# Cold-Inducible RNA-Binding Protein Modulates Circadian Gene Expression Posttranscriptionally

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# Background

- **生物节律（rhythms)**：节律性振荡
  - 生物钟（clock):位于SCN
  - 节律基因（Circadian Gene）

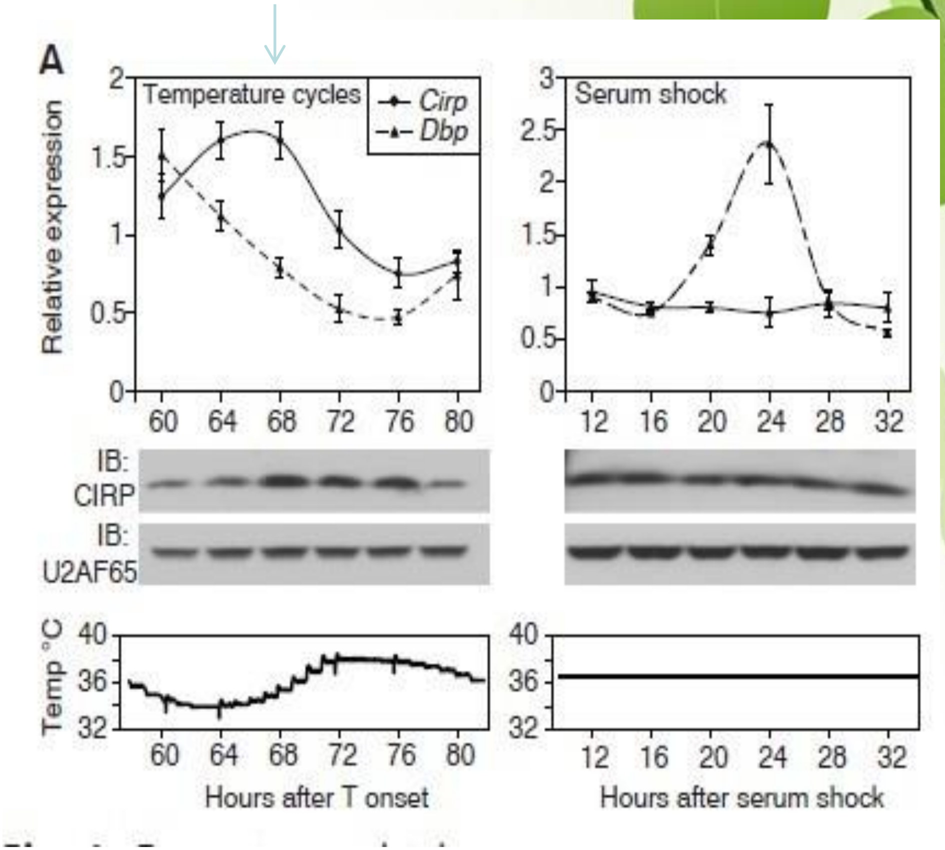
    or 钟基因（clock gene）：

    *Clock*、*Bmal 1*、*Per*、*Dbp*
- **CIRP（ Cold-Inducible RNA-Binding Protein）**

# Results

- expression of CIRP follows a diurnal rhythm that is controlled by systemic cues rather than local oscillators（振荡器）
- expression of Dbp,which is controlled by core clock transcription factors, oscillated under both conditions

