Molecular analysis of the C-terminal fragments of Methoprene tolerant and Germ cell-expressed receptors from *Drosophila melanogaster*

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ABSTRACT

Methoprene tolerant (Met) and *Germ cell-expressed* (Gce) proteins are considered as the juvenile hormone (JH) receptors in *Drosophila melanogaster*. Both proteins mediate the hormone action and, additionally, enable the crosstalk between signaling pathways regulating the basic insects' physiological processes. Bioinformatic analyses assign Met and Gce to the family of bHLH-PAS transcription factors, responsible for the regulation of important developmental and physiological processes in mammals and insects. However, their amino acid sequence similarity is limited to defined domains in the N-terminal region, while their long C-terminal fragments (MetC, GceC, respectively) show significant differences in the primary structure. The main hypothesis of the project is based on the assumption, that the differences in the molecular characteristics of MetC and GceC may be essential for their function and subcellular distribution diversity during *Drosophila melanogaster* development. The aim of our studies is to characterize the MetC and GceC fragments structurally. The results of this project are extremely important, since Met and Gce proteins are the first described hormone receptors in the bHLH-PAS family.

The results presented in the thesis indicate that the MetC and GceC exhibit properties of intrinsically disordered proteins (IDPs). IDPs are characterized by the highly extended, ellipsoidal conformation and the lack of stable tertiary structure. GceC, with unstructured-like (U-like) IDP characteristics, shows a slightly higher degree of asymmetry as premolten globule-like (PMG-like) MetC. Simultaneously, that GceC has more molecular recognition motifs (MoREs) and has a greater tendency to take the structure. Therefore, it can be considered that GceC interacts with more physiological partners and its function and localization can be regulated in a much more complex manner than for Met.

The MetC and GceC, as IDPs, can be expected to interact with multiple protein partners. The extensive literature studies allowed to designate two candidates for interactions: the nuclear receptor Fushi Tarazu factor-1 (Ftz-F1) and the regulatory protein 14-3-3. According to literature data, the interaction between MetC or GceC and Ftz-F1 changes the nuclear receptor

activity and enable expression of specific JH-dependent genes. In turn, the interaction with 14-3-3, predicted only for GceC, can modulate JH receptor functions by changing its subcellular localization, structure, stability and molecular contacts with physiological partners. The results presented in the thesis clearly show, that the best way to analyze the weak interactions of IDPs and IDRs is the NMR spectroscopy. The obtained results confirmed the interactions between GceC and LBD Ftz-F1, GceC and 14-3-3 as well as MetC and LBD Ftz-F1, in the absence of interaction between MetC and 14-3-3. The established interactions can directly affect the function and activity of full-length proteins.