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We tried to identify the sumoylation site(s) in Prod by disrupting each potential site one by one, and expressing the HA-tagged mutant proteins in transfected S2 cells. Next we immunoprecipitated the mutant proteins with anti-HA antibody, and tested their molecular weight and sumoylation on Western blots. In one of the mutant proteins the high molecular weight sumoylated band seemd to disappear, and the S2 cells expressing this protein showed an altered Prod chromosomal immunostaining pattern. This indicates that the Prod protein is sumoylated at the 123rd lysine aminoacid.

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The role of the small GTPase LIP1 in the function of the plant circadian network

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The circadian clock is a biological timing mechanism that provides rhythmicity to gene expression, metabolism, and physiology with ~24h periodicity. The central oscillator of eukaryotic clocks is based on the network of clock genes and proteins, which are interconnected by transcriptional/translational negative feed-back loops.

Current models of the plant circadian clock postulate three interlocked feedback loops. A pair of single Myb-domain transcription factors, *CIRCADIAN CLOCK ASSOCIATED1 (CCA1)* and *LATE ELONGATED HYPOCOTYL (LHY)*, plays central roles in two loops. In one loop, *CCA1* and *LHY* repress the expression of the Pseudo-Response Regulator gene *TIMING OF CAB EXPRESSION 1 (TOC1)*. *TOC1* closes the first loop by inducing *CCA1* and *LHY* transcription for the next cycle. In a second loop, *PRR7* and *PRR9*, are induced by *CCA1* and *LHY*. *CCA1* and *LHY* are subsequently repressed by *PRR7* and *PRR9*. In a third loop, *GIGANTEA (GI)* and, possibly, *PRR5* are positive regulators of *TOC1*. *GI* is negatively regulated by both *CCA1/LHY* and *TOC1* (McClung 2008).

The *lip1-1* (light insensitive period 1) mutant isolated from *Arabidopsis thaliana* displays novel circadian phenotypes. *lip1-1* was isolated as an early-phase mutant based on the expression pattern of *CAB2:LUC* circadian output marker in constant darkness. In wild-type plants, period length shortens with increasing light fluence rates and the phase of rhythms can be shifted by light pulses administered to darkadapted plants. In *lip1-1*, period length is nearly insensitive to light intensity and larger phase shifts can be induced during the subjective night (Kevei et al 2007).

The first aim of our work was to determine the molecular mechanism by which LIP1 affects the plant circadian clock. Transcript levels of clock genes were determined by quantitative real-time PCR in *lip1* mutants. Our data show that LIP1 affects the expression of *GI*, *PRR9* and *TOC1*. The effect on *GI* expression was supported by the analysis of *gi-lip1* double mutant plants.

We generated promoter:LUC+ reporter gene constructs for each core clock genes in *lip1* mutant background and we could prove that the transcription of all core clock components is affected by the mutation.

Our second aim was to identify how the function of LIP1 is controlled. LIP1 is a plant-specific atypical small GTPase. Small GTPases are molecular switches shuttling between the GDP-bound inactive and the GTP-bound active states. For this process they require downstream signaling elements (effectors) and upstream signaling elements (e.g. GEFs) (Berken et al 2005). We found that LIP1 interacts with a member of the plant specific family of RopGEFs, RopGEF7 in yeast two-hybrid system. However, the insertion mutant allele of *RopGEF7* showed no circadian phenotype in planta. The family of RopGEFs consists of 14 members. We tested the circadian phenotype of insertion mutants for all of them and found that a mutant allele of *RopGEF2* has a *lip1*-like circadian phenotype. *lip1* mutant plants show stress phenotype also, they are sensitive to salt. *RopGEF2* mutant plants display a *lip1*-like salt phenotype. RopGEF2 might be the member of the RopGEF family which promotes LIP1 function.

Previous data showed that LIP1 is localized in the cytosol, nucleus and in cell compartments as well. We tested the function of *nuclear export signal* (NES) or *nuclear localization signal* (NLS) tagged YFP-LIP1 fusion proteins in *lip1* mutant background to see if any of the *lip1* phenotypes could be complemented. YFP-LIP1-NLS fusion proteins could restore the circadian phenotype. Neither of the constructs could restore the salt sensitivity phenotype. These data indicate that LIP1 affects the circadian clock in the nucleus, but nucleo-cytosolic shuttling is required to fulfill its role in tolerating salt stress.

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