Supporting Information

Rodriguez et al. 10.1073/pnas.1219969110

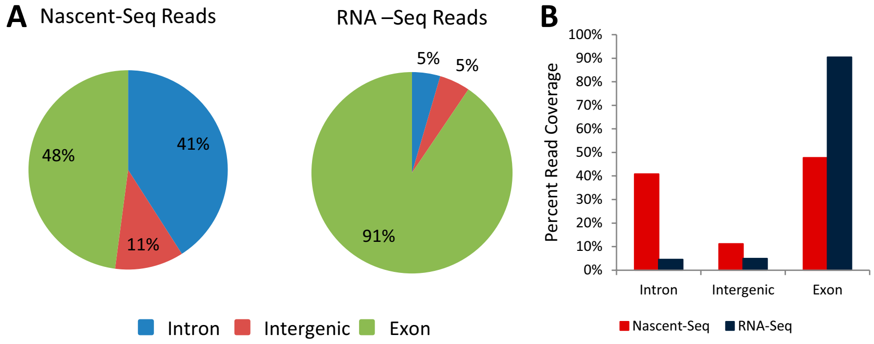

Fig. S1. Nascent-Seq enriches for intron signal. Nascent-Seq enriches for intron signal by ninefold compared with RNA-Seq. (*A*) Forty-one percent of Nascent-Seq reads fall within introns, whereas fewer than 5% of RNA-Seq reads fall within introns. Percent read coverage per base pair within introns and exons is plotted. (*B*) Percent read coverage in intron, intergenic, and exon regions plotted as a bar graph.

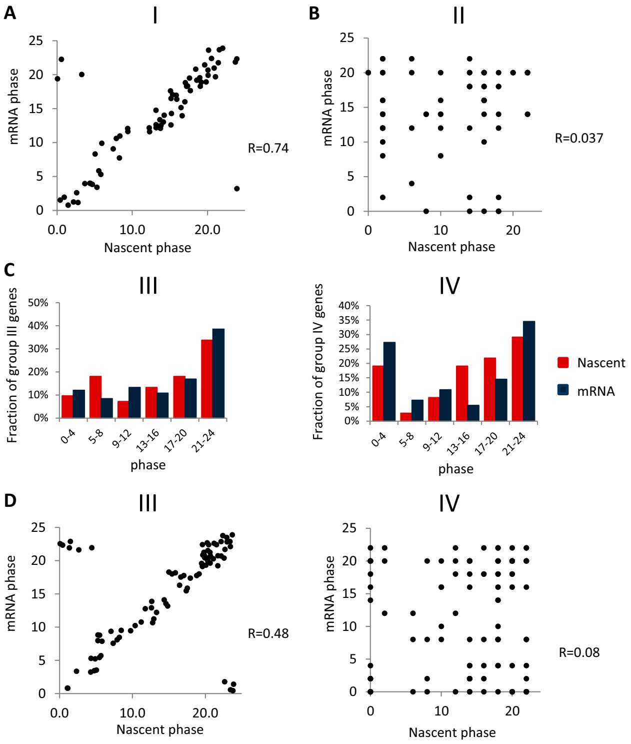

Fig. S2. Nascent vs. mRNA phase distributions for all cycling groups. (*A*) Individual group I phase distributions are highly correlated. Phases determined by Fourier analysis are plotted. (*B*) Individual group II phase distributions are poorly correlated. Phases were determined by assigning the time point with the average maximum reads per base pair for the Nascent-Seq and RNA-Seq datasets, respectively. (*C*) Group III and group IV Nascent-Seq (red) and RNA-Seq (blue) phase distributions. Group III phases were determined by Fourier analysis. Group IV phases of both datasets were set to the time point with the maximum average reads per base pair. (*D*) Individual group III phase distributions are highly correlated in contrast to individual group IV phase distributions, which are not correlated. Group III phases were determined by Fourier analysis. Group IV phases of both datasets were set to the time point with the maximum average reads per base pair.

