Network balance via CRY signalling controls the Arabidopsis circadian clock over ambient temperatures

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Background

- The circadian clock is an endogenous 24h timer, found throughout nature. It allows an organism to temporally orchestrate metabolic, physiological, biochemical and developmental processes.

- A defining feature of circadian clocks is the remarkable control of the clock's pace (the circadian period) over a physiological range of constant temperatures——temperature compensation.

Background

- It is unclear whether temperature compensation is owing to specific molecular mechanisms.

- The obvious alternative is that there are no special molecular mechanisms but that the clock gene network has evolved so that the many, milder effects of temperature upon its components produce an overall balance.

Introduction

As the previous data put, we believe the latter hypothesis is more convinced.(in standard laboratory conditions close to 22℃)

- **Model 1**

As precedent shows that there is a so-called ‘isoform switch’ mechanism proposed for the Neurospora(脉孢菌) clock; and a recent model argues that that their effects are network-balanced.

- **Model 2**

The **genetic circuit** of the Arabidopsis circadian clock, in common with other organisms, comprises several interlocking feedback loops. A recent mathematical model proposes a three-loop structure as a framework for analysis.

- the morning loop
- the evening loop
- A third loop is formed between morning and evening loops

Introduction

- In classical studies of temperature compensation, the focus is on the period of the free-running oscillator(振荡器), under constant conditions.

- However, in most physiologically relevant situations, the clock is entrained by daily environmental cycles, comprising **both light and temperature fluctuations.**

Introduction

- In this work, we hypothesised that daily cycles of light and temperature should not disrupt each other's entrainment effects, and that this requirement might be most simply satisfied if the two signals acted through the same machinery.
- We tested the hypothesis that light and temperature share common input mechanisms to the clock.

- Outputs included the scoring of circadian period in wild-type (WT) and Arabidopsis photoreceptor mutants.
- We applied linear regression and mixed-effects models to period estimates and identified significant variation in light, genotype, temperature and marker in our data.
- Using statistical modelling, we identified a strong interaction between temperature and BL in the control of circadian period.

Introduction

- We decided to test the hypothesis that temperature control is not delivered through dedicated molecular mechanisms as in the initial hypothesis, but is instead by network balancing through the BL input pathway.

- This led to the development of a fully temperature-compensating model of the Arabidopsis circadian oscillator simply by adding passive temperature effects through the known BL input pathways (Pokhilko et al, 2010);
- Remarkably, this model was not only capable of matching multiple non-trivial features of our data, but could also predict an unexpected and previously unobserved change in the abundance of a key clock protein with temperature.

Materials and methods

◆ Plant material

The CCR2:LUC+ transgene was transformed into both mutant and WT plants using an Agrobacterium(农杆菌)-mediated floral dip method. For each mutant, at least three independent lines were characterised.

