Cell

A Genome-wide RNAi Screen for Modifiers of the Circadian Clock in Human Cells

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Journal talk
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Introduction

Circadian Clock

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suprachiasmatic nuclei (SCN)

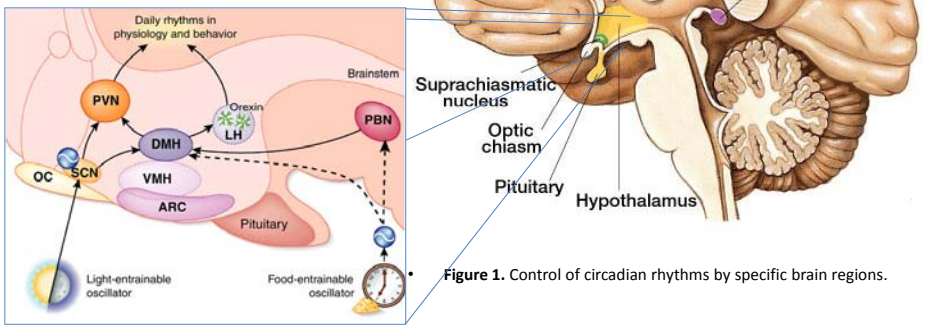


Figure 1. Control of circadian rhythms by specific brain regions.

Circadian Clock in Mammals

- RORE.
  NR1Ds and RORs either repress or activate gene transcription from ROR elements.
- D-box binding elements.
  bZIP, DBP, TEF either repress or activate gene transcription from D-box.

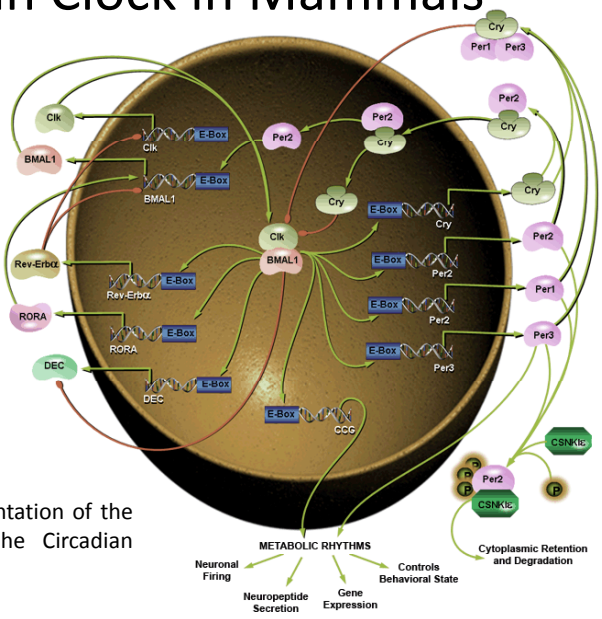
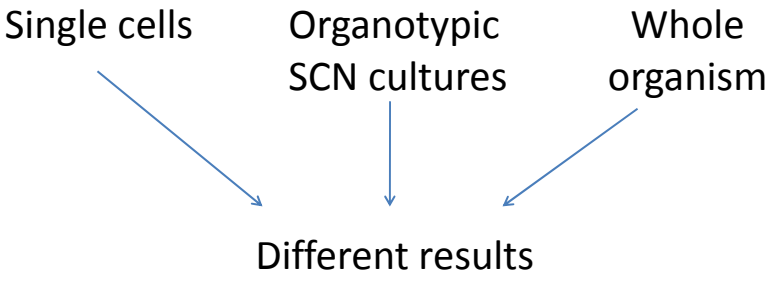


Figure 2. Schematic representation of the principal components of the Circadian Clock in Mammals

Genes Function

- Knockout mice of *Rev-erbα*, *Rorα* o *Rorβ*
  - Lower amplitude rhythms
  - Abnormal period lenghts
  - interindividual variability in both phenotypes
- Knockout mice of *Pgc1α*
  - Long-period locomotor activity
- Dose-dependent knockdown of ROR and REV-ERB in vitro
  - potent effect on the baseline and the amplitude of circadian gene expression

Complex Interaction



Intercellular coupling in generating response

Materials

- U2SO cells *Bmal1-dLuc* and *Per2-dLuc* reporter cell lines
- siRNA libraries for the primary and secondary screens were purchased from QIAGEN.
- Additional siRNAs from Invitrogen and Dharmacon used as controls in target validation experiments. GL2 and GL3 siRNAs from QIAGEN Screen controls purchased from Invitrogen included CRY1-HSS102308, CRY2-HSS102311, and BMAL1 or ARNTL-HSS100703. CRY1 and NR1D1 siRNAs used in dose-dependent experiment were previously described.

