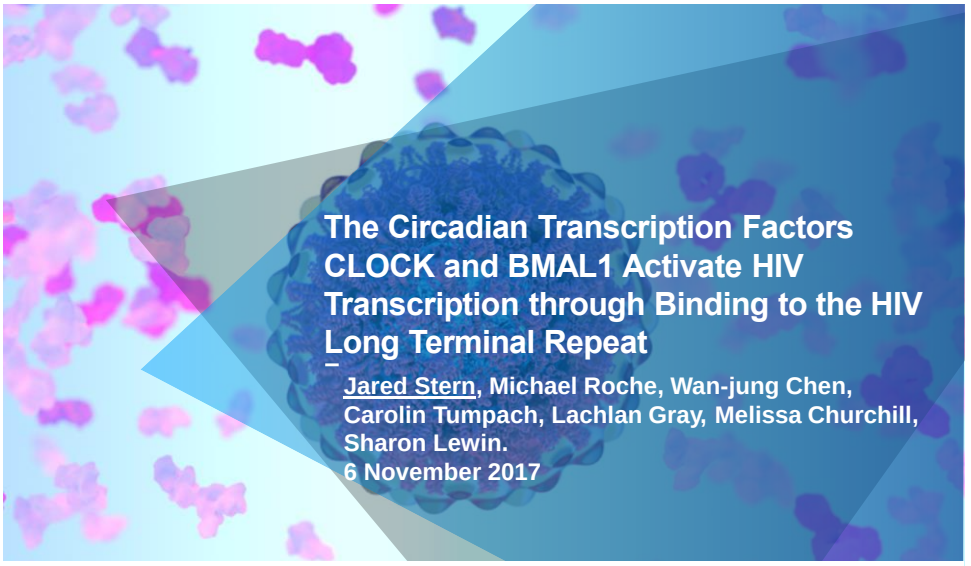


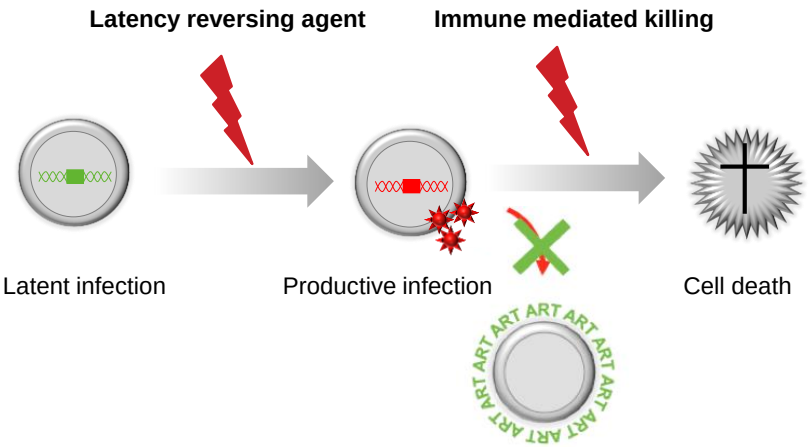
The Circadian Transcription Factors CLOCK and BMAL1 Activate HIV Transcription through Binding to the HIV Long Terminal Repeat

Jared Stern, Michael Roche, Wan-jung Chen, Carolin Tumpach, Lachlan Gray, Melissa Churchill, Sharon Lewin.

