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Less Is More: How Protein Degradation Regulates Muscle Development

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Abstract. The organization of sarcomeric structures during muscle development involves regulated multistep assembly pathways. The myosin assembly factor UNC-45 functions both as a molecular chaperone and as an Hsp90 co-chaperone for myosin throughout muscle thick-filament formation. Consequently, mutations in *unc-45* result in paralyzed worms with severe myofibril disorganization in striated body wall muscles. Our data suggest that functional muscle formation in *Caenorhabditis elegans* is linked to ubiquitin-dependent UNC-45 turnover, regulated by the E3 enzymes UFD-2 and CHN-1 in cooperation with the ubiquitin-selective chaperone CDC-48 (also known as p97 in human). Missense mutations in the gene encoding p97 are known to cause a dominant, late-onset hereditary inclusion body myopathy. Remarkably, we identified a conserved role of CDC-48/p97 in the process of myofiber differentiation and maintenance, which appears to have important implications for understanding defects in muscle formation and maintenance during pathological conditions.

The assembly of myosin into thick filaments during muscle development is still a largely unexplored phenomenon (Barral and Epstein 1999). Recent data suggest that the organization of myosin into sarcomeric structures is the result of a regulated multistep assembly pathway that requires additional factors. Candidates for this process are

members of a protein family containing a UCS (*U*NC-45/*C*RO1/*S*he4p) domain, which have been indicated to be necessary for proper myosin function (Hutagalung et al. 2002). One founding member of this family is UNC-45, for which homologs have been identified in a variety of organisms, from yeast to humans (Hutagalung et al. 2002). 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 (Barral et al. 2002). 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* (Epstein and Thomson 1974) result in paralyzed animals with severe myofibril disorganization in striated body wall muscles (Barral et al. 1998).

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 (Hoppe et al. 2004; Janiesch et al. 2007). 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 (Hoppe 2005; Koegl et al. 1999). The human CHN-1 ortholog CHIP was identified both as a co-chaperone of Hsc70 and Hsp90 and to be an E3 enzyme (Ballinger et al. 1999; Connell et al. 2001). 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 (Cyr et al. 2002; Murata et al. 2003).

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 (Hoppe et al. 2004). 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 (Janiesch et al. 2007). 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 (Fig. 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 (Rouiller et al. 2000). 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 (Rape et al. 2001; Ye 2006). 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 (Janiesch et al. 2007). This observation suggests that the formation of the CDC-48/UFD-2/CHN-1 complex could be developmentally regulated by muscle-specific co-expression.

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) (Watts et al. 2004). Direct binding and co-localization between p97 and the mammalian UFD-2 and CHN-1 homologs, Ufd2a and CHIP, indicate regulation of myosin assembly by an evolutionarily conserved p97/Ufd2a/CHIP complex (Fig. 2a) (Janiesch et al. 2007). Consistent with the hypothesis that such a complex could be required for vertebrate muscle formation, Ufd2a and CHIP have been implicated in cardiac and skeletal myogenesis or cardiotoxic resistance, respectively (Ballinger et al. 1999; Kaneko et al. 2003; Mahoney et al. 2002).

IBM associated with Paget disease of bone and frontotemporal dementia (IBMPFD) is an inherited disorder that produces adult-onset muscle wasting and weakness and is characterized by muscle pathology

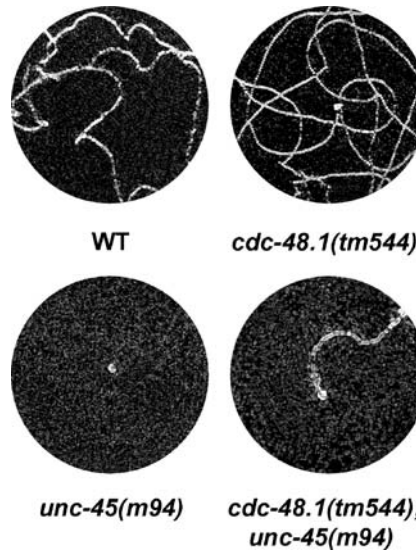

Fig. 1. CDC-48 regulates the myosin chaperone UNC-45, suppressing the movement defect of temperature-sensitive *unc-45(m94)* worms. The bacterial lawns on the plates show traces of temperature shifted worms, *cdc-48.1(tm544)*, *unc-45(m94)*, double mutants, and wild-type (*WT*), after crawling for 1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

including cytoplasmic and nuclear aggregates in skeletal and cardiac muscle (Watts et al. 2004). 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 (Janiesch et al. 2007). Therefore, p97 seems to regulate UNC-45 levels during the process of myofiber differentiation and muscle maintenance, which is abolished during pathological conditions, resulting in the accumulation of aggregated proteins.

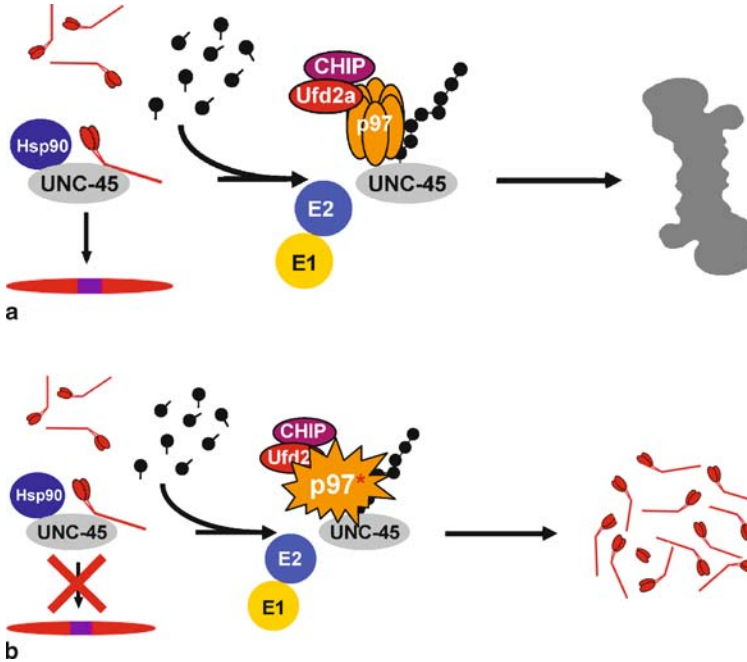

Fig. 2a,b. Model for UNC-45-dependent myosin assembly. The myosin-directed chaperone UNC-45 binds myosin and Hsp90 simultaneously in muscle thick-filament assembly. **a** The conserved p97/Ufd2a/CHIP complex directly multiubiquitylates UNC-45, leading to subsequent degradation by the 26S proteasome. 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

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 (Martinsson et al. 2000; Tajsharghi et al. 2005). 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 (Fig. 2b). Our future studies will address the molecular mechanism underlying the process of inclusion-body formation and hopefully help us understand the connection between protein degradation and muscle development.

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