Overview of Web-Based Data Resource in Circadian BioGPS

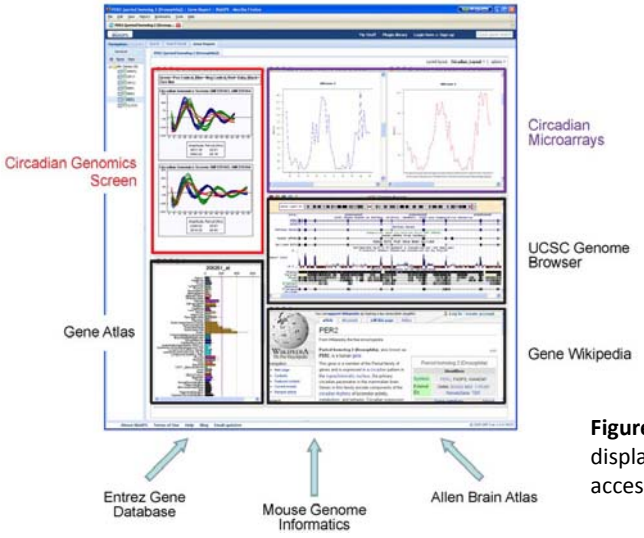


Figure 3. Screening data displayed in BioGPS open-access database

Methods

- RNA interference
- Inverse transfection on microarray
- LumiCycle assay
- Q-PCR

RNA interference

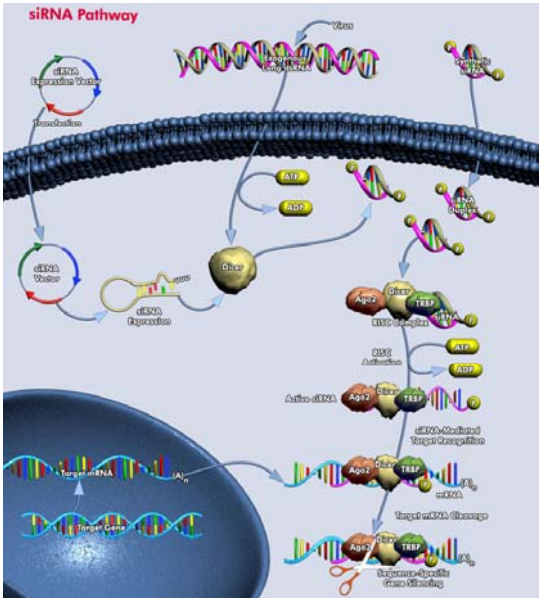


Figure 4. Schematic representation of the pathway for the mechanism of RNA interference

Results

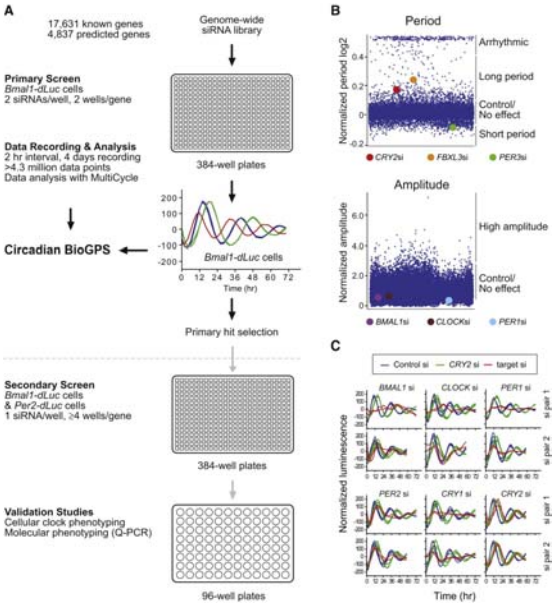


Figure 5. A cell-based genome-wide siRNA screen for circadian clock modifiers

(A) A schematic diagram of the genome-wide siRNA screen including the primary screen, data mining, hit selection, secondary screen, and validation of several

selected targets. In the primary screen, reporter cells were transfected with siRNA in 384-well plates followed by kinetic bioluminescence recording.

(B) Distribution of circadian parameters of the entire primary screen.

(C) Cellular clock phenotypes of siRNA knockdown of known clock genes. Plots of cellular oscillations upon knockdown of BMAL1, CLOCK, PER1, PER2, CRY1,

or CRY2 by two independent pairs of siRNAs in the primary screen are presented. The spikes of initial 10 hr bioluminescence readings resulted from media change

and were removed from the plot.