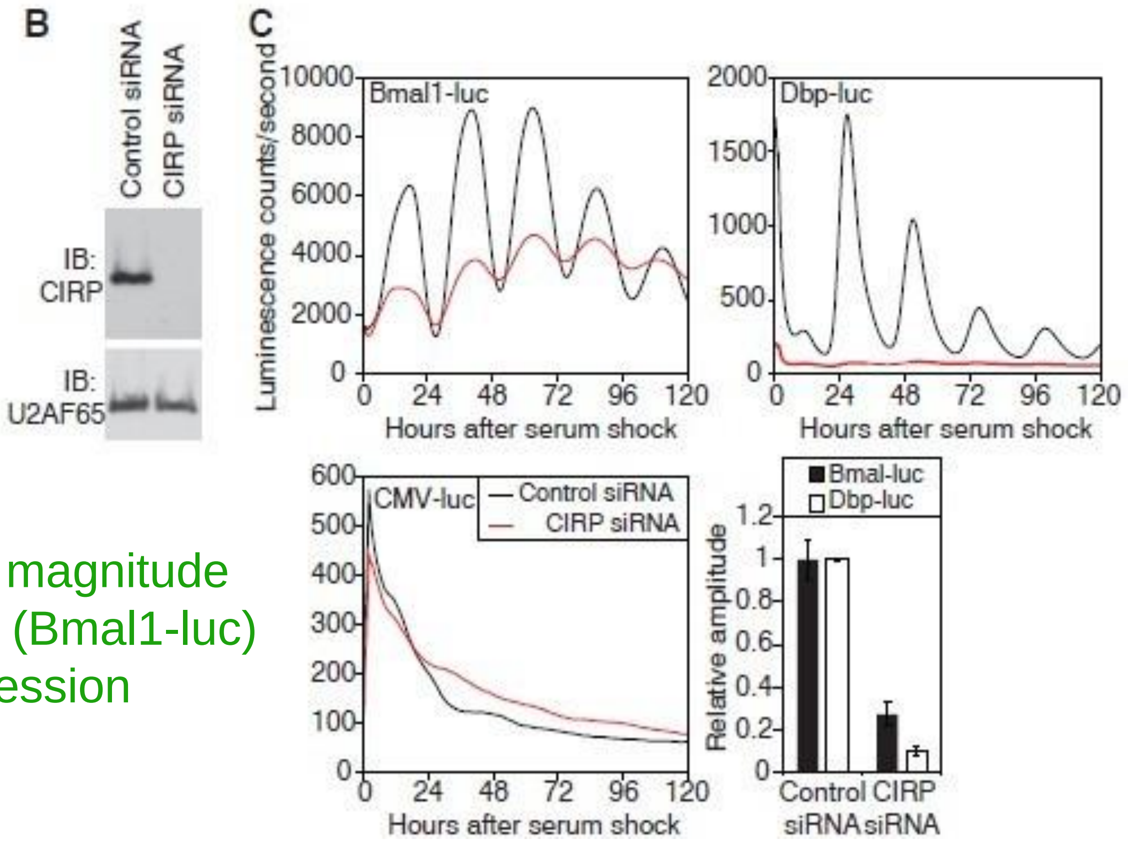
Dbp expression appeared to be regulated by the core oscillator