◆ Growth conditions and rhythm analysis

◆ RNA analysis

Total RNA was extracted & qRT-PCR reactions

◆ Protein analysis

CRY and LHY proteins were extracted & western blot

◆ Computational methods

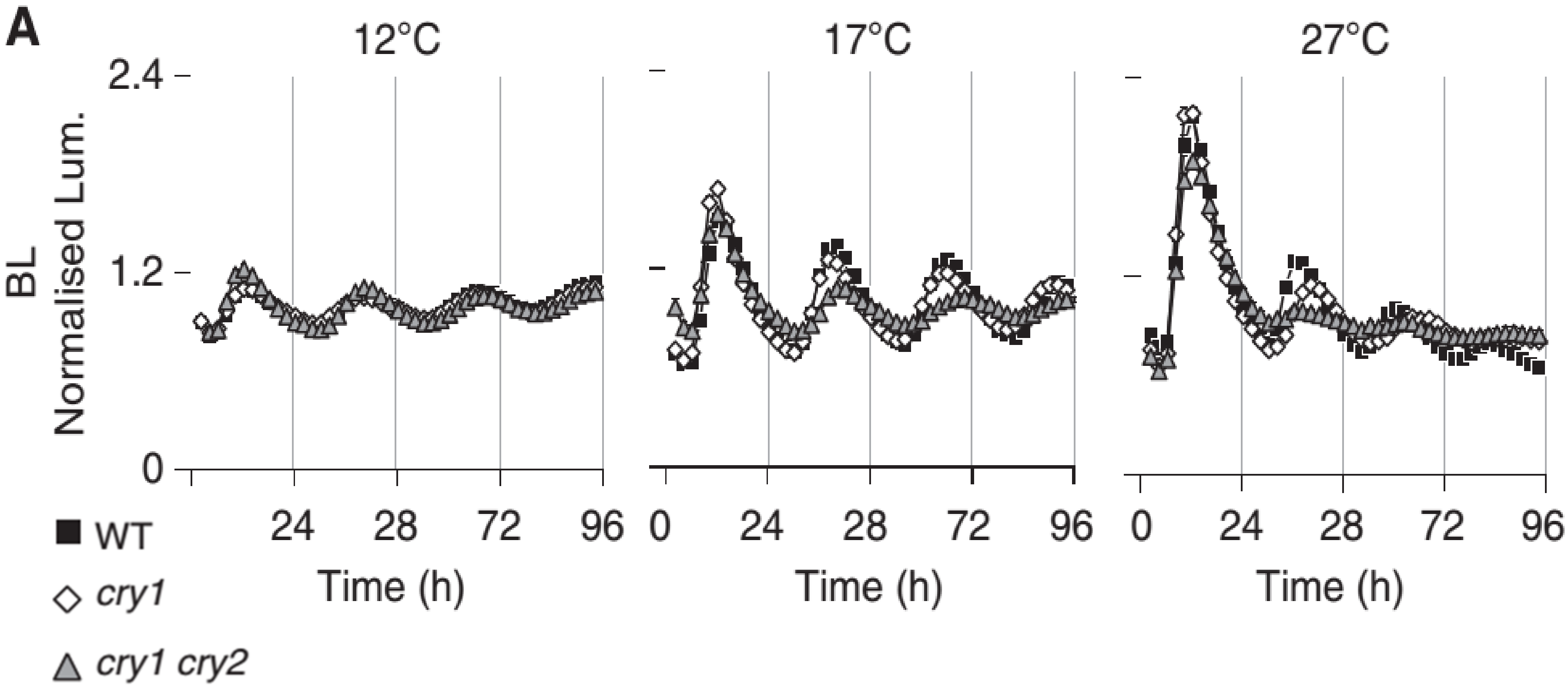
Sensitivity analysis & period estimation for simulations

