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Characterization of molting defective gene in the ecdysone production of Drosophila larvae

Bernadett Tomisa

Department of Genetics, University of Szeged, Hungary

Steroid hormone ecdysone (E) mediates a wide variety of developmental events in insects. Therefore, understanding the function and the regulation of ecdysone signalling pathway is essential for insect biology. During Drosophila larval life the source of ecdysone is the endocrine organ, the ring gland (RG). Ecdysone is synthesized from cholesterol via a series of hydroxylation steps catalyzed by cytochrome P450 enzymes.

Known genes encoding Ecdysone synthesis enzymes (spook, phantom, disembodied, shadow and shade) are members of the „Halloween" class of genes. Mutants of this class share a characteristic phenotype: thin, unstructured embryonic cuticle with dorsal and anterior holes therefore caused embryonic lethality. Although several studies reported details of ecdysone synthesis, little is known about the regulation of the process. Here we report how the *molting defective* gene (*mld*) is involved in the regulation of ecdysone production.

Mld encodes a nuclear zinc finger protein that is required for ecdysone production. Since *mld* does not regulate the expression of known ecdysone synthesis genes, it might control the expression of steroid biosynthesis. Mutations of *mld* cause an arrest of development in the first larval instar. This defect can be rescued by providing ecdysone.

Mld homozygous mutant larvae have two main phenotypes. On the one hand it has an enlarged ring gland and on the other hand it is lack of ecdysone.

We show that the hypertrophy of RG is due the increased polytheny of PG cells. Using somatic mosaics we also showed that RG hypertrophy is not autonomous. The PG hypertrophy is due to the low ecdysone level in the mutant, and suppressed if treated with ecdysone. This effect is inhibited if ecdysone receptor gene (EcR) is silenced in PG cells by RNAi. Silencing EcR isoforms in RG specific manner in wild type also has an organism specific effect, resulting enlarged RG and smaller adult body size.

We conclude from these results that *mld* gene is a larval specific regulator of ecdysone production. We found, the phenocritical period of *mld* mutant phenotype is in the second part of the first larval stage, the time when ecdysone production is needed, also supports the larva specific role the gene. Furthermore the results of epistasis experiments with *mld*, *sad* (E synthesis gene), and mld, *kkv* (ecdysone dependent cuticle biosynthetic gene) show that *mld* does not play role in embryonic development. Studies with germ line mosaics of amorphous *mld* alleles indicate the lack of maternal effect of mld gene.

We found that the classic ecdysone deficient mutation DTS3 (dominant temperature sensitive-3) is an *mld* allele. In order to better understand the DTS phenotype we determined the mutation sites of mld^{DTS3} by sequencing the mutant allele. Two point mutations were found in exon-three, one resulting a premature stop codon, and an other resulting P to S amino acid change.

Supervisor: Péter Maróy
Email: betti.tomisa@gmail.com

Influence of DNA damage and repair, on the ability of cyanobacterial cells to repair UV-B radiation-induced damage to the Photosystem II complex

István-Zoltán Vass

Laboratory of Molecular Stress- and Photobiology, Institute of Plant Biology, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

Two of the most significant primary effects of UV-B irradiation in cells of photosynthetic organisms are the damage to DNA and the impairment of active protein complexes, of which the most pronounced one is the inactivation of Photosystem II mainly due to damaging the D1 protein. We have investigated the correlation of Photosystem II protein damage and its repair with the concomitant DNA damage and its repair. As model organisms the cyanobacterium *Synechocystis* PCC6803 wild type (WT), as well as its photolyase lacking mutant (ΔphrA) were used for this purpose. We found that during exposure to UV-B radiation the ΔphrA cells accumulated a significant number of DNA damages concomitant with a radical decrease in Photosystem II activity, and D1 protein levels. After terminating the UV-B illumination the ΔphrA cells showed no repair of damaged DNA, and only a limited capacity to repair the damaged Photosystem II centers. The WT cells, however, didn't suffer significant damages to their DNA. In these cells PSII activity as well as repair capacity, including effective turnover of the D1 protein pool, was maintained under the same UV-B irradiation conditions. These data show that the repair capacity of Photosystem II is directly influenced by the ability of cells to repair UV-B damaged DNA.

Supervisors: Péter B. Kós, Imre Vass
E-mail: vassiz@gmail.com