Application Note

USING THE NIGHTSHADE IN VIVO PLANT IMAGING SYSTEM TO STUDY THE CIRCADIAN CLOCK

Abstract

The circadian clock regulates many different biological functions in most eukaryotes and some prokaryotes. The study of these functions requires tools that can dynamically assess gene expression over long periods of time and in a controlled environment. This application note describes how the NightSHADE In Vivo Plant Imaging System was used to monitor the activity of the CCA1 and LHCB promoters and hypocotyl elongation in controlled lighting conditions.

Introduction

The alternance between day and night is of great importance for most living beings because many factors that are important for life can change dramatically between day and night: temperature, availability of food, presence of predators, and others. Responding to the differences between day and night requires many physiological, biochemical, and developmental processes to be synchronized with light and darkness cycles. The circadian clock is an endogenous 24-h timer that is found in most eukaryotes and even some prokaryotes (photosynthetic bacteria). It is responsible for synchronising biological processes with the day/night cycle [1]. In plants, many aspects of growth and development are regulated by the circadian clock and light signalling.

Studying the influence of the circadian clock on the regulation of gene expression requires tools that allow to assess gene expression dynamically over long periods of time (at least several days) in a controlled growth environment and in a non-destructive way. This is possible with the combination of reporter constructs with firefly luciferase (LUC) under the control of promoters of interest and sensitive imaging systems, such as the NightSHADE *In Vivo* Plant Imaging System.

In addition to reporter genes, the measurement of growth rate of the seedlings and the hypocotyl [2, 3] is another approach to investigate the circadian clock that can be performed using in vivo imaging.

In this application note we show two examples of the use of in vivo imaging for the study of the circadian clock: monitoring of the expression of genes involved in circadian rhythms for several days, and the measurement of hypocotyl lenghts.

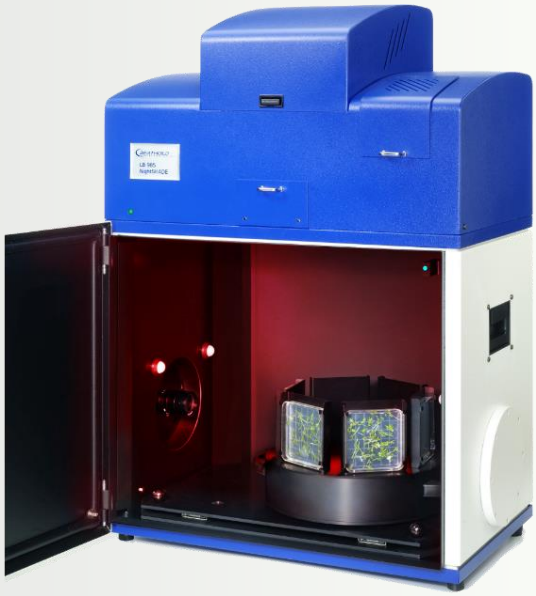
Rainer Kembügler, Charles Schmidt, Bernd Hutter & Francesc Felipe

Berthold Technologies – www.berthold.com

The Berthold Technologies NightSHADE evo LB 985N In Vivo Plant Imaging System

The NightSHADE evo LB 985N In vivo Plant Imaging System is a modular, easy to use optical imaging system dedicated to in vivo analysis of plants. Equipped with an absolutely light-tight cabinet and a cooled CCD camera it enables sensitive luminescence and fluorescence monitoring in tissues, seedlings, and whole plants.

The camera can be attached either to the ceiling or the side walls of the dark room – the sample chamber – to facilitate imaging from above and from the side. The latter position of the camera enables processing of multiple seedlings in parallel while growing plants vertically oriented to enable observation of the complete plant. Furthermore, key environmental conditions like temperature or humidity as well as daylight can be simulated to provide a controlled growth environment.



Materials and Methods

- NightSHADE evo In Vivo Plant Imaging System, from Berthold Technologies.
- Transgenic *A. thaliana* lines (prCCA1::LUC, CS9382; prCAB::LUC, CS9381; Col-0, CS28168), from TAIR stock center.
- IndiGO™ software, from Berthold Technologies, for image acquisition and quantification of bioluminescence.
- ImageJ software, from NIH, to measure hypocotyl lengths.

After stratification, seeds were germinated [2] and grown on plates containing MS medium.

Seedlings were entrained under short day conditions (8 h light/16 h dark) for 7 days (bioluminescent reporter experiment) or 3 days (hypocotyl elongation experiment). Light was provided by cool white fluorescents, 50 µmol m^{-2} s^{-1}.

For the bioluminescent reporter experiment, seedlings were sprayed with luciferin and imaged in Luminescence mode every hour for 3 days under dim constant light using the CCD camera of the NightSHADE.

For the hypocotyl elongation experiment, seedlings were imaged in Photo mode using the CCD camera of the NightSHADE in the side position (Fig. 1), every 4 hours. The indiGO™ software was used to control the CCD camera, the rotor, and light/dark conditions. Images were imported in the ImageJ software to measure hypocotyl lengths.

