MPhil
Project Proposal

For Feng Geng at the Wigge Group, SLCU

7 May 2018

Key reference

- Ezer, D. *et al.* (2017) The evening complex coordinates environmental and endogenous signals in Arabidopsis. *Nat Plants* 3, 17087 [Link]

- Describes how the **evening complex (EC)** integrates temperature and light information in *Arabidopsis*.

nature plants

ARTICLES

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The evening complex coordinates environmental and endogenous signals in *Arabidopsis*

Daphne Ezer[1], Jae-Hoon Jung[1], Hui Lan[1], Surojit Biswas[1†], Laura Gregoire[2], Mathew S. Box[1], Varodom Charoensawan[1,3], Sandra Cortijo[1], Xuelei Lai[1,2], Dorothee Stöckle[1], Chloe Zubieta[2], Katja E. Jaeger[1] and Philip A. Wigge[1*]

Plants maximize their fitness by adjusting their growth and development in response to signals such as light and temperature. The circadian clock provides a mechanism for plants to anticipate events such as sunrise and adjust their transcriptional programmes. However, the underlying mechanisms by which plants coordinate environmental signals with endogenous pathways are not fully understood. Using RNA-sequencing and chromatin immunoprecipitation sequencing experiments, we show that the evening complex (EC) of the circadian clock plays a major role in directly coordinating the expression of hundreds of key regulators of photosynthesis, the circadian clock, phytohormone signalling, growth and response to the environment. We find that the ability of the EC to bind targets genome-wide depends on temperature. In addition, co-occurrence of phytochrome B (phyB) at multiple sites where the EC is bound provides a mechanism for integrating environmental information. Hence, our results show that the EC plays a central role in coordinating endogenous and environmental signals in *Arabidopsis*.

Plants are sensitive to their environment, and the distribution and phenology of plants has already altered in response to climate change[1,2]. Such growth and developmental changes require the integration of multiple environmental signals, such as light and temperature, into endogenous gene expression programmes. The circadian clock plays a key role in this process by enabling plants to anticipate future events such as sunrise and darkness as well as gating responses to environmental information according to time of day[3–5]. The expression levels of many circadian clock genes vary in response to changes in the environment[6], but the underlying mechanisms by which these signals are integrated are not known. The circadian clock in *Arabidopsis* contains multiple interlocking loops with transcriptional and post-translational regulation. Three circadian clock genes, *EARLY FLOWERING3, 4 (ELF3 and 4)* and the MYB transcription factor *LUX ARRYTHMO (LUX)* together comprise the evening complex (EC)[5,7], and are expressed at the end of the day. The EC coordinates elongation growth in *Arabidopsis* seedlings, as it directly represses the expression of the bHLH transcription factor *PHYTOCHROME INTERACTING FACTOR 4 (PIF4)* (refs 7–9). *PIF4* is necessary for warm temperature-mediated elongation growth[10], and *pif4* and *pif4,5* mutants also display a reduced induction of flowering in response to warm temperature[11–14]. Natural variation in the activity of the EC is responsible for differences in thermal responsiveness and warmer temperatures reduce the binding of the EC to target promoters, resulting in increased *PIF4* expression[15,16].

