Interactions between the circadian clock and temperature responses in *Arabidopsis thaliana*

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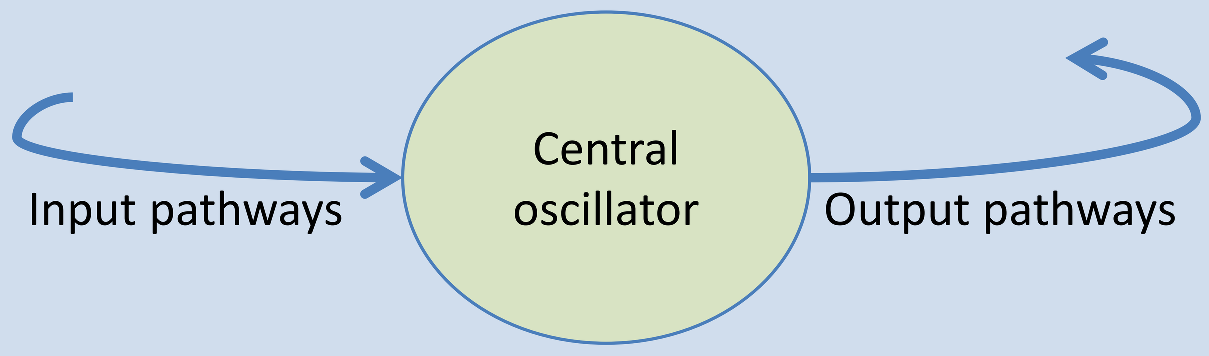
Arabidopsis thaliana
A good model plant, due to its small size, short life cycle and prolific seed production

Circadian rhythms and the circadian clock

Organisms have evolved to adapt to the 24-hour rotation of the earth around its axis: they do not just respond, but anticipate periodic environmental stimuli, such as light, and adjust their biology accordingly.
Circadian rhythms are defined by the following three properties:

- they are **endogenous** and they persist with a period of roughly 24h in the absence of external stimuli
- they are **entrainable** by light and temperature cycles, allowing organisms to adapt to local time
- they exhibit **temperature compensation,** meaning that they persist with roughly the same period over a wide range of physiological temperatures

Examples: sleep/wake cycles in humans and photosynthetic activity in plants



Graphical representation of the circadian clock

What drives circadian rhythms?

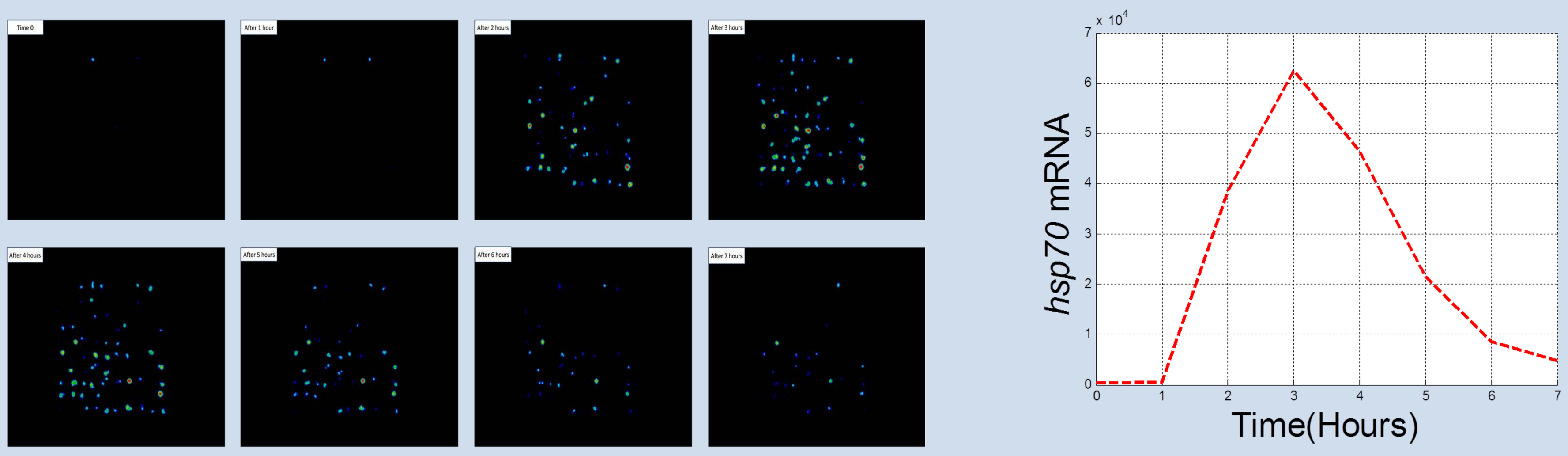
A biochemical oscillator, known as the **circadian clock**. It consists of:

- a **core oscillator**, which is a network of interacting genes influencing each other's expression
- **input pathways**, which serve for the entrainment of the clock
- **output pathways**, which control circadian rhythms throughout the organism at different phases of the clock

