Bin F. et al: Circadian Enhancers Coordinate Multiple Phases of Rhythmic Gene Transcription In Vivo

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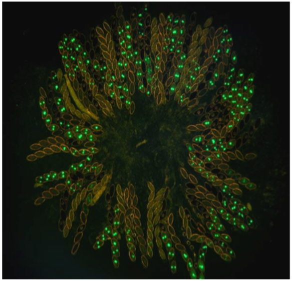
Outline

1. Background
2. Results
3. Conclusion
4. Related Works

Circadian Rhythms

Circadian: about 1 day (24hour)

Most organisms from Bacterial to Human have circadian rhythms.



Neurospora

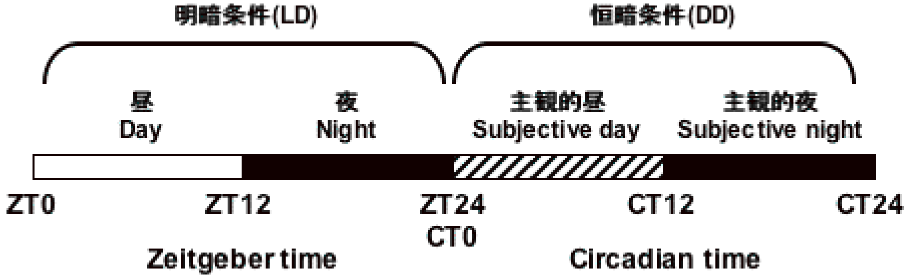
[redacted]

Mouse

Human

Basic concepts

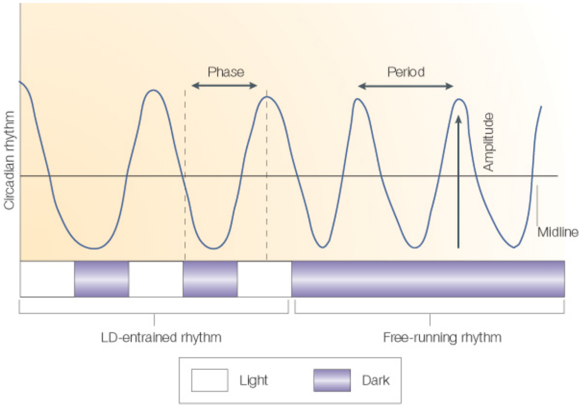
Circadian hour n. The unit of time corresponding to 1/24 of the duration of a circadian cycle.



Circadian time n. A standard of time based on the free-running period of a rhythm (oscillation). Note: By convention, the onset of activity of diurnal organisms defines circadian time zero (**CT 0**). The onset of activity of nocturnal organisms defines circadian time twelve (CT 12).

Zeitgeber time n. A standard of time based on the period of a zeitgeber. Note: Under standard light-dark cycles, the time of lights on usually defines zeitgeber time zero (**ZT 0**) for diurnal organisms and the time of lights off defines zeitgeber time twelve (ZT 12) for nocturnal animals.

Basic concepts

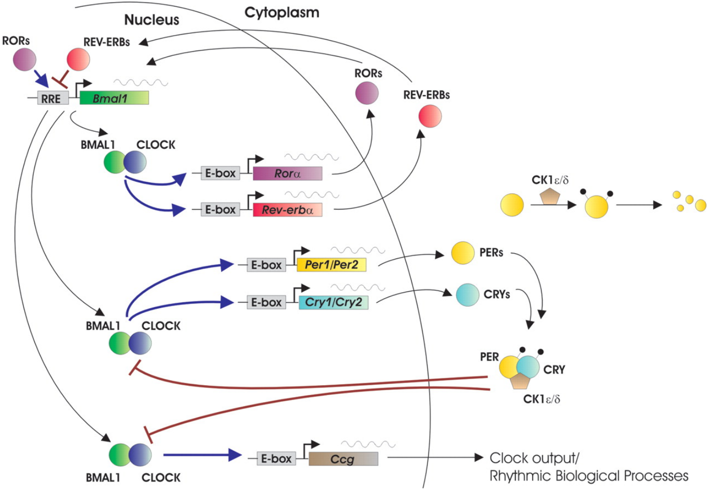


Nature Reviews | Genetics

In-phase: The same/similar phase
Antiphase: A phase shows 12 hour or half of circadian cycle difference.

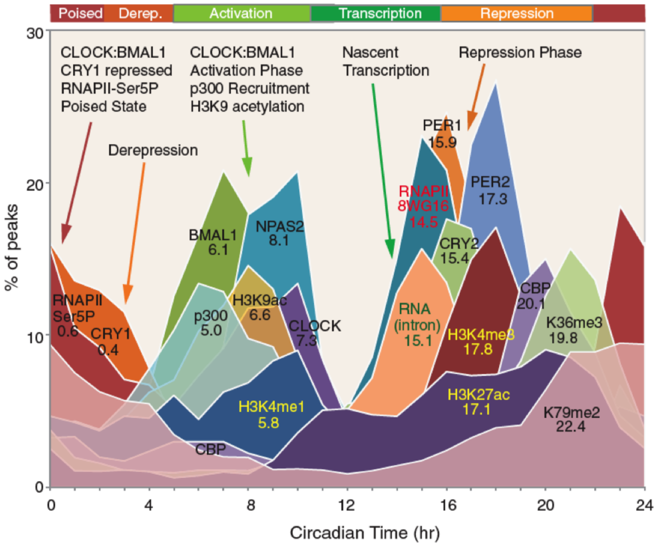
Known circadian TFs and Pathway

A network of transcriptionaltranslational feedback loops constitutes the mammalian circadian clock