6 November 2017

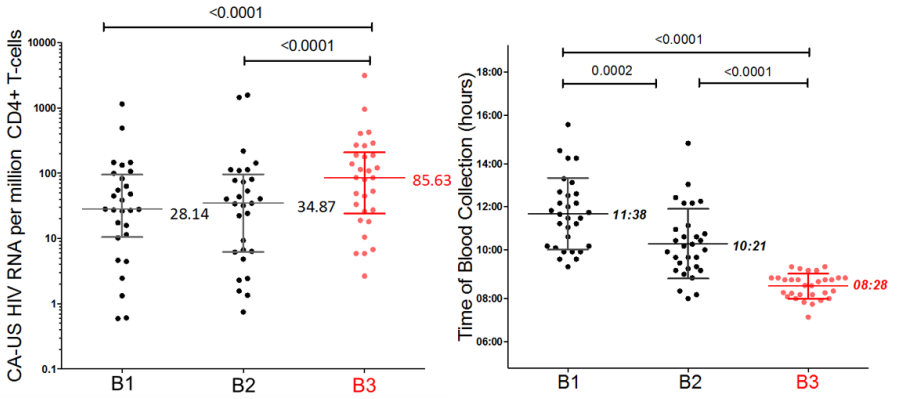
Doherty Institute
A joint venture between The University of Melbourne and The Royal Melbourne Hospital
The University of Melbourne
The Royal Melbourne Hospital

Activating HIV Latency as a Strategy for Cure



Archin et al., Nature 2012; Elliot et al., PLoS Pathog. 2014; Rasmussen and Lewin, Curr. Opin. HIV AIDS 2016

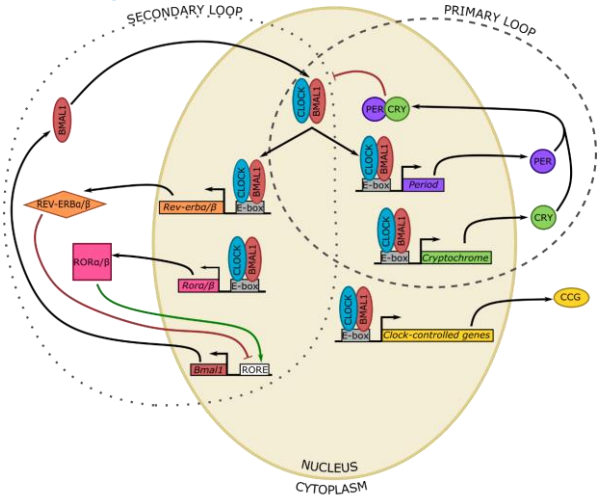
Preliminary Findings – Disulfiram Clinical Trial



N=30 participants in a randomised clinical trial. B1 = screening; B2 = between screening and enrolment; B3 = day of administration of disulfiram

Elliott et al., Lancet HIV 2015

Circadian Cycles

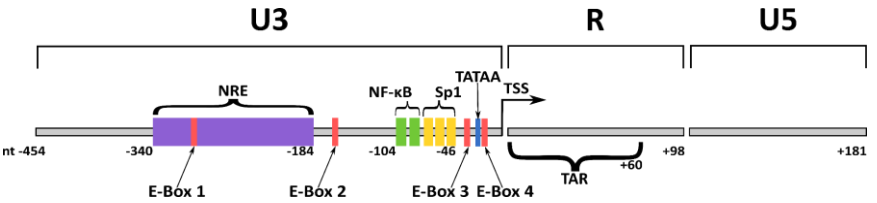


- CLOCK and BMAL1 heterodimers bind to an E-box to drive transcription
- Control is mediated through *Per* and *Cry* genes

Gekakis et al., Science 1998; Hogenesch et al., PNAS 1998; Kume et al., Cell 1999; Jin et al., Cell 1999