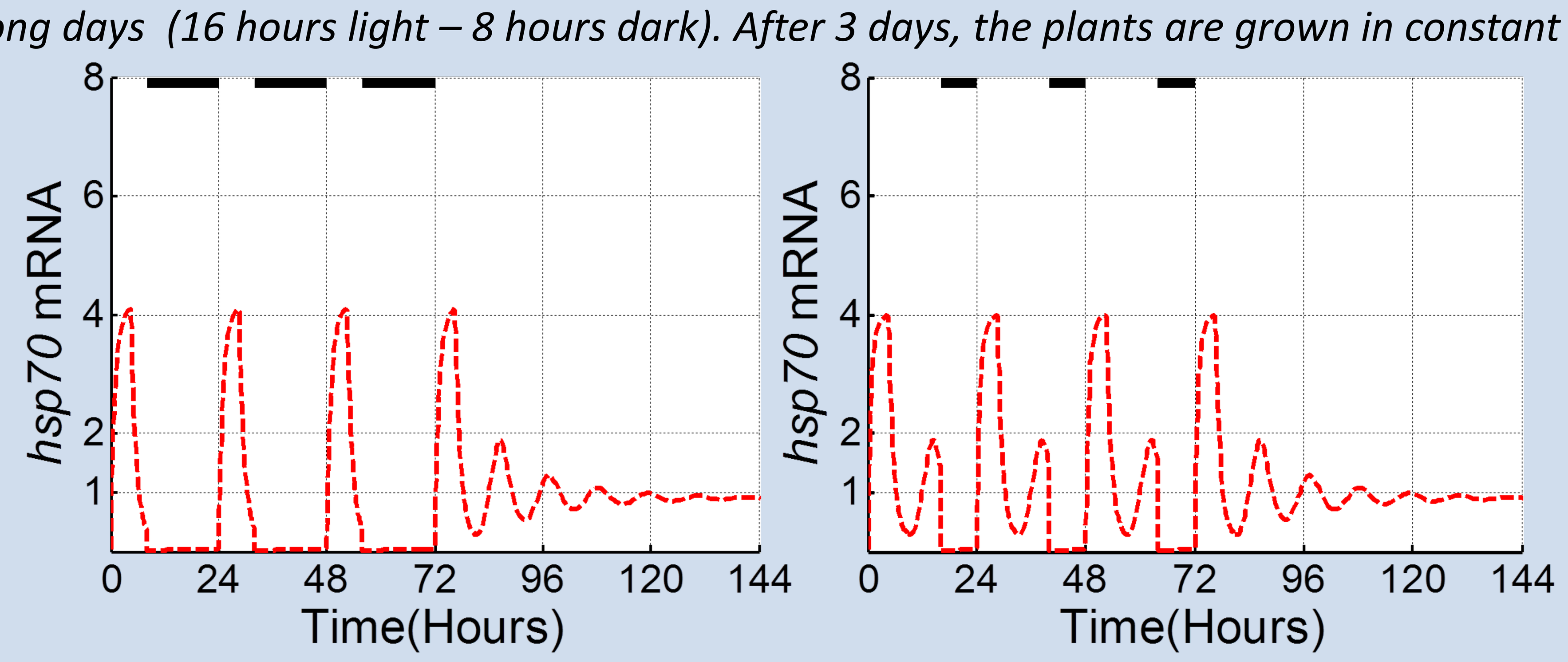
Experiment and modelling approach

In order to study gene expression dynamics, we used **luciferase reporter assays**:
Luciferase is an enzyme which exists in many organisms (we used firefly luciferase). In the presence of its substrate, **luciferin**, it catalyses a chemical reaction during which light is emitted. When this happens *in vivo*, the phenomenon is known as **bioluminescence**.
To this end:

- plants are genetically engineered, so that the promoter of the gene of interest followed by the luciferase gene are incorporated in their genome, a process known as **transformation**
- the promoter of a gene controls its transcription, and hence its expression, so the above construct ensures that the luciferase gene is going to be expressed with the same pattern as the gene whose expression we want to study
- the plants are sprayed with luciferin
- the bioluminescence is measured with a luminometer at regular time intervals



Above: Bioluminescence measured over a 7-hour interval, starting from subjective dawn, and the corresponding graph of bioluminescence against time
Below: Model predictions for HSP70 expression in short days (8 hours light – 16 hours dark) and in long days (16 hours light – 8 hours dark). After 3 days, the plants are grown in constant light.



Why study this?

Temperature compensation is a very important property. Intuitively, the circadian clock is the organism's way of keeping track of time, hence it should clearly not depend on the ambient temperature. In addition, temperature cycles can reset the clock.
Despite its importance, the way temperature signals are integrated in the circadian clock is poorly understood (in contrast to light signals).

Our link between the circadian clock and temperature is a gene called HSP70 (HSP stands for " Heat Shock Protein"):

- it exhibits a (possibly clock driven) diurnal rhythm
- it responds strongly to temperature steps

Directions for future research

- Investigate further the molecular basis of temperature compensation and temperature sensing in *Arabidopsis*
- Take an integrated approach to understand how light and temperature together modulate responses in *Arabidopsis*, either directly or through the circadian clock
- Extend this study using real environmental data
- Investigate what differentiates responses in plants in spring and in autumn, despite roughly the same photoperiods and temperature ranges