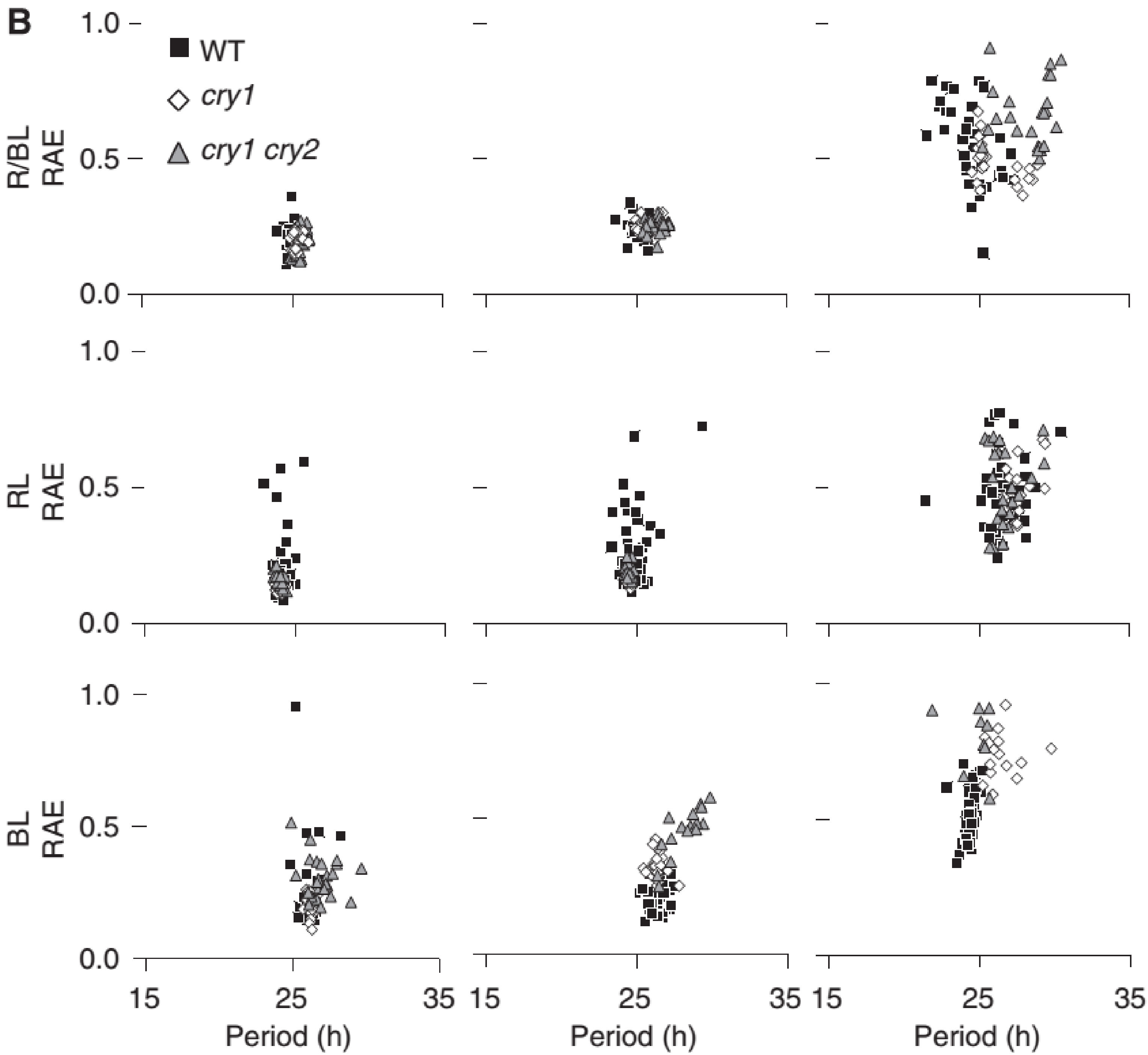
◆ Identifying sources of variation

standard linear regression models & linear mixed-effects models & the open source statistical programming environment R

