

Experimental validation of a predicted feedback loop in the multi-oscillator clock of *Arabidopsis thaliana*

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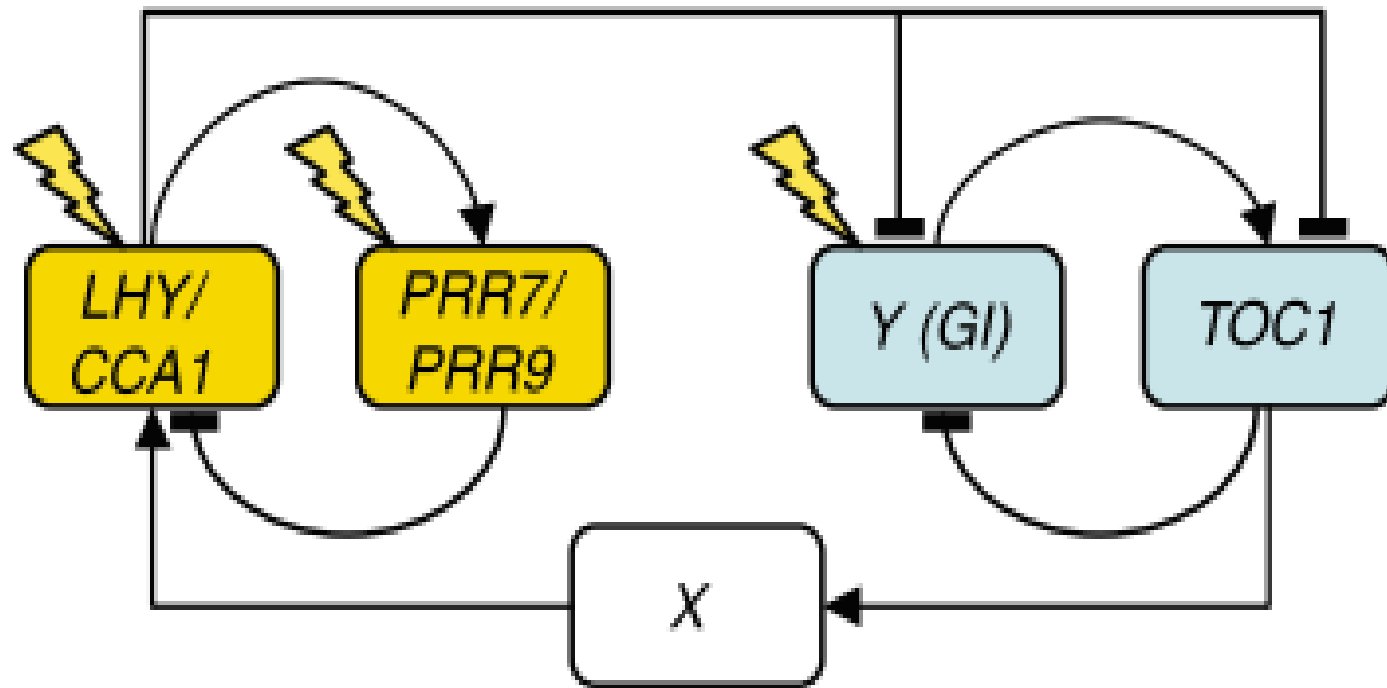
- Introduction
- Results
- Model validation against existing and new experimental data
- GI is a component of Y
- Regulatory properties of the three-loop network.
- Discussion

Introduction

1. Circadian rhythms are generated by a central network of 6–12 genes that form interlocked feedback loops. (Glossop et al, 1999)
2. The circadian clock generates 24-h rhythms in most eukaryotes and in cyanobacteria. (Dunlap et al, 2003)
3. Two partially redundant genes, LATE ELONGATED HYPOCOTYL (*LHY*) and CIRCADIAN CLOCK ASSOCIATED 1 (*CCA1*), repress the expression of their activator, TIMING OF CAB EXPRESSION 1 (*TOC1*) in *Arabidopsis*. (Alabadi et al, 2001)