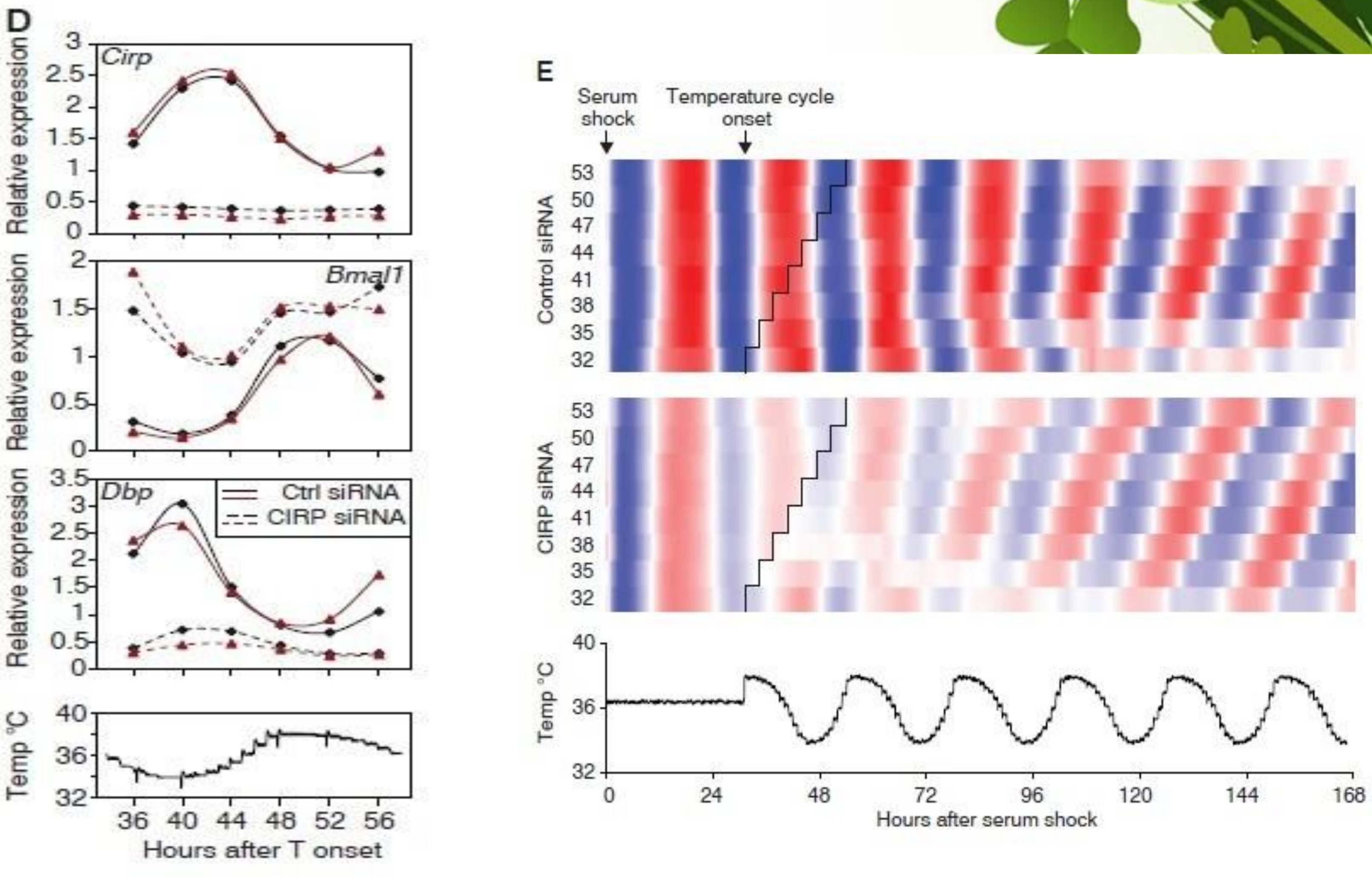
# Whether CIRP affect core clock function or not?

- CIRP-depleted cells
(transfected with siRNAs)

- Control cells
(transfected with control siRNAs)

