The role of HY5 and HYH transcription factors in the regulation of the plant circadian clock

Abstract of Ph.D. dissertation

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INTRODUCTION

One of the major criteria for evolutionary success is that organisms can overcome the challenges of their environment with minimal energy loss. Because of the rotation of the Earth, changes in several important environmental parameters (such as light, temperature) show a rhythmic nature, so organisms that can predict these changes in advance, and thus timing their metabolic processes and behaviors, can save a significant amount of energy.

The time between regular events is called a period, and biological rhythms can be divided into several groups. Circadian rhythms - according to the Latin words "circa" (approximately) and "diem" (day) - are rhythms with a period of approximately one day (20-28h), dictated by the internal circadian clock of the organisms. The circadian clock itself is made up of three main units.

The core of the clock, the "central oscillator", is a recurring transcription / translation network in which clock genes and their clock proteins are involved. The interactions between the clock elements take place without any external influence, so the physiological processes connected to the central oscillator operate rhythmically even when the organism is placed under constant

environmental conditions. The second important unit of the clock is the output. The gene products of this unit (and the metabolic processes and behavioral patterns they control) do not affect the rhythm of the central oscillator. Their common feature is that their continuous expression would be a huge waste of resources, because their gene products have an optimal utilization at a particular time of the day, outside of which the rate of return on production is below maximum. By limiting gene expression to an appropriate time window, part of this energy can be saved. The third unit, the input, is a set of signaling networks that monitor significant daily environmental signals, the so-called "zeitgebers", and adjust the pace and phase of the central oscillator according to the environmental light and thermal cycles. This synchronization, also called "entrainment", at least in part occurs at the molecular level by stimulating or inhibiting the transcription / translation of certain clock elements, resulting in a change in the level of clock components and hence, in the pace of the clock.

For plants, light is the most important zeitgeber, as light is not only a source of information but also a source of energy, so monitoring and adjusting to this parameter is crucial. Identification of members of the light-signaling chain that transmit

information to the circadian clock is an area of intensive research. In recent years, many receptors and signaling intermediates have been identified in this regard, but little is known about the input elements that directly affect the expression of clock genes and thus act as a direct link between the input and the central oscillator.

One potential candidate for this role is the HY5 (ELONGATED HYPOCOTYL 5) bZIP transcription factor, which binds to promoters containing the ACGT motif (ACE element). It has some overlapping functions with its homologue, HYH (HY5 HOMOLOG), but the role of HY5 appears to play a more prominent role in plant physiology: as an essential positive regulator of photomorphogenesis, it is an integrator of several light and hormonal signaling pathways. It also plays an important role in inducing flavonoid and terpenoid synthesis, acclimatizing the plant to cold, and assimilating various nutrients. In order to avoid disruption of the simultaneous operation of all these functions, HY5 is likely to act as a member of different complexes, as suggested by the fact that, unlike traditional transcription factors, HY5 does not possess a transcriptional activation domain.

RESEARCH OBJECTIVES

As a central component of light signalling, and as a transcription factor, HY5 has been shown to be an ideal candidate for direct control of clock light tuning and synchronization by altering the expression of certain clock genes, but contradictory studies can be found in the literature. It has been demonstrated that HY5 binds to several clock gene promoters (*TOC1, CCA1, LHY, ELF4*) in white light and also activates transcription of the clock gene *ELF4*. However, in other studies, the circadian rhythm of the *hy5 hyh* mutant line did not differ from the wild type. In my dissertation, I set out to investigate and resolve these inconsistencies to determine

- whether HY5 and its homologue are capable of influencing plant circadian clock function and,
- if they have clock-associated function, identify the molecular mechanism and environmental conditions under which they operate.

RESEARCH METHODS

- Molecular cloning techniques
- Creation and maintenance of transgenic *Arabidopsis thaliana* plants
- *In vivo* luciferase enzyme activity measurements in intact seedlings
- Chromatin immunoprecipitation (ChIP)
- Plant genomic DNA extraction
- Plant total RNA extraction
- Quantitative Real-Time PCR assay
- Total plant protein isolation
- Western-blot
- Electrophoretic Mobility Shift assay (EMSA)

RESULTS

Prior to our work, the period of *hy5* mutant and wild-type lines was examined in continuous white light. As we know, white light, as a mixture of multiple wavelengths of light, can activate many plant receptors and signalling pathways, and HY5 is involved in the regulation of all these signalling pathways simultaneously and is able to integrate their information. For this reason, we hypothesized that the different wavelength compositions of the white light used in the studies may have explained the different results.