Introduction

4. A short-period rhythm persists for several cycles in *lhy;cca1* and *toc1* mutant plants (Mas et al, 2003a, Locke et al, 2005a)
5. Mathematical modelling proposes a new circuit comprising two interlocking feedback loops which predicted the existence and expression patterns of two hypothetical components **X** and **Y**. **GI** was identified as a candidate for Y. (Locke et al, 2005b)



PRR7/PRR9–LHY/CCA1 feedback loop

Y–TOC1 feedback loop

LHY/CCA1–TOC1–X loop

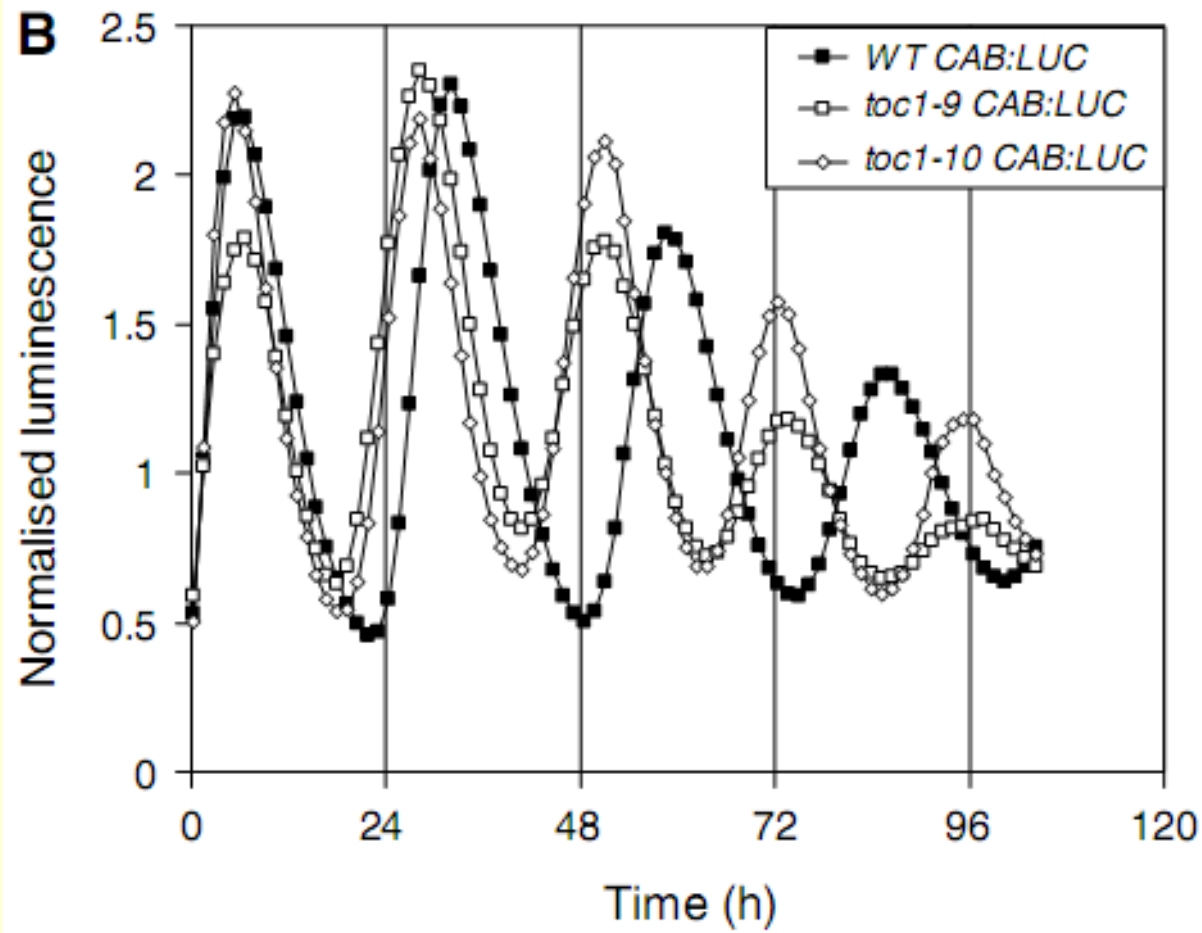
mutants

- toc1 RNAi lines
- TOC1 deletion mutant
- lhy;cca1 double mutant
- prr7;prp9 double mutant
- gi mutant(reducing Y translation by 70%)
- gi;lhy;cca1 triple mutant
- x mutant