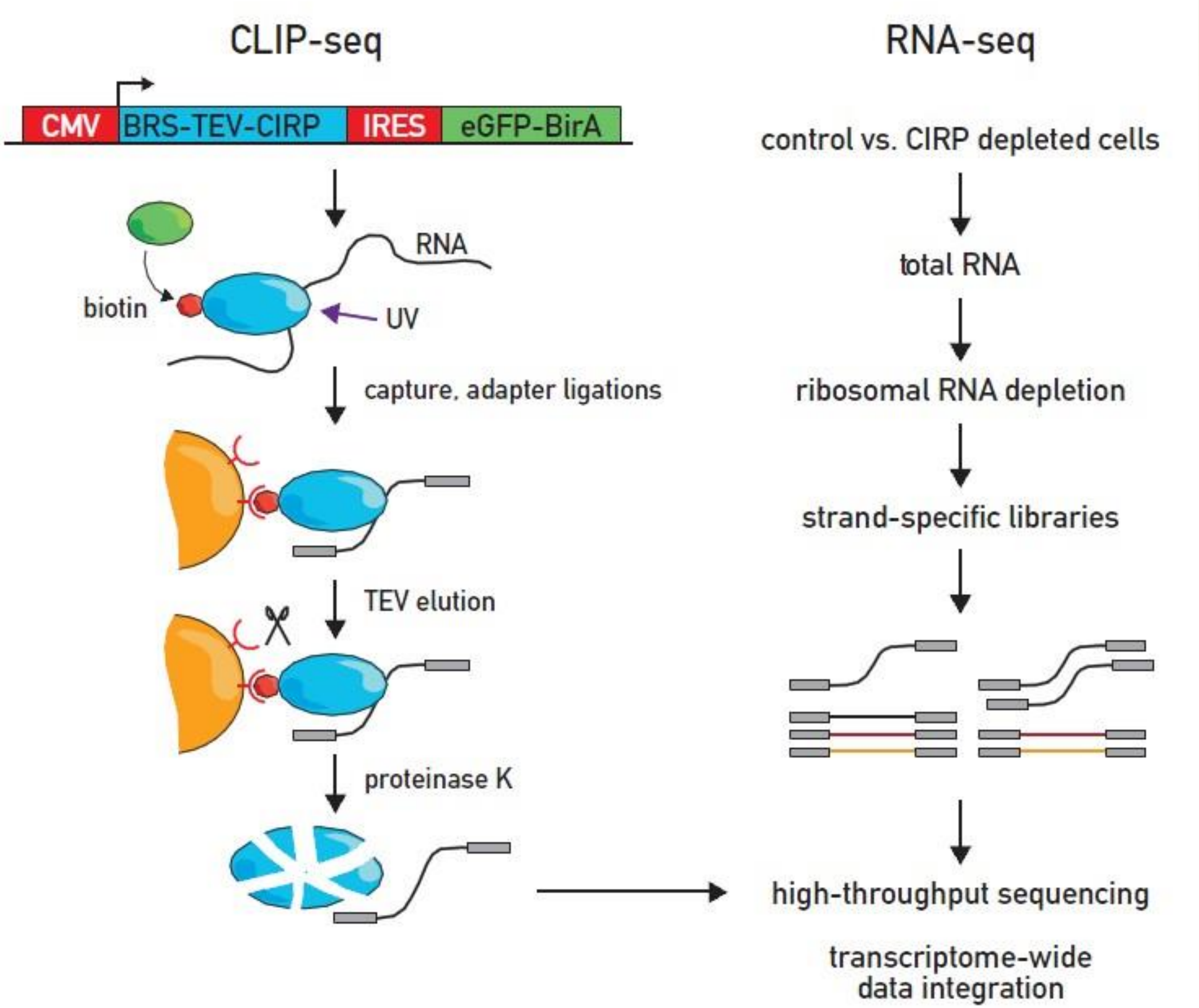


CIRP depletion decreased the magnitude and amplitude of Bmal1-luciferase (Bmal1-luc) and Dbp-luciferase (Dbp-luc) expression