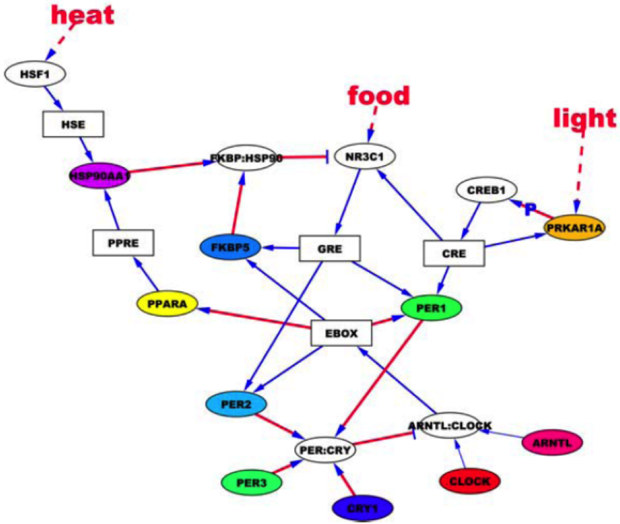
(Caroline 2006)

Koike Science 2012



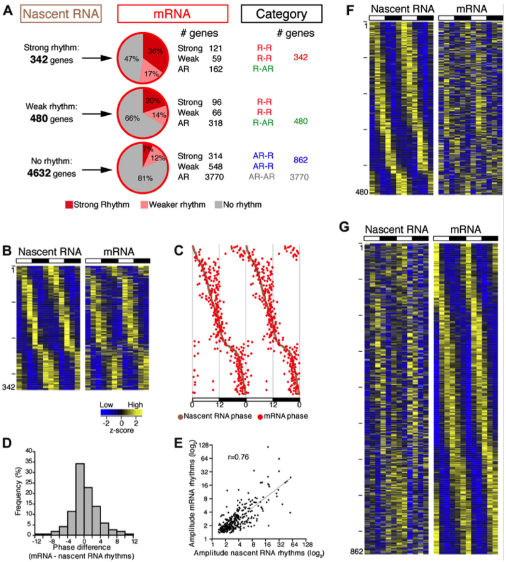
Circadian landscape of the cistrome and epigenome of the liver (Koike etc, Science 2012), RNA-Seq data

Yan et al. 2008 PlosCB



Mostly are microarray data; Contains circadian information from 14 different tissues

Menet et al. elife 2012



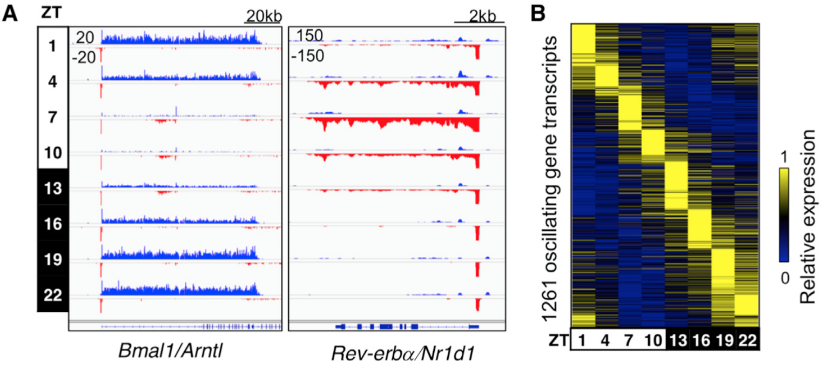
Post-transcriptional events account for a significant fraction of rhythmic gene expression in the mouse liver, RNA-Seq data

Motivatioin

- Phases of targeting gene do not correlate with circadian TF's binding phase
- A substaintial portion of circadian TF binding far from TSS
- High degree of overlap between core clock TF with competing effect, such as BMAL1 and Rev-erbα
- Delayed phase relative to BAML1/CLOCK

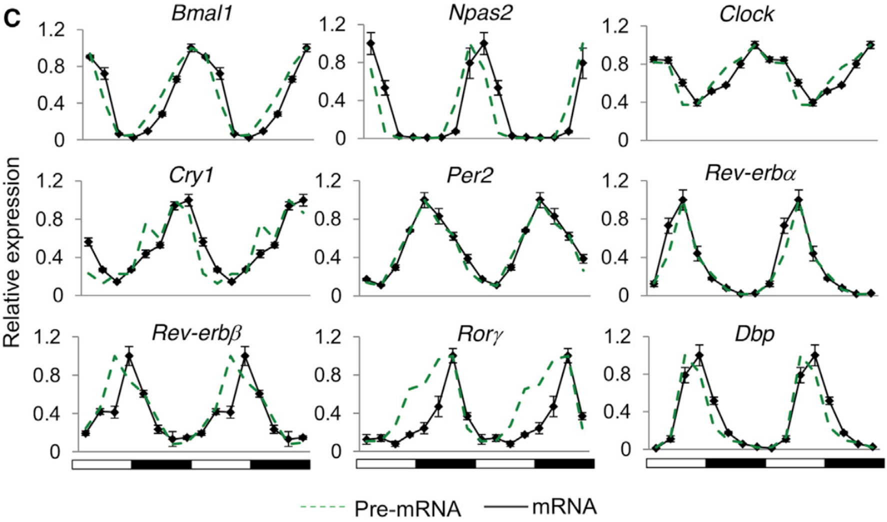
How the interaction of multiple regulators at the genome, particularly at distal enhancer elements, produces distinct phases of circadian transcriptional activity.

Circadian Transcription in Mouse Liver



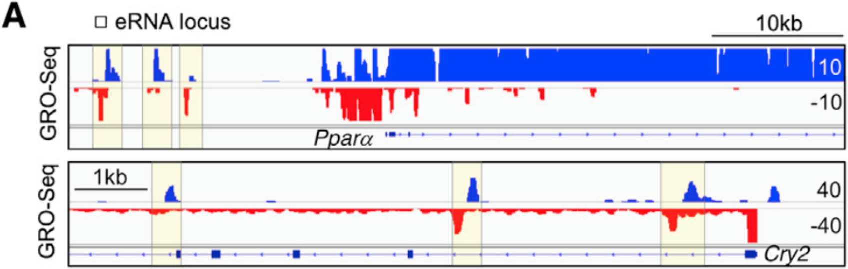
Three-hour interval GRO-seq, show robust oscillation patterns, BMAL1 and Rev-ERBa;total 1261 circadian genes sorted by the phase

