

Proteins In Balance: Muscle Development As An Example

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Proteins, made of linear chains made of amino acids, are essential parts of cells and organisms that catalyze biochemical reactions and are vital to metabolism. Beside catalytic activities, proteins also have structural or mechanical functions, such as actin and myosin in muscle. Folding, stability, activity, and function of certain proteins are often regulated by post-translational modification of specific amino acid residues.

Folding and stability of all cellular proteins (the proteome) is essential for cells and organisms throughout their lifetime. To adjust protein biogenesis to the demands of growth, differentiation, environmental stress and ageing, all cells possess a highly complex quality control network that balances synthesis, folding, translocation, assembly/disassembly, and turnover of proteins. The stability of the proteome (proteostasis) is challenged by exposure to proteotoxic environmental conditions, oxidant-induced covalent modifications, inherited polymorphisms, and misfolding.

The maintenance of protein homeostasis involves molecular chaperones and the ubiquitin-proteasome system (UPS) to refold or degrade unfolded proteins, respectively. Molecular chaperones have diverse roles to regulate protein conformation, and are essential to protect nascent polypeptides from misfolding. Proteotoxicity is a longterm challenge for cells and organisms increasing with age. A relatively small number of molecular chaperones contribute to a general protein folding homeostasis. Consequently, their overexpression enhances lifespan, whereas reduced abundance shortens lifespan. Chaperone-mediated adaptation to proteotoxicity is complemented by ubiquitin-dependent protein degradation. Despite its role in proteostasis, protein ubiquitylation turned out to be a key posttranslational control mechanism providing different fates of targeted substrates in diverse cellular processes including cell-cycle progression, signal transduction and development. The covalent attachment of the small polypeptide ubiquitin to internal lysine residues of protein substrates, required for degradation by the 26S proteasome, involves

ubiquitin-activating E1 enzymes, ubiquitin-conjugating E2 enzymes, ubiquitin protein E3 ligases, and sometimes polyubiquitylation factors (E4 enzymes). Since several ubiquitin ligases have been implicated in longevity, the UPS seems to contribute prominently to the regulation of cellular aging.

The arrangement of contractile proteins in striated muscles provides an impressive example how proteostasis coordinates synthesis, assembly and organisation of structural and motor proteins. Recent data suggest that the organization of myosin into sarcomeric structures is the result of a regulated multi-step assembly pathway that requires additional factors. Candidates for this process are members of a protein family containing a UCS (*UNC-45/CRO1/She4p*) domain, which have been indicated to be necessary for proper myosin function. One founding member of this family is *UNC-45*, for which homologs have been identified in a variety of organisms, from yeast to humans. It was demonstrated that the UCS domain of *UNC-45* interacts with muscle myosin and exerts chaperone activity onto the myosin head, whereas its N-terminal TPR domain (*tetratricopeptide repeat*) binds the general molecular chaperone Hsp90. Thus, *UNC-45* functions both as a molecular chaperone and as an Hsp90 co-chaperone for myosin during muscle thick filament assembly. Consequently, mutations in *C. elegans unc-45* result in paralyzed animals with severe myofibril disorganization in striated body wall muscles.

Our recent work revealed that protein levels of the myosin chaperone *UNC-45* are subject to stringent regulation, which appears to be dependent on *UFD-2* and *CHN-1* ubiquitylation activity. *UFD-2* is an ortholog of yeast *UFD2* known to bind oligoubiquitylated substrates to catalyze the addition of further ubiquitin moieties in the presence of E1, E2 and E3 enzymes. Thus, *UFD2* defines a novel enzymatic activity that mediates multiubiquitin chain assembly, needed for subsequent proteasomal degradation and thus was termed E4 enzyme. The human *CHN-1*

ortholog CHIP was identified both as a co-chaperone of Hsc70 and Hsp90 and to be an E3 enzyme. Thus, CHIP probably acts as a protein quality-control ubiquitin ligase that selectively leads abnormal proteins recognized by molecular chaperones to degradation by the 26S proteasome.

We were able to show that either UFD-2 or CHN-1 alone, in collaboration with E1 and E2, conjugates UNC-45 with one to three ubiquitin moieties. Therefore, both CHN-1 and UFD-2 work independently as E3 enzymes in this pathway. However, in combination, CHN-1 and UFD-2 increase the ubiquitylation of UNC-45. Movement defects of *unc-45* thermosensitive (*ts*) mutants are suppressed in animals lacking CHN-1 or UFD-2 most likely due to stabilization of the corresponding UNC-45 (*ts*) proteins. Interestingly, analysis of body wall muscle cells by polarized light microscopy showed that the muscle structure of *chn-1* and *ufd-2* knockout worms is comparable to that of wild-type, however overexpression of transgenic *unc-45* leads to strong sarcomeric assembly defects. Therefore, the amount of UNC-45 protein present in the muscle cells is critical for proper thick filament function.