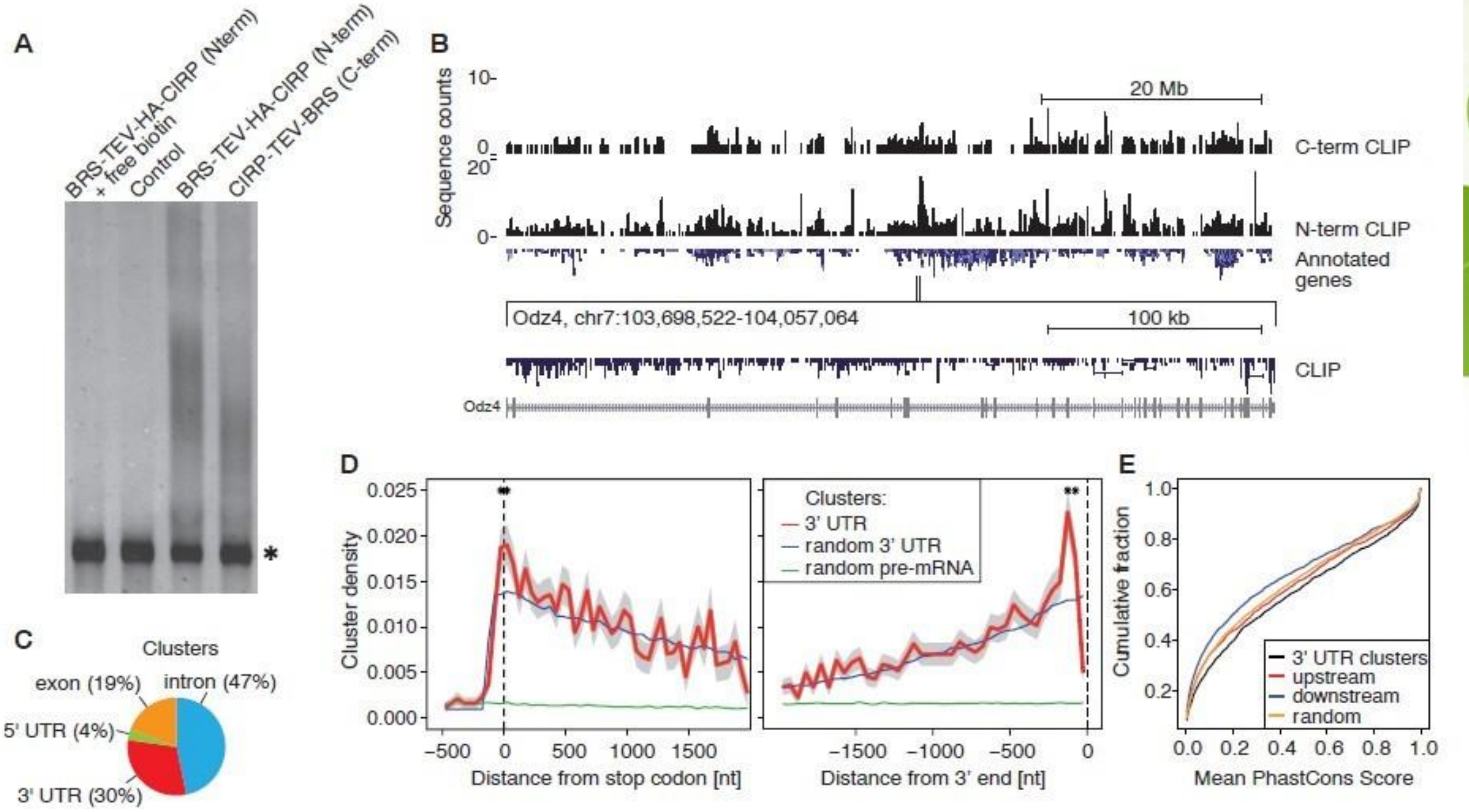


Loss of CIRP furthermore affected the phase-entrainment kinetics of oscillators to body temperature rhythms

