

The molecular mechanism of entrainment of the plant circadian clock by light

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At the core of the eukaryotic circadian network, clock genes/proteins form multiple transcriptional/translational negative feed-back loops and generate a basic ~24h oscillation, which provides daily regulation for a wide range of processes. This temporal organization enhances the fitness of the organism only if it corresponds to the natural day/night cycles. Light is the most effective signal in synchronizing the oscillator to environmental cycles. Light signals mediated by photoreceptors are forwarded to the oscillator and cause an acute change in the level/activity of certain clock components that eventually results in a phase shift of the oscillation (Devlin and Kay 2001). Our aim is to reveal the molecular details of this process (also called entrainment or resetting) in *Arabidopsis thaliana*.

The plant circadian oscillator is supposed to consist of three inter-locked feedback loops (Locke et al. 2006). In the first loop the morning-expressed CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)/LATE ELONGATED HYPOCOTYL (LHY) transcription factors inhibit the expression of the TIMING OF CAB EXPRESSION 1 (TOC1) gene; conversely, the evening-expressed TOC1 positively regulates the transcription of CCA1/LHY. In the second loop GIGANTEA (GI) induces TOC1 expression during the afternoon/evening, while TOC1 represses GI during the night. Recent data suggested the operation of a third loop, where CCA1/LHY up-regulate the PSEUDO RESPONSE REGULATOR 7/9 (PRR7/9) genes (homologs of TOC1) in the morning and PRR7/9 proteins down-regulate CCA1/LHY expression during the day. In *Arabidopsis*, CCA1/LHY, GI and PRR9 may represent the primary targets of resetting light signals, merely based on the fact that these clock genes are acutely light-inducible. However, the role of their light induction in phase resetting has not been tested directly.

The primary elements of the plant light input pathway are the red/far-red light absorbing phytochromes (PHYA, B, D, E) and blue light absorbing cryptochromes (CRY1,2; Devlin and Kay 2000). However, the molecular links between phytochrome signalling and the core clock components are still missing.

In the first set of experiments we studied the function of the red/far-red absorbing phytochrome B (PHYB) photoreceptor in the resetting process. Our data show that the *phyb-9* mutation affects different parameters (phase and/or period) of rhythmic expression of components of the multi-loop circadian oscillator in *Arabidopsis*. This could be explained by decoupling of the different loops of the oscillator in *phyb-9*. However, we showed that genetic manipulation of a single loop has the same effect on the other loops in wild type and *phyb-9*, which indicates that absence of PHYB does not separate the individual loops. Rather, our data suggest the existence of tissue-specific clocks, which are regulated by PHYB in different ways.

The circadian oscillator responds with characteristic phase shifts to short light pulses. In the second set of experiments we investigated the effect of light induction of certain clock components on the magnitude of such phase shifts. Our data showed that the pattern of light induction of a single clock component does not correspond directly to particular phase responses, but high level of CCA1 and GI induction coincides with strong phase delays, while high level of GI and PRR9 induction coincides with strong phase advances. Phase response curves in single clock mutants indicate that among the light inducible clock components, CCA1, LHY and GI are negative elements of resetting during the subjective night, but PRR9 is a positive element of resetting during the subjective day.

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Study of the genetic relationships of oribatid mites (Acari, Oribatida) using nucleotide sequences

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Oribatid mites play important role worldwide in soil life, due to their high abundance and soil dwelling lifestyle. They are the most species rich order in the subclass Acari. Members of different groups of oribatid mites show very different and peculiar morphological appearance, however they belong to single taxonomical unit. There are almost 10 000 described species on the world, from which 523 species are reported in the area of Hungary to date. The species living in Hungary represent 5 major taxonomical units, divided in 77 families and 191 genera. The system of oribatid mites based on morphological features.

