# *DROSOPHILA* AS A MODEL SYSTEM TO STUDY IRON METABOLISM

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Ferritin is a 24-subunit iron-storage complex assembled of H and L chains. In this study, we examined systemic and cellular ferritin regulation and trafficking in *Drosophila*. *In situ* mRNA hybridization experiments showed that ferritin H and L transcripts are co-expressed during embryogenesis. Mutation of either the H or the L subunits resulted in embryonic lethality, indicating that both subunits are essential for embryonic development. We could demonstrate that the ferroxidase activity of ferritin H-chain is essential and that a GFP-ferritin fusion protein can sustain normal development in fruit flies and permits live imaging of the major iron-storage protein complex in this organism. Analysis of flies carrying mutant H or L chains, or flies overexpressing either one of the subunits revealed a constant ratio of ferritin H and L subunits, pointing to tight post-transcriptional regulation. This regulation is in agreement with recent structural data from the secreted ferritin of *Trichoplusia ni* (Hamburger *et al.*, 2005). We generated transgenic animals for ferritin overexpression and showed a requirement of both chains for successful overexpression of insect ferritin. Ferritin overexpression impaired the survival of iron-deprived flies, further supporting a need for ferritin regulation. However, radioactive iron tracing experiments pointed to the presence of a regulatory system, which determines *in vivo* the quantity of iron loading into ferritin. Iron-feeding experiments also demonstrated that specialized and distinct intestinal cell populations metabolize nutritional iron and copper sources separately. We characterized the time-response of ferritin induction upon iron feeding and describe the subcellular dynamics of this response. *In vivo* expression of GFP-tagged holoferritin confirmed that iron-loaded ferritin molecules traffic through the Golgi organelle and are secreted into hemolymph. Furthermore, our study revealed both conserved features and insect-specific adaptations of ferritin regulation and trafficking. It is currently unknown if specialization for metal sequestration is conserved in mammals, but concentration of different metals in different cellular populations was recently reported in plant seeds (Kim *et al.*, 2006). Iron Regulatory Protein (IRP) regulates ferritin expression in both *Drosophila* and humans by binding to the Iron Responsive Element (IRE). However, the mechanistic details of this regulation differ between the two organisms. We have reported how fly IRPs compare to the human homologs (Lind *et al.*, 2006). Here, we highlight the similarities of the IREs between the two species, show the conservation of IRE in *Fer1HCH* mRNAs from the entire *Drosophila* genus and explain how it is possible to regulate total ferritin levels in *Drosophila* by regulating a single ferritin chain (as *Drosophila* L-chain mRNA lacks an IRE). Other significant aspects of our study include the proof-of-principle that live imaging of ferritin trafficking is possible, because it is unknown how ferritin is secreted in the circulatory system of humans and little is known about ferritin trafficking in general. The question of how ferritin iron loading occurs *in vivo* in the mammalian or the insect systems also remains unknown. Importantly, our results suggest that an iron-delivery system for ferritin loading exists in insects.