Primary Hit Selection

- 1028 short-period hits → 76 double hits
- 4230 long-period hits → 274 double hits
- 493 high-amplitude hits → 18 double hits
- No low-amplitude traces, poor curve fitting and inconsistent period length data.

Primary Hit Selection:

254 genes + 3 S.D. from the mean.

89 single siRNA pair hits in duplicate well.

Total 343 genes selected.

Secondary Confirmation Assay

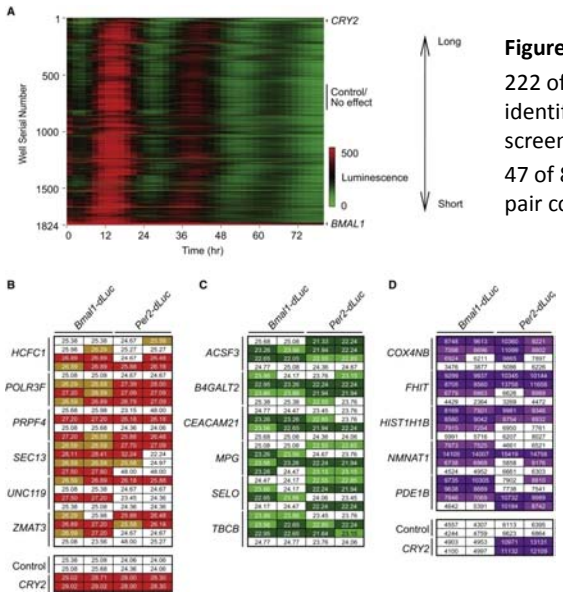


Figure 6. BMAL1 assay.

222 of 238 genes identified in secondary screen

47 of 89 single siRNA pair confirmed.

Dose-Dependent Phenotypic Validation

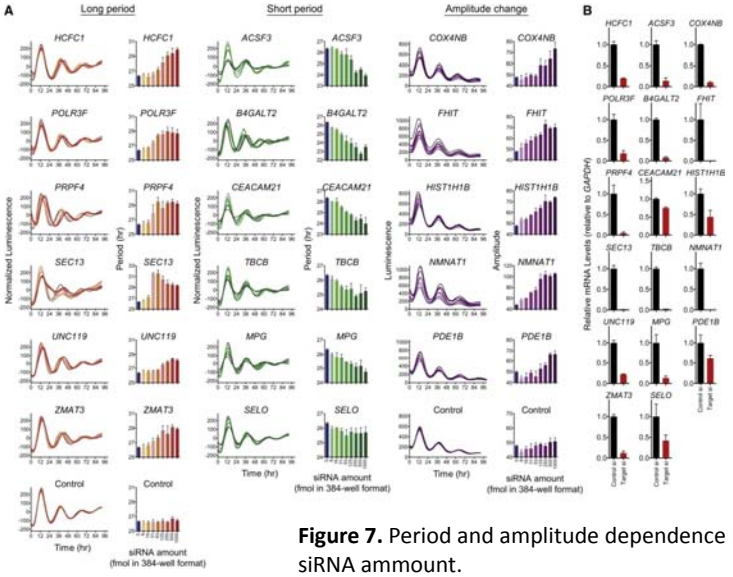
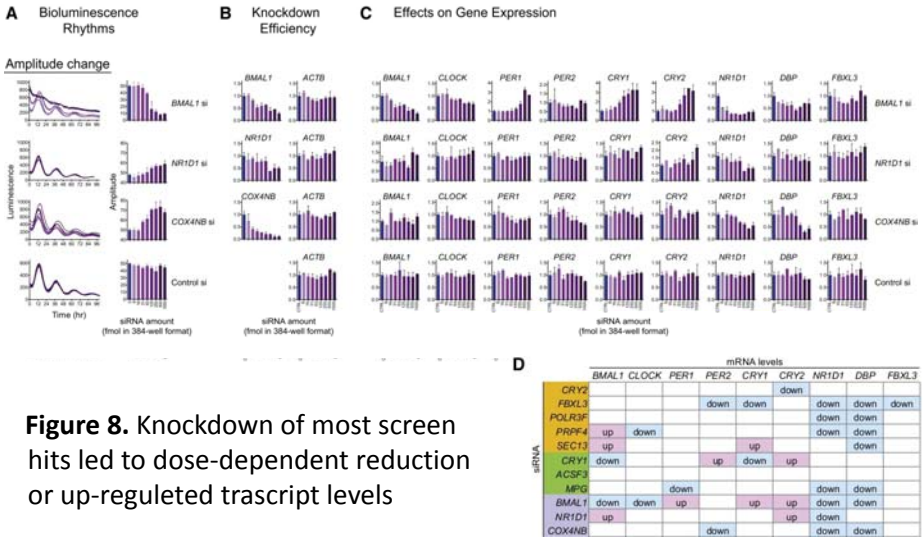


Figure 7. Period and amplitude dependence by siRNA ammount.

