A ROLE FOR UNC-45 IN MAINTAINING MYOSIN DURING AGING

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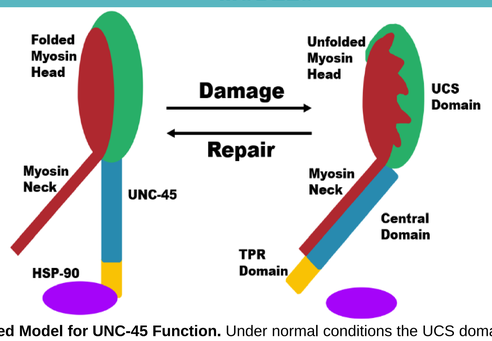
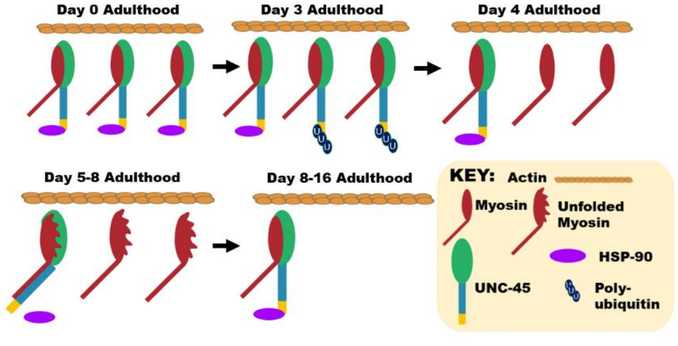
ABSTRACT

UNC-45 is a chaperone required for the folding of functional myosin heads and the proper assembly of myosin into thick filaments. UNC-45 was first discovered in *C. elegans* and later found to be conserved in all animals. UNC-45 consists of a TPR domain that interacts with HSP-90, a central domain, and a UCS domain that interacts with and folds the myosin head. In addition to its essential role in muscle development, we hypothesize that UNC-45 has a role in mature muscle, to re-fold myosin heads damaged from physical, thermal, or oxidative stress. One type of stress is aging, as a popular theory is that the decline in cellular function found in aging is due to an accumulation of damage to macromolecules that occurs with time. Sarcopenia is the decrease in muscle mass and function seen in the elderly in the absence of any underlying disease. Herndon et al. (2002) showed that *C. elegans* can serve as a model for sarcopenia. Using immunostaining with antibodies to myosin heavy chain A (MHC A), we show that there is a gradual decline in motility beginning at day 4 and the number of A-bands (a measure of thick filament assembly) beginning at day 8 adults. By day 12 and especially day 16, there is also disorganization of A-bands. This disorganization appears similar to that of *unc-45* ts mutants grown at the restrictive temperature. We have found that in *C. elegans* a decline in *hsp-90* mRNA (day 2) directly precedes a decline in HSP-90 protein (day3), which directly precedes a decline in UNC-45 protein (day4), which then in turn precedes a decline in Myosin B (day8), the main client of UNC-45. *unc-45, unc-54,* and *myo-3* mRNA decline immediately after reaching maturity (day 1) and remain stable, though low – which is expected for proteins assembled into the sarcomere. We also observe a decrease in UNC-45 protein, but not mRNA, in an *hsp-90* loss of function mutant, suggesting a role for HSP-90 in UNC-45 regulation and/or protein stabilization. We also observe early onset of sarcopenia when UNC-45 is lost during adulthood and an increase in an unknown UNC-45 post translational modification with age. This leads us to investigate the possibility that **during aging a loss of HSP-90 leads to UNC-45 degradation, possibly through exposing the protein for an unknown modification, which then leads to a loss of muscle mass and function**.

MODELS



Proposed Model for UNC-45 Function. Under normal conditions the UCS domain of UNC-45 is bound to the myosin head and the TPR domain is bound to HSP-90. Under stress conditions, HSP-90 detaches from the TPR domain, causing a conformational change in UNC-45 that allows the Central domain to bind to the myosin neck resulting in inhibition of the myosin power stroke while the UCS domain protects/re-folds the myosin head. HSP-90 can then rebind the TPR domain, causing the Central domain to release the myosin neck, allowing movement of the myosin motor. Note that only the myosin head and neck are shown for simplicity of illustration.



Model to be Tested: HSP-90 declines at day 3, leading to the poly-ubiquitination and degradation of UNC-45. Because of this loss of UNC-45, when myosin becomes unfolded it cannot be refolded and is lost. Due to low *myosin* and *unc-45* mRNA levels, the protein is not replenished once lost. Based on this model, retaining UNC-45 levels would lead to better maintenance of Myosin in the thick filament.