results

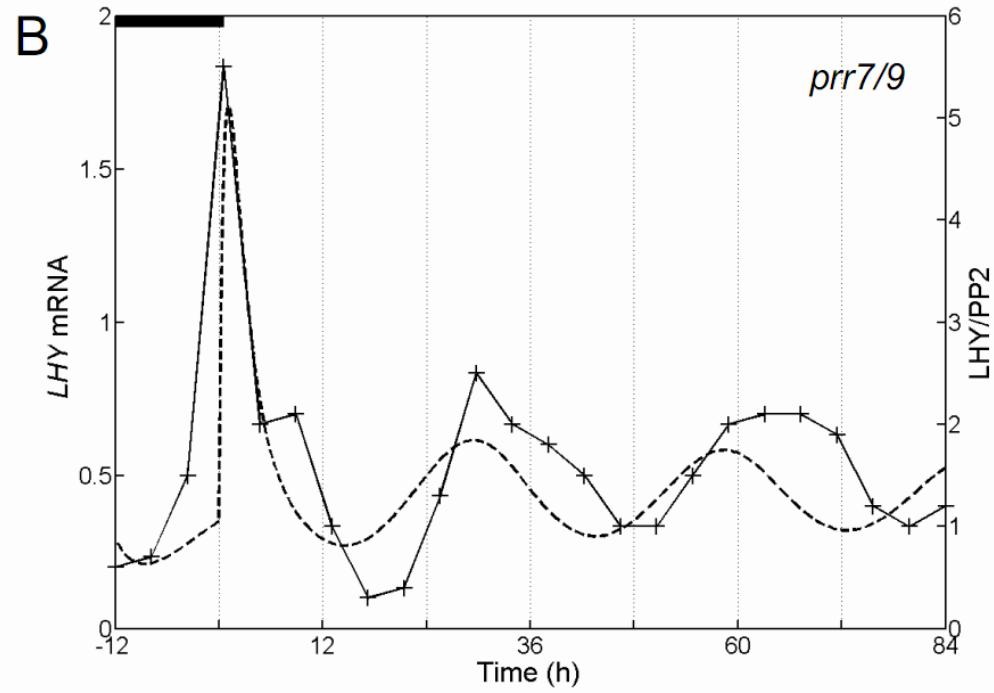
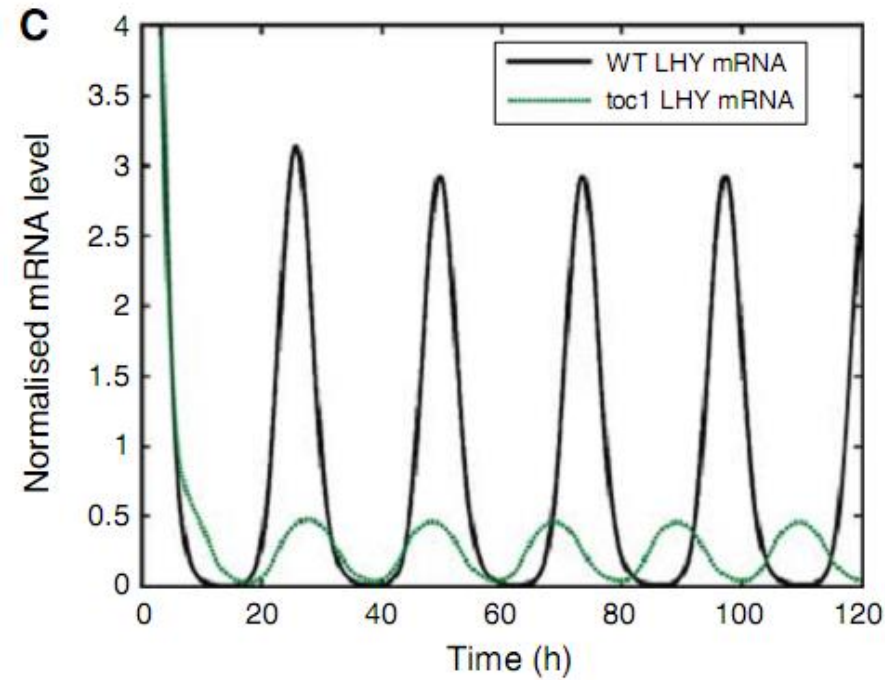
1. Model validation against existing and new experimental data
 - A short-period rhythm can exist in Mutants with reduced TOC1 (Alabadi et al, 2001; Mas et al, 2003a)