Circadian Transcription in Mouse Liver



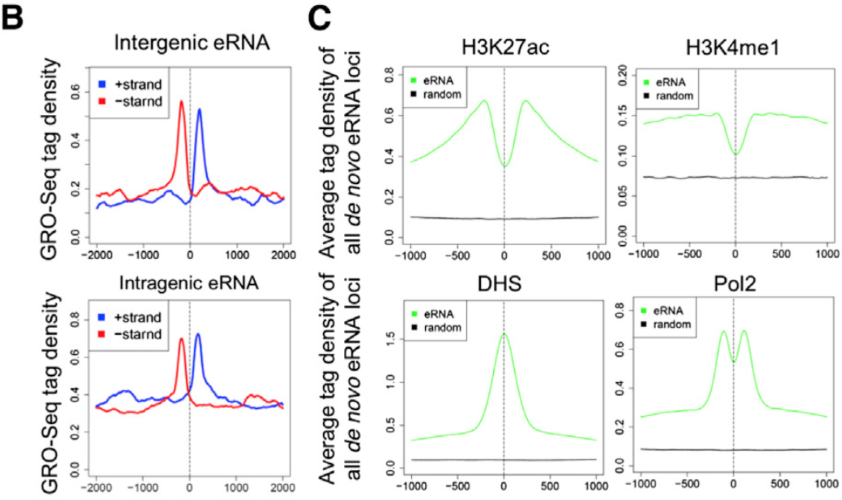
De Novo Identification of Circadian Liver Enhancer RNAs

Both bidirectional and undirectional, intergenic and intragenic peaks are considers



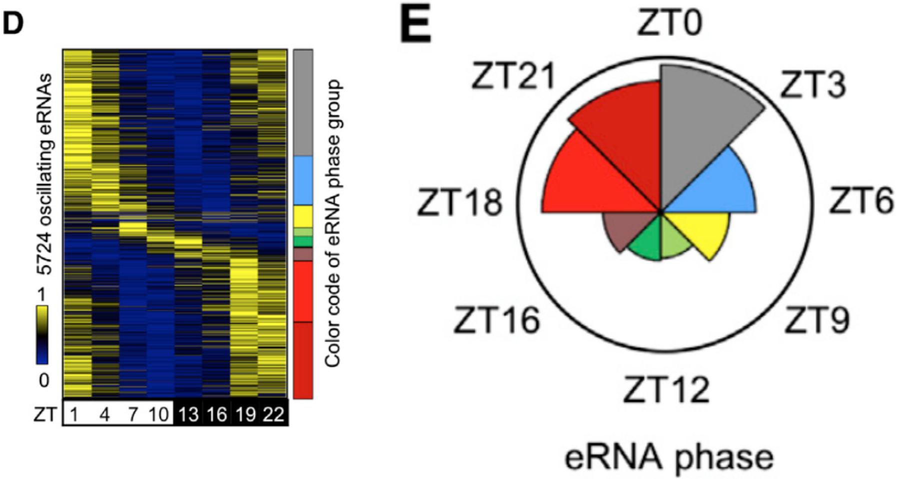
Example of eRNA locus, from 19086 high confidence loci

De Novo Identification of Circadian Liver Enhancer RNAs



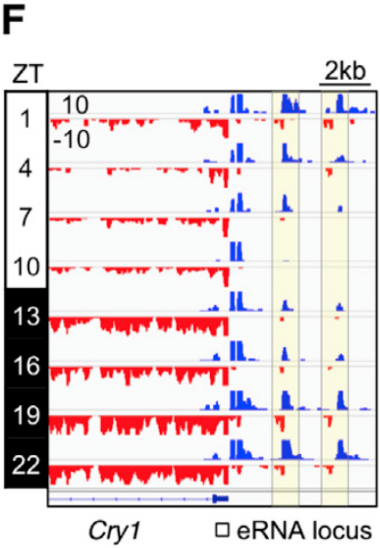
Bimodal profile and enrichment of epigenomic features

De Novo Identification of Circadian Liver Enhancer RNAs



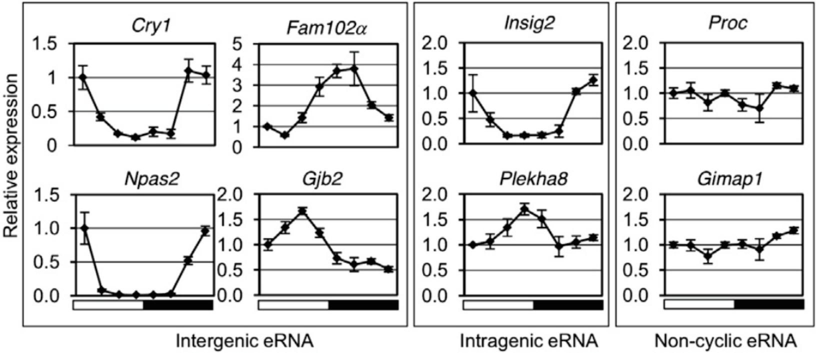
5724(30%) circadian. Define 8 group phases; 71% with a phase between ZT18 and ZT3, 29% in other phases

De Novo Identification of Circadian Liver Enhancer RNAs

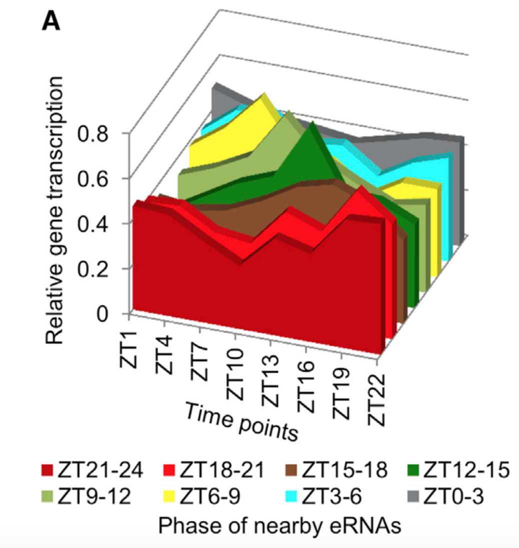


ZT22 Cry1 and RT-qPCR

De Novo Identification of Circadian Liver Enhancer RNAs



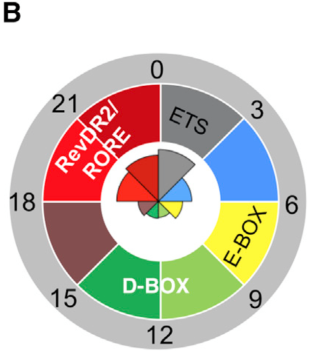
Phase-Specific Transcription Factors at Circadian Enhancers



gene expr vs closest oscillating eRNA (within 200kb from TSS) (3a), show patterns in phase