Of the core EC components, only LUX is recognisable as a

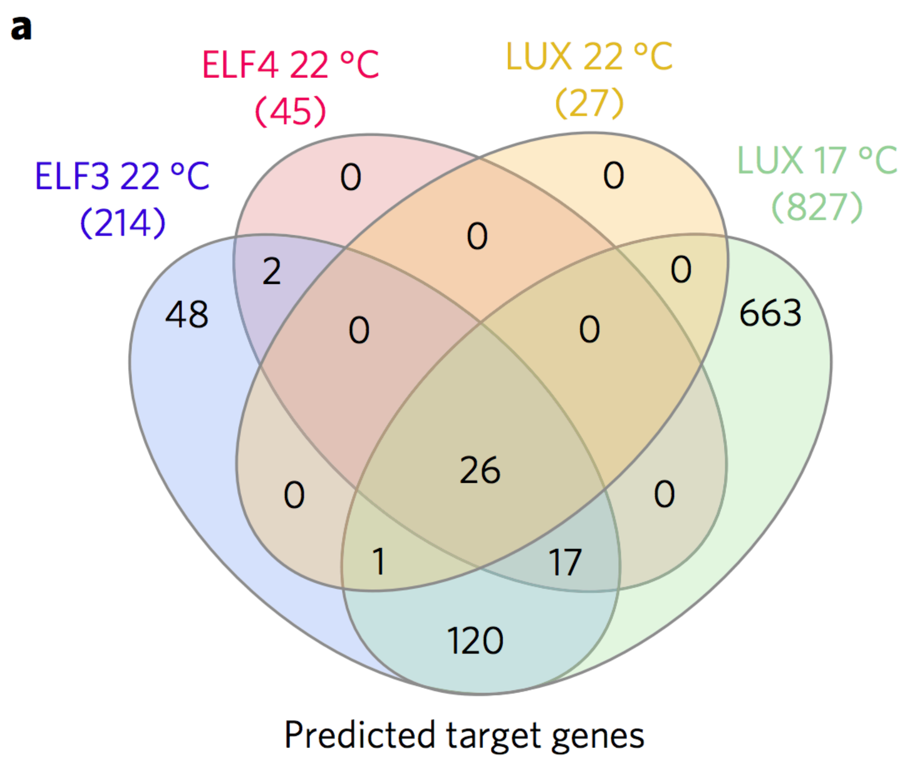
Although the role of the EC has been studied at specific circadian loci, the system-wide impact of the EC is not known, since its global binding pattern and regulatory effects are not yet characterized. In addition, the mechanisms by which the EC is able to provide environmental responsiveness to target loci and integrate environmental signals into the circadian clock are poorly understood. In this study, we demonstrate that the EC regulates key nodes controlling photosynthesis, the circadian clock, growth, phytohormones and temperature signals. We show that the EC directly integrates temperature information and we describe a mechanism by which the cobinding of phytochrome B (phyB) with the EC to target loci enables environmental signals to be directly integrated into the circadian clock.