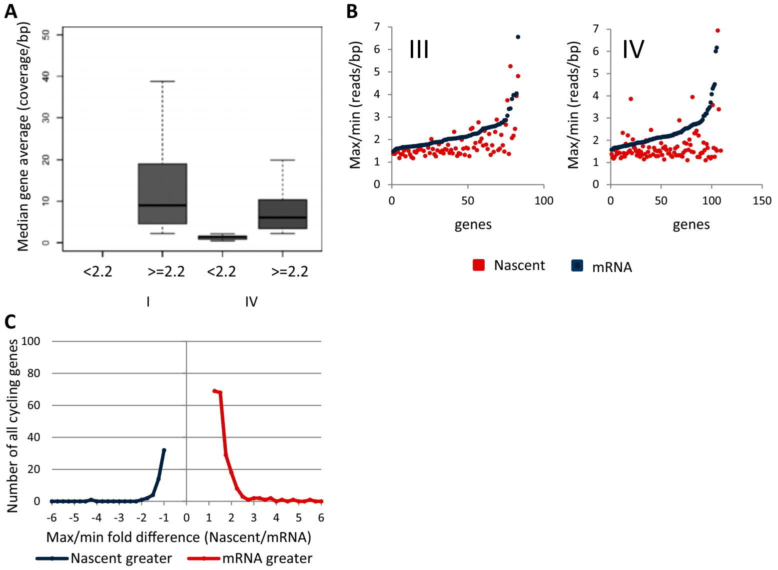

Fig. S3. Group IV genes contain posttranscriptional cyclers. (*A*) Almost 80% of group IV genes have nascent expression levels above the threshold required for group I, indicating that expression or sequencing depth is not the only factor responsible for the observed lack of cycling in group IV genes. (*B*) Most group III and group IV genes have higher mRNA cycling amplitudes relative to their nascent cycling amplitudes (maximum/minimum ratios). (*C*) The distribution of ratios between the mRNA and nascent cycling amplitudes is shown for all mRNA cyclers. For 24 genes the mRNA amplitude is twofold higher than the nascent amplitude; only two genes have twofold higher nascent than mRNA amplitude. The data therefore indicate a significant contribution of posttranscriptional regulation to circadian gene expression.

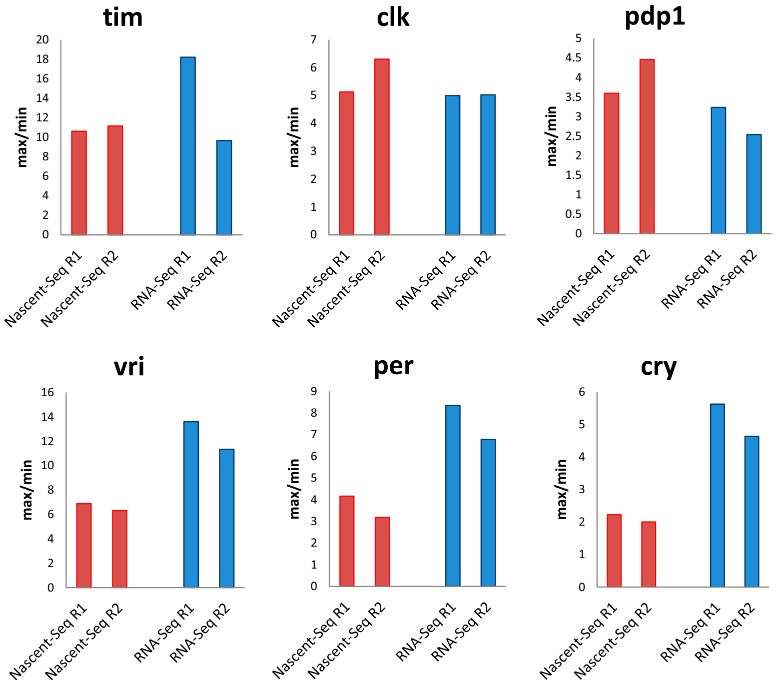

Fig. S4. Several core clock genes exhibit higher mRNA amplitudes than nascent amplitudes. Plotted are the average ratios of maximum/minimum reads per base pair within exons across all time points. Nascent-Seq replicate (R1, R2) ratios are in red; RNA-Seq replicate (R1, R2) ratios are in blue.

