# Discovery of clock genes in plants that regulate the circadian rhythm

March 23 2016

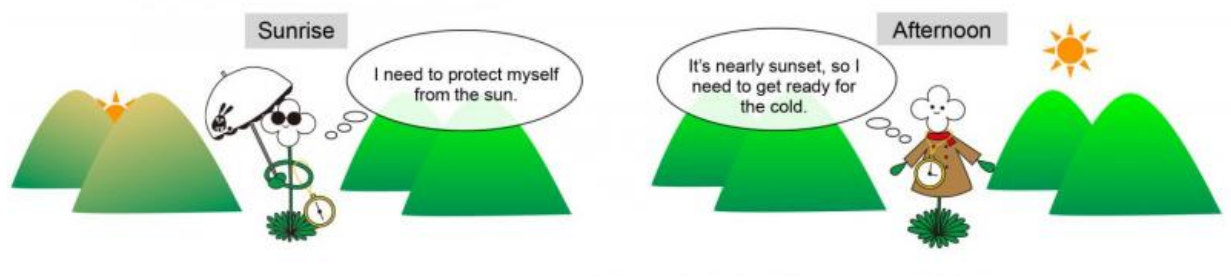


Figure 1. Plants use their circadian clock to predict time-based changes in the environment.

Plants prepare for cold evenings by triggering biological processes, such as closing of their stomata and synthesizing wax to prevent water loss. Biologists have shown that these processes, which are induced by genes expressed in the evening, are actually regulated by clock proteins that are produced during sunrise. Further understanding of these clock-mediated processes could promote growth of plants in climates that are different from their origin.

In order to adapt to the alternation between day and night caused by the rotation of the earth, many organisms possess a circadian clock (biological clock) that is regulated by their genes. Nevertheless, the interplay of genes that are related to the plant's circadian clock is still not

fully understood.

In a new study, published online in the journal *Plant Cell*, a team of biologists led by Norihito Nakamichi at the Institute of Transformative Bio-Molecules (WPI-ITbM) in Nagoya University, has uncovered that the clock genes produced during the evening are regulated by clock proteins produced in the morning.

The team has also discovered that these evening clock genes are responsible for plants to carry out biological processes to respond to the environment during the evening. In order to prepare for the cold temperature during the evening, plants prepare responses to drought stress, transmit signals from plant hormones, regulate the opening and closing of their stomata, and produce wax to prevent loss of water.

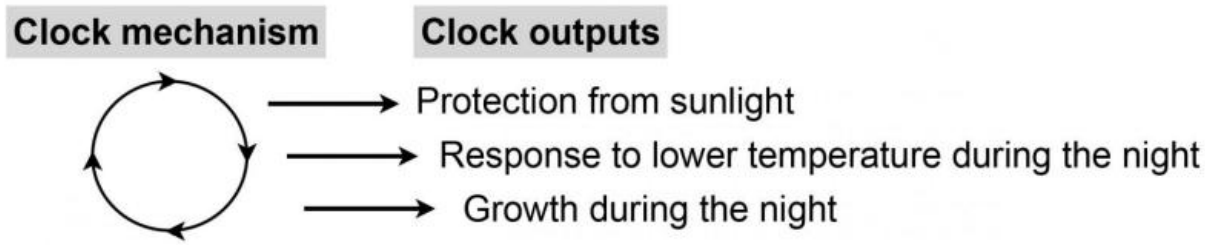


Figure 2. Various biological processes are controlled by the plant's circadian clock.

Many of the crops that we see nowadays are selected species with optimized properties that are regulated by their circadian clock. For example, in Japan, early flowering wheat cultivars are grown so that it can be harvested before the rainy season arrives. Early flowering cultivars have been generated as a result of modulation of circadian

clock-associated genes. Nakamichi and his colleagues' discovery on a set of key clock genes for drought resistance in plants may make it possible to generate optimized plant species to grow in specified environments through modification of the circadian clock.

The circadian clock in many organisms consists of an approximately 24-hour cycle. In plants, they use their circadian clock so that the appropriate biological processes occur at the right time of the day. For example, sudden daylight will generate reactive oxygen species that are toxic to plants, so the plants start to synthesize molecules that will remove the reactive oxygen species before sunrise.

During the afternoon, plants make themselves ready to deal with the cold temperatures that will follow sunset. In this manner, plants use their biological clock to respond in beforehand to the changes in their surrounding environment that are caused by variation in time.