# CIRP-bound transcripts

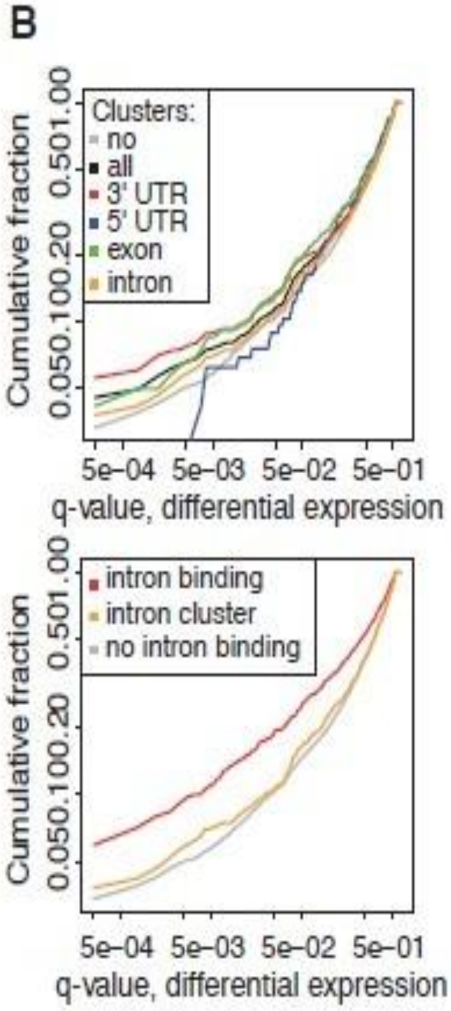


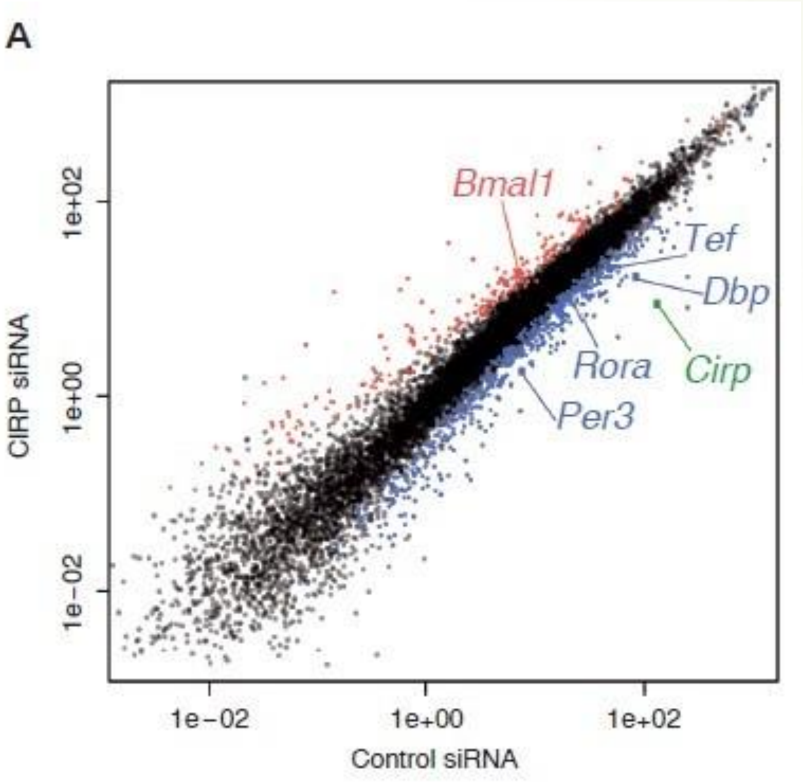
# To identify CIRP interaction sites in transcripts, we extended an algorithm detecting locally enriched CLIP sequences (clusters)



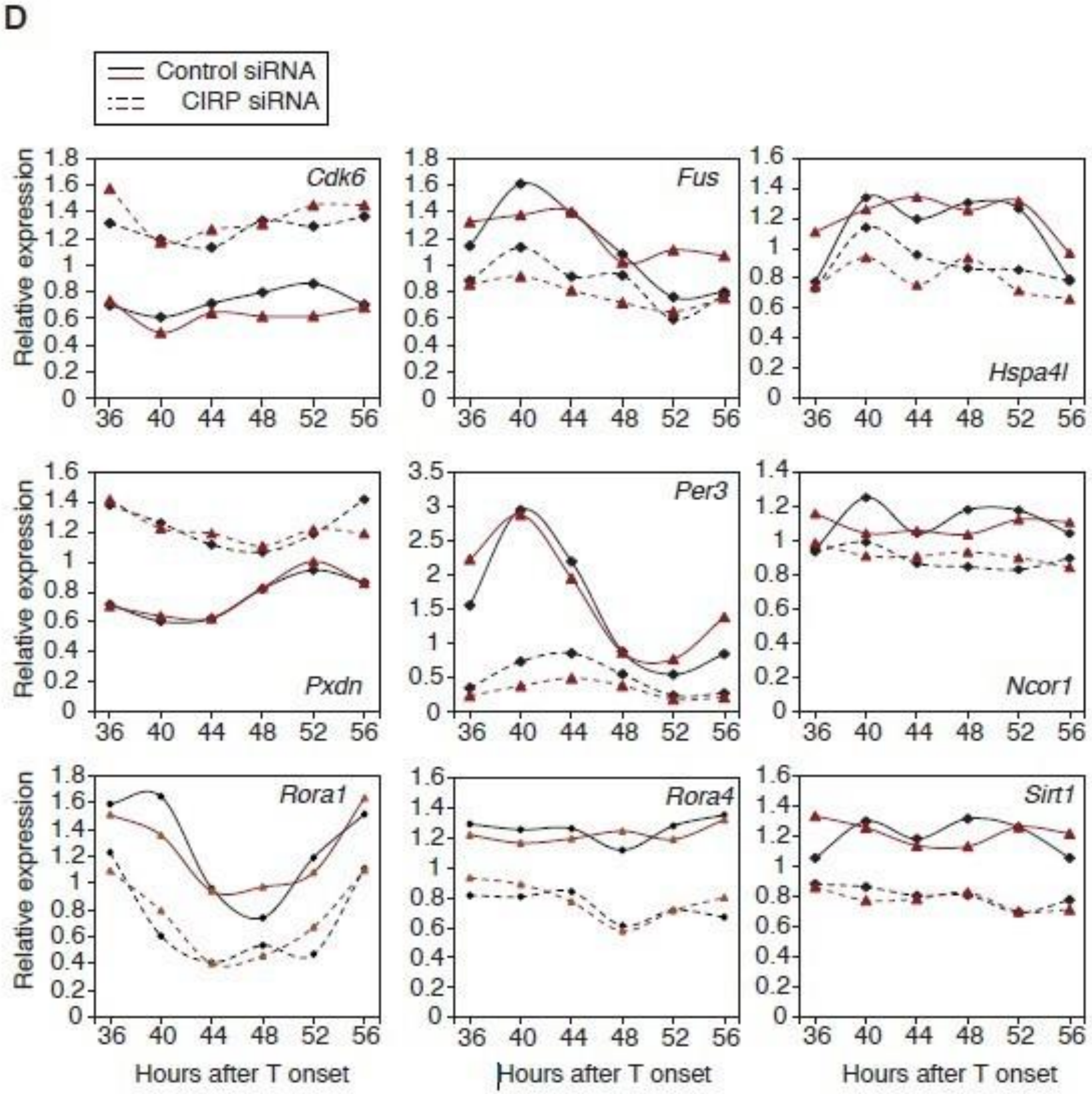
- Clusters in 3′ UTRs were particularly enriched close to stop codons and ~150 nucleotides upstream of poly(A) sites.
- CIRP binding sites in 3′ UTRs were more conserved than simulated control clusters

