

# Chaperoning myosin assembly in muscle formation and aging

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The activity and assembly of various myosin subtypes is coordinated by conserved UCS (UNC-45/CRO1/She4p) domain proteins. One founding member of the UCS family is the *Caenorhabditis elegans* UNC-45 protein important for the organization of striated muscle filaments. Our recent structural and biochemical results demonstrated that UNC-45 forms a protein chain with defined periodicity of myosin interaction domains. Intriguingly, the UNC-45 chain serves as docking platform for myosin molecules, which promotes ordered spacing and incorporation of myosin into contractile muscle sarcomeres. The physiological relevance of this observation was demonstrated in *C. elegans* by transgenic expression of UNC-45 chain formation mutants, which provokes defects in muscle structure and size. Collaborating with the molecular chaperones, Hsp70 and Hsp90, chain formation of UNC-45 links myosin folding with myofilament assembly. Here, we discuss our recent findings on the dynamic regulation of UNC-45 structure and stability in the context of muscle regeneration mechanisms that are affected in myopathic diseases and during aging.

## UNC-45-a Myosin-Directed Chaperone

Assembly and function of striated muscle depends on the precise organization of structural and motor proteins into contractile repeats called the sarcomeres (Fig. 1). These are highly ordered units providing a periodic framework that coordinates gliding between thin actin and thick myosin filaments with muscle contraction.<sup>1,2</sup> The incorporation of myosin into contractile

muscle thick filaments requires precise temporal and spatial control afforded by the general chaperones Hsp70 and Hsp90,<sup>3-5</sup> and UCS (UNC-45/CRO1/She4p) domain proteins.<sup>6-8</sup> One founding member of the UCS family is the myosin-directed chaperone UNC-45, which was originally identified in *C. elegans*.<sup>9-11</sup> According to its role in myosin assembly, mutations in UNC-45 result in disorganized striated body wall muscles and drastic movement defects of worms.<sup>6,9,10</sup> The existence of UNC-45 homologs in vertebrates indicates a conserved requirement for myosin-specific chaperones in muscle formation. Vertebrate genomes contain two UNC45 isoforms, namely UNC45a and UNC45b, which have different expression patterns and functions.<sup>12</sup> *UNC45a* is expressed in all tissues implicated in progesterone receptor maturation, cell proliferation, oncogenesis, and formation of the circulatory system.<sup>12-15</sup> In contrast, *UNC45b* expression is limited to myogenic processes in cardiac and skeletal muscles, respectively.<sup>12</sup> Downregulation by RNA interference (RNAi) or loss of function mutants of *UNC45b* results in myosin assembly defects and disorganization of sarcomeric structures.<sup>4,16</sup>

## UNC-45 Chains Coordinate Folding and Assembly of Muscle Myosin

Whereas the role for Hsp90 in myosin folding is well described in different organisms, the mechanistic requirement for UNC-45 is less clear.<sup>3,17,18</sup> UNC-45 directly binds to muscle myosin through its UCS domain in vitro. This interaction seems to be important for correct incorporation of myosin into sarcomeric structures since mutations

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in the UCS domain result in paralyzed worms with severe myofibril disorganization.<sup>6,9,10</sup> Moreover, UNC-45 cooperates throughout the process of myosin folding and assembly with Hsp70 and Hsp90, which bind to its TPR (tetratricopeptide repeat) domain. Thus, UNC-45 provides substrate specificity for the partner chaperones during late stages of sarcomere formation and myofibrillogenesis.

To clarify how UNC-45 coordinates both myosin folding and muscle thick filament assembly, we teamed up with the laboratory of Dr Tim Clausen (IMP) and combined biochemical and structural analyses with physiological studies in *C. elegans*. Besides the known N-terminal TPR domain, the central region, and the C-terminal UCS domain, the crystal structure of the full-length UNC-45 protein showed an additional neck domain.<sup>19</sup> The neck region links TPR and central domains with the UCS domain, which together form a mouth-like structure (Fig. 1). Interestingly, additional co-crystallization studies demonstrated that Hsp70 and Hsp90 interact with the N-terminal TPR domain, indicating that indeed both chaperones support UNC-45 in myosin folding and assembly.

