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DISSERTATION SUMMARY

# Molecular and genetic analysis of the Rpt1 subunit of *Drosophila* 26S proteasome

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The eukaryotic 26S proteasome defines the final end of the lifetime of many proteins by recognizing the multiubiquitin tag attached to the appropriate protein and thereafter executing its destruction through its deubiquitylation, unfolding, translocation to the peptidase sites and lastly, cleavage of the protein backbone after every 8-10 amino acids. Within the proteasome, the activity of the core protease particle is regulated by one or two regulatory complexes (RC). The RC-s are responsible for the above mentioned first steps of the destruction. The RC is comprised of two well-defined protein subcomplexes, the lid and the base. The base contains six AAA-type ATPases, similar to each other in sequence, which are assumed to function as reverse chaperones, unfolding the substrate and translocating it through the gated orifice of the core peptidase right to the protease sites.

Despite their similarities, several unique features were discovered concerning these ATPases, even though their direct functional pathways and their interactions with other proteins are still unknown. One of them, p48B/Rpt1 has remained largely uncharacterized until now in *Drosophila*. The ease of mutagenesis with P-elements and subsequent mutation detection in this organism aided us in characterizing three P-element insertions in the 5'-untranslated region (UTR) of the Rpt1 gene. These insertions proved to be recessive lethals, two of them dying in second larval and one in pupal stage as homozygotes. One larval lethal allele was modified by transposase-induced P-element excision and the resulting white-eyed flies were examined further. These mutants could be sorted into two lethality groups, namely second larval and pupal stage lethals. After sequencing their appropriate genomic regions, pupal lethality was found to be associated with short (30-35 bp) while the larval lethality with long (more than 600 bp) remaining P-element sequences at the original insertion site, which were formed due to imprecise P element excision.

Several tests were performed to verify the relation between the mutation in the gene and the lethality stage, considering the possibility that a second-site mutation could be responsible for the phenotype. The effect of the mutation on the subunit expression and modification was investigated and we discovered profound changes both at mRNA and protein levels in case of Rpt1 mutants compared to the wild-type. RT-PCR revealed a decrease in full-length Rpt1 mRNA expression in one pupal lethal mutant as well as immunoblot against the Rpt1 protein showed reduced subunit expression in the mutant. At molecular levels we found several ubiquitin-proteasome system-related abnormalities in the mutant animals. Native gel electrophoresis demonstrated that the two well-defined forms of 26S proteasome (the singly-capped form: RC-20S proteasome, or the doubly- capped form: RC-20S proteasome-RC) disappear in pupal lethal mutants, and the amount of free 20S proteasome increases. The mass of ubiquitylated proteins is also increased in the mutants in comparison with the wild-type. We found that the ratio of the two isoforms of Rad23, which is a nucleotide excision repair factor and, in addition, a multiubiquitylated substrate shuttling protein associated to the proteasome, is also affected: the higher molecular weight form termed A, which is 10-fold less abundant in the wild type than the B one, is almost as abundant as the B form in the mutant pupae. The expression of proteasome subunits other than Rpt1 seems to be only slightly impaired. To sum up our results we analyzed several mutant alleles of one proteasomal ATPase gene and our findings indicate that this subunit is essential for the proper functioning of the 26S proteasome and for the normal development of the fruitfly *Drosophila*, furthermore this investigation provided us interesting insights into the function and regulation of the proteasome regulatory complex.

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