Phase-Specific Transcription Factors at Circadian Enhancers

Hypothesis: Specific cTF responsible for the different phase of gene expr by driving the transcription of diversly phased eRNA

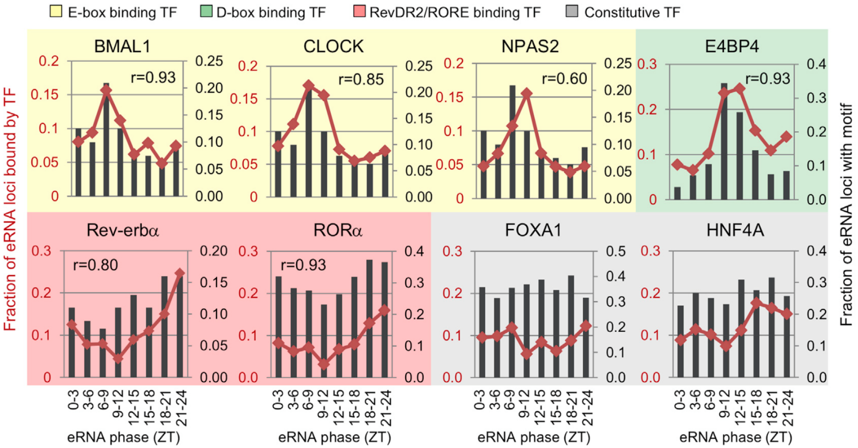


Phase specific motifs in enhancer groups

Phase-specific	**Annotated motif**	**P-value***
RevDR2		6e-7
RORE		2e-8
E-box		5e-10
D-box		4e-16
ETS		1e-9
Constitutive	**Annotated motif**	**P-value****
Forkhead		1e-13
HNF4		1e-60

* Hypergeometrc test, in-phase eRNAs vs. out-of-phase eRNAs. ** Highest enrichment in phased eRNAs vs. matched genomic control.

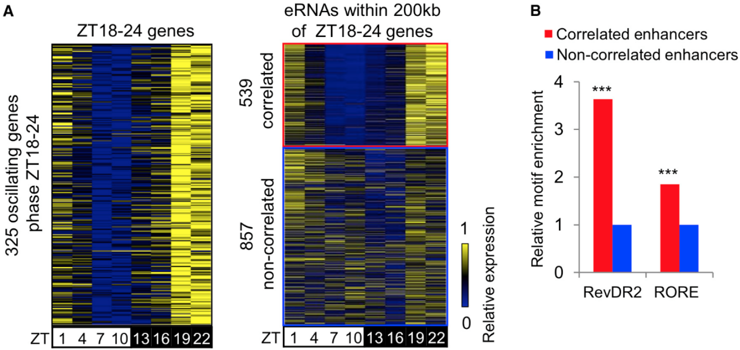
Phase-Specific Transcription Factors at Circadian Enhancers



Correlation of motif occurrence and TF binding (Question: Fraction of both Tf ound and with motif?)

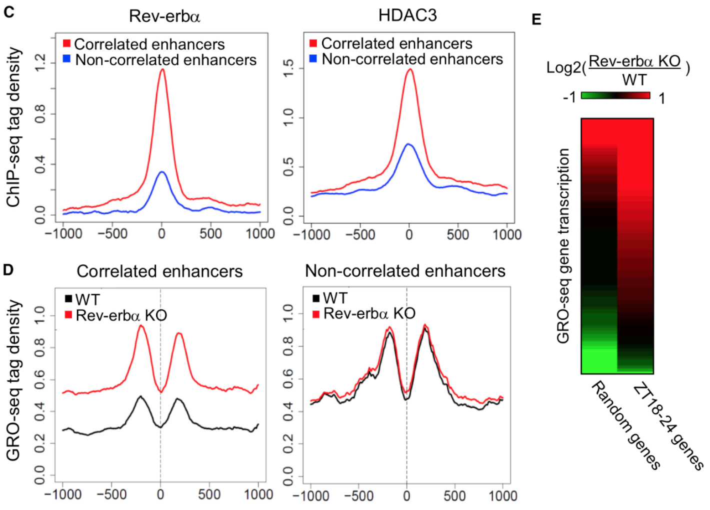
Phase Correlation between eRNA and Gene Body Transcription Marks Functional Enhancers of Circadian Genes

Whether specific TF found to bind at circadian enhancers were driving transcription of nearby in-phase gene:



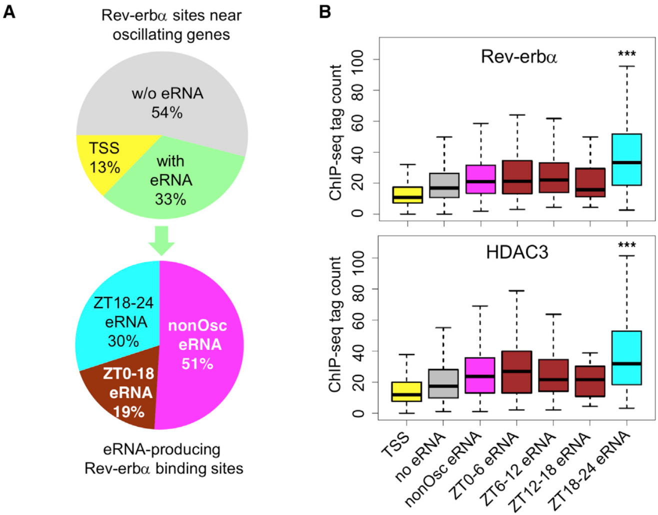
ZT18-24: 325 circGene with 539 neighboring eRNA correlated, while 857 not correlated