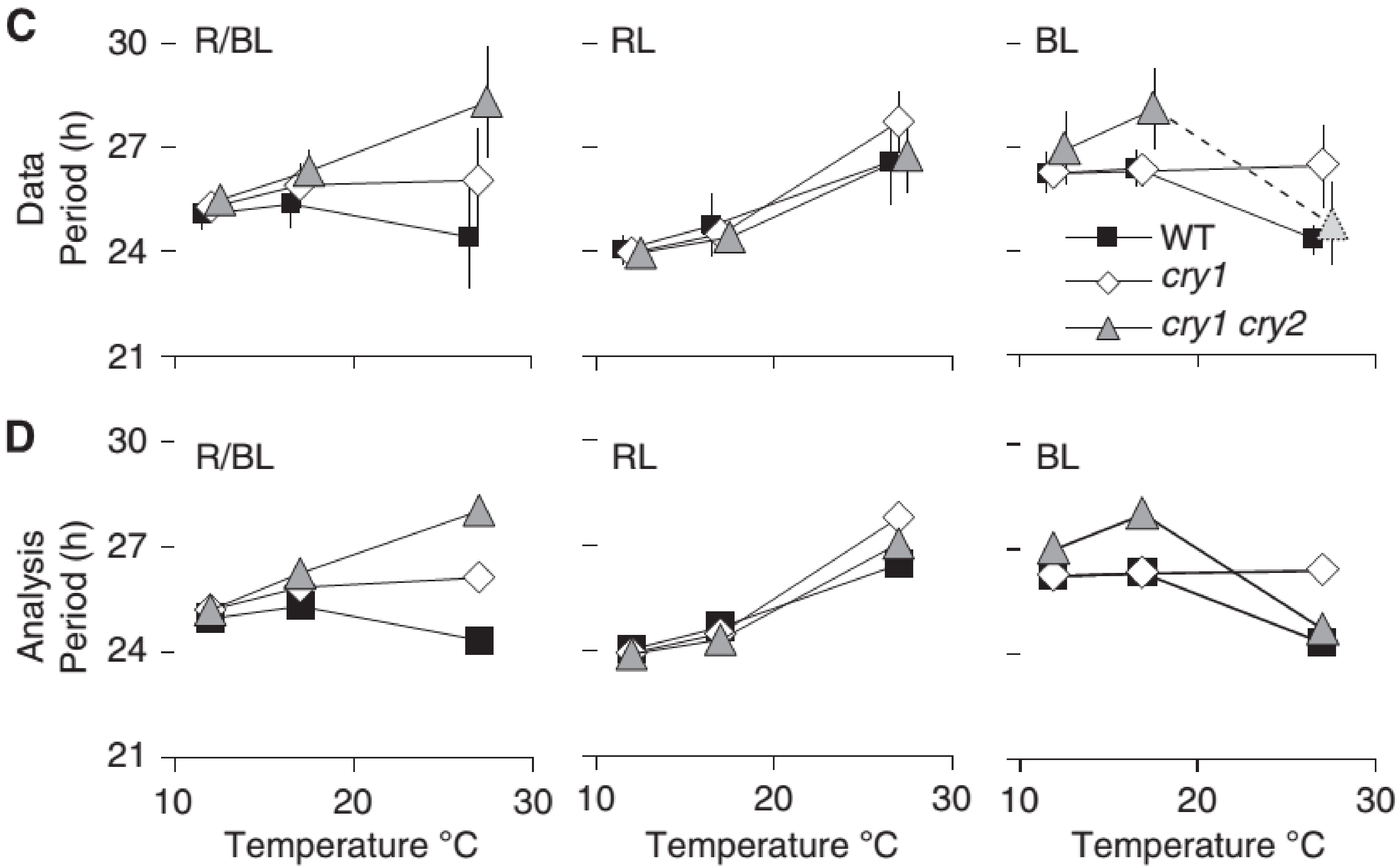
Result

- Temperature compensation requires blue light and cryptochromes




B
WT
cry1
cry1 cry2
R/BL RAE
RL RAE
BL RAE
1.0
0.5
0.0
15
25
35
Period (h)
Period (h)
Period (h)

