Moreover other fluorescent pseudomonads such as *Ps. agarici*, *Ps. constantinii* and *Ps. gingeri* can cause various symptoms as well. The same degree of discoloration may be caused by dissimilar species of pseudomonads, suggesting that the factors are not exclusive to a particular pseudomonad species (Godfrey et al. 2001).

Many investigations have been carried out to find an appropriate method for preventing or controlling this disease. There are trials to use of chemical wash formulations, including such chemicals as calcium chloride, sodium hypochlorite, hydrogen peroxide, bronopol and antibiotics in watering mushrooms, but none of them has been found to be fully effective and non-toxic to humans (Wong and Preece 1985).

Biological control by competition has also been investigated. Potential bacterial control agents have been isolated and shown to be active antagonists (Nair and Fahy 1972).

In this study, the *Pseudomonas* strains of an infected oyster mushroom farm in Hungary were investigated. Sixty strains were isolated on Gould's S1 medium, which consistently gives high selectivity and good recoveries of fluorescent pseudomonads with samples obtained from a variety of habitats. S1 medium has several advantages over other media used for the isolation of fluorescent pseudomonads. The identification of the strains was carried out by sequencing a part of the *rpoB* gene or in some other cases a part of the 16S rDNA gene. The *rpoB* gene codes for the RNA polymerase β-subunit, it is a highly conserved essential gene, so it could be used for bacterial identification. The pathogenicities of the strains were tested on yeast extract media in Petri-dishes, in direct confrontation tests.

To find and effective antagonistic agent, we isolated and evaluated fluorescent pseudomonads, bacilli and lytic bacteriophages, against *Pseudomonas tolaasii*, and other pathogenic *Pseudomonas* strains.

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Interacting protein partners on Drosophila telomeres

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The *proliferation disrupter* (*prod*) gene in *Drosophila* encodes a 346- amino-acid protein that localizes strongly to the centric heterochromatin of the second and third chromosomes as well as to >400 euchromatic sites, and all telomeres. In *Drosophila melanogaster*, three telomeric domains can be distinguished by DNA sequence and by proteins associated with them: the end of the chromosomal DNA molecule (capping complex), the retrotransposon array consisting of three non-long terminal repeat retrotransposons, *HeT-A*, *TART*, and *TAHRE* (HTT), and the subtelomeric repetitive telomere associated sequences, repetitive region (TAS). Chromosome length in *Drosophila* is maintained by targeted transposition of the three telomere-specific non-long terminal repeat retrotransposons, HTT, to the chromosome end.

Immunofluorescence stainings of different mutant telomeres clearly showed, that Prod binds to the *HeT-A* element of the HTT array. We could also show that Prod binding represses *HeT-A* transcription, nevertheless does not influence HTT length. Reduction of Prod levels in heterozygous $prod^{k08810}$ null mutant flies results in elevated levels of *HeT-A* transcripts in ovaries as well as in third instar larvae, while it has no effect on genomic *HeT-A* copy number, which we used as a measurement for HTT length.

To identify proteins that may interact with Prod, we performed a yeast two-hybrid screen, using the Clontech Matchmaker cDNA library and the entire *prod* cDNA as bait. About 120 000 cDNA clones were tested and about 100 potential Prod interactors were identified. Prod was found 4 times, suggesting that Prod interacts with itself. We also identified Z4 as one of the interacting proteins, which is one of the few proteins known to be associated with the HTT telomeric domain. The Chromator protein, which has previously been shown to co-immunoprecipitate with Z4 and co-localize with Z4 in interbands of polytene chromosomes and at some telomeres, was also found to interact with Prod. We could verify the latter interaction with co-immunoprecipitation experiment.

Proteins Uba2 and Lesswright were the strongest interactors of Prod in the yeast two hybrid screen.Uba2 is the E1 SUMO activating enzyme regulating the initial steps of sumoylation, - a posttranslational protein modification system modifying protein activity - while the second step is performed by the E2 ubiquitin-conjugating enzyme encoded by the lesswright gene in *Drosophila*. Computer sequence analysis showed that Prod (and Chromator) have potential sumoylation sites. This raised the possibility that either Prod itself is sumoylated or sumoylation enzymes are recruited by Prod to sumoylate Prod-associated proteins. Our immunostainings confirm this notion, demonstrating that most if not all sumoylated proteins on telomeres are located on the HTT array.

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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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