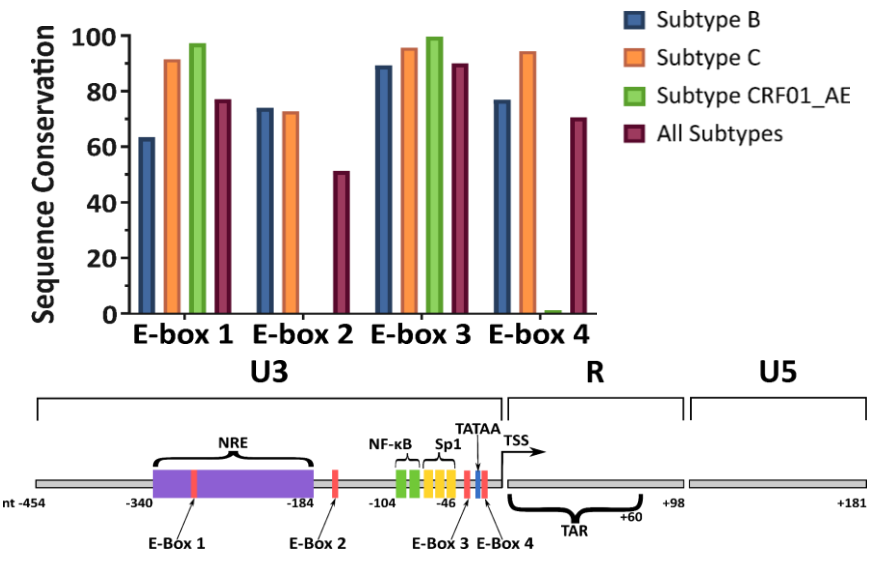
HIV and the Circadian Cycle

- CLOCK/BMAL1 has been found to bind to LTR
- Knockdown of CLOCK led to a log decrease in HIV infectivity



Terme et al., Retrovirology 2009; Tacheny et al., Nucleic Acids Res. 2012; Konig et al., Cell 2008

Sequence Conservation in the Long Terminal Repeat



Hypothesis

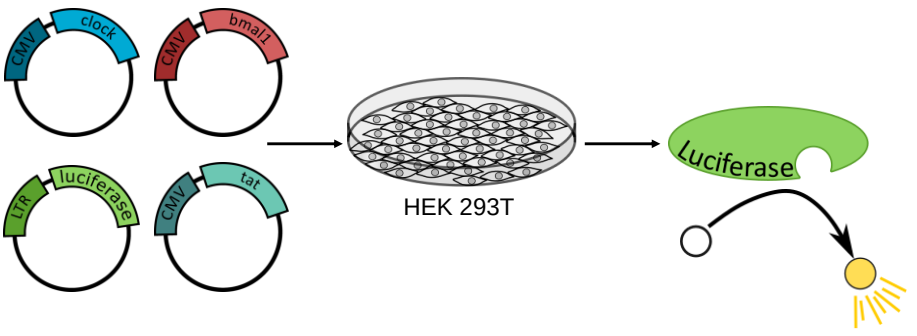
- In latently infected cells, HIV is under transcriptional control by the cell-autonomous circadian cycle.

Aims

- To determine whether the circadian proteins CLOCK and BMAL1 bind to the the HIV LTR to activate latent infection
- To exploit the circadian cycle of latently infected cells to identify new drug targets and optimise potency of other latency reversing agents

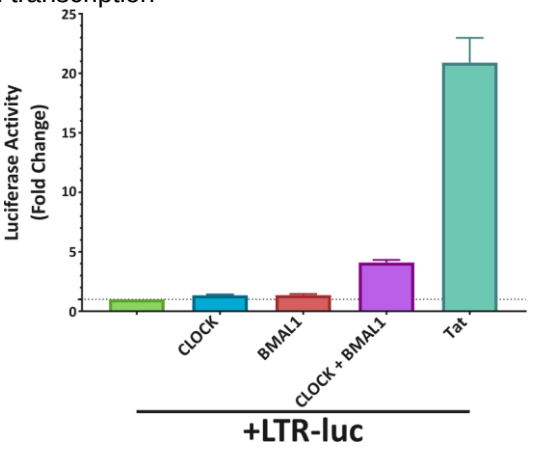
Effect of CLOCK/BMAL1 on HIV Transcription

- Genes encoding the human circadian transcription factors, CLOCK and BMAL1, were cloned into a pcDNA3.1 expression vector
- These proteins' effect on HIV transcription were assessed using an LTR-driven luciferase reporter plasmid (LTR-luc)

