A.R.). We thank Joseph Takahashi and Seung-Hee Yoo for *mPer2^{Luc}* mice. We also thank Hajime Tei and Akiko Hida for *Per1-luciferase* mice.

APPENDIX

Tissue Culture and Luminescence Recording

Heterozygous *mPer2^{Luc}* mice (Yoo et al., 2004), aged 2 to 10 months (mean age, 4.7 ± 0.53 SEM months) and born after 11 to 15 generations of back-crossing with the C57BL/6J strain, were maintained in a 12:12-h light/dark cycle. One hour before lights-off, cultures of SCN, pituitary gland, adrenal gland, cornea, lung, and liver were prepared as previously described (Yamazaki and Takahashi, 2005). The tissues were maintained at 28.6, 30.7, 32.7, 34.8, and 36.8 °C (referred to as 29, 31, 33, 35, and 37 °C, respectively), as recorded by a temperature data logger (HOBO H8 Pro; Onset, Pocasset, MA) inside the LumiCycle. The temperature remained stable throughout the course of the experiments, with fluctuations of ± 0.02 °C. Bioluminescence was monitored in real-time with the LumiCycle, and photon counts were integrated over 10-min intervals.

Data Analysis

LumiCycle software (Actimetrics Inc., Evanston, IL) was used to subtract the 24-h moving average from the raw luminescence data and to smooth the data by 0.5-h adjacent averaging. The first peak value of PER2::LUC expression measured was reported as the phase. To determine period, the baseline-subtracted and smoothed data was exported to ClockLab (Actimetrics Inc.) and a regression line was drawn through the peaks of 4 to 6 cycles. At all temperatures examined, the period of the male pituitary gland was approximately 0.5 h longer than the female pituitary. Adrenal glands harvested from males (26 of 28 males), but not from

females (2 of 25 females), were rhythmic from 31 to 37 °C. Tissue samples that were rhythmic for less than 3 cycles were not analyzed. To compare the waveform at 31 and 37 °C, baseline-subtracted and smoothed data were averaged, normalized, and plotted for each tissue. Normalization was achieved by dividing each averaged plot by the maximum y-value of its first peak. From the slope of the linear trend line fitted to the plot of period as a function of temperature, the Q_{10} for each tissue was calculated as follows: $Q_{10} = (R_2/R_1)^{10/(T_2 - T_1)}$, where R is rate and T is the temperature measured by the logger (i.e., 28.6 rather than 29 °C).

In contrast to the previous report that the SCN of *mPer2^{Luc}* mice have an average period of 23.5 h at 36.5 °C (Yoo et al., 2004), we measured an average period of 24.8 h. This may be attributed to the use of new recording media (the media used previously was discontinued by the manufacturer) or to strain differences since we used tissues from mice congenic with the C57BL/6J strain, while mice of a mixed genetic background (C57BL/6J and 129Sv) were used in previous experiments.

To examine the functionality of the PER2::LUC fusion protein with specific regard to temperature compensation, we measured the period of the SCN of *mPer1-luc* and *mPer2^{Luc}* mice at 33 and 37 °C. The period of the SCN did not differ between *mPer1-luc* and *mPer2^{Luc}* mice, suggesting that the PER2::LUC fusion protein does not alter normal temperature compensation.

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Figure 1.

Central and peripheral oscillators are temperature compensated. The mean periods (hours) of SCN, pituitary, cornea, adrenal glands, lung, and liver harvested from *mPer2^{Luc}* mice are expressed as a function of temperature (°C). Data are expressed as the mean period $\pm$ SEM of 4 to 7 replicates.

Figure 2.

The waveform of PER2::LUC expression in various tissues is not dependent on temperature. SCN, pituitary, lung, and cornea from *mPer2^{Luc}* mice were cultured at 31 or 37 °C. The baseline-subtracted data for each tissue was averaged, normalized, and plotted as a function of days in culture.

Figure 3.

Temperature-dependent phase of PER2::LUC expression in the SCN. The circadian time in hours (CT12 is lights-off) of the first peak of PER2::LUC expression (phase) in the SCN was averaged (expressed as the mean $\pm$ SEM) and plotted as a function of temperature (°C).