In order to study genetic relationships at large and small scale taxonomical levels we chose molecular markers. We used the 160 base pair long part of the 28S D3 nuclear ribosomal DNA coding domain and the 680 base pair long partial sequence of mitochondrial cytochrome-oxidase I (Cox1) subunit. 96 species were collected and determined from different localities of Hungary. 36 species, representing the main

taxonomical groups were selected, and used for the molecular studies. All species used were documented with light and scanning electron microscope images. Distance and likelihood based methods were used in combination with our own and GenBank sequences to determine the genetic similarity between oribatid mites at family level.

Our results suggest that the similarity of 28S ribosomal DNA sequences support the monophyly of lower, but not higher oribatid groups. Cytochrome-oxidase (Cox1) sequences are known to be useful at taxonomical level of genera. We introduced Cox1 sequence in the identification of larva stages.

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Kynurenines: neuroactive compounds in the central nervous system: An *in vitro* study

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Kynurenic acid (KYNA) is a neuroprotective endogenous tryptophan metabolite produced by astrocytes and neurons via the kynurenine pathway in both humans and rodents. At non-physiological concentrations, KYNA is an excitatory amino acid receptor antagonist that can partially act at both the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid and N-methyl-D-aspartate subunits of the glutamate receptors (Stone 1993).

In the brain, KYNA is synthesized in astrocytes from its bioprecursor L-kynurenine (KYN) and is then rapidly released into the extracellular compartment. Previous studies have indicated that rat cortical slices have also the ability to synthesize KYNA from exogenously added KYN (Turski et al. 1989). The synthesis of KYNA from KYN is catalysed by kynurenine aminotransferase I and II (KAT I and II) (Schwarcz et al. 2002).

The use of KYNA as a neuroprotective agent is rather restricted, however, because KYNA has only a very limited ability to cross the blood-brain barrier (Fukui et al. 1991). In contrast, KYN and different synthetic KYNA derivatives cross this barrier more readily (Giles et al. 2003).

In the course of the experiments on rat brain slices, the Schaffer collaterals were stimulated and field excitatory postsynaptic potentials (fEPSPs) were recorded in the pyramidal layer of the hippocampal CA1 region. To test the effects of KYNA, we used an *in vitro* pentylenetetrazole (PTZ) model. PTZ, a chemical convulsant frequently utilized in the study of seizures (Yudkoff et al. 2006), exerts its effects by binding to the picrotoxin binding site of the post-synaptic GABA-A receptor (Macdonald et al. 1977). PTZ administered *in vitro* at 1 mM induced a considerable increase in the amplitude of the fEPSPs recorded from the hippocampal CA1 region. When applied locally in an extremely high concentration (20 mM), PTZ resulted in characteristic wavelets. However, KYNA administration not only decreased the amplitude of the hippocampal CA1 responses evoked by Schaffer collateral stimulation, but also afforded protection from the PTZ-induced response enhancement.

The KYNA precursor KYN also blocked the development of the PTZ-induced high increase in amplitude. To prove that the KYN→KYNA conversion did take place in our experiments and that it was KYNA which afforded the protection against the effects of PTZ, we applied N-omega-nitro-L-arginine, an inhibitor of KAT I and II (Rózsa et al. 2008).

SZR-72, a synthetic kynurenic acid derivative, applied *in vitro*, proved to be also effective in preventing the high increase in fEPSP amplitudes, generated by PTZ.

These findings show that treatment with KYN, or the synthetic kynurenic acid derivative, SZR-72, even at very low concentration, has an effect on enhanced neural excitability and thus support the hypothesis that manipulations of the kynurenine pathway might be a rewarding target in different neuronal disorders affected by neuronal hyperexcitation.

Additionally we have shown, that KYNA in submicromolar concentration range has a positive neuromodulatory effect. In nM concentrations, kynurenic acid does not give rise to inhibition, but in fact facilitates the field excitatory postsynaptic potentials, recorded from the hippocampal CA1 region (Rózsa et al. 2008).

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