- TOC1 deletion mutant



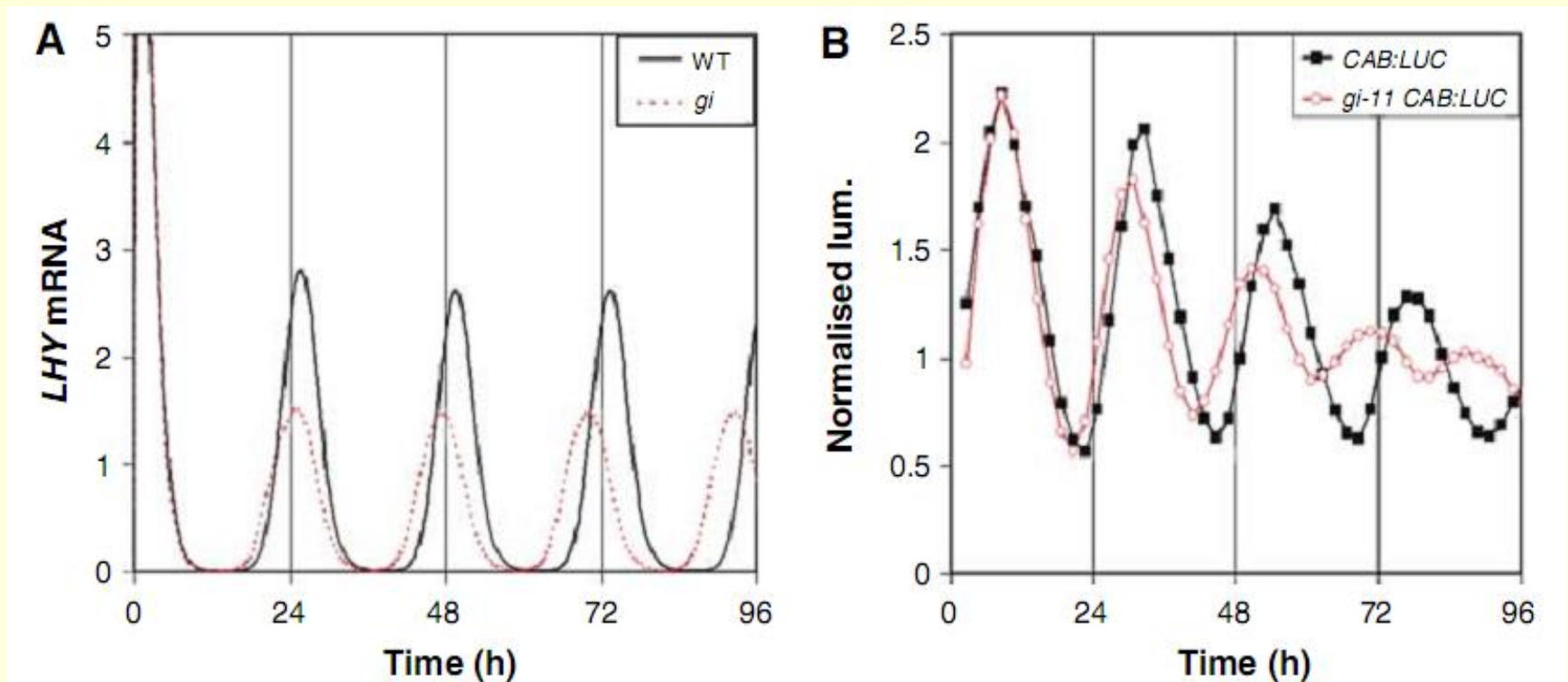
Conclusion: A TOC1-independent oscillator can persist in toc1 plants.