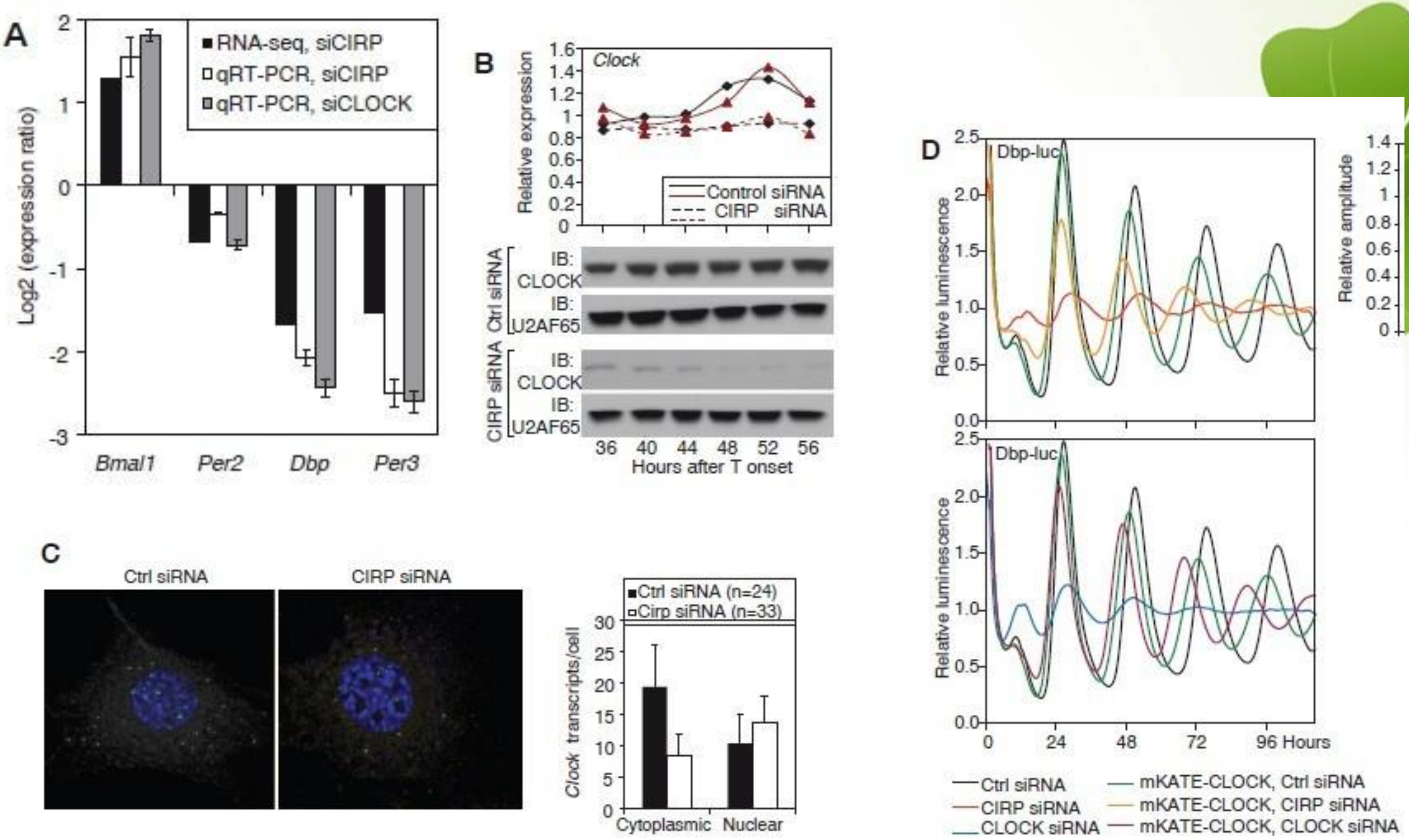
# Comparison of the accumulation of transcripts with and without CIRP clusters in control and CIRP-depleted cells by RNA-seq