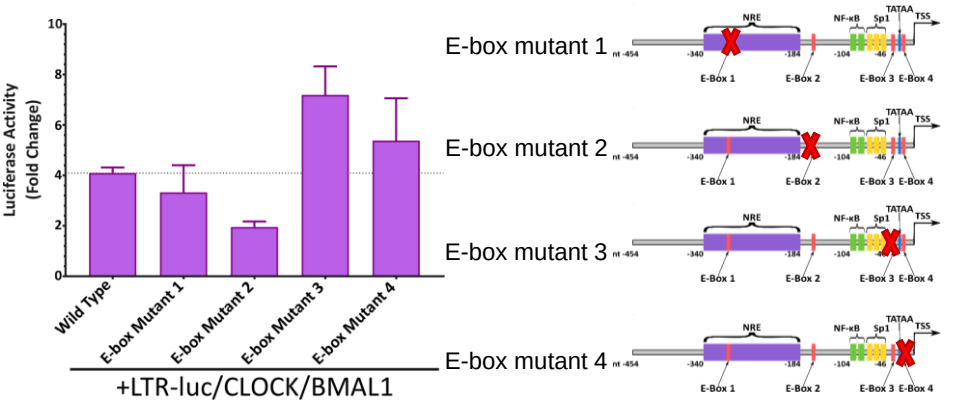


CLOCK/BMAL1 Together Increase LTR Activity

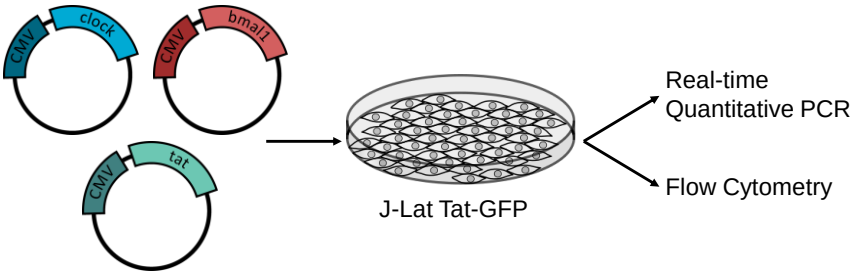
- CLOCK and BMAL1 alone had little effect on LTR-driven transcription
- CLOCK and BMAL1 together resulted in a 4.09-fold increase in LTR-mediated transcription



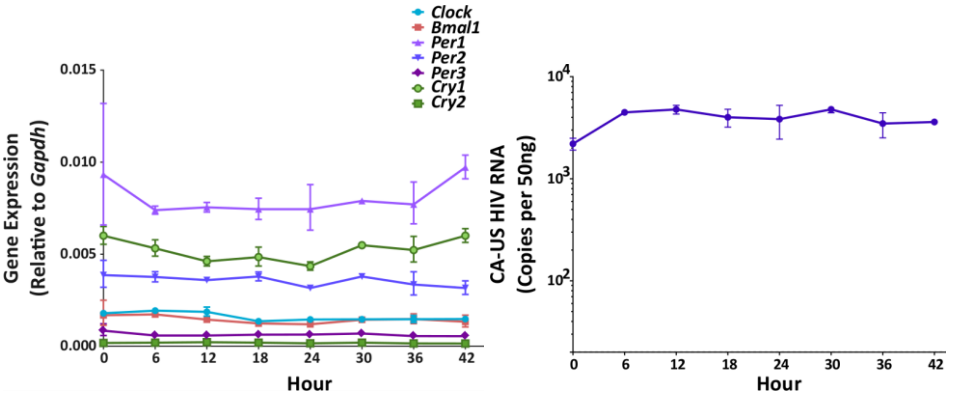
CLOCK/BMAL1 interact with at least one E-box



