Abstract

Tropomyosin (Tpm) is an actin-binding protein that plays central role in the regulation of the thin filament. Tpm extends along the filament and binds seven consecutive actin subunits through periodic repeats P1-P7. Together with the troponin (Tn) complex, Tpm controls actin-myosin interactions in the Ca^{2+}-dependent manner. In addition, Tpm influences thin filament stability and turnover, processes regulated by actin-binding proteins, including cofilin (Cof), which promotes actin-filament severing and depolymerization, and tropomodulin (Tmod), which caps the filament's pointed end and limits actin subunit exchange. Missense mutations in Tpm genes are associated with several congenital myopathies and distal arthrogryposes. The molecular mechanisms linking local structural changes in Tpm to altered thin filament regulation and disease phenotypes remain incompletely understood.

The aim of this thesis was to determine how two actin-binding periodic repeats of Tpm2.2, P1 and P5, which participate in interactions with actin and Tn and contribute to the regulation of myosin binding, affect functional communication among actin, myosin, Tn, Cof, and Tmod. To achieve this goal, four disease-associated pathogenic mutations in the *TPM2* gene, encoding the skeletal muscle Tpm isoform Tpm2.2 were selected: substitutions D20H and E181K, associated with hypercontractile phenotypes and located in the N-terminal halves of P1 and P5, and E41K and N202K, associated with hypocontractile phenotypes and located in the C-terminal halves of these periods. Thin filaments reconstituted from recombinant and muscle-isolated proteins were used in *in vitro* assays to examine the roles of the P1 and P5 regions in protein–protein interactions, actin polymerization, filament length and stability, and Cof2-dependent severing and depolymerization.

None of the pathogenic variants substantially altered the affinity of Tpm2.2 neither for F-actin alone nor with Tn, excluding loss of stable filament association as the disease mechanism. However, the hypercontractile variants changed orientation of Tpm2.2 on the filaments, which released inhibition of the actomyosin ATPase. In contrast, the hypocontractile variants orientation on the filament was not changed, so that they inhibited ATPase activity to the same extent as the wild type. The mutation-dependent phenotypes were also evident in the presence of Tn complex ($\pm Ca^{2+}$). The hypocontractile variants failed to fully activate ATPase activity while retaining their inhibitory capacity, whereas the hypercontractile Tpm2.2 variants produced excessive activation

but exhibited reduced inhibition of actomyosin ATPase activity. Single-turnover experiments revealed no significant effects of mutations on ATP binding or actomyosin dissociation and reassociation. However, opposite effects on product release were observed. Hypercontractile variants accelerated ADP release, whereas hypocontractile variants slowed it, providing a direct kinetic basis for the altered motor performance. In the *in vitro* motility assay, the hypercontractile variants increased myosin-driven actin translocation, whereas the hypocontractile variants reduced it, consistent with the hyper- and hypocontractile clinical phenotypes. These phenotypic differences were also reflected in Ca^{2+} sensitivity, which was increased by the hypercontractile variants and decreased by the hypocontractile variants. Independently of the phenotype, mutations in P1 enhanced the direct interaction between Tpm2.2 and Tn in the absence of actin, whereas mutations in P5 had no effect, indicating that changes in the affinity of Tn for Tpm2.2 did not determine the phenotypic differences between Tpm2.2 variants.

The mutations did not affect actin polymerization kinetics and the filament length at the steady-state. Analysis of filament length under mechanical load generated by ATP-driven actomyosin interactions showed that the mutations affected filament stability in a context-dependent manner. The presence of Tn strongly protected the filaments against Cof2-induced severing and depolymerization; however, the effects of the mutations again depended on their location within Tpm2.2 and on the presence of Tmod1. Because the mutations did not alter the affinity of either Cof2 or Tmod1 for actin, the observed effects on filament length and stability are likely to result from the interplay between multiple factors.

In conclusion, the primary function of actin-binding periodic repeats P1 and P5 is to regulate contraction by controlling actin–myosin interactions. The effects of pathogenic mutations within these repeats closely correlate with the hyper- and hypocontractile phenotypes observed in patients. These mutations also destabilize the filaments and alter their length, which may contribute to filament disarray and the formation of abnormal structures observed in patients' muscle cells.