Phase Correlation between eRNA and Gene Body Transcription Marks Functional Enhancers of Circadian Genes



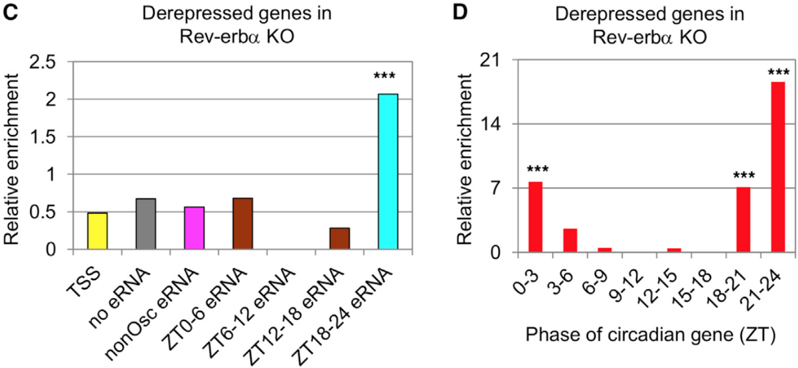
WT and KO(Rev-erbα ZT10. However, in KO mice, there are also down-regulated genes identified

Circadian eRNAs Reveal the Functional Rev-erb Cistrome at Oscillating Genes



High ChIP-seq signal of Rev-erbα and HDAC3, resulting in the descresing of H3K9ac

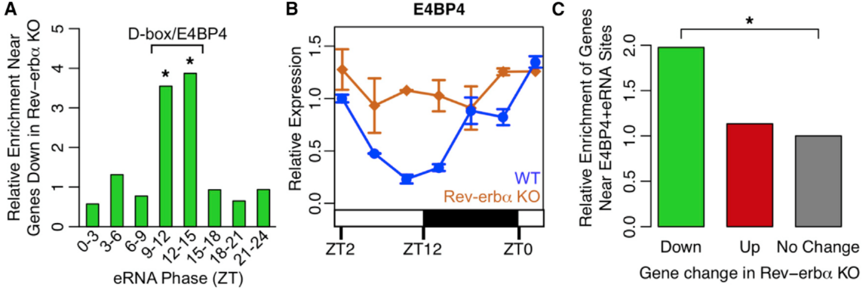
Circadian eRNAs Reveal the Functional Rev-erb Cistrome at Oscillating Genes



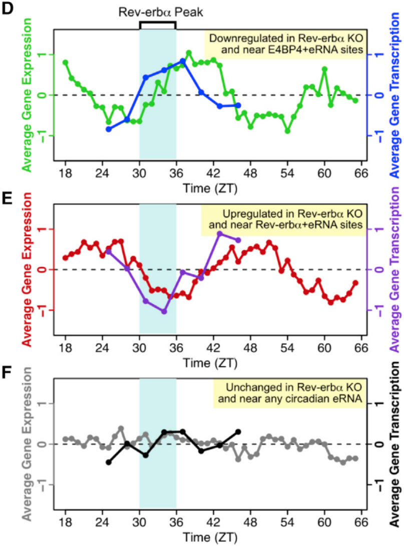
Enrichment of de-repressed circ Gene in KO mice ¿3fold near site producing ZT18-24 ; de-repressed gene also highly in ZT18-24

eRNA Analysis Identifies E4BP4 as a Key Mediator of Gene Activation by Rev-erb

There is a substantail set of genes paradoxically down-regulated at ZT10 in Rev-erbα KO mice

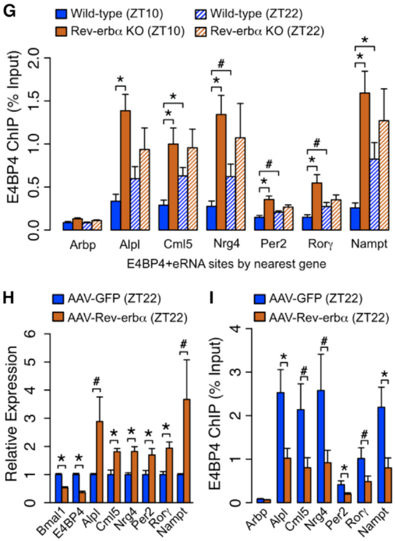


eRNA Analysis Identifies E4BP4 as a Key Mediator of Gene Activation by Rev-erb



E4BP4+eRNA (E4BP4 putative target genes) were generally circadian with peak and trough expression in phase with Rev-erbα (D). antiphase with the gene repressed by Rev-erbα

eRNA Analysis Identifies E4BP4 as a Key Mediator of Gene Activation by Rev-erb

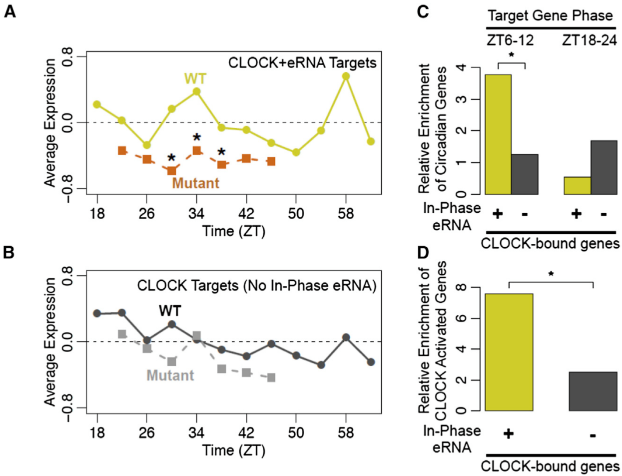


ChIP-qPCR of E4BP4 binding at genes downregulated in KO livers at ZT10;