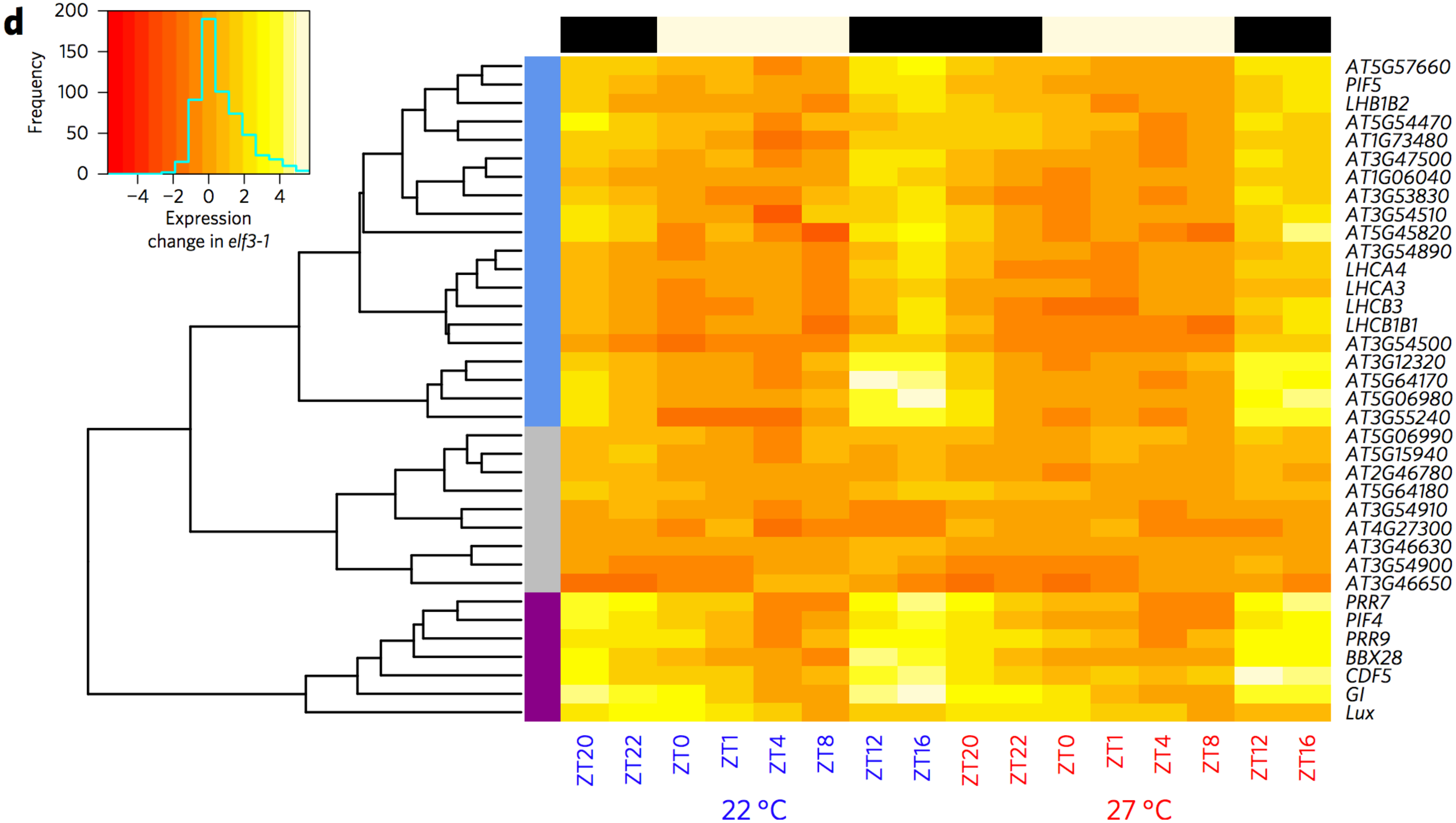
Results

G-box (CACGTG) motifs are highly enriched at EC binding sites. To understand how the EC may control plant responses to the environment, we sought to determine its binding genome-wide. Although the LBS is found at more than 10,000 sites in the *Arabidopsis* genome, typically only a subset of the theoretical transcription factor binding sites are occupied *in vivo*. It is also not known to what extent the target sites of the separate EC proteins overlap genome-wide. To resolve these questions we mapped the LUX, ELF3 and ELF4 target sites *in vivo* using ChIP coupled to sequencing (ChIP-seq) using epitope tagged versions of these genes expressed under their own promoters (see Supplementary Fig. 1 for westerns, Supplementary Fig. 2 for complementation and Supplementary Table 1 for sequencing read counts).

Evening Complex in *Arabidopsis*

- Consists of circadian clock genes: **ELF3, ELF4** and **LUX**
- **Temperature** sensitive binding at genes important for light and temperature response, as well as circadian growth (PIF5, LUX).
- EC binding cooccurs with **phytochrome B** — integrate **light** information





d
200
150
100
50
0
Frequency
−4
−2
0
2
4
Expression change in *elf3-1*
AT5G57660
PIF5
LHB1B2
AT5G54470
AT1G73480
AT3G47500
AT1G06040
AT3G53830
AT3G54510
AT5G45820
AT3G54890
LHCA4
LHCA3
LHCB3
LHCB1B1
AT3G54500
AT3G12320
AT5G64170
AT5G06980
AT3G55240
AT5G06990
AT5G15940
AT2G46780
AT5G64180
AT3G54910
AT4G27300
AT3G46630
AT3G54900
AT3G46650
PRR7
PIF4
PRR9
BBX28
CDF5
GI
Lux
ZT20
ZT22
ZT0
ZT1
ZT4
ZT8
ZT12
ZT16
ZT20
ZT22
ZT0
ZT1
ZT4
ZT8
ZT12
ZT16
22 °C
27 °C

“Can we decipher/decode the equivalent temperature + light sensing mechanism in grass plants?”

Brachypodium distachyon

- **Model** system for genomic studies in **grasses**.
- Related to cereal grain species (**wheat**, **barley**)
- Small diploid genome (~355Mbp)
- Short life cycle and small physical size
- **Long day** plant

What we think/know

- Elements of the EC pathway is conserved, notably **ELF3**.
- ELF3 is a **repressor** of flowering activity, and is degraded rapidly in the light.
- ELF3 levels is modulated by phytochrome **PHYC**, which can accumulate to high levels in long days.
- Loss-of-function *phyC* mutants flower **late**. Same with *ELF3-OX* plants.
- Loss-of-function *elf3-1* mutants flower **early**.

