Correspondences

Peripheral circadian oscillators require CLOCK

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In mammals, the circadian system is hierarchical — a brain pacemaker located within the suprachiasmatic nucleus (SCN) is responsible for regulating locomotor activity rhythms and for synchronizing peripheral oscillators [1,2]. Recent genetic evidence in mice indicates that the bHLH transcription factors CLOCK and NPAS2 have partially redundant functions within the SCN [3,4]. To further examine the roles of CLOCK and NPAS2, we generated CLOCK-deficient (*Clock*$^{-/-}$), NPAS2-deficient (*Npas2*$^{-/-}$) and double-mutant (*Clock*$^{-/-}$;*Npas2*$^{-/-}$) mice carrying the *mPer2*^{Luciferase} reporter gene [5]. We monitored the bioluminescence rhythms of tissue explants in culture and found that while CLOCK or NPAS2 is able to maintain SCN bioluminescence rhythmicity (Supplemental Data) [4], peripheral oscillators are arrhythmic without CLOCK. Thus, there are fundamental differences between the clock machinery of different tissues.

We determined CLOCK's role in peripheral oscillators by focusing on liver and lung tissue. The circadian oscillator in the liver has been useful for understanding the biochemical interactions that comprise the mouse circadian clockwork, e.g., [6,7]. Moreover, the livers of CLOCK-deficient mice rhythmically express *mPer1* and *mPer2* mRNAs and proteins during the first day in constant darkness [3]. This suggested that the circadian clock in the liver might also be maintained in the absence of CLOCK due to the partially redundant function of NPAS2.

Surprisingly, however, bioluminescence profiles of liver and lung explants from Clock$^{-/-}$ mice are arrhythmic (Figure 1A),

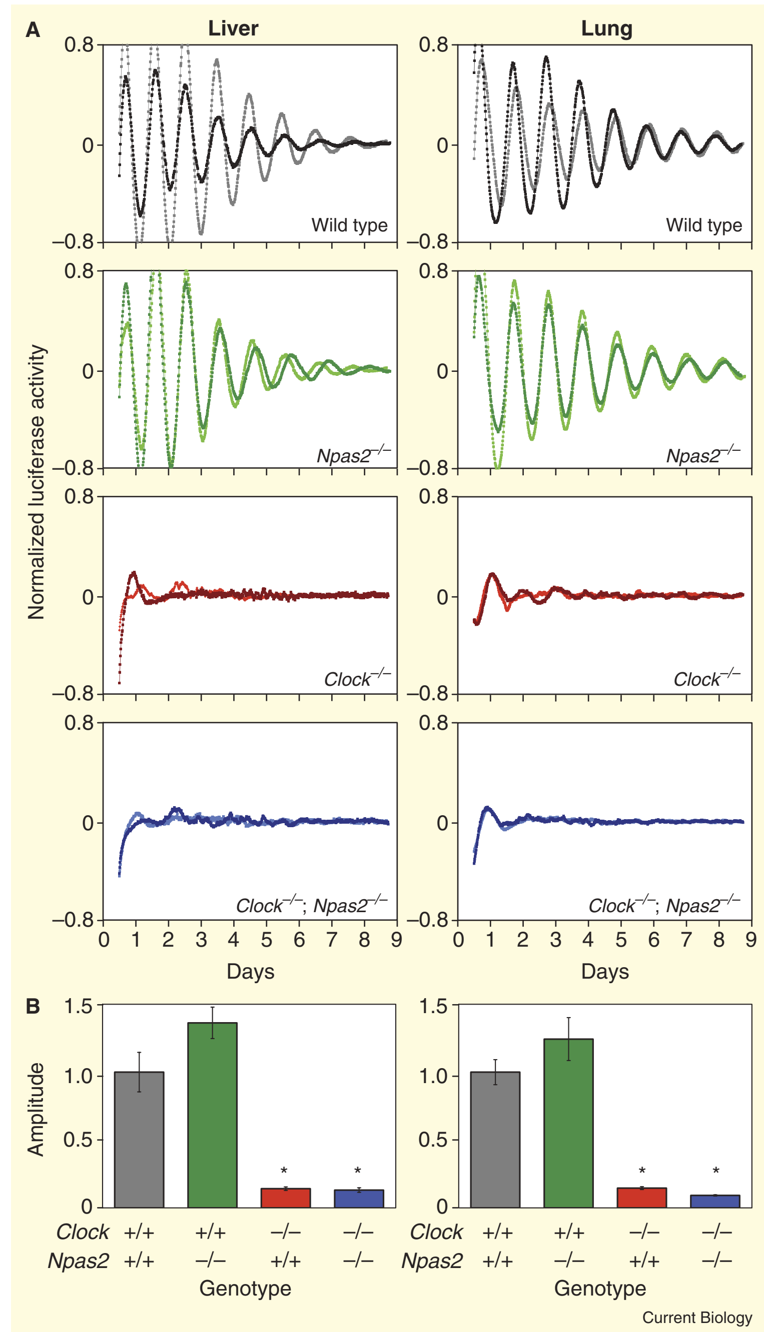

Figure 1. Analysis of clock function in cultured tissue explants.