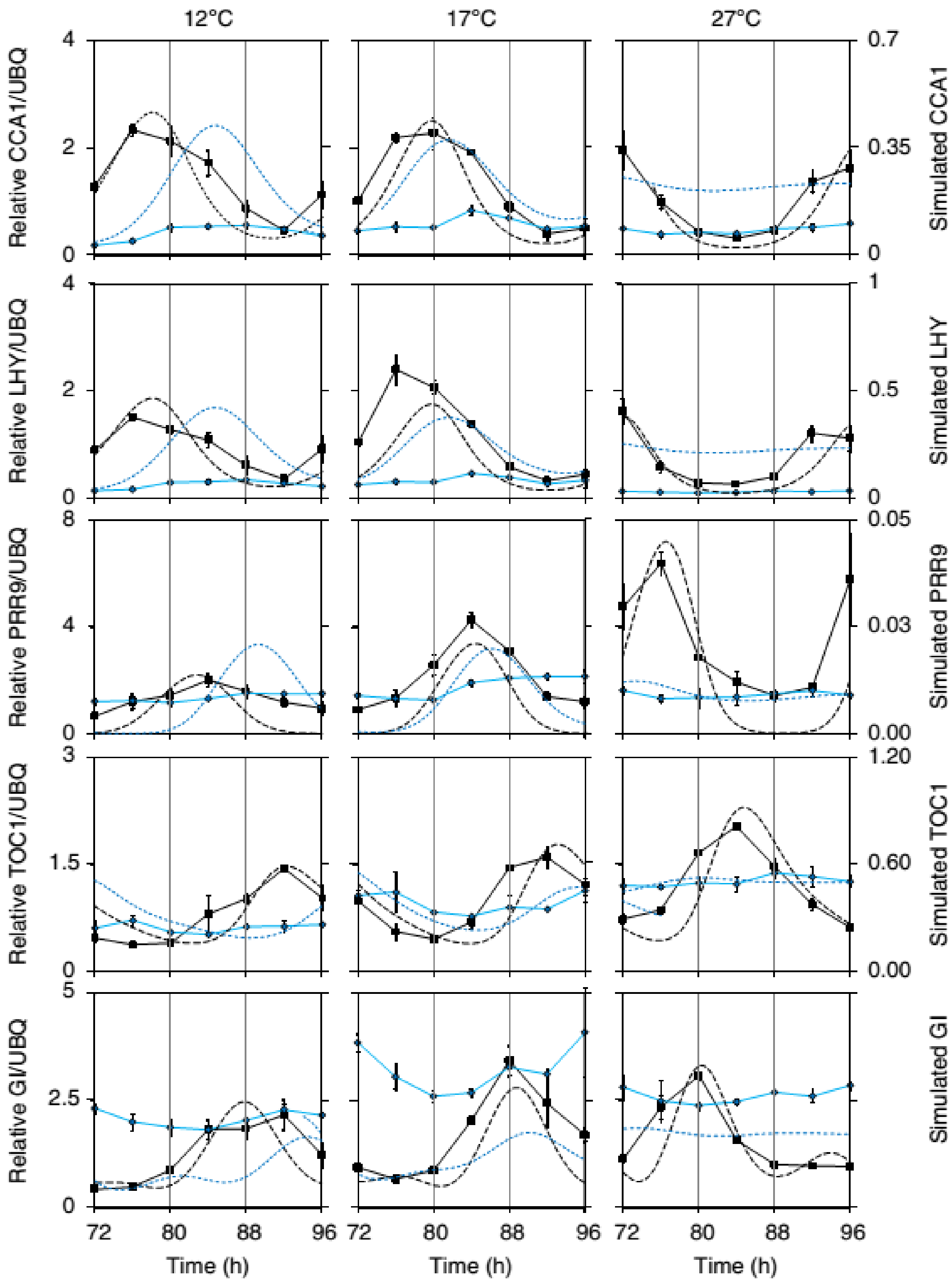
Result



C
D
R/BL
RL
BL
Data
Analysis
Period (h)
30
27
24
21
10
20
30
Temperature °C
WT
cry1
cry1 cry2

Result

- Temperature does not affect CRY abundance in Arabidopsis
- The expression of clock genes change little with temperature
- A temperature-compensated model of the Arabidopsis circadian oscillator



Temperature-specific effects of cry photoreceptors on the expression profiles of clock genes are matched in the temperature-dependent model.

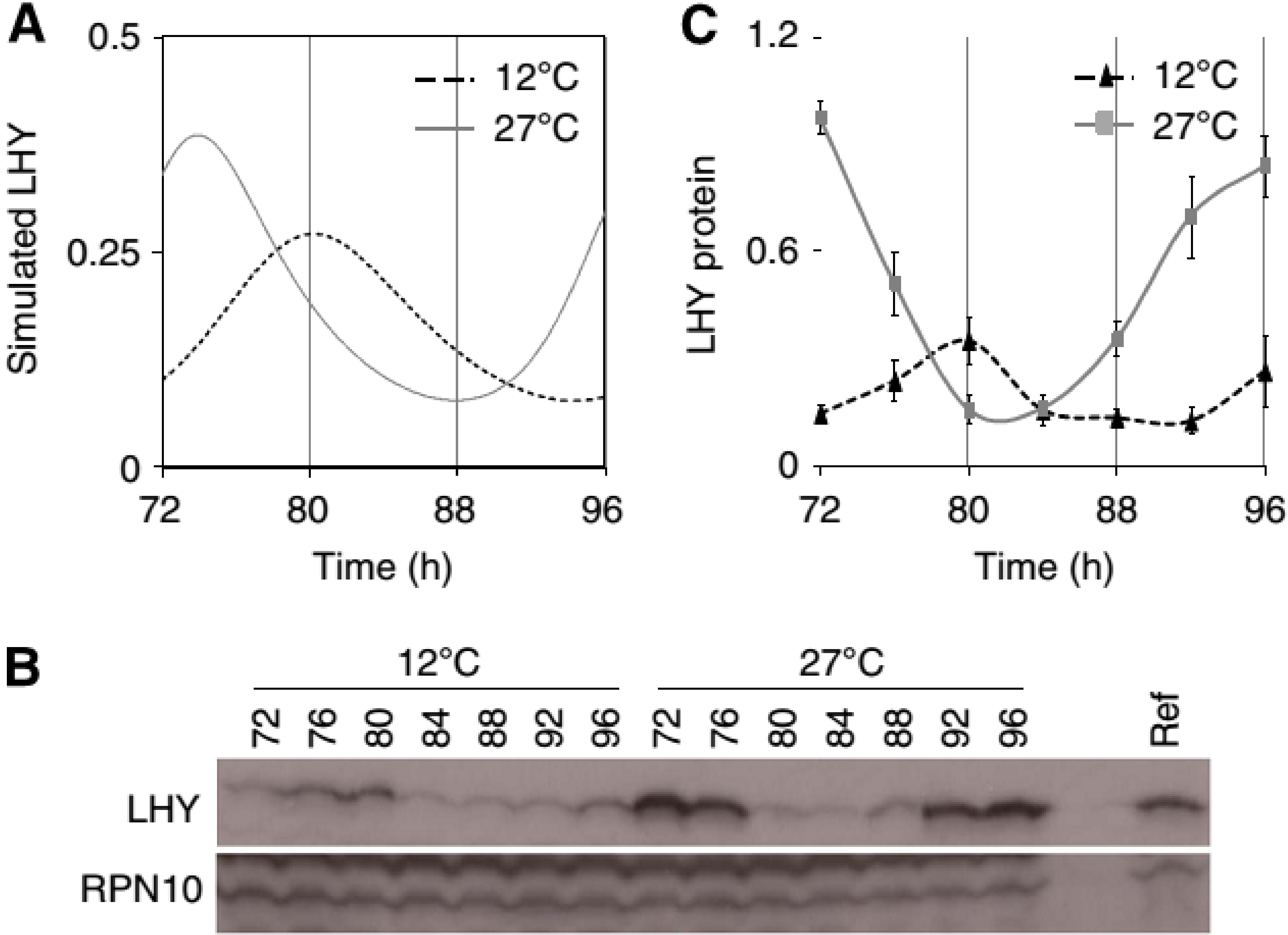
- Measured (solid lines, left axis)
- Simulated (dashed lines, right axis)

