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DISSERTATION SUMMARY

Isolation of circadian clock mutants in *Arabidopsis thaliana*

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It is a well known phenomenon that some biological processes exhibit periodic changes on a daily time-scale. Some of these maintain periodicity even in the absence of external periodic stimuli which suggests that an internal pacemaker or "clock" exists in the organism. Indeed, genetic mutations were identified which cause defects in the mechanism producing circadian rhytmicity. In several model organisms the molecular basis for circadian clockwork is described in detail. Surprisingly, circadian clock seems to be a good example of convergent evolution: there are no conserved clock elements among organisms in different taxa. However, it is generally accepted that in all organisms special clock proteins generate circadian rhythms through a transcription/translation feedback loop, parts of which are unrelated, but the functions and the architecture of the system is similar. The rhythm generated by the circadian clock is received by several output elements like photosynthetic activity or gene expression. To keep in phase with the external 24 h cycle of the day, the clock can be reset by several environmental factors which percepted and transmitted to the clock by input receptors.

Despite of the large amount of information available on the molecular nature of circadian pacemaker in other organisms, little is known about the clock mechanism in plants. Therefore we decided to identify the elements of the clock system in *Arabidopsis thaliana* by searching for mutants displaying altered circadian phenotype. We used transgenic lines carrying a Cab2:luc reporter gene for mutagenesis by EMS and T-DNA. We screened for mutants by continuously measuring the luciferase enzyme activity in individual plants over two days in constant darkness after a 7-day long entrainment by 12 h dark-12 h light period. Progeny of individual mutants was tested for herited mutant phenotype either in constant darkness and red light. Mutants exhibited any kind of circadian phenotype were kept for further analysis and mapping. We started the genetic mapping of the mutants showing the most robust phenotype.

After screening of 60,000 EMS mutagenized seedlings we obtained 37 potential mutants. Eight of these were selected for genetic mapping. From the T-DNA screen 5 of the 5,000 line tested were selected as a putative mutant. In none of the 5 cases could we show cosegregation of the T-DNA tag with the mutant phenotype, therefore these mutants will be mapped. After obtaining a rough map position from some EMS mutants we found that it is the same in 3 of our mutants and it is the position where an already described clock gene – ZTL – is located. Therefore, we decided to sequence that gene in the 3 mutants. All of them proved to be new alleles of the ZTL clock gene. ZTL is an F-box protein, which probably plays a role in the ubiquitin-mediated destruction of clock proteins. It contains a LOV/PAS domain, which is tought to bind flavin cofactors or mediate protein-protein interactions and can be found in several clock-associated protein. It contains the F-box which docks target proteins to the E3 ubiquitin ligase complex, and a Kelch-repeat domain which is a common protein-interacting domain and expected to bind the target protein. In one of our mutants, there is an early STOP codon in the last Kelch-repeat, and probably it causes a loss-of-function phenotype. The second ZTL mutant contains a mutation in the 4. Kelch-repeat which causes a non-conservative Gly->Asp amino-acid change, and it is likely a loss-of-function allele, also. The third ZTL mutant mutation is located in the LOV/PAS-domain causing a Leu->Ser amino-acid change. It has an interesting phenotype, because like other ZTL mutants it has a long-period but the difference in period length is constant over a large scale of light intensity which is not true in the case of null-alleles of ZTL. This indicates that in this mutant light-sensitivity remains unaffected, but the function of the molecule is damaged in some extent. Several mutants have not been mapped yet, but have interesting phenotypes and therefore we can expect them to have mutations in unknown clock genes.

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