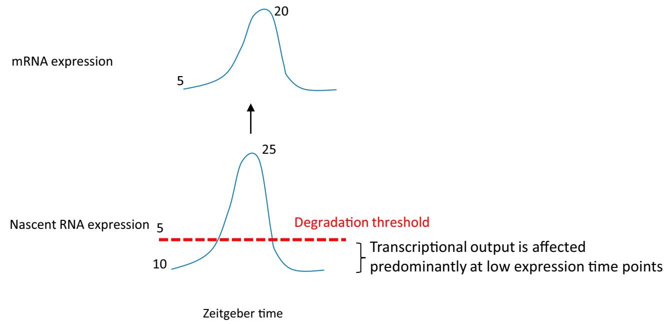

Fig. S5. Degradation threshold model. A constant degradation expression threshold could explain the higher mRNA than nascent cycling amplitudes without invoking a clock-controlled (temporally variable) posttranscriptional mechanism. The model posits that the trough values of cycling transcription are near this threshold, so degradation mechanisms can degrade their targets (e.g., within the nucleus) efficiently. After this threshold is substantially breached, a larger fraction of mRNA is stable, thereby creating a larger mRNA cycling amplitude than transcriptional amplitude. In the diagram, a constant degradation of five transcripts reduces the mRNA trough to 5 and the mRNA peak to 20, giving rise to a mRNA amplitude of 4 compared with a transcriptional amplitude of 2.5.

Table S1. Top 50 microarray cycling genes

Refseq identifier	Gene symbol	No. of microarray cycles	In annotation from nascent pA data?	Nascent or RNA cycler group
CG6571	*rdgC*	6	Yes	
CG6622	*Pkc53E*	6	Yes	
CG9552	*rost*	6	Yes	
CG4899	*Pdh*	6	Yes	
CG4784	*Cpr72Ec*	6	Yes	IV
CG14029	*vri*	6	Yes	I
CG3234	*tim*	6	Yes	I
CG5798	*CG5798*	6	Yes	I
CG17352	*CG17352*	6	Yes	I
CG2121	*CG2121*	6	Yes	I
CG18345	*trpl*	6	Yes	I
CG7811	*b*	6	Yes	I
CG6649	*Ugt35b*	6	Yes	I
CG11143	*Inos*	5	Yes	
CG13305	*CG13305*	5	Yes	
CG11407	*CG11407*	5	Yes	
CG7365	*CG7365*	5	Yes	
CG9023	*Drip*	5	Yes	
CG9027	*CG9027*	5	Yes	
CG9837	*CG9837*	5	Yes	
CG4962	*CG4962*	5	Yes	
CG1572	*CG1572*	5	Yes	
CG4779	*hgo*	5	Yes	
CG1982	*Sodh-1*	5	Yes	III
CG18111	*Obp99a*	5	Yes	III
CG10175	*CG10175*	5	Yes	III
CG14528	*CG14528*	5	Yes	III
CG14275	*CG14275*	5	Yes	II (cycles in Nascent and RNA, but different phases)
CG17386	*CG17386*	5	Yes	I
CG7391	*Clk*	5	Yes	I
CG1441	*CG1441*	5	Yes	I
CG10553	*CG10553*	5	Yes	I
CG6772	*Slob*	5	Yes	I
CG6730	*Cyp4d21*	5	Yes	I
CG42329	*CG42329*	5	Yes	I
CG32858	*sn*	4	Yes	
CG15162	*MESR3*	4	Yes	
CG11958	*Cnx99A*	4	Yes	
CG18598	*CG18598*	4	Yes	
CG2681	*CG2681*	4	Yes	IV
CG3966	*ninaA*	4	Yes	IV
CG13848	*pinta*	4	Yes	III
CG3672	*Cpr67B*	4	Yes	III
CG11796	*CG11796*	4	Yes	III
CG14996	*Chd64*	4	Yes	III
CG17888	*Pdp1*	4	Yes	I
CG2647	*per*	4	Yes	I
CG11529	*CG11529*	4	Yes	I
CG11853	to	4	Yes	I
CG9363	CG9363	4	Yes	I

Table S2. Primers used for validation

Name	Primer used for quantitative RT-PCR validation
CG4784_F_PTF	ACAAACGGTTCGCTATGAGG
CG4784_R_PTF	AAAGCTTTCTCCCTGGCTTC
CG10006_F_PTF	TCATCGTGAACCACAACGAT
CG10006_R_PTF	TTCATAAAAGCTCGCGGAGT

Other Supporting Information Files

Dataset S1 (XLS)