- PRR7/PRR9–LHY/CCA1 feedback loop

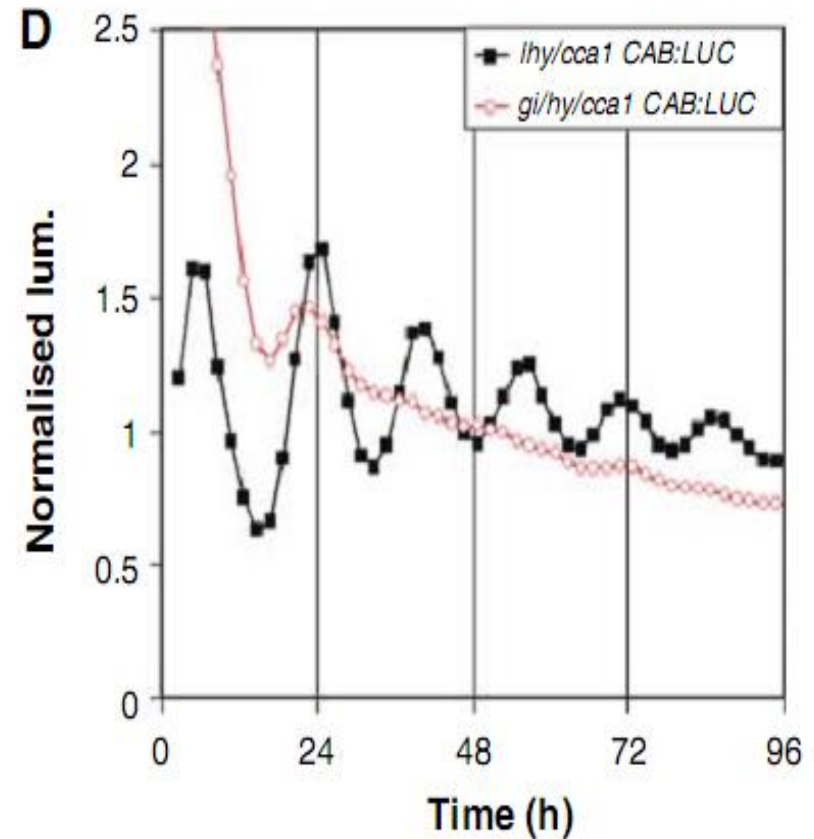
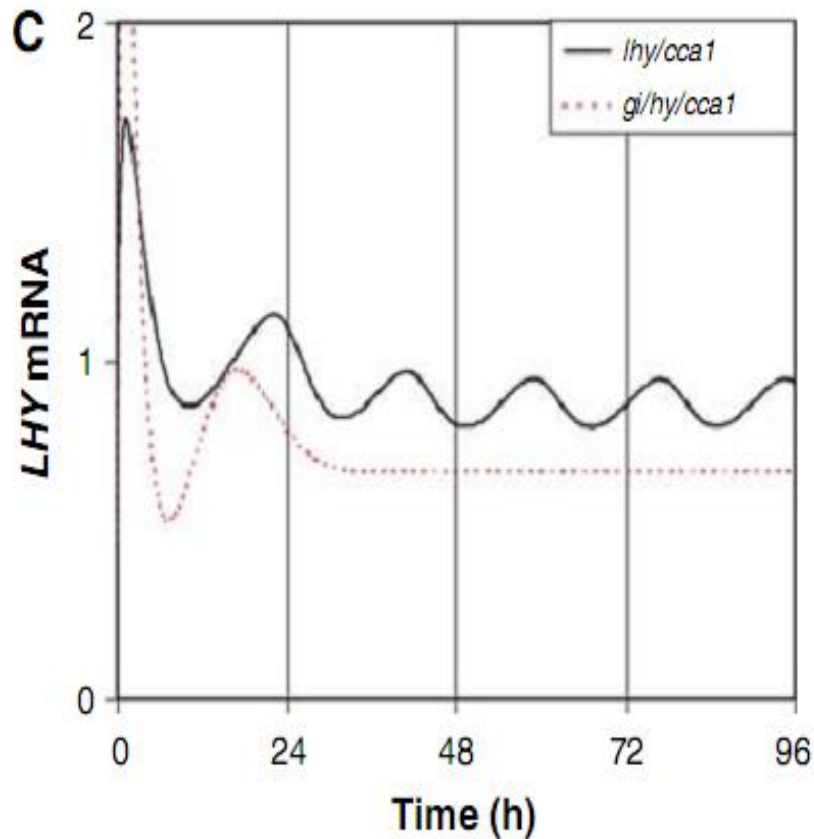


