Drosophila Importin-7 is required for proper muscle attachment site formation

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Importin 7 (Ipo7) belongs to a family of proteins whose basic function is in the import of proteins into the nucleus for the induction of gene expression. Ipo7 is upregulated in cancer and blocking Ipo7/Dim7 function affects cell proliferation. There are several closely related Ipo7 proteins in mammals, but only one encoded in the Drosophila genome, encoded by the moleskin (msk) gene. Studying this single Drosophila importin7 (Dim7) ortholog in the fly model has the advantage of less functional redundancy to uncover its molecular role. Previous work has found that Dim7 is a dynamic protein that regulates the nucleocytoplasmic shuttling of proteins in the early embryo, but accumulates at muscle attachment sites (MASs) in late embryogenesis once the muscle and tendon cells are formed. Moreover, our lab found that mutations in msk exhibit defects in embryonic muscle attachment. Thus, the overall goal of this proposal is to understand which regions of Dim7 are important for the differential subcellular localization in muscle. Completion of my overall project will answer two questions: (1) Which region of Dim7 is essential for the nuclear and/or MAS localization in muscle cells?; and (2) What proteins are responsible for the MAS localization of Dim7 in contractile muscle?

Dim7 has an N-terminal Importin-beta, (IBN_N) domain, an internal exportin Cse1-like (Cse1) domain, followed by an uncharacterized C-terminal region with no identifiable domains. To determine how Dim7 gets recruited into the nucleus or MAS, we generated YFP-tagged deletion mutants that remove regions of Dim7. Briefly, the Dim7-YFP deletion constructs were recombined into a *msk* mutant background and examined for the distribution of Dim7 in muscle tissue under the *mef2* promoter. Expression of the IBN_N domain (Dim7ΔA) alone in *msk* mutants resulted in the strong nuclear accumulation of Dim7 without apparent muscle attachment defects. Removal of the entire N-terminus, including the IBN_N and Cse1 domains (Dim7ΔE) did not alter the overall distribution of Dim7 in muscles, but reduced the ability of the attachment site to form. These data, taken together suggest that the nuclear function of Msk may be sufficient to initiate muscle-tendon attachment, possibly through the previously identified epidermal growth factor (Egf) signaling pathway. For all Dim7 deletion constructs muscle attachment was rescued, but not the localization of Dim7 to the attachment sites. A possible solution for this could be that the Dim7 protein functions as a dimer and requires multiple domains for complete function and localization to the muscle attachment site. Additional work has shown that overexpression of respective Dim7 deletion constructs cause disruption of nuclear positioning in larval muscle and MAS defects in adult indirect flight muscles. Further directions include identifying how Dim7 is responsible for MAS formation, whether through direct interactions or transcription factor association, and uncovering the role of Dim7 in larval and adult muscle.

