The effects of myopathy-causing mutations in *TPM2* on tropomyosin-dependent regulation of the thin filament length

Importance of the thin filament length maintenance in muscle contraction

The intricacies of muscle contraction have long fascinated scientists. Due to numerous studies many groundbreaking discoveries were made. However, not all aspects of the regulation of muscle contraction are fully understood. The contraction of the sarcomere, the basic contractile unit of muscle cells, is driven by the cyclic interactions between myosin heads protruding from the thick filaments and actin thin filaments. One of the main questions that are still not answered is how is maintained the optimal filament length, which directly impacts the number of myosin heads that bind to actin. It is important because the more myosin heads bind to actin, the more force is produced during contraction. Interestingly, while the lengths of myosin thick filaments are uniform, the lengths of thin filaments vary within certain limits in different muscle fibers. Thus, what is the molecular mechanism that maintains the thin filament length?

Tropomyosin (Tpm) is an elongated protein that polymerizes along the entire thin filament forming long chains. Tpm binds troponin (Tn), a protein which can shift the position of Tpm chains on the filament in the Ca^{2+}-dependent manner. Together, Tpm and Tn control access of other ligands, including cofilin (Cof), and tropomodulin (Tmod), two proteins which play a role in maintaining the length of thin filaments. Tpm assists in binding of Tmod to one of the ends of the filament. When Tmod is strongly bound, the filaments elongation is inhibited. On the other hand, Tpm regulates Cof ability to cut and depolymerize actin filaments. Through these interactions, Tpm can modulate muscle contraction by maintaining specific thin filament length.

Mutations in the *TPM2* gene encoding Tpm2.2 – the muscle isoform of Tpm, have been implicated in severe muscular disorders known as congenital myopathies and arthrogryposes. These disorders are manifested by a variety of symptoms, including muscle weakness or excessive contraction. It remains unclear whether mutations in *TPM2* cause variations in thin filament length and whether these changes contribute to the development of pathological symptoms.

Aims of the project

By investigating the effects of *TPM2* mutations on thin filament length, I intend to find a molecular mechanism that potentially links variations of the thin filament with etiology of congenital muscle diseases. I am planning to collect experimental data which will explain whether Tpm2.2 is involved in the thin filament length regulation and whether disease-causing mutations disrupt these functions. More specifically, I will examine effects of the mutations on the actin filament stability, binding of Tmod to actin and its ability to inhibit actin filament elongation, and regulation of severing and depolymerizing activity of Cof.

Description of the research

To investigate the effects of mutations in *TPM2*, I will purify actin, Tn and myosin from chicken muscle. The other proteins will be recombinant, which means that genes encoding the proteins will be transformed into bacterial cells and bacteria will be induced to produce high amounts of the proteins. Mutations found in patients diagnosed with myopathies have already been introduced in the DNA encoding Tpm2.2. The purified muscle and recombinant proteins will be combined together to reconstitute the thin filaments. Biochemical and fluorescence microscopy assays will be used to see how the mutations affect Tpm2.2 functions and regulation of the then filament length by Tmod and Cof.

Substantial results expected

Execution of this project will uncover new mechanisms involved in congenital muscle diseases and will provide information on the mechanisms responsible for development of congenital muscle diseases. The studies will ensure a comprehensive analysis of the interplay between actin, Tpm2.2, Tn, Cof, and Tmod in regulating thin filament length. This study will help in understanding the molecular bases development of the so far incurable congenital muscle diseases.