The most intriguing finding of our work established the assembly of linear UNC-45 protein chains by interaction between neck region and TPR domain. The UCS domain is not involved in UNC-45 polymer formation and sticks out of the UNC-45 backbone providing a periodic sequence of myosin binding sites. The multimeric structural organization of TPR and UCS domains thus ensures functional independence in binding co-working chaperones and client substrates. Binding of Hsp70/90 to the TPR domain of UNC-45 facilitates the coordinated folding of myosin molecules with defined periodicity. Intriguingly, the structural distance between the UNC-45 tandem modules would allow them to act on dimeric myosin heads that extend from the coiled-coil backbone of growing muscle thick filaments. The structural prediction that multimeric UNC-45 chains assist the assembly and growth of muscle sarcomeres is indeed agreeably verified in *C. elegans*. Transgenic expression of UNC-45 mutants defective in chain assembly causes dominant-negative defects in the

sarcomere organization and myofibrillogenesis of wild-type worms.

Therefore, our structural and biochemical analyses demonstrated that UNC-45 forms linear protein chains offering a multisite docking platform for molecular chaperones and substrate proteins, which is highly compatible with the formation of polar myosin filaments. These findings provide first ideas how myosin molecules might get incorporated into contractile sarcomeric structures during growth and maintenance of striated muscle.

### Dynamic Regulation of UNC-45 Chain Stability

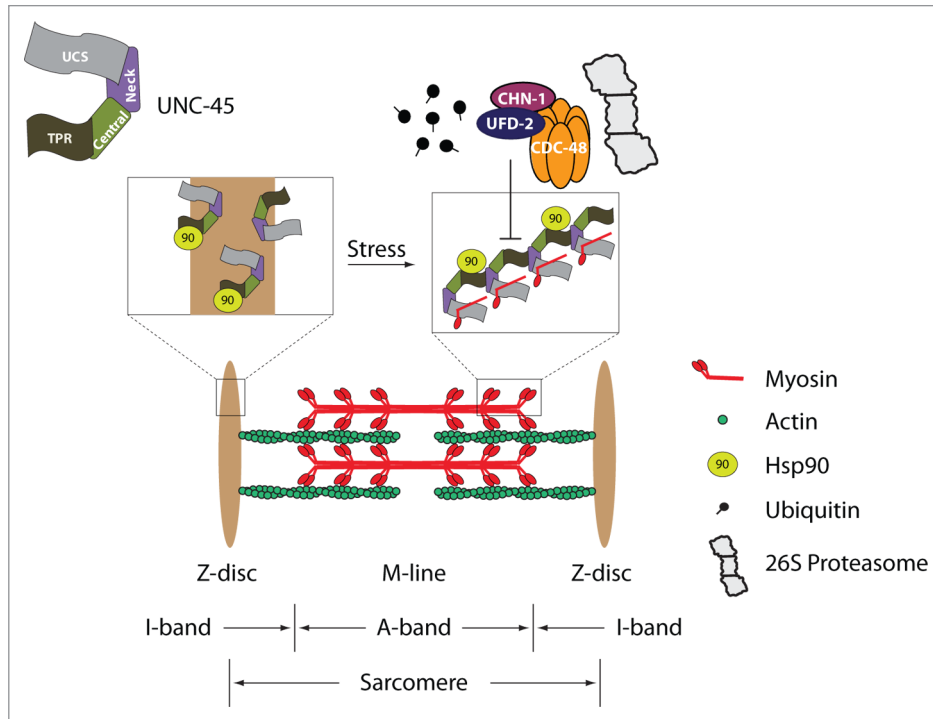
The formation of UNC-45 chains seems to be a highly dynamic process. The small binding interface of the oligomer might facilitate rapid (dis)assembly of UNC-45 chains, which usually contain not more than two to five subunits in vitro. The transient nature of UNC-45 polymers is additionally reflected by the dominant-negative effect of UNC-45 interface mutants in vivo, which could terminate the oligomerization process when incorporated into the growing chaperone chain. Thus, UNC-45 is arranged in short assembly lines providing multisite binding scaffolds to act specifically on dimeric myosin heads along growing myofilaments rather than forming unlimited multimeric structures. In addition to muscle development, UNC-45 also supports myosin integrity under stress conditions.<sup>20</sup> This role in muscle regeneration requires rapid relocation of UNC-45 between different sarcomeric regions, which would be hindered by stable UNC-45 filaments (Fig. 1).