Network Effects



siRNA	BMAL1	CLOCK	PER1	PER2	CRY1	CRY2	NR1D1	DBP	FBXL3
CRY2						down			
FBXL3				down	down		down	down	down
POLR3F							down	down	
PRPF4	up	down					down	down	
SEC13	up				up			down	
CRY1	down			up	down	up			
ACSF3									
MPG			down				down	down	
BMAL1	down	down	up		up	up	down	down	
NR1D1	up					up	down		
COX4NB				down			down	down	

Figure 8. Knockdown of most screen hits led to dose-dependent reduction or up-reguleted trascript levels

The Expanded Clock Gene Network

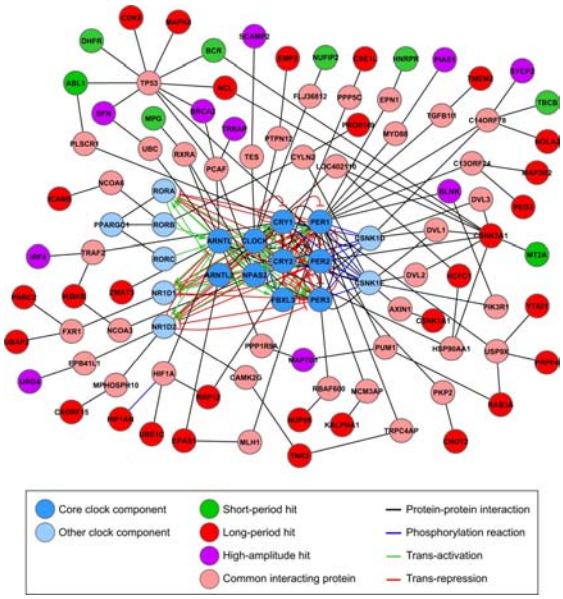


Figure 9. List of interactions identified in primary siRNA screen.

Interconnectedness between the clock and other biological processes

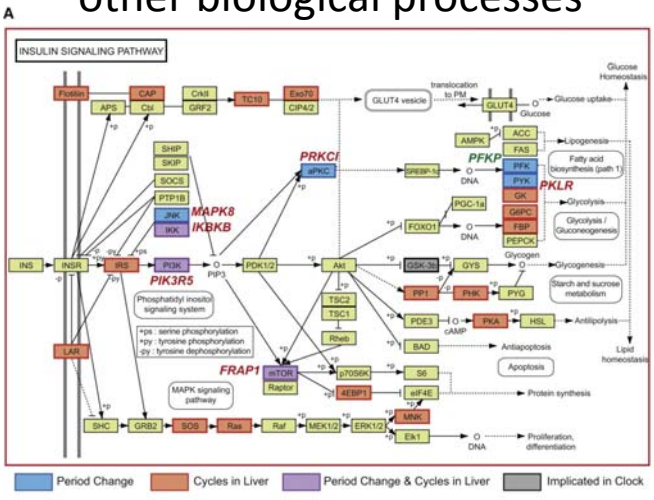


Figure 10A. Regulation of cellular circadian clock by components in the insulin signaling pathway.

Interconnectedness between the clock and other biological processes

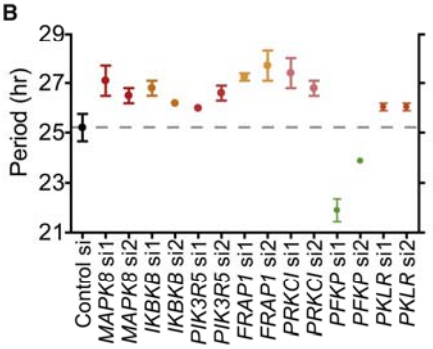


Figure 10B. Effect of siRNAs against genes involved in insulin signaling.

Conclusions

- 1000 genes whose knockdown resulted in low amplitude circadian oscillations.
- 100 genes whose knockdown led to long or short period length of oscillation.
- protein interaction network analysis showed that some factors directly or indirectly interact with core clock components.
- the clock is interconnected with many biological pathways.

Thanks for the attention!!!