mRNA expression for the overexpression of Rev-erbα

ChIP-qPCR of E4BP4 binding for over-expr of Rev-erbα

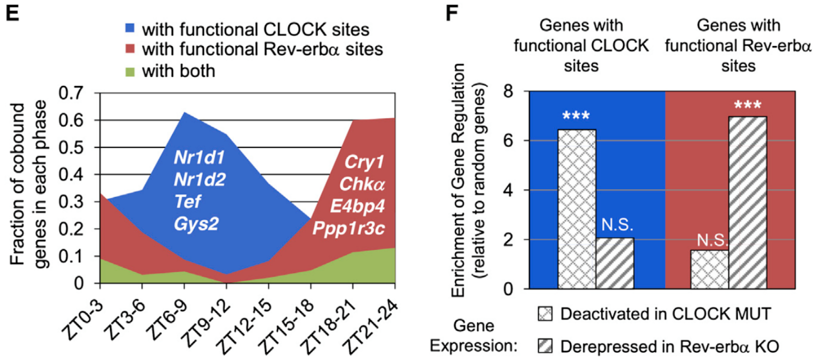
Circadian eRNAs Define Functional Cistromes that Distinguish CLOCK and Rev-erb Target Genes



Rev-erb and Clock maximal binding to genome the same time window ZT8-10

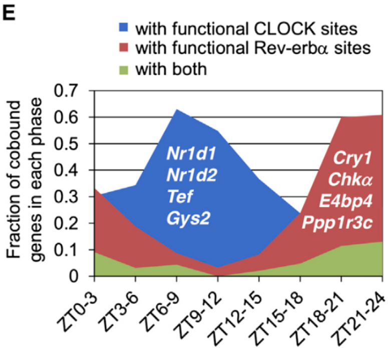
CLOCK with in-phase eRNA, stronger reduction in mutatant vs WT, than binding site with non-phase eRNA. ZT6-10

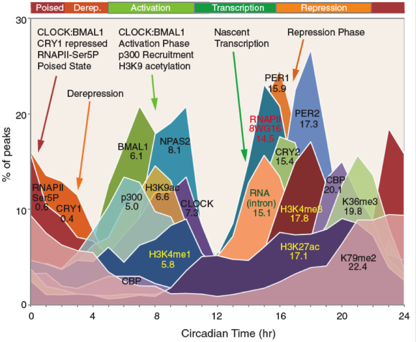
Circadian eRNAs Define Functional Cistromes that Distinguish CLOCK and Rev-erb Target Genes



Target circ Gene for Clock and Rev-erb shows enrichment in ZT6-9 and ZT18-24 respectively.

Circadian eRNAs Define Functional Cistromes that Distinguish CLOCK and Rev-erb Target Genes



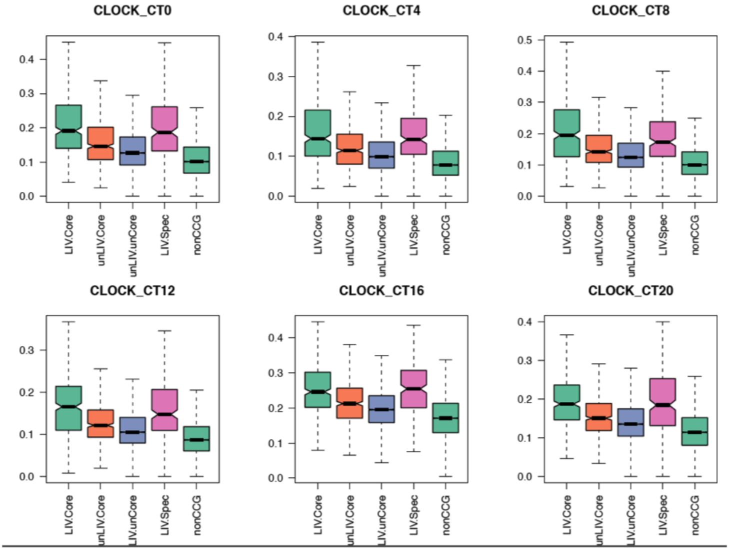


(Koike et al. 2012)

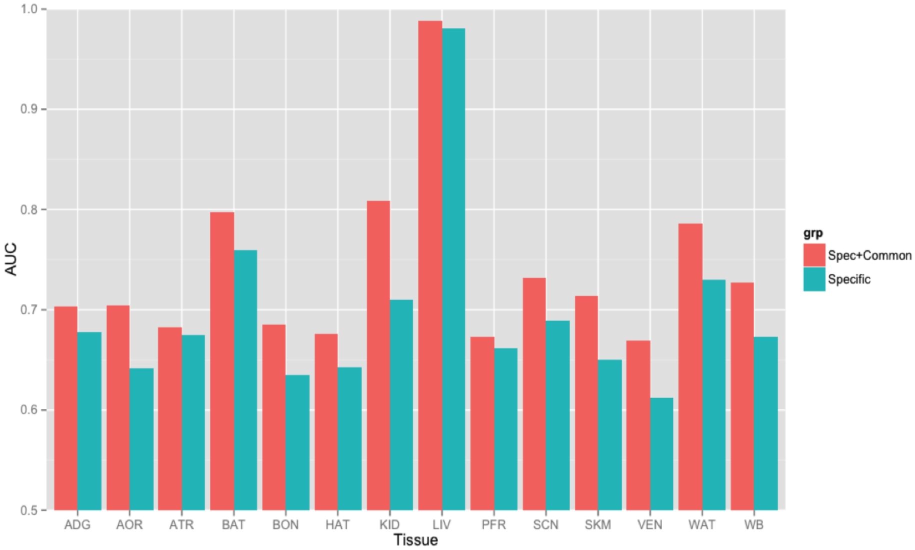
Summary

- Identified oscillating enhancers with varying peak activity times
- Specific phases of oscillation are associated with distinct regulatory motifs and TF binding patterns
- eRNA oscillations are highly predictive of the rhythmicity and phase of transcription at nearby genes
- Circadian eRNA can both identify the TF coordinating specific phases of gene transcription and distinguish the functional binding sites within a circadian cistrome