(A) Representative bioluminescence profiles of tissue explants from liver (left column) or lung (right column). Data were normalized to the average luciferase activity produced over the duration of the recording and are plotted as the difference from the centered 24 hour moving average (Supplemental Data). The two representative profiles shown for each genotype are from separate experiments. Genotypes are indicated in the lower right of each panel. Our animal studies were approved by the Institutional Animal Care and Use Committee of the UMass Medical School. (B) Normalized amplitudes of the bioluminescence profiles from liver (left panel) or lung (right panel) explants. See Supplemental Data for a detailed description of amplitude determination. Genotypes are indicated below each panel. Each bar is the mean ± sem with the following *n*'s (*n*(explants)/*n*(animals)): Liver: *Clock*$^{+/+}$;*Npas2*$^{+/+}$ = 19/9, *Clock*$^{+/+}$;*Npas2*$^{-/-}$ = 10/4, *Clock*$^{-/-}$;*Npas2*$^{+/+}$ = 23/10, *Clock*$^{-/-}$;*Npas2*$^{-/-}$ = 8/3. Lung: *Clock*$^{+/+}$;*Npas2*$^{+/+}$ = 18/9, *Clock*$^{+/+}$;*Npas2*$^{-/-}$ = 7/4, *Clock*$^{-/-}$;*Npas2*$^{+/+}$ = 21/10, *Clock*$^{-/-}$;*Npas2*$^{-/-}$ = 6/3. Asterisks indicate significant differences from wild type (ANOVA $p < 0.001$, Tukey's HSD *post hoc*). Note, amplitude measurements were not significantly different between *Clock*$^{-/-}$;*Npas2*$^{+/+}$ and *Clock*$^{-/-}$;*Npas2*$^{-/-}$ using the same test ($p > 0.98$).

despite a significant elevation in Npas2 expression in the livers of Clock$^{-/-}$ mice [3]. Comparison of rhythm amplitudes confirmed that bioluminescence profiles of Clock$^{-/-}$ liver and lung explants are indistinguishable from those of arrhythmic Clock$^{-/-}$;Npas2$^{-/-}$ mice (Figure 1B). Arrhythmicity was not due to low luciferase activity; explants from either Clock$^{-/-}$ or Clock$^{-/-}$;Npas2$^{-/-}$ mice produced levels of bioluminescence that were comparable to those of explants from wild-type mice (Supplemental Data).

The arrhythmic phenotype of CLOCK-deficient peripheral oscillators could result from rapid desynchronization of a population of cells each containing a functional intracellular oscillator. To examine this possibility, we exposed explants from Clock$^{-/-}$ mice to a media change, a stimulus known to resynchronize individual oscillators and thereby restore population rhythm amplitude [5,8]. A media change on the 9th day in vitro was sufficient to produce an acute increase in luciferase activity in all explant cultures, indicating that the explants were healthy and responsive (Figure 2). Media change reinstated rhythmicity in liver and lung explants from wild-type and Npas2$^{-/-}$ animals (Figure 2). In Clock$^{-/-}$ and Clock$^{-/-}$;Npas2$^{-/-}$ explants, however, a media change produced only a single peak within about 24 hours and bioluminescence levels returned to approximately the mean level by the second day (Figure 2). Notably, similar bioluminescence patterns after initial placement in culture or after media change were also observed with SCN explants from arrhythmic Clock$^{-/-}$;Npas2$^{-/-}$ mice (Supplemental Data and data not shown). Thus, the mPer1 and mPer2 mRNA and protein rhythms previously detected in CLOCK-deficient livers in vivo [3] are most likely being driven by systemic cues secondary to the SCN-based rhythmicity in these animals, as recently described [7].

We conclude that —unlike the circadian pacemaker in the SCN— peripheral oscillators in liver and lung are dependent on CLOCK. NPAS2 maintains rhythmic SCN function in the absence of CLOCK, but NPAS2 alone is unable to maintain rhythmicity in peripheral tissues. This could be due to fundamental differences in the clock mechanism between tissues or due to differences at the level of intercellular interactions. There may be a small subpopulation of cell-autonomous, NPAS2-dependent oscillators in the SCN of *Clock*$^{-/-}$ mice, which drives rhythmicity in the SCN as a whole due to networking among SCN neurons [9,10]. Peripheral tissues probably lack robust within-tissue networking, and thus, may lack the ability to disseminate a rhythmic signal from a small subset of cells throughout the entire tissue.

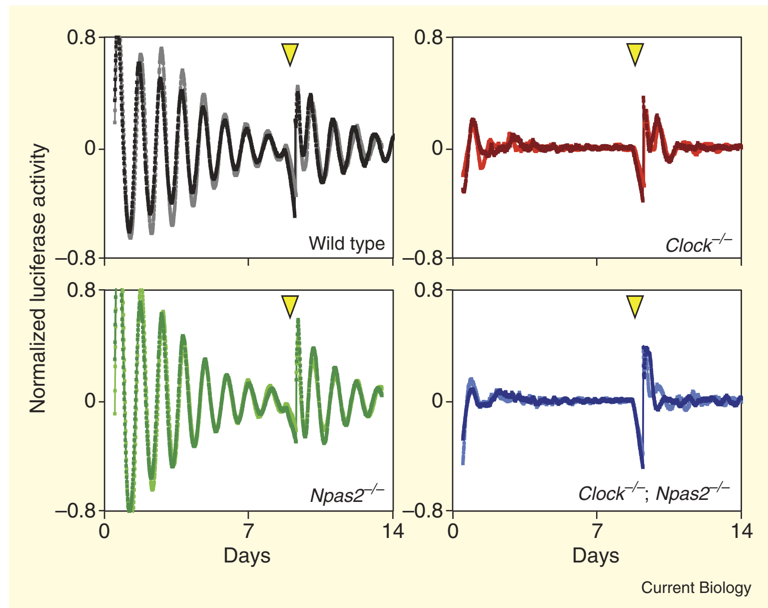

Figure 2. A media change does not restore rhythmicity to CLOCK-deficient peripheral oscillators.

Each panel contains bioluminescence profiles from lung explants, plotted as described for Figure 1. Genotypes are indicated in the lower right of each panel. The yellow arrowhead indicates the time of the media change.

Supplemental data

Supplemental data including experimental procedures are available at http://www.current-biology.com/cgi/content/full/17/14/R538/DC1

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