Another factor that we identified to be involved in targeting the myosin assembly chaperone UNC-45 for degradation is the ubiquitin-selective chaperone CDC-48 (Figure 1). Its homologs Cdc48p in yeast and p97 in mammals belong to the family of AAA-type ATPases and form homohexameric rings with chaperone-like activity. CDC-48/p97 is intimately linked to the ubiquitin pathway because its central role is to bind and segregate ubiquitylated proteins to extract these from their binding partners for substrate recruitment and ubiquitin chain assembly. In *C. elegans*, we found that CDC-48 forms a complex together with UFD-2 and CHN-1 to regulate UNC-45 protein levels. This trimeric complex links turnover of UNC-45 to functional muscle formation. Our recent work showed an upregulation of *ufd-2*, *chn-1*, and *cdc-48* transcripts during larval stages in which body-wall muscle development mainly

occurs. This observation suggests that the formation of the CDC-48/UFD-2/CHN-1 complex could be developmentally regulated by muscle-specific co-expression.

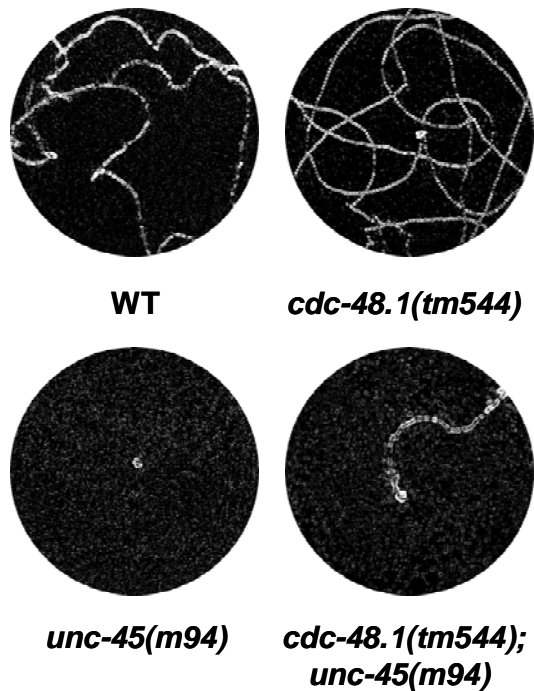


Figure 1. CDC-48 regulates the myosin chaperone UNC-45. Suppression of the movement defect of temperature sensitive *unc-45(m94)* worms. The bacterial lawns on the plates show traces of temperature shifted worms after crawling for 1 h at 22°C for *cdc-48.1(tm544)* and *unc-45(m94)* single and double mutants, and wild-type (WT). Ten young adults were assayed for each strain and all displayed similar motility.

Intriguingly, a similar pathway required for muscle development might exist in humans as well, since mutations in p97 are known to cause a dominantly inherited form of inclusion body myopathy (IBM). Direct binding and co-localization between p97 and the mammalian UFD-2 and CHN-1 homologs, Ufd2a and CHIP, indicates regulation of myosin assembly by an evolutionarily conserved p97/Ufd2a/CHIP complex (Figure 2A). Consistent with the hypothesis that such a complex could be required for vertebrate muscle formation, both Ufd2a and CHIP have been implicated in cardiac and skeletal myogenesis or cardiotoxic resistance, respectively.