Related work



Related work



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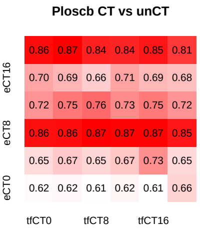
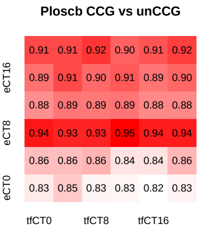


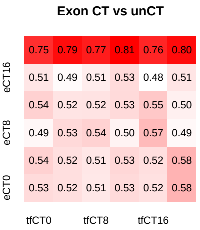
Ploscb CT vs unCT

	tfCT0		tfCT8		tfCT16	
	0.86	0.87	0.84	0.84	0.85	0.81
eCT16	0.70	0.69	0.66	0.71	0.69	0.68
	0.72	0.75	0.76	0.73	0.75	0.72
eCT8	0.86	0.86	0.87	0.87	0.87	0.85
	0.65	0.67	0.65	0.67	0.73	0.65
eCT0	0.62	0.62	0.61	0.62	0.61	0.66



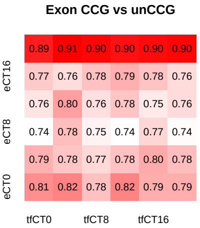
Ploscb CCG vs unCCG

	tfCT0		tfCT8		tfCT16	
	0.91	0.91	0.92	0.90	0.91	0.92
eCT16	0.89	0.91	0.90	0.91	0.89	0.90
	0.88	0.89	0.89	0.89	0.88	0.88
eCT8	0.94	0.93	0.93	0.95	0.94	0.94
	0.86	0.86	0.86	0.84	0.84	0.86
eCT0	0.83	0.85	0.83	0.83	0.82	0.83



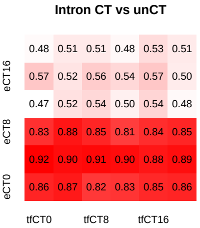
Exon CT vs unCT

	tfCT0		tfCT8		tfCT16	
	0.75	0.79	0.77	0.81	0.76	0.80
eCT16	0.51	0.49	0.51	0.53	0.48	0.51
	0.54	0.52	0.52	0.53	0.55	0.50
eCT8	0.49	0.53	0.54	0.50	0.57	0.49
	0.54	0.52	0.51	0.53	0.52	0.58
eCT0	0.53	0.52	0.51	0.53	0.52	0.58



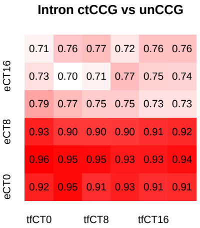
Exon CCG vs unCCG

	tfCT0		tfCT8		tfCT16	
	0.89	0.91	0.90	0.90	0.90	0.90
eCT16	0.77	0.76	0.78	0.79	0.78	0.76
	0.76	0.80	0.76	0.78	0.75	0.76
eCT8	0.74	0.78	0.75	0.74	0.77	0.74
	0.79	0.78	0.77	0.78	0.80	0.78
eCT0	0.81	0.82	0.78	0.82	0.79	0.79



Intron CT vs unCT

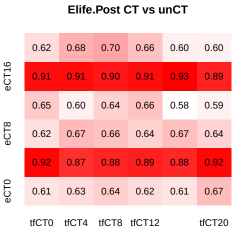
	tfCT0		tfCT8		tfCT16	
	0.48	0.51	0.51	0.48	0.53	0.51
eCT16	0.57	0.52	0.56	0.54	0.57	0.50
	0.47	0.52	0.54	0.50	0.54	0.48
eCT8	0.83	0.88	0.85	0.81	0.84	0.85
	0.92	0.90	0.91	0.90	0.88	0.89
eCT0	0.86	0.87	0.82	0.83	0.85	0.86



Intron ctCCG vs unCCG

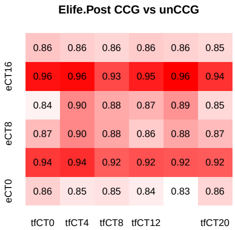
	tfCT0		tfCT8		tfCT16	
	0.71	0.76	0.77	0.72	0.76	0.76
eCT16	0.73	0.70	0.71	0.77	0.75	0.74
	0.79	0.77	0.75	0.75	0.73	0.73
eCT8	0.93	0.90	0.90	0.90	0.91	0.92
	0.96	0.95	0.95	0.93	0.93	0.94
eCT0	0.92	0.95	0.91	0.93	0.91	0.91

Related work



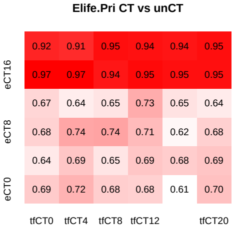
Elife.Post CT vs unCT

	tfCT0	tfCT4	tfCT8	tfCT12		tfCT20
	0.62	0.68	0.70	0.66	0.60	0.60
eCT16	0.91	0.91	0.90	0.91	0.93	0.89
	0.65	0.60	0.64	0.66	0.58	0.59
eCT8	0.62	0.67	0.66	0.64	0.67	0.64
	0.92	0.87	0.88	0.89	0.88	0.92
eCT0	0.61	0.63	0.64	0.62	0.61	0.67



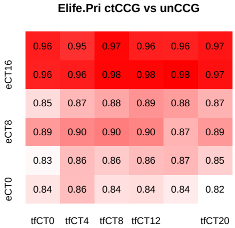
Elife.Post CCG vs unCCG

	tfCT0	tfCT4	tfCT8	tfCT12		tfCT20
	0.86	0.86	0.86	0.86	0.86	0.85
eCT16	0.96	0.96	0.93	0.95	0.96	0.94
	0.84	0.90	0.88	0.87	0.89	0.85
eCT8	0.87	0.90	0.88	0.86	0.88	0.87
	0.94	0.94	0.92	0.92	0.92	0.92
eCT0	0.86	0.85	0.85	0.84	0.83	0.86