Besides the structural conditions, the elusive appearance of UNC-45 chains might be further influenced by post-translational modifications and/or the amount of UNC-45. Interestingly, the stability of UNC-45 is tightly controlled at the protein level, which is important for muscle function.<sup>21-23</sup> Our previous work revealed that polyubiquitylation of UNC-45, mediated by the E3 enzymes UFD-2 and CHN-1 together with the ubiquitin-selective chaperone CDC-48, results in its degradation by the 26S proteasome.<sup>21,23</sup> Consistently, overexpression of UNC-45 in *cdc-48.1*, *ufd-2*, and *chn-1* deletion mutants induces

strong sarcomeric assembly defects in worms.<sup>21,23</sup> Thus, the regulation of UNC-45 protein levels in muscle cells appears to be critical for correct myofilament organization.<sup>21-23</sup> The developmental timing of UNC-45 turnover is defined by muscle-specific assembly of the CDC-48/UFD-2/CHN-1 ubiquitylation complex.<sup>21</sup>

A similarly conserved proteolytic pathway appears to be critical for myosin assembly in humans as well since functional p97, the human homolog of CDC-48, is required for UNC45b turnover.<sup>21,24</sup> Consistently, certain missense mutations in p97 are directly linked to inclusion body myopathy (inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia, called IBMPFD) causing muscle wasting and protein aggregates in skeletal and cardiac muscle.<sup>25</sup> Myopathy-related p97 mutations are not able to replace CDC-48 in UNC-45 degradation in worms. Moreover, the turnover of human UNC-45 is abrogated by the same IBMPFD-associated p97 mutations, resulting in severely disorganized myofibrils and sarcomeric defects in patient myotubes.<sup>21</sup> 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. 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.<sup>26</sup> Sure enough, UFD2a and CHIP have been implicated in cardiac and skeletal myogenesis or cardiotoxic resistance.<sup>27,28</sup> The pathological consequence of increased UNC45b levels on sarcomere formation is emphasized by the observation that human UNC45b is highly upregulated upon ischemic heart failure.<sup>29</sup>

According to the transient nature of UNC-45 polymers, the chain assembly process might be modulated by the CDC-48/p97-dependent ubiquitylation pathway. UNC-45 ubiquitylation either reduces the pool of monomeric UNC-45 at the muscle sarcomere available for chain formation. Alternatively, ubiquitylation of specific site chains within the binding interface block direct interaction between UNC-45



**Figure 1.** Model for the regulation of UNC-45 chain formation in muscle maintenance. The sarcomeric unit is defined by the distance between two Z-discs, including the A-band, I-band, and M-line. The A-band is the region of interdigitation between thick myosin (red) and thin actin (green) filaments, whereas the I-band mainly contains thin filaments. UNC-45 (different domains indicated) composes tandem modules that allow the simultaneous binding of Hsp70/Hsp90 and myosin, enabling the folding and assembly of myosin in regular spacing. In the fully developed muscle, monomeric UNC-45 might be stored at the Z-disk, which anchors the thin actin filaments of the I-band. Under stress conditions, UNC-45 is relocated to damaged myosin filaments of the A-band and might assemble into short chaperone chains to maintain the sarcomeric structure especially during muscle regeneration and aging. The process of UNC-45 chain formation might be influenced by the conserved CDC-48/UFD-2/CHN-1 ubiquitylation complex. The ubiquitylation of UNC-45 either reduces the pool of the monomeric form available for chain formation or inhibits UNC-45 polymerization directly by modifying the binding interface.

