IDENTIFYING BINDING SITES FOR THE βFTZ-F1 PROTEIN WITHIN THE *E93* GENE OF *DROSOPHILA MELANOGASTER*

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Steroid hormones control a wide range of developmental processes in eukaryotes including humans. They bind to nuclear receptor proteins thereby regulating the expression of target genes in the right tissues (tissue-specific gene induction) and at the right times (stage-specific gene induction). The goal of our research is to answer one of the central questions of developmental biology – how can a single hormonal signal elicit different responses at different times and in different tissues during development?

Using *Drosophila melanogaster*, with its fluctuating levels of the steroid hormone ecdysone, as a model, we want to unravel the molecular basis of metamorphosis: the dramatic transition from larva to adult fly. We are studying genes that are regulated by ecdysone during *Drosophila* metamorphosis, focusing on the β*FTZ-F1* gene, which encodes a nuclear receptor that appears to play a central role in directing stage-specific genetic and developmental responses triggered by ecdysone.

During metamorphosis, the expression of β*FTZ-F1* is specific to the middle of the prepupal stage and it is vital for the stage-specific expression of the *E93* gene in the late prepupal stage. βFTZ-F1 protein provides the early gene *E93* with the competence to respond to ecdysone in late prepupae. E93 functions as a stage-specific transcription factor causing the induction of programmed cell death in the larval salivary gland.

We hypothesize that βFTZ-F1 protein provides competence to *E93* through a mechanism that involves direct binding of βFTZ-F1 protein to the *E93* gene. The main goal of the project is to produce a soluble radioactively-labeled βFTZ-F1 protein by *in vitro* translation and to identify the regulatory targets as well as binding sequences of the βFTZ-F1 protein in the *E93* gene.

Previous attempts using a bacterial expression system have failed to yield a soluble βFTZ-F1 protein for binding assays. Thus, I introduced by PCR an optimal Kozak sequence, which is essential for an efficient initiation of translation, in the already existing expression vector for βFTZ-F1 protein. Capped mRNA was *in vitro* transcribed from the modified vector which was subsequently translated in a reticulocyte lysate system into ^{35}[S]methionine-labeled βFTZ-F1 protein. The exact DNA sequence to which βFTZ-F1 protein binds will be identified by gel-shift assays (EMSA) using the *in vitro* synthesized protein.