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„Molecular characterization of calponin-like Chd64 and immunophilin FKBP39"

Two major lipophilic hormones regulate insect development and growth, a steroid called 20-hydroxyecdysone (20E) and the sesquiterpenoid juvenile hormone (JH). The initiation of molting and other essential biological processes are controlled by 20E, whereas JH works as a modulator of the ecdysteroid-induced gene expression cascade. In contrast to 20E, whose mode of action is well-known, the biological mechanism of gene regulation by JH is poorly understood. Consequently, the cross-talk between the two signaling pathways remains a puzzle.

Recently, Li *et al.* suggested that two proteins, the 21-kDa calponin-like protein (Chd64) and the 39-kDa FK506-binding protein (FKBP39), are key components of a dynamic, multiprotein complex that crosslinks these two hormonal signalling pathways. This putative complex also includes ecdysone receptor (EcR), *Ultraspiracle* (Usp) and *methoprene-tolerant* (Met) protein and can bind to the juvenile hormone response element (JHRE). However, the molecular basis of the interactions between FKBP39, Chd64 and the other components of the multiprotein complex is not understood. Until now, Chd64 and FKBP39 have not been biochemically or structurally characterized. The aim of this work was to identify the structural features that would provide understanding of the role of Chd64 and FKBP39 in multiple and dynamic complex that cross-links the signaling pathways.

In this work, we demonstrate the results of *in silico* and *in vitro* analyses of the structural organization of Chd64 and FKBP39 from *Drosophila melanogaster*. These analyses revealed that both proteins have an asymmetrical, elongated shape. Chd64 is a monomeric protein and exhibits dual structural organisation, i.e. it has a globular core, which corresponds to the CH (calponin homology) domain and disordered tails

that outflank the globular core. Monitoring the unfolding of Chd64 revealed a highly complex process. Chd64 unfolds and forms of a molten globule (MG) - like intermediate state. FKBP39, as well as Chd64, possesses a dual nature. It consists of N-terminal NPL (nucleoplasmin –like) domain, C-terminal FKBD (FK506-binding domain) and a long, highly charged, disordered linker. In contrast to current thought regarding the preserved pentameric structure of proteins possessing an N-terminal NPL domain, the quaternary structure of the full-length FKBP39 is tetrameric. The NPL domain forms a stable, tetrameric core and FKBD are linked to it by intrinsically disordered, flexible chains that form tentacle-like segments. Our results revealed, that Chd64 and FKBP39 may exist in discrete structural forms, indicating that these proteins are pliable and capable of easily acquiring different conformations. The plasticity of Chd64 and FKBP39 and the existence of IDRs (intrinsically disordered regions) may be crucial for multiple interactions with various partners and constitute the foundation for their regulatory function.