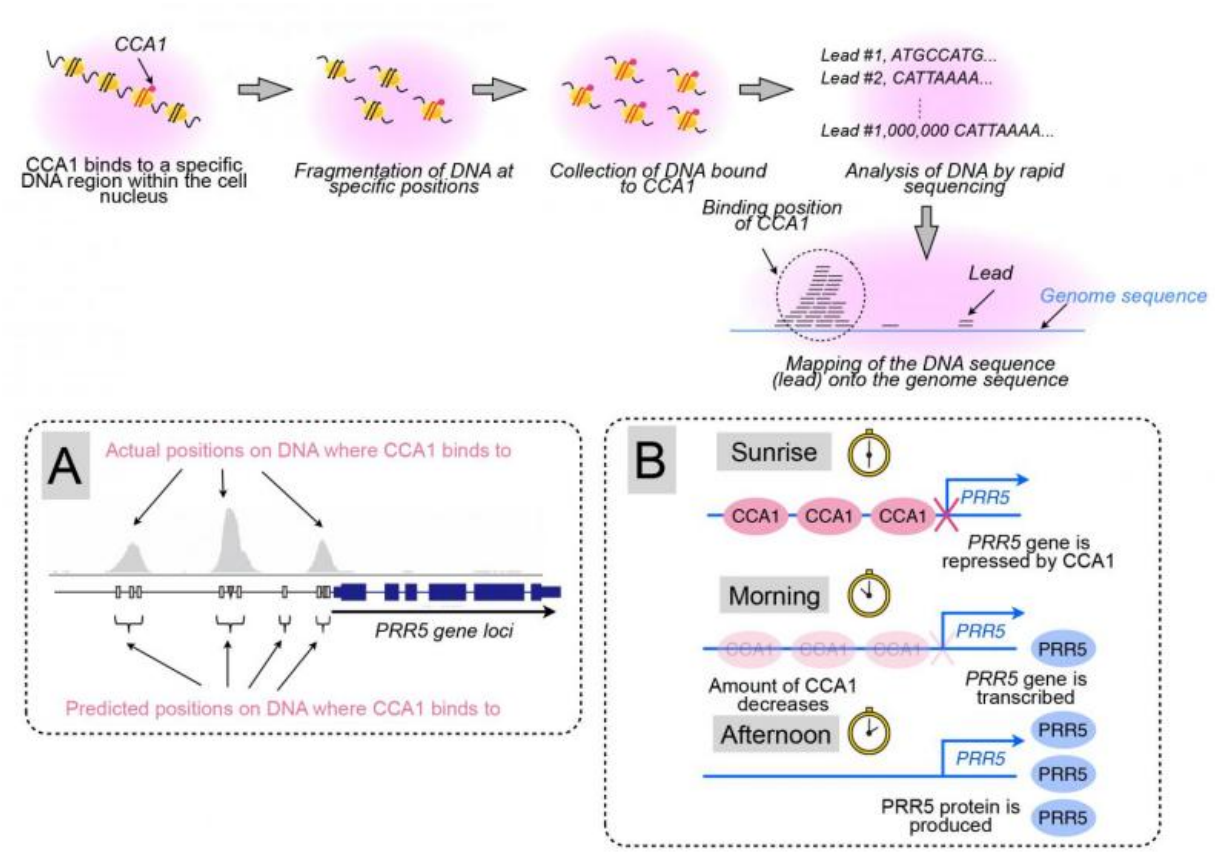


Figure 3. CCA1 clock protein binds near the PRR5 gene within the cell.

The circadian clock is considered to consist of mutual regulation between multiple genes, but this complicated network of genes and molecules has not been fully clarified. Norihito Nakamichi, an associate professor at ITbM and a leader of this study who has been carrying out plant circadian clock since 2004, decided to look into how various biological events are regulated by the clock. "The reason why I am interested in studying the biological clock is because I really like the logic behind how various biological processes are brought about by the plant's complex clock network," speaks Nakamichi.

"Since 2011, we have been trying to find the key factor that regulates the

expression of the gene that is transcribed during the afternoon," says Nakamichi. The group used PSEUDO-RESPONSE REGULATOR 5 (PRR5), which is a clock gene of the model plant, Arabidopsis thaliana.

"We first hypothesized that CCA1, which is a clock protein that is generated during sunrise, binds to a specific DNA sequence that is involved in the expression of the target gene PRR5," describes Nakamichi. "We collected the CCA1 protein bound to DNA by a technique called Chromatin immunoprecipitation (ChIP), and analyzed the DNA sequence by rapid DNA sequencing." Although rapid DNA sequencing is a well-known technique, comprehensive analysis of the DNA sequences can be a rather complicated process.