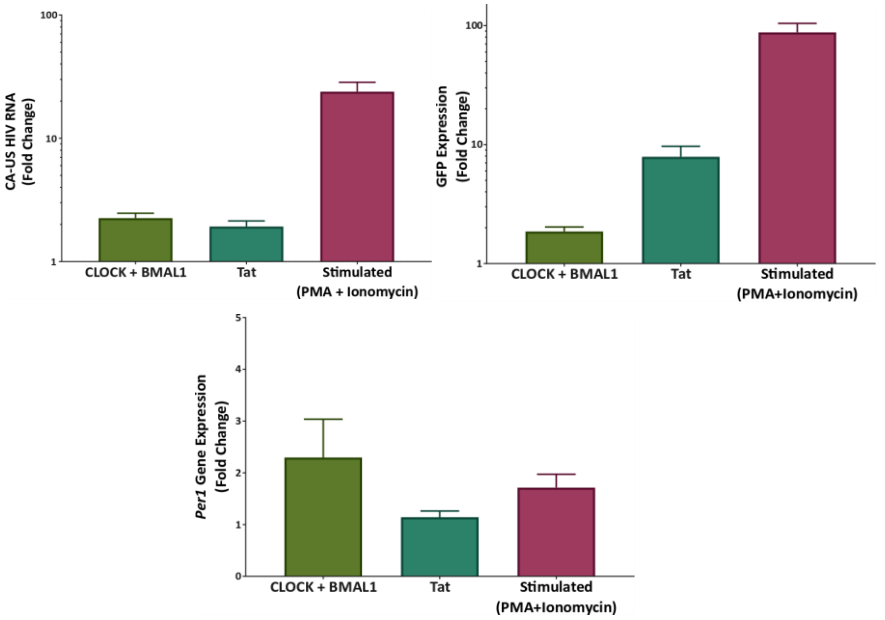
Method: Nucleofection of CLOCK and BMAL1



J-Lat Tat-GFP Cell Lines Lack Circadian Cycling in vitro



CLOCK:BMAL1 Activate Proviral Transcription in vitro



Summary

- We observed a relationship between time and cell associated unspliced HIV RNA in individuals living with HIV on ART
- The circadian transcription factors, CLOCK and BMAL1, upregulate HIV LTR-mediated transcription 2-7 times above basal transcription using reporter cell lines
- This upregulation required at least one E-box motif
- These results suggest that latent HIV transcription is controlled by circadian rhythms and this presents a novel pathway to exploit for latency reversal

Future directions

- Knock out multiple E-boxes simultaneously to determine if redundancy exists
- Demonstrate an effect in primary T-cells from HIV-positive individuals on ART
- Determine the effects of circadian-altering drugs on activation of latent HIV infection
- Determine the interaction of CLOCK/BMAL1 on the effects of other latency reversing agents (LRA) which would have implications for the best time of day to administer an LRA