proteins (Fig. 1). In fact, preliminary data suggests that mainly lysine residues within the neck region and the UCS domain are targets for ubiquitylation, which might interfere with both UNC-45 chain formation and myosin binding. Post-translational modifications and/or cellular factors that fine-tune UNC-45 chain assembly thus add an additional layer of regulation for the coordination of myosin assembly and muscle functionality.

### Impact in Muscle Maintenance and Aging

In contrast to the well-established role of UNC-45 in muscle development, the contribution in sarcomere integrity and muscle maintenance is less defined. UNC-45 remains associated with fully functional and folded myosin in matured thick filaments of the body wall muscle in worms, indicating that UNC-45 is still needed in the fully assembled sarcomere.<sup>30</sup> Moreover,

zebrafish UNC45b persists at a particular sarcomeric structure called the Z-disk after completion of myosin assembly. Muscle damage induces UNC45b translocation to thick filaments where it stabilizes and maintains myosin molecules.<sup>20</sup> Based on our model, UNC-45 might be stored on the Z-disk in its monomeric form whereas stress-induced relocation of UNC-45 to growing or damaged myofibrils might facilitate the assembly of polymeric UNC-45 chains (Fig. 1). Thus, the dynamic regulation of UNC-45 chain formation could provide the flexibility to rapidly respond to physiological changes and stress conditions associated with muscle development and regeneration mechanisms that are affected in myopathic diseases and during aging. The formation of stable UNC-45 chains of infinite length would otherwise impede relocation within sarcomeric substructures and, thus, limit the functionality of UNC-45 during muscle maintenance. Ubiquitin-dependent spatial and temporal control of

UNC-45 chains (dis)assembly might further help to reduce interference with the myosin folding and assembly process.

The process of muscle aging called sarcopenia is characterized by the accumulation of misfolded proteins and a reduction of muscle mass, which involves sarcomeric disintegration and disorganization. In line with the reported upregulation of the ubiquitin proteasome system (UPS) upon sarcopenia, aged muscles contain higher levels of p97 and CHIP. This upregulation correlates with the decline of UNC45b, suggesting defects in assembly of new myofibrils and limited maintenance of old ones.<sup>31</sup> Similarly, silencing *unc-45* expression in the *Drosophila* heart leads to reduced myosin levels and short lifespan, whereas the opposite effect is caused by overexpression.<sup>32</sup> Studying the effect of UNC-45 filament mutants blocking chain formation will clarify how myosin folding and assembly is coordinated with muscle maintenance and during aging.

## Concluding Remarks

The precise organization of structural and motor proteins in contractile sarcomeric repeats involves temporal and spatial coordination of myosin folding and incorporation into growing myofilaments. The myosin-directed chaperone UNC-45 appears to be a central regulator of this multistep assembly process. UNC-45 forms an oligomer which resembles the spacing of myosin motor domains in muscle fibers. Thus, UNC-45 chaperone chains might function as a “template” defining the geometry, regularity, and periodicity of myosin arranged into muscle thick filaments. The dynamic oligomerization of UNC-45 polymers is well suited to respond upon mechanic and age-related stress conditions and might be modulated by a conserved ubiquitylation pathway (Fig. 1). Our structural and mechanistic data provide first ideas how myosin folding and thick filament assembly are coordinated under different physiological conditions, including development, regeneration, and aging of the muscle. These results identify UNC-45 as a prime candidate for chronic age-related diseases, including muscle wasting and heart failure.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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