mRNA expression profiles of clock genes in

- WT (black lines)
- cry1 cry2 double-mutant plants (blue lines)

under constant BL.

Result



A
0.5
0.25
0
Simulated LHY
72
80
88
96
Time (h)
12°C
27°C
C
1.2
0.6
0
LHY protein
72
80
88
96
Time (h)
12°C
27°C
B
12°C
27°C
72
76
80
84
88
92
96
72
76
80
84
88
92
96
Ref
LHY
RPN10

Result

- Experimental testing of the temperature dependent clock model

- Using the model to test the temperature-dependent effects of the cry mutants

- Simulating the temperature-dependent effects of other clock mutants

Discussion

- Here, we adopted **a different systems-level approach** that produced a temperature-dependent model of the Arabidopsis clock.

- This validation provides evidence that temperature compensation in plants is based on the network-balancing model, rather than relying on unusually temperature-(in)sensitive components.

Discussion

- Our experimental results showed an unexpected interaction between light quality and temperature regulation. At warmer temperatures, we found that BL was **essential for maintenance** of period, and that WT plants had a 2 h longer period in RL than BL(Figure 1).

- There might be an evolutionary advantage in the WT plant's controlled change of period.

- Cryptochromes were absolutely required for robust oscillatory behaviour at 27℃.

Discussion

- **One surprising observation** was that clear circadian rhythms in the clock outputs CCR2 and CAB2 were observed at 12 and 17℃ under BL in the cry1 cry2 double mutant (Figure 1).

- The molecular rhythms of clock component RNAs were more strongly affected (Figure 2), though LHY and CCA1 retained rhythmicity with only slightly lower fold amplitude than in WT.

Discussion

- Our mathematical analysis showed that the **gene circuit** of the clock model could support the period control observed in WT plants.

- The more general hypothesis:
  - light-responsive processes were the most important temperature inputs. Added justification is the fact that almost all the genes previously implicated in the control of period by temperature (LHY, CCA1, GI, PRR9 and PRR7) are light-responsive at either the transcriptional or, for PRR7, the protein level.

- **However**, in suggesting this hypothesis, we are not ruling out that other components of the clock are temperature dependent, indeed we would expect this to be the case.

Discussion

- That LHY protein levels would increase with rising temperature. Remarkably, we confirmed this temperature-induced change (Figure 3).

- The increased LHY protein at 27℃ is presumably active, as it correlated with higher levels of its target gene, PRR9 (Figure 2).

- It does so without any unusual temperature-dependent steps, suggesting that the plant clock **is network-balanced**.

Discussion

- Increasing LHY levels are expected to lengthen period, However, rising temperature increased LHY levels in the plants (Figure 3), but shortened period (Figure 1).
- PRR9 over expression can shorten period but also reduces LHY and CCA1 mRNA levels in constant light. But, the fact is that LHY and CCA1 mRNA levels did not change with temperature (Figure 2) strongly, suggesting that another temperature-dependent process abrogated the effect of increased PRR9 expression at 27℃.
- Thus temperature effects on multiple processes were also required to explain the data, in line with the **network-balancing hypothesis**.

Thank you!