Acknowledgements

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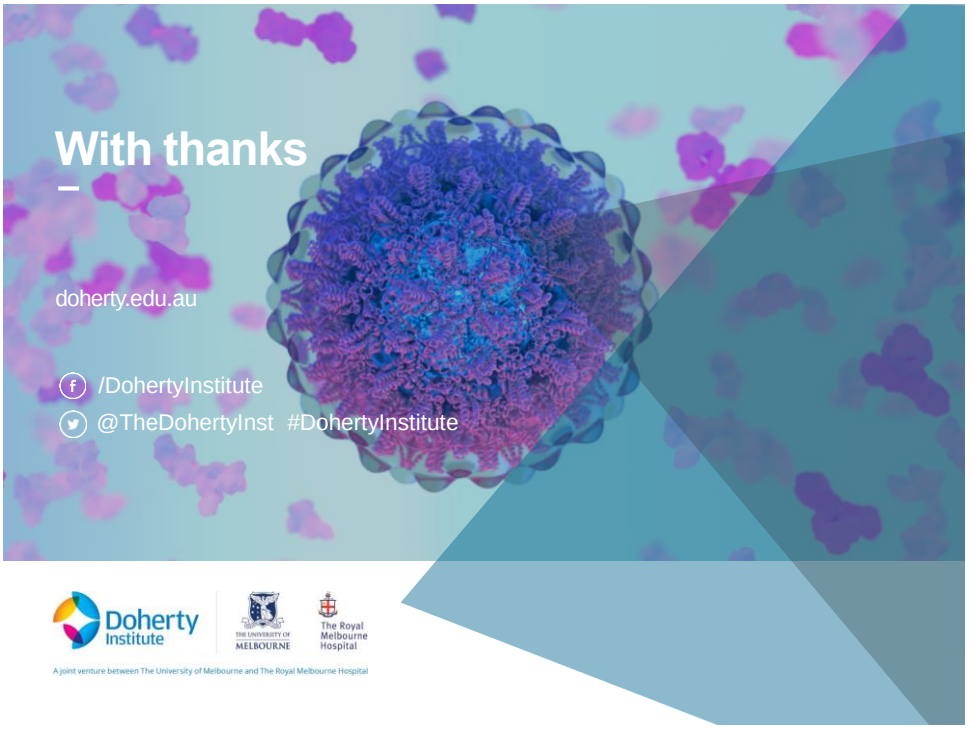
NHMRC


With thanks
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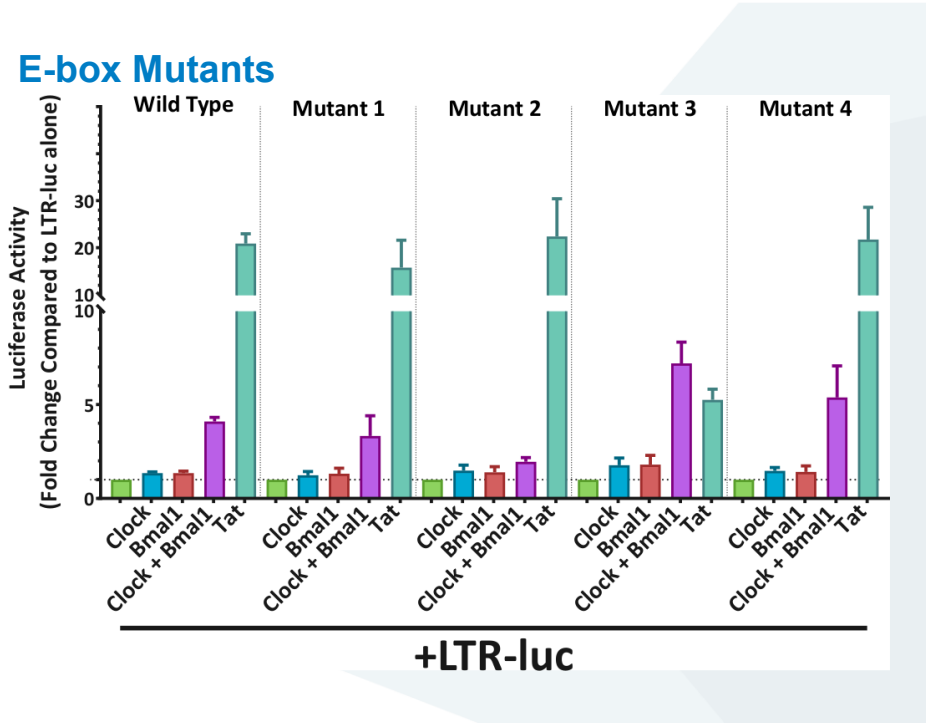
E-box Mutants
Wild Type
Mutant 1
Mutant 2
Mutant 3
Mutant 4
Luciferase Activity
(Fold Change Compared to LTR-luc alone)
30
20
10
10
5
0
Clock
Bmal1
Clock + Bmal1
Tat
+LTR-luc

Nucleofection