2.GI is a component of Y

- *gi* mutation (modelled by reducing Y translation by 70%)
- *gi* null mutant



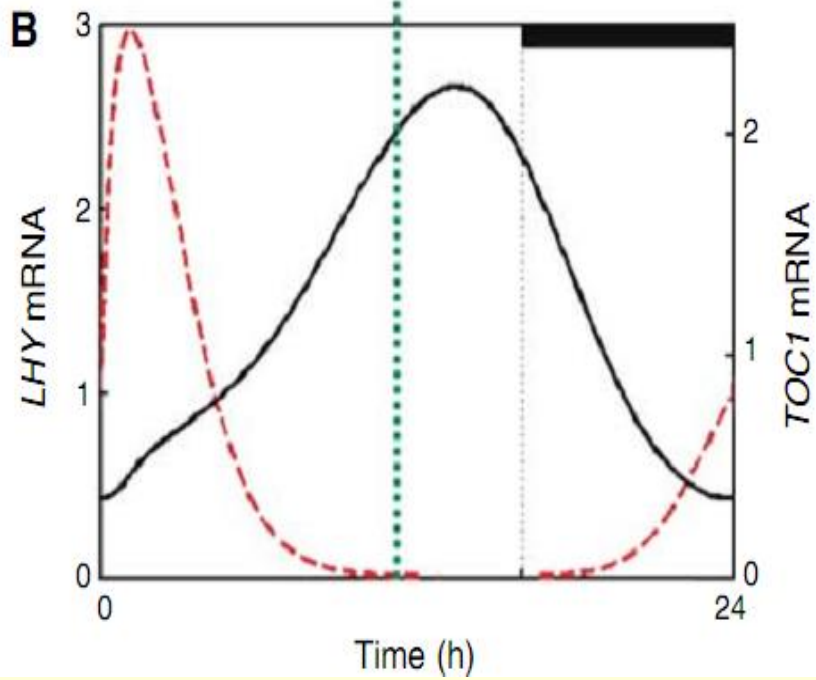
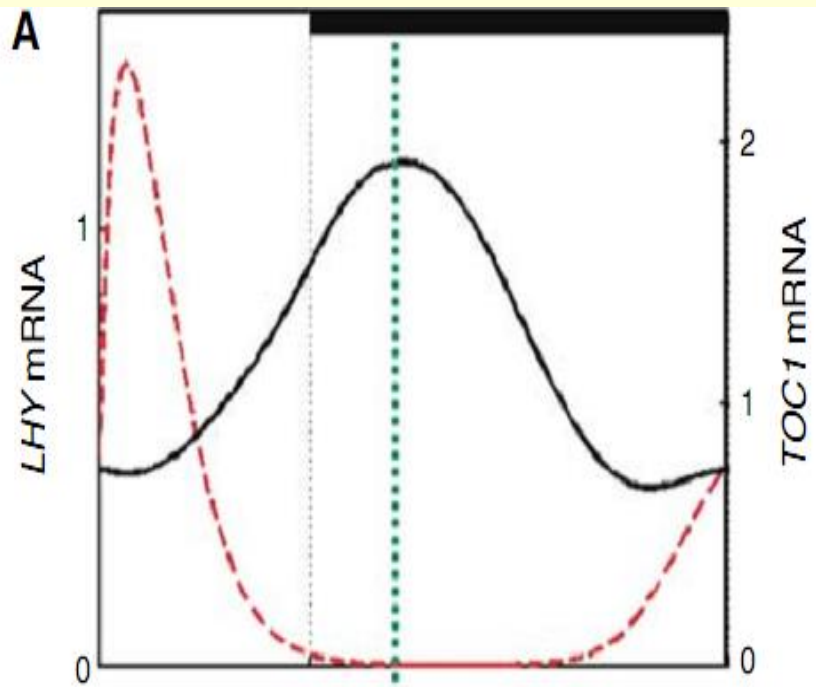
Y-TOC1 feedback loop



