Revealing the molecular mechanisms of muscle weakness in an inherited myopathy

Inherited myopathies are a newly discovered class of muscle diseases normally present at birth. They are diverse but often characterized by weakness in facial, masticatory, limb and respiratory muscles [1]. This class of diseases is due to mutations in different genes encoding proteins involved in muscle contraction [1]. Among the related genes, there are *TPM2* and *TPM3*. They encode tropomyosin which is a 284-residue coiled-coil protein composed of two α-helical chains, forming a rod-shaped coiled-coil dimer. This protein binds head-to-tail along the length of actin filaments and plays a key role in force generation [2]. In fact, when Ca^{2+} binds to the troponin complex, tropomyosin moves toward the inner domain of actin filaments, exposing sites on actin that allow weak binding of myosin cross-bridges (displacement from the B-state to C-state, both being considered as off-states). Weak-to-strong transition induces a further tropomyosin movement (to the M-state, known as on-state), exposing more sites on actin filaments, permitting additional binding of myosin cross-bridges and, ultimately, leading to force generation (Fig. 1) [2].

Recently, a novel mutation in *TPM2* has been discovered and has been associated with an inherited myopathy, limb muscle weakness and distal arthrogryposis (joint contractures) [3]. This gene mutation changes just one DNA nucleotide and results in the substitution of just one amino acid in tropomyosin (Arg133Trp). This raises the question of the molecular mechanisms by which such minor change induces limb muscle weakness. To answer this question, limb muscle biopsies from individuals carrying the Arg133Trp mutation and from healthy controls were taken (using protocols approved a local ethics committee). Single muscle fibers were subsequently isolated, mounted in a special chamber and low-angle X-rays were applied at beamline **BL45XU**. Tropomyosin (far off-meridional part of the second actin layer line at 1/19 nm^{-1}) and actin (sixth and seventh actin layer line at 1/5.9 and 1/5.1 nm^{-1}, respectively) intensity changes were monitored during contraction (Fig. 2) under two conditions: first, when no overlap between actin and myosin filaments

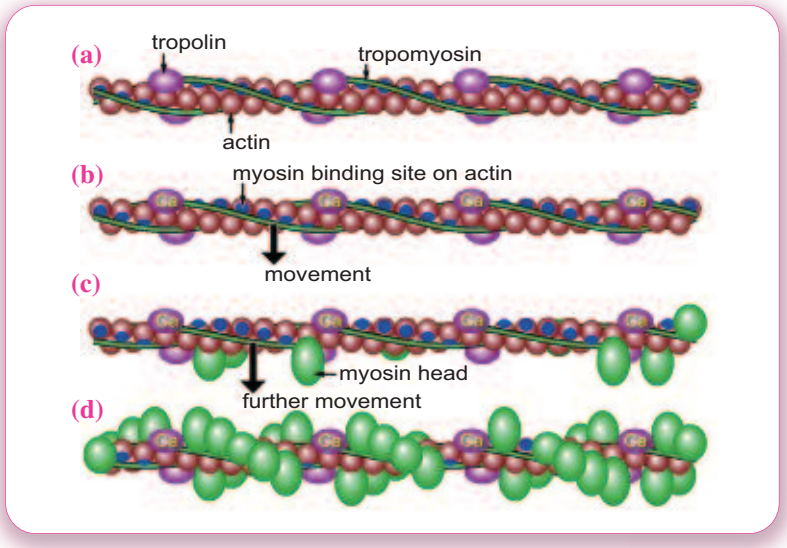

Fig. 1. Tropomyosin B-state: because tropomyosin completely covers the binding sites on actin, myosin cross-bridges cannot be formed **(a)**. Tropomyosin C-state: when Ca^{2+} binds to troponin, this allows the movement of tropomyosin **(b)**; binding sites on actin are then exposed and permit the formation of weak myosin cross-bridges **(c)**. Tropomyosin M-state: weak-to-strong myosin cross-bridge transition induces a further tropomyosin movement, exposing more binding sites on actin, permitting additional binding of myosin cross-bridges and force production **(d)**.

exists; second, when actin and myosin filaments fully overlap [4].

During contraction, when no overlap exists between actin and myosin filaments, the intensity change of the second actin layer line (1/19 nm^{-1}) was weaker in muscle fibers from individuals carrying the Arg133Trp mutation when compared with controls. This directly proves that the mutation partially hinders tropomyosin movement from the B-state to C-state over the surface of actin. In addition, when actin and myosin filaments fully overlap, intensity changes of the second, sixth (1/5.9 nm^{-1}) and seventh (1/5.1 nm^{-1}) actin layer lines were even more attenuated in cells from persons expressing the Arg133Trp mutation when compared with controls. This demonstrates that the tropomyosin displacement between the C-state and M-state is also disturbed and this phenomenon inhibits activation-induced actin conformational changes. One obvious reason for such dysfunction is that the Arg133Trp mutation, located in a highly conserved tropomyosin region, modifies the structure of the protein and the affinity for other surrounding molecules, provoking a local unusual stability and flexibility and altering the normal equilibrium positions (B-, C- and M-states). Hence, fewer myosin binding sites can be exposed on actin filaments and fewer myosin cross-bridges can exist, ultimately leading to a large deficit in force generation and muscle weakness [4].

There is an increasing awareness in the scientific community concerning the importance and need of a detailed understanding of muscle weakness in inherited myopathies. However, to date, basic and clinical research has primarily focused on characterizing the causative genes, ignoring the molecular mechanisms underlying frailty. The above use of low-angle X-rays appears as a powerful methodological tool and, therefore, opens up a new field of investigation in order to design future therapeutic interventions in inherited myopathies.

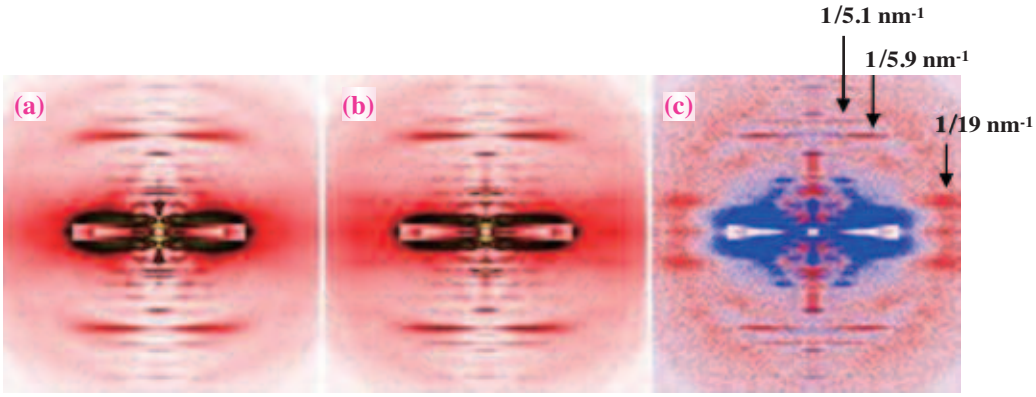

Fig. 2. X-ray diffraction patterns of human control fibers where actin and myosin filaments fully overlap. These patterns were recorded at the BL45XU beamline during relaxation **(a)** and contraction **(b)**. Differences in intensity profiles between relaxation and contraction also appear **(c)**. Tropomyosin (far off-meridional part of the second actin layer line at 1/19 nm^{-1}) and actin (sixth and seventh actin layer line at 1/5.9 and 1/5.1 nm^{-1}, respectively) are shown with arrows.

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References

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