Accordingly, the white light was subdivided into different ranges that could be interpreted individually by the plants, and the function of the clock (e.g. free-running period) was monitored in *hy5*, *hyh*, *hy5 hyh* mutants and wild-type (WT) lines in continuous blue (BL), red (RL), and distant red (FR) light. Our experimental method (tracing of luciferase markers with clock-driven expression) did not allow us to compare the circadian rhythms of these lines in FR light, but we successfully demonstrated that the period of *hy5* and *hyh* mutants in blue light is shorter than that of the WT plants. This indicates that HY5 and HYH decelerate the

circadian clock in blue light. In red light, the difference in periods was negligible, and in white light a moderate phenotype was obtained, as expected, given that white light contains blue and red wavelengths. This suggests that HY5 and HYH are indeed involved in light control of the circadian clock, and the proportion of different wavelength ranges is important.

As transcription factors, HY5 and HYH are most likely bound to the promoter region of clock genes and affect the rate of plant circadian rhythm by altering their transcription rate. This hypothesis was addressed by a ChIP-seq approach. One of our interesting observations was that HY5 was found to be associated with the promoters of almost all clock and clock-associated genes. This observation was further refined by our *in vitro* EMSA experiment demonstrating that HY5 is likely to bind to specific G-box-like *cis*-elements located in these promoters. Another important result of the ChIP experiments is that although HY5 binds to the promoter of almost the same gene set in red and blue light, the strength of association is significantly greater in blue light. To explain this phenomenon, we examined the transcriptional and post-transcriptional regulation of HY5 and HYH accumulation in blue and red light, which showed that blue light

enhances the expression of HY5 and HYH at both levels. The resulting higher levels of HY5 and HYH protein may also explain the stronger association with chromatin and the blue-specificity of the circadian phenotype of *hy5 hyh* mutants.

Although HY5 is present in the promoter regions of almost all clock and clock-associated genes, our mRNA expression studies indicate that HY5 only affects the transcription rate of three clock genes (*PRR5*, *LUX*, *BOA*) negatively. This result does not fit for the first time with our previous results, since mathematical modelling of the circadian network of the *hy5 hyh* mutant (i.e. increased transcription of *PRR5, LUX* and *BOA*) predicted a long-period phenotype, which is the opposite of the short period we observed. The same model also estimated increased levels of *CCA1* mRNA, which was not detected in the *hy5 hyh* mutant under any experimental conditions. Interestingly, when the *CCA1* mRNA levels were set to WT-like levels in the simulation program (along with increased transcription of *PRR5*, *LUX* and *BOA*), the simulation gave a shorter period than for the WT plant, consistent with our results. This information may suggest that HY5 actually affects *CCA1* gene transcription, but this interaction is not detectable, since HY5 simultaneously regulates other parts of the

circadian network that suppress, in general, the effect of HY5 on *CCA1*. If this line of reasoning is correct, this may also be the case with other elements of the circadian clock, meaning that HY5 can connect the blue light dependent input to the circadian oscillator through a number of other points besides the *PRR5*, *LUX* and *BOA* genes.

Another important observation of the basic experiment in which our work was based was that the period shortening in the *hy5 hyh* mutants was measurable especially in plants grown on low-sucrose media, while in plants grown on higher sucrose, the difference in period was much smaller. Because the plant's sugar supply monitoring system also provides information toward the circadian clock, we suspected that the sugar input may interfere at some point with the HY5-dependent branch of the light input. To test this idea, we examined transcriptional and post-transcriptional control of HY5 and HYH expression as well as chromatin association of HY5 in plants grown on high and low sucrose media. According to our results, the presence / absence of sugar did not affect the function of HY5 in any aspect, therefore, further experiments are needed to explain the sugar dependence of the *hy5* circadian phenotype.

LIST OF PUBLICATIONS

Publication used in the thesis:

Hajdu A*, **Dobos O***, Domijan M, Bálint B, Nagy I, Nagy F, Kozma-Bognár L.(2018): ELONGATED HYPOCOTYL 5 mediates blue light signalling to the Arabidopsis circadian clock. Plant Journal doi: 10.1111/tpj.14106.

Other publications:

Hajdu A, Terecskei K, Gyula P, Ádám É, Nyakó A, **Dobos O**, Kozma-Bognár L (2019): LIP1 regulates the plant circadian clock via the oscillator component GIGANTEA. Genes, 10 (accepted for publication)

Hajdu A, Ádám É, Sheerin DJ, **Dobos O**, Bernula P, Hiltbrunner A, Kozma-Bognár L, Nagy F.(2015): High-level expression and phosphorylation of phytochrome B modulates flowering time in Arabidopsis. Plant Journal doi: 10.1111/tpj.12926.

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