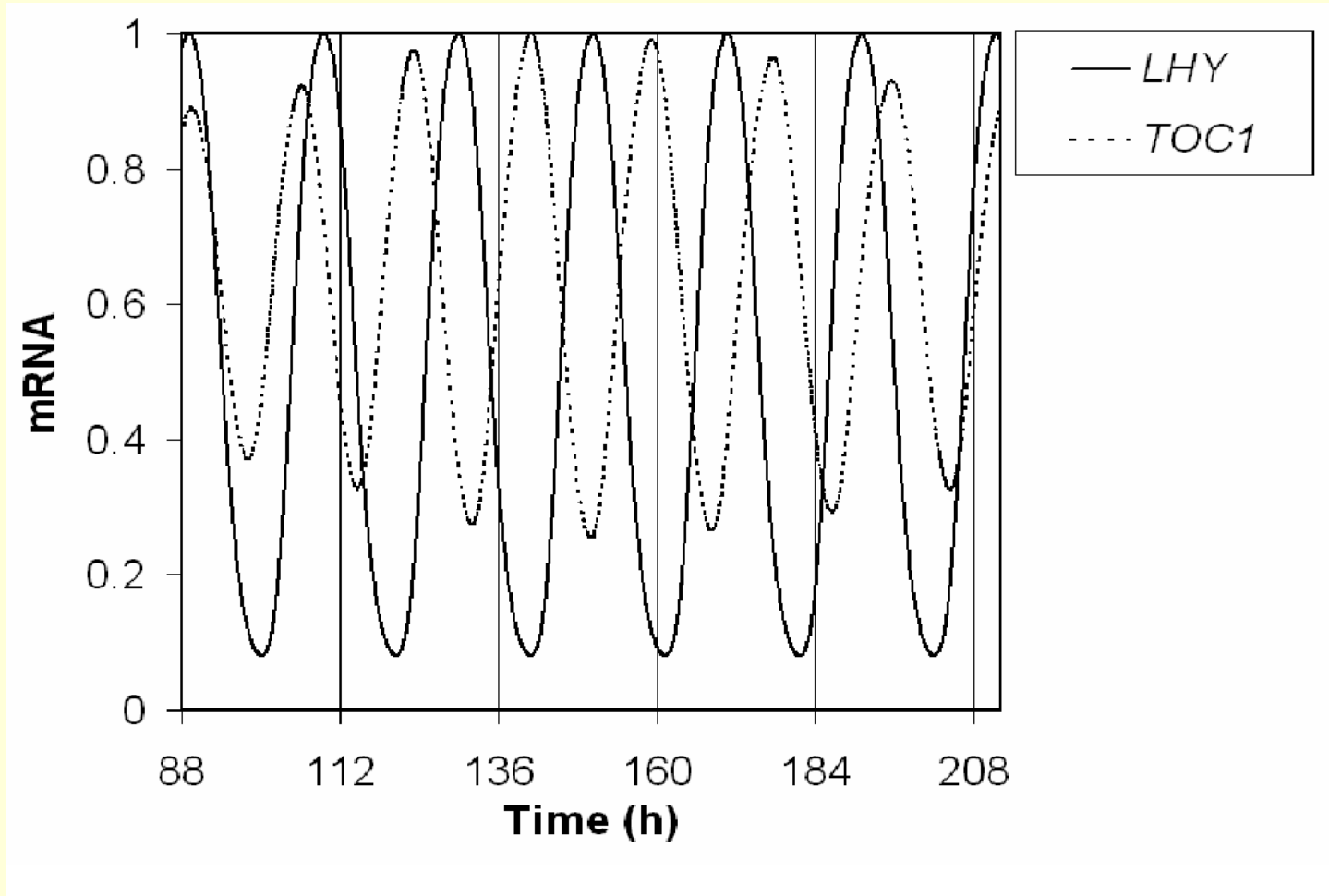
Conclusion: Y-TOC1 feedback loop generates the 18 h rhythm.