C. ELEGANS DEVELOP SARCOPENIA AND HAVE DECREASED THICK FILAMENT ASSEMBLY DURING AGING.

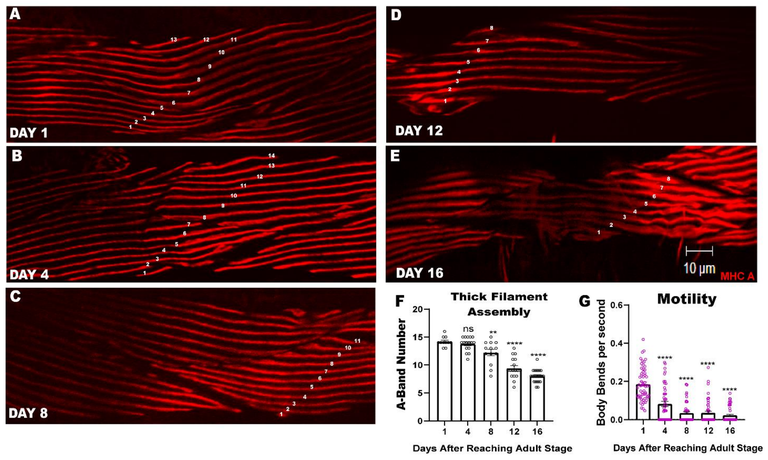


Figure 1. Muscle Thick filament assembly and whole nematode motility declines with age. A-E) are representative images of body wall muscle near the vulva immunostained with α-MHC A at different ages of adulthood (day1, 4, 8, 12, 16) with an A-band count depicted as white numbers along the filaments. F) is the quantification of A-band number at different ages of adulthood. G) is the quantification of crawling motility assays at different ages of adulthood. Statistic depicted are that day of adulthood (4,8,12, or 16) compared to day 1 of adulthood. There was no statistical difference between days 8,12 and 16 motility. **p-value < 0.005, ***p-value < 0.0005**** p-value < 0.0001.

UNC-45 DEGRADATION MAY BE INCREASED IN AGING MUSCLES.

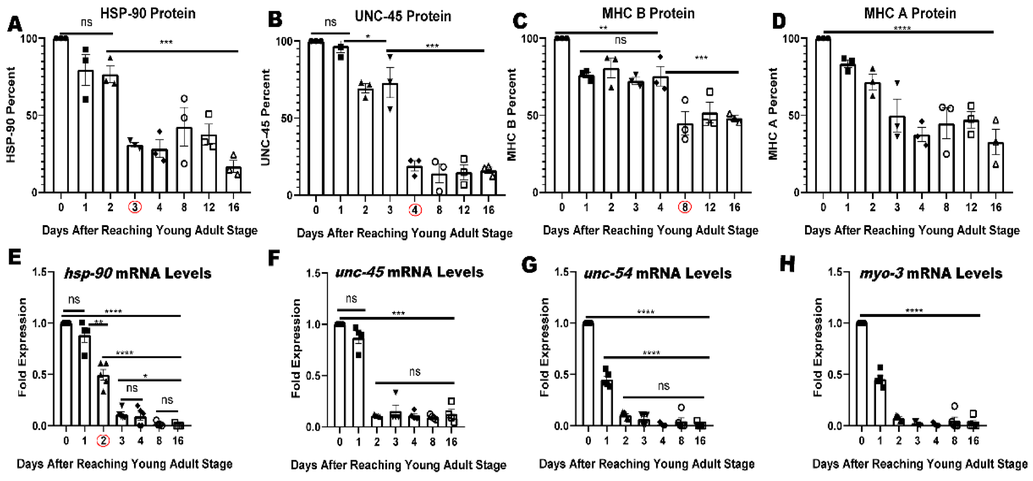


Figure 2. The Sequential Decline of HSP-90, UNC-45, and Myosin with Age A-D) Graphical quantification of steady state protein levels of HSP-90, UNC-45, MHC B, and MHC A (Myosin isoforms). Data are shown as a percentage of protein relative to Histone H3 protein. E-H) Steady state mRNA fold expression of *unc-45, hsp-90, unc-54* (MHC B), and *myo-3* (MHC A) during aging relative to *gpdh-2* (GAPDH). Days of significant protein or mRNA decline are circled with red circles. * p-value < 0.05 **p-value < 0.005, ***p-value < 0.0005**** p-value < 0.0001

DECREASE IN FUNCTIONAL HSP-90 PROTEIN RESULTS IN DECREASED UNC-45 PROTEIN.

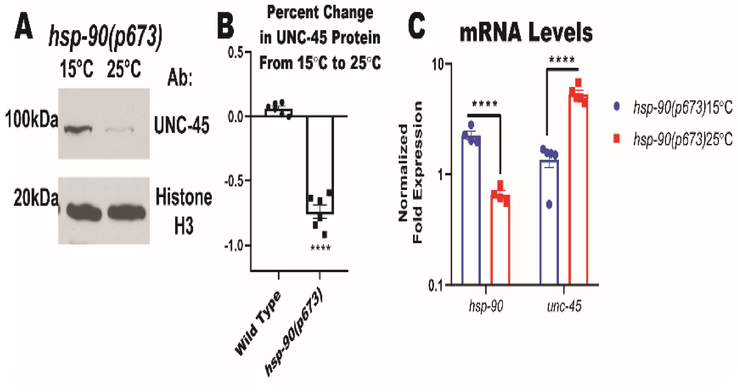


Figure 3. ***hsp-90*** **Loss of Function Temperature Sensitive Mutant Has Decreased UNC-45 Protein, but Not Transcript.** A) Western Blot of UNC-45 steady state protein levels. Histone H3 was used as the protein loading control B) shows quantification of UNC-45 protein percent reduction at 25°C relative to 15°C from wild type and *hsp-90(p673)*. C) shows the relative fold expression of *unc-45* and *hsp-90* mRNA levels of the *hsp-90(p673)* strain grown at 15°C and 25°C. *ges-1* (gut esterase) was used to normalize expression. * p-value<0.05 **** p-value < 0.0001.

