Abstract

The establishment and maintenance of myofibrillar structures is a highly complex process, which is also controlled via the regulated, proteasomal degradation of involved components. In the nematode Caenorhabditis elegans the myosin-specific chaperone UNC-45 plays a central role in the assembly of thick filaments during muscle development. Recent findings suggest that UNC-45 is a subject of stringent regulation by the two ubiquitin ligases CHN-1 and UFD-2.
In regard of the mechanistic process of UNC-45 ubiquitination and the question why two E3 ligases are required for this regulation, a special focus of this work was the analysis of CHN-1 and its ability to dimerize. It was shown that dimerization of CHN-1 is not a prerequisite for its primary ubiquitination activity, in contrast to the published assumption. Instead, evidence was found that the dimerization could have an impact on the nature of ubiquitin linkages made by the E3 ligase. This is particularly interesting in view of the fact that the homologous protein CHIP in addition to its role as a Co-chaperone in protein quality control also interacts with other ubiquitin ligases and is thereby involved in the regulation of a variety of proteins. In this regard evidence arose from this study that the functionality of CHN-1 in different complexes could be determined by its quaternary structure. It was also shown that in case of UNC-45 ubiquitination monomeric CHN-1 seems to be suffcient. From the data presented here, a possible model of UNC-45 regulation was developed and discussed.
Another focus of this work was the structure-function analysis of the myosin chaperon UNC-45. For this purpose, a protocol for purification of UNC-45 was established and a polyclonal antibody was raised against purified protein. From the detailed biochemical analysis of phenotypically relevant UNC-45 mutations could be concluded, that UNC-45 is present in an oligomeric state, which seems to be a prerequisite for its correct function. In cooperation with the research group of Dr. Tim Clausen from the IMP in Vienna, the first complete structure of UNC-45 protein has been solved.