3. Morning and evening oscillators allow tracking of dawn and dusk

- The model predicts that two short-period oscillators, the morning-expressed PRR7/9–LHY/CCA1 loop and the evening-expressed TOC1–Y/GI loop, are coupled together by the LHY/CCA1–TOC1–X loop



- x mutant



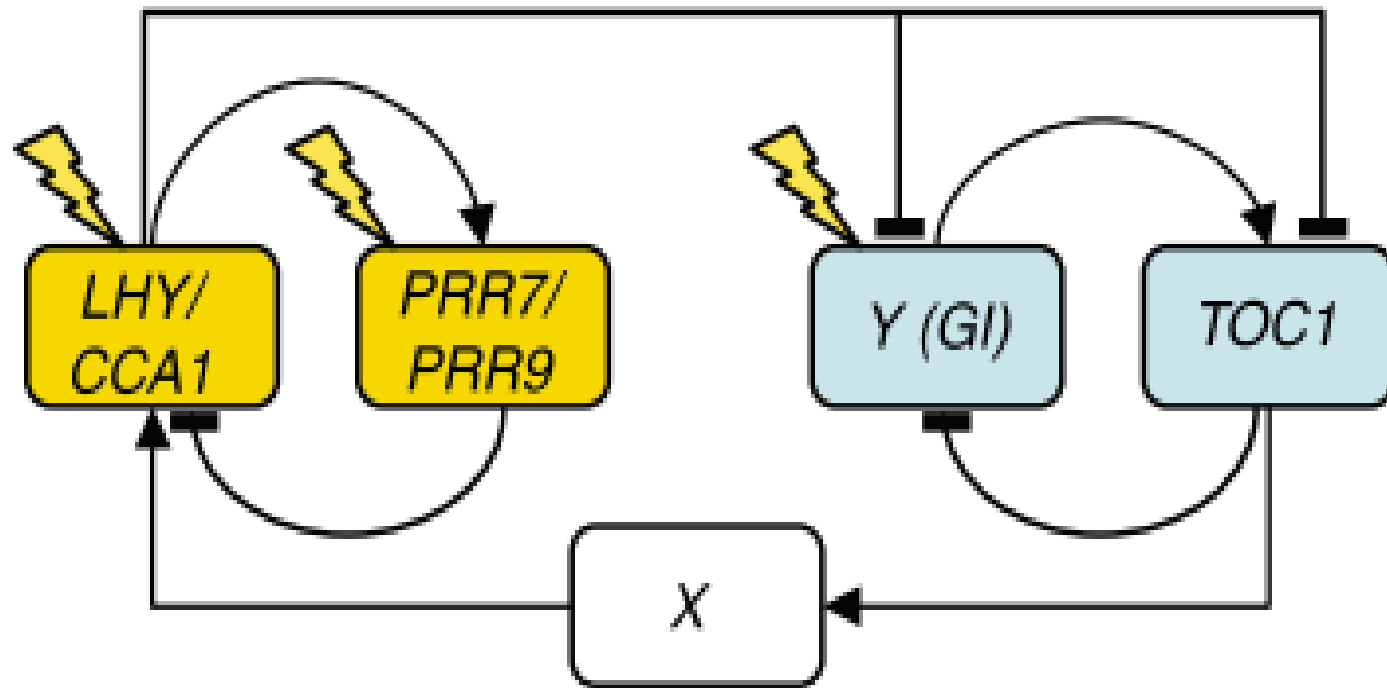
Conclusion: LHY mRNA levels oscillated with a 20.4 h period under LL conditions and TOC1 levels oscillated with a 17.3 h period.

Discussion

1. This marks an advance in systems biology, because GI was identified as a candidate gene in this loop using experiments based directly on predictions from mathematical modelling.
2. The three-loop model is not yet complete, as it does not incorporate known clock-affecting genes such as *PRR3*, *PRR5*, TIME FOR COFFEE (*TIC*), EARLY FLOWERING 4 (*ELF4*) and LUX ARRHYTHMO (*LUX*) (reviewed by McClung, 2006)

3. Analysis of the three-loop network suggests new avenues for experiments. X mutation could lead to desynchronisation of two short-period clocks.

4. It will now be important to understand the role and balance of the light inputs into each of the feedback loops of the clock.



PRR7/PRR9–LHY/CCA1 feedback loop

Y–TOC1 feedback loop

LHY/CCA1–TOC1–X loop

Thank you!