- the former were more frequently misregulated than the latter
- Amounts of mRNAs of transcripts with dispersed CIRP binding in introns were more likely to be misregulated in CIRP-deficient cells than those of pre-mRNAs devoid of CIRP binding


D
Control siRNA
CIRP siRNA
Cdk6
Fus
Hspa4l
Pxdn
Per3
Ncor1
Rora1
Rora4
Sirt1
Relative expression
Hours after T onset
36
40
44
48
52
56
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
0.5
1.5
2
2.5
3
3.5


A
Log2 (expression ratio)
2
1
0
-1
-2
-3
■ RNA-seq, siCIRP
□ qRT-PCR, siCIRP
▨ qRT-PCR, siCLOCK
Bmal1
Per2
Dbp
Per3
B
Relative expression
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Clock
Control siRNA
CIRP siRNA
Ctrl siRNA
CIRP siRNA
IB: CLOCK
IB: U2AF65
IB: CLOCK
IB: U2AF65
36
40
44
48
52
56
Hours after T onset
C
Ctrl siRNA
CIRP siRNA
■ Ctrl siRNA (n=24)
□ Cirp siRNA (n=33)
Clock transcripts/cell
30
25
20
15
10
5
0
Cytoplasmic
Nuclear
D
Dbp-luc
Relative luminescence
2.5
2.0
1.5
1.0
0.5
0.0
Relative amplitude
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Dbp-luc
Relative luminescence
2.5
2.0
1.5
1.0
0.5
0.0
0
24
48
72
96 Hours
Ctrl siRNA
CIRP siRNA
CLOCK siRNA
mKATE-CLOCK, Ctrl siRNA
mKATE-CLOCK, CIRP siRNA
mKATE-CLOCK, CLOCK siRNA

# Conclusion

- we identified transcripts bound to cold-inducible RNA-binding protein CIRP in a transcriptome-wide fashion
- established a role for CIRP in circadian clock function
- low-amplitude oscillators are more sensitive to Zeitgeber cues than high amplitude oscillators
- CIRP enhances the amplitude of circadian oscillators by increasing

  the cytoplasmic accumulation of mRNAs encoding CLOCK and, perhaps, other regulators relevant for the circadian timing system, such as RORa, NCOR1, SIRT1, and PER3.

# Thank you ！