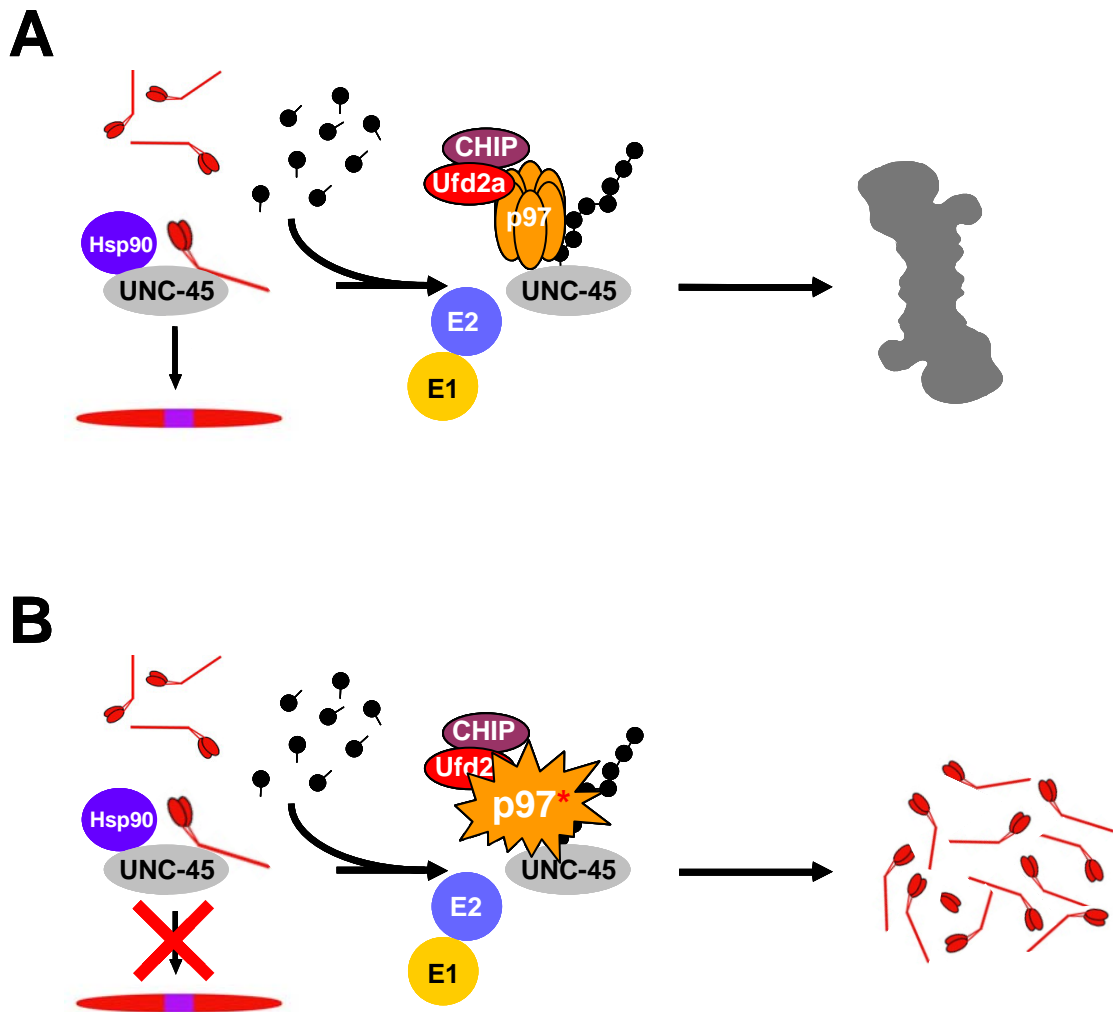


Figure 2. Model for UNC-45-dependent myosin assembly. The myosin-directed chaperone UNC-45 binds myosin and Hsp90 simultaneously in muscle thick filament assembly. (A) The conserved p97/Ufd2a/CHIP complex directly multiubiquitylates UNC-45, leading to subsequent degradation by the 26S proteasome. Development specific assembly of the multiubiquitylation complex seems to connect UNC-45 turnover to functional muscle formation. (B) IBMPFD-causing mutations in human p97 disrupt the ubiquitylation process resulting in increased levels of UNC-45. The stabilization of UNC-45 probably disturbs the integration of myosin into sarcomeric structures or supports their disassembly. High amounts of unassembled myosin might then induce protein aggregation in muscle cells.

IBM associated with Paget's disease of bone and frontotemporal dementia (IBMPFD) is an inherited age-related disorder that produces adult-onset muscle wasting and weakness and is characterized by muscle pathology including cytoplasmic and nuclear aggregates in skeletal and cardiac muscle. We demonstrated that in contrast to wild-type, mutations in p97 known to cause myopathy are not able to replace CDC-48 throughout the UNC-45-dependent myosin assembly pathway in worms. Moreover, the degradation of human UNC-45 is abrogated by the same IBMPFD-associated p97 mutations, resulting in severely disorganized myofibrils and sarcomeric defects. Therefore, p97 seems to regulate UNC-45 levels during the process of myofibre differentiation and muscle maintenance, which is abolished during pathological conditions resulting in the accumulation of aggregated proteins during aging.

The pathogenic mechanisms that cause muscle weakness in IBM, and IBMPFD in particular, might be related to the aggregation of stabilized or misassembled proteins. How these protein aggregates and finally inclusion bodies are formed in the presence of p97 mutations is not clear. Interestingly, another dominantly-inherited form of IBM is caused by mutations in the head region of fast myosin IIa (MYH2), which render MYH2 to aggregate. Consistent with such a myosin-based inclusion body formation, stabilization of UNC-45 may disturb the integration of myosin into sarcomeric structures or support their disassembly. The resulting accumulation of unassembled myosin in the cytosol might then induce protein aggregation in both skeletal and cardiac muscle (Figure 2B).

This example of myosin assembly regulation pointed out that the striated muscle as a multiprotein complex depends on a defined balance between folding, assembly and proteolysis. Consequently, a decline in proteostasis capacity during aging or pathophysiological conditions might contribute to decreased muscle filament

stability and protein aggregation. Given that missense mutations in general are masked by the proteostasis network in early life, could provide an explanation for the age-dependancy of many heritable protein aggregation diseases, such as Parkinson's and Huntington's disease.