Our Data: RNA-Seq

2 weeks old:
eight timepoints (ZT0, 1, 4, 8, 12, 16, 20, 22)

- WT (Bd21-3), long day: 150R
- WT (Bd21-3), short day: 148R
- *elf3-1*, short day: 149R

3 weeks old:
six timepoints (ZT0, 4, 8, 12, 16, 20)

- WT (Bd21-3) and *elf3-1*, <u>short</u> day: 144R

4 weeks old — set 1:
Three timepoints (ZT0, ZT8, ZT16)

- WT (Bd21-3) and *phyC*, <u>long</u> day: 143R

4 weeks old — set 2:
Three timepoints (ZT0, ZT4, ZT12, ZT20)

- WT (Bd21-3) and *ELF3-OX*, <u>long</u> day: 169R

Our Data: ChIP-Seq

ELF3-OX

- Already available: short-day (**SD**) <u>ZT4</u>; long-day (**LD**) <u>ZT0</u> and continuous light (**LL**): <u>ZT0</u>

- ChIP-Seq will be repeated for **SD, LD, LL** — <u>ZT16, ZT0, ZT4</u> (9 samples in total). To be sequenced this month, should be ready by early June.

- Plans for ChIP-Seq for PHYC and PPD1: plants need two months to grow. The project will not involve these by default.

The aim of the project

Identify a set of target genes for the “evening complex” in *Brachypodium*

- This should be the overlap between genes whose expression are misregulated in mutants AND exhibit differential binding of ELF3.
- Which **timepoints** are interesting for comparison?
- Which combination of **mutants** and **photoperiods**?

Project overview
~10 weeks

1. **Process and map next-gen reads**
    - RNA-Seq: HISAT + StringTie
    - ChIP-Seq: BowTie2
2. **Evaluation of sample quality**
    - PCA and correlation plots to check comparability

3. **Differential expression**
    - DeSeq or edgeR packages
    - Which timepoints/mutants/photoperiods?
4. **Derive differentially bound genes**
    - Peak calling with MACS2
    - Motif enrichment of the peaks (HOMER)
5. **Compute overlap between gene sets**
    - Gene Ontology and pathway enrichment (Reactome)

6. **Clustering analysis**
    - Hierarchical clustering versus CLUST algorithm
7. **Possible further analyses**
    - Simple linear models
    - Integrate with chromatin accessibility data (DNase I hypersensitivity sites/ATAC-Seq)

Useful references

Recommended (especially the first one!)

- Woods, D.P. *et al.* (2014) PHYTOCHROME C is an essential light receptor for photoperiodic flowering in the temperate grass, Brachypodium distachyon. *Genetics* 198, 397–408 Link

- Huang, H. and Nusinow, D.A. (2016) Into the Evening: Complex Interactions in the Arabidopsis Circadian Clock. *Trends Genet.* 32, 674–686 Link

Worth a look (read the introduction/discussion)

- Yang, Y. *et al.* (2013) OsELF3 is involved in circadian clock regulation for promoting flowering under long-day conditions in rice. *Mol. Plant* 6, 202–215 Link

- Higgins, J.A. *et al.* (2010) Comparative genomics of flowering time pathways using Brachypodium distachyon as a model for the temperate grasses. *PLoS One* 5, e10065 Link

- Abu-Jamous, B. and Kelly, S. 13-Feb-(2018) , Clust: automatic extraction of optimal co-expressed gene clusters from gene expression data. , *bioRxiv*, 221309 Link

Some terminologies

In preparation, it is worth reading up what these terms mean on Wikipedia/Google /YouTube:

- *Arabidopsis* is a **dicot**; *Brachypodium* is a **monocot**.

- What is a **long-day** and **short-day** plant? *Arabidopsis* and *Brachypodium* are both long day plants.

- **Zeitgeber Time (ZT):** what does it mean experimentally?

- Genes to know by heart: **ELF3**, **PHYC, FT**, **PPD1** (PRR37 in Arabidopsis), CONSTANS, LUX, GI