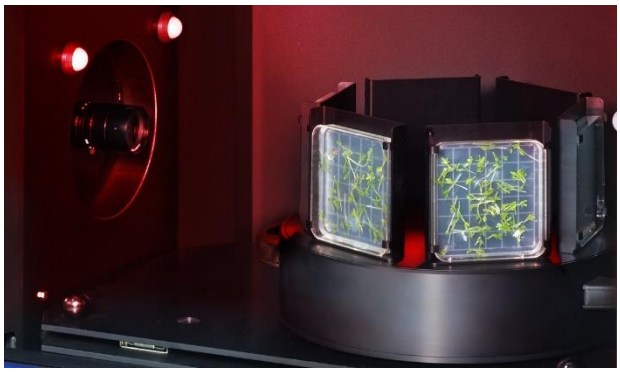


***Figure 1**: Rotating plattform inside the NightSHADE holding square plates to be imaged using the side position of the CCD camera.*

Results and discussion

The signal corresponding to the activation of the promoter of CCA1 (Circadian Clock Associated 1) started at a very high level in the first data point available (2 h after starting the first light period) but declined quickly (Fig. 2). Several peaks were visible around t = 11 h, t = 23 h, t = 26 h, t = 50 h, and t = 72 h, and valleys around t = 20 h, t = 24 h, t = 42 h and t = 67 h.

Similarly, the signal of CAB (chlorophyll a/b-binding protein, also known as LHCB, light-harvesting chlorophyll a/b-binding protein) showed peaks around t = 11 h, t = 22 h, t = 30 h, t = 59 h and t = 72 h, and valleys around t = 19 h, t = 24 h, t = 45 h and t = 67 h. The amplitude of the changes was maximal in the first 24 h cycle and smaller in the following cycles.

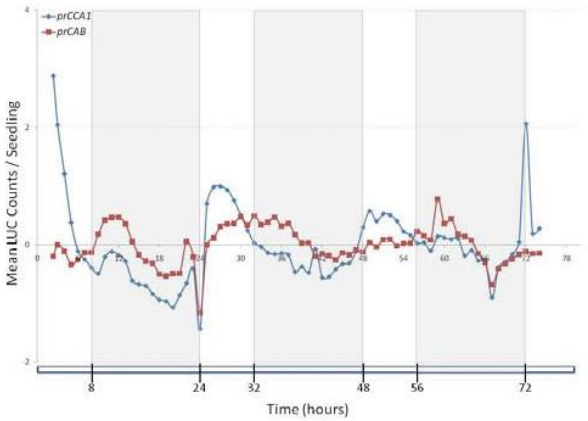


***Figure 2**: Bioluminescent signal corresponding to the activation of the promoter of CCA1 (blue) and CAB (red). Seedlings (prCCA1::LUC & prCAB::LUC) were entrained under 8-h-light (cool white fluorescents, 50 µmol m-2 s-1) / 16-h dark photoperiods for 7 days, sprayed with luciferin and imaged for bioluminescence every hour for 3 days under dim constant light in the NightSHADE.*

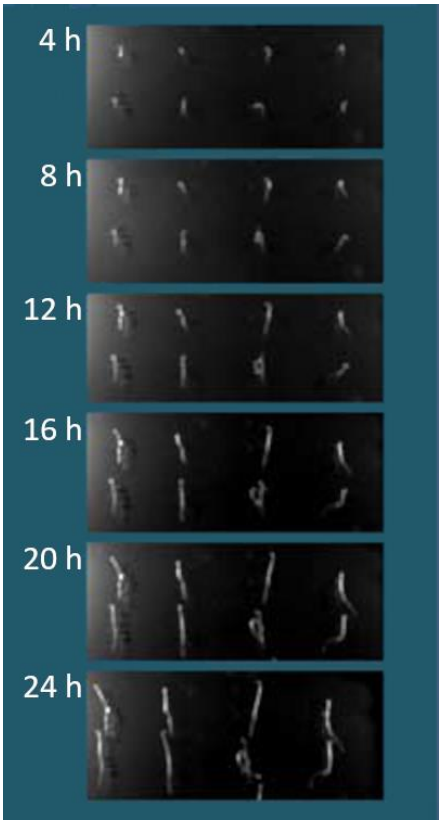


***Figure 3**: A. thaliana (Col-0) seedlings were grown as described and imaged in the NightSHADE every 4 hours. Only the top-half of the plate is shown in each image.*

CCA1 is involved in the central feedback loop in Arabidopsis and is thought to act around dawn to activate CAB gene expression [4]. Thus, the coincidence in time of most peaks and valleys in the signal of CCA1 and CAB is consistent with the activation of CAB by CCA1. In this experiment it is not always possible to see the change in CCA1 signal preceding that of CAB. However, it should be noted that in this experiment both signals are not measured in the same seedlings, but each line expresses a different construct. This could explain timing of the expression being not exactly as expected.

Elongation of the hypocotyl after 24 h was evident (Fig. 3) and easy to measure using ImageJ on the images acquired with indiGO™ (data not shown).

Conclusions

The NightSHADE evo in vivo plant imaging system offers various tools for the investigation of the circadian clock, which are presented in this application note: first, the quantification of the signal of bioluminescent reporter genes in seedlings of different lines of *A. thaliana*. Second, the measurement of hypocotyl length in seedlings using the lateral camera position combined with the ImageJ software and third, the control of lighting conditions during the whole experiment (several days). The combination of all three features constitutes a very powerful toolbox for the study of the expression of genes under the control of the circadian clock or the influence of photoperiod or light quality and quantity.

References

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Berthold Technologies GmbH & Co. KG
Calmbacher Straße 22
75323 Bad Wildbad
GERMANY
Phone: +49 7081 177 0
Email: bio@berthold.com

www.berthold.com