A MUTANT WITH REDUCED LEVELS OF UNC-45 WHEN GROWN AT 25°C EXPERIENCES EARLY ONSET OF SARCOPENIA.

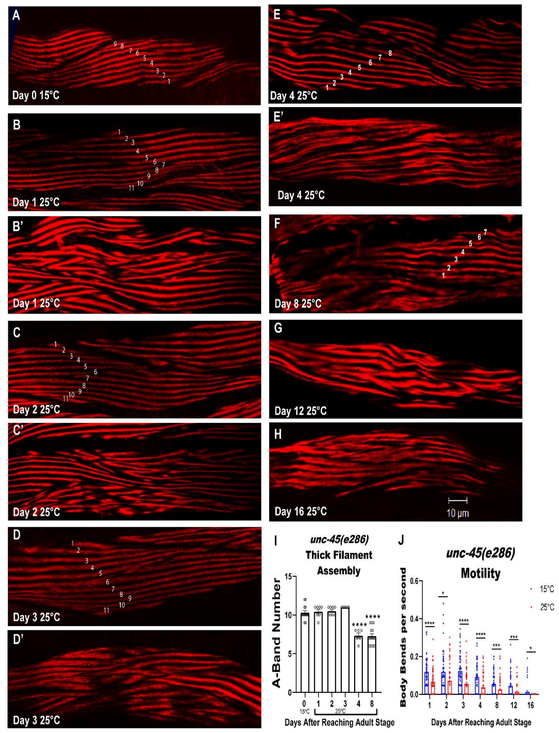


Figure 4. UNC-45 has a role in maintaining assembled thick filaments and nematode motility during adulthood The canonical unc-45 temperature sensitive mutant, e286, was allowed to develop normally at the permissive temperature of 15°C and shifted to the restrictive temperature of 25°C on day 0 of adulthood A) is a representative image of body wall muscle immunostained with α-MHC A at day 0 of adulthood after the animal was allowed to develop at 15°C B-H) are representative images or body wall muscle from animals grown at 25°C immunostained with α-MHC A at different adult ages (day1, 4, 8, 12, 16) with an A-band count depicted as white numbers for cells with parallel thick filaments (A-F). A'-E') are representative images of muscle cells that have perturbed thick filaments and for which A-band number could not be counted. I) is the quantification of A-band number at different ages of adulthood. J) is the quantification of crawling motility assays at different adult ages at the permissive (15°C) and restrictive (25°C) temperatures. **p-value < 0.005, ***p-value < 0.0005**** p-value < 0.0001

AN UNKNOWN UNC-45 POST TRANSLATIONAL MODIFICATION INCREASES WITH AGE

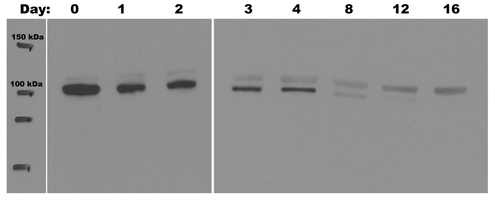


Figure 5. An Unknown UNC-45 PTM may Increase with Age. Western Blot with samples from different ages (0-16) blotted with an antibody to UNC-45. UNC-45 is 107 kDa, but an extra band, the size of modified UNC-45, can be seen above. This higher band increases with age as the lower band at 107 kDa decreases.

CONCLUSION

- **At day 2** ***hsp-90*** **mRNA declines. At day 3 HSP-90 protein declines. At day 4 UNC-45 protein declines. By day 8 MHC B protein has declined.**
- ***unc-45, unc-54*****, and** ***myo-3*** **mRNA all show a decline in mRNA immediately after reaching the young adult stage, which is typical of sarcomeric proteins with a low turnover rate.**
- **UNC-45 Ubiquitination and degradation may increase with age.**
- **HSP-90 may play an important role in UNC-45 regulation.**
- **First evidence that UNC-45 plays a pivotal role after development in adult muscle.**
- **Loss of UNC-45 during adulthood is sufficient to cause early onset of sarcopenia.**
- **An unknown UNC-45 modification increases with age and may contribute to the increase in degradation with age.**

- **FUTURE DIRECTIONS:**
  - **Determine if increasing UNC-45 in older animals is sufficient to alleviate the sarcopenic phenotype**
  - **Measure the UNC-45 decay rate in the hsp-90 mutant and in older animals**
  - **IP UNC-45 from day 0-16 animals and Blot with anti-ubiquitin to determine if UNC-45 ubiquitination increases with age**
  - **IP UNC-45 from older ages (day 12-16) and use mass spec. to determine PTM**