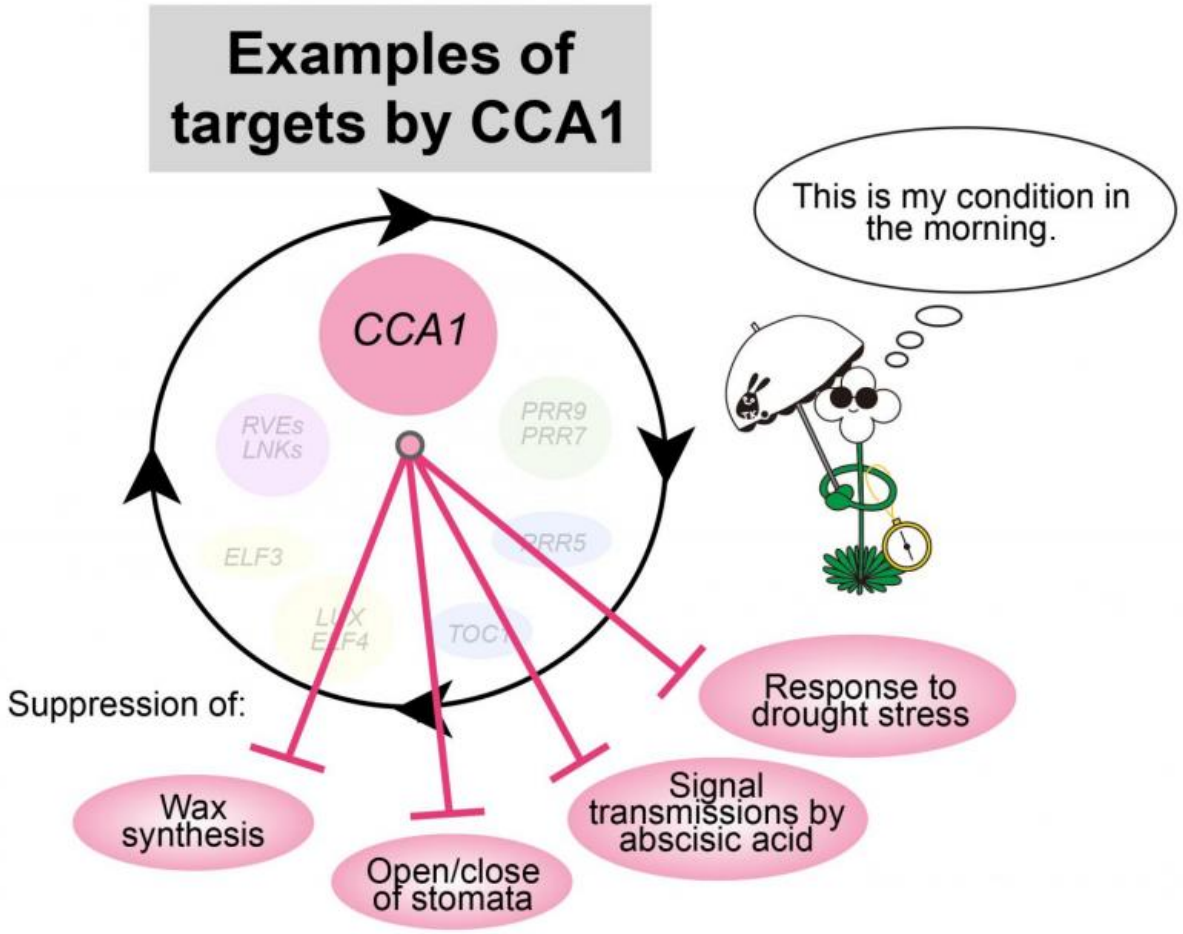

Figure 4. Elucidating the target gene of the CCA1 protein.

"We actually had to go back and forth many times until we were able to identify that the PRR5 gene appears at the regulatory region at a high frequency," continues Nakamichi. "I was extremely excited when I saw the data suggesting that the CCA1 protein directly acts towards the regulatory region of the PRR5 gene and has a major effect on it."

In addition, the group found in the plant cell's chromosome, the target DNA region of the CCA1 clock protein. "We found many genes that are expressed in the evening nearby the DNA region that CCA1 binds to," explains Nakamichi. Some of these genes are responsible for the plant's responses to drought stress, transmission of the signals from the plant hormone, abscisic acid, regulation of the opening and closing of stomata, and production of wax. "The results of our studies suggests that the CCA1 protein induces these biological processes to occur at a specific time during the evening."

"Plants have a sophisticated clock system where the appropriate biological processes occur at the correct time of the day. If the CCA1 protein did not function in the morning, the plant will start preparing for the cold and prevent water loss during the day," says Nakamichi. "By being able to switch on and off the function of the clock proteins at the correct time, this may facilitate growth of plants that are adaptable in different climates," he continues.

"The genes related to the circadian clock are common in many plants. Many of the crops that have been selected so far are actually ones that had sudden changes, either naturally or artificially, in the genes that

control their circadian clock," says Nakamichi. "We believe that our work has contributed to further understanding of the molecular mechanism of the circadian clock in Arabidopsis thaliana, and we hope that this will help to enable the appropriate tuning of the circadian clock in many other plant species."

**More information:** Mari Kamioka et al. Direct repression of evening genes by CIRCADIAN CLOCK-ASSOCIATED 1 in Arabidopsis circadian clock, *The Plant Cell* (2016). DOI: 10.1105/tpc.15.00737

Provided by Nagoya University