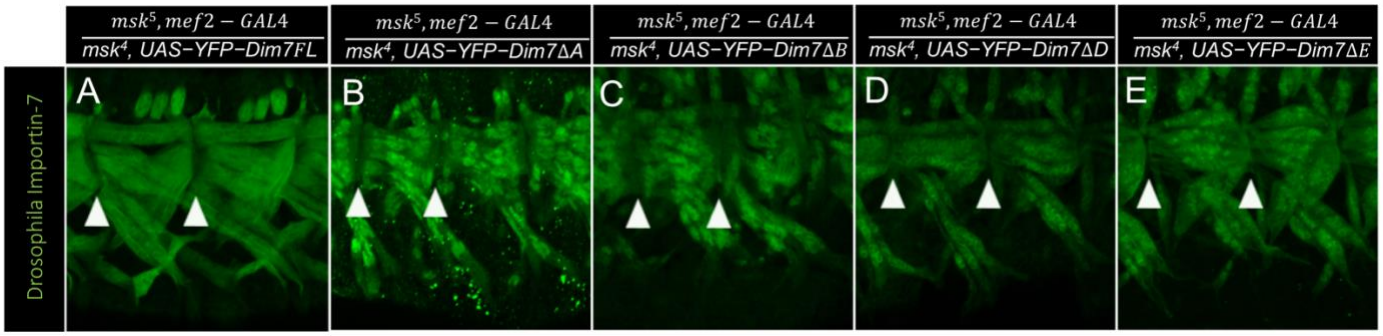

Figure 1 Rescue experiments were performed by expressing the indicated Dim7 deletion constructs in a *msk-/-* mutant background. (A) Dim7FL was able to rescue MAS formation in the *msk* mutant background. (B) Expression of the Dim7DA construct indicates that the Cse1-like domain is necessary for nuclear export of Dim7. (C and D) Expression of Dim7DB and Dim7DD show recovery of proper MAS formation but no localization of Dim7 to the MAS. (E) Deletion of the N-terminal region of Dim7 (Dim7DE) shows varied Dim7 localization, where some embryos appear WT, while others show a decrease of Dim7 at the MASs. None of the individual rescue experiments were able to localize Dim7 to MASs suggesting Dim7 function is more complex and may require multiple domains for complete function.

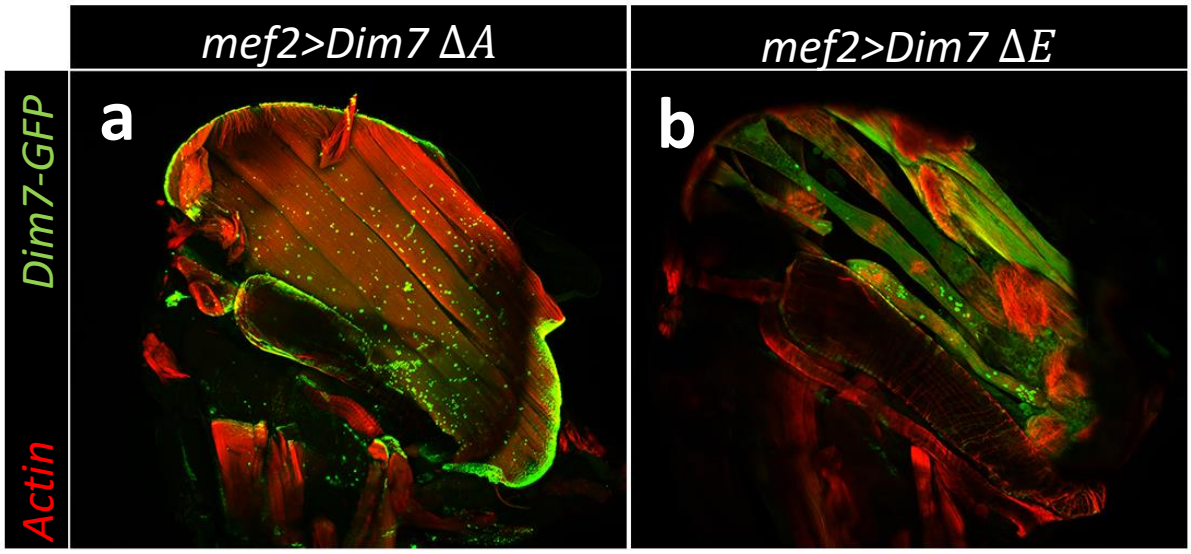

Figure 2 Dim7 deletion constructs were over expressed and their effect on survival throughout the *Drosophila* lifecycle was recorded. Looking at the flight ability of the surviving adults, Dim7DA showed WT flight ability and Dim7DE showed decreased flight activity. The indirect flight muscles (IFMs) of these flies were examined. The thoraces of 9 day old flies were bisected to examine the IFMs. Using confocal microscopy, the localization of Dim7 and effect on IFM structure was examined. (a) mef>Dim7DA showed WT muscle formation. (b) mef>Dim7DE showed decrease muscle size and improper muscle formation.