Elife.Pri CT vs unCT

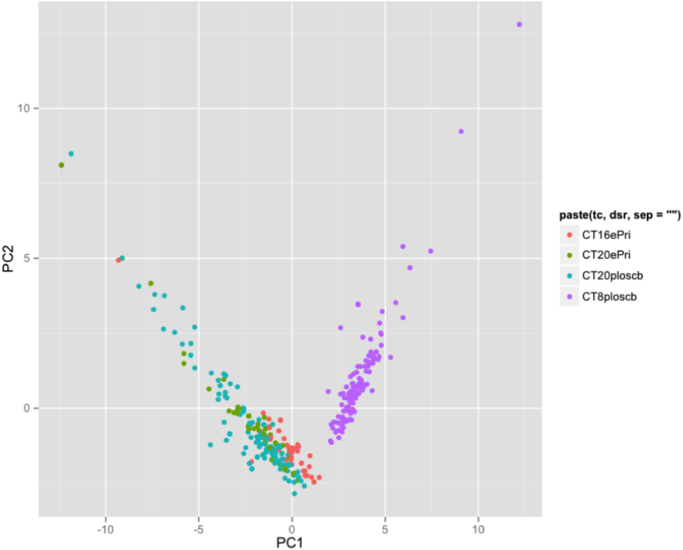
	tfCT0	tfCT4	tfCT8	tfCT12		tfCT20
	0.92	0.91	0.95	0.94	0.94	0.95
eCT16	0.97	0.97	0.94	0.95	0.95	0.95
	0.67	0.64	0.65	0.73	0.65	0.64
eCT8	0.68	0.74	0.74	0.71	0.62	0.68
	0.64	0.69	0.65	0.69	0.68	0.69
eCT0	0.69	0.72	0.68	0.68	0.61	0.70



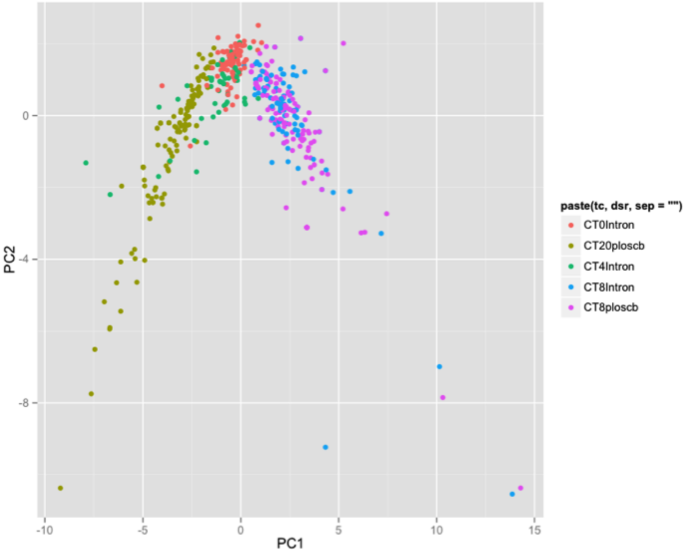
Elife.Pri ctCCG vs unCCG

	tfCT0	tfCT4	tfCT8	tfCT12		tfCT20
	0.96	0.95	0.97	0.96	0.96	0.97
eCT16	0.96	0.96	0.98	0.98	0.98	0.97
	0.85	0.87	0.88	0.89	0.88	0.87
eCT8	0.89	0.90	0.90	0.90	0.87	0.89
	0.83	0.86	0.86	0.86	0.87	0.85
eCT0	0.84	0.86	0.84	0.84	0.84	0.82

Related work

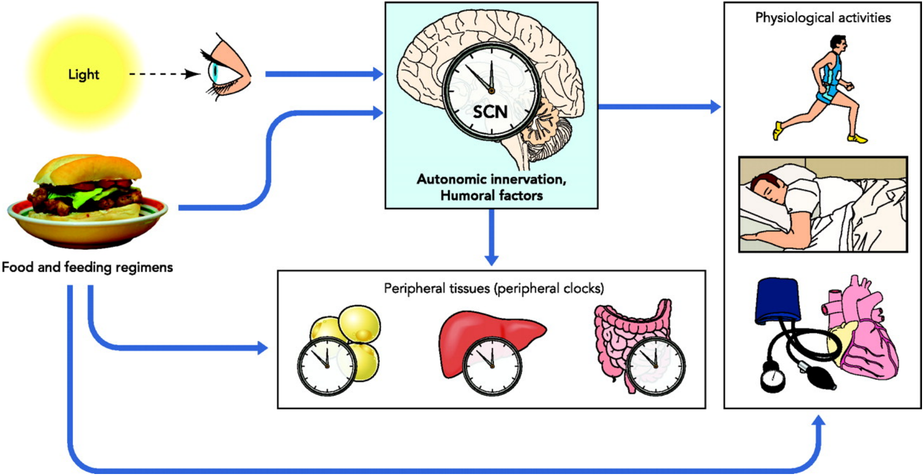


Related work



Brain circadian

The circadian rhythmic regulation in brain, so consider this effect when analyzing Brainspan project?



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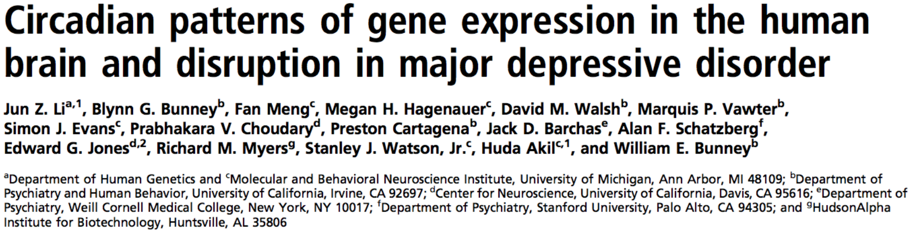

Circadian patterns of gene expression in the human brain and disruption in major depressive disorder

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The phasing of known circadian genes was consistent with data derived from other diurnal mammals. Cyclic patterns were much weaker in the brains of patients with MDD due to shifted peak timing and potentially disrupted phase relationships between individual circadian genes