The force-generating attachment of myosin heads (cross-bridges) to the actin filaments is controlled differently in fast- and slow-muscle fibre types

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The dynamic functional diversity of skeletal muscle fibres is generally thought to be predominantly related to the molecular species of the myosin heavy chain (MHC) isoform present, which determines the speed of myosin head (cross-bridge) pulling cycles. The molecular mechanisms of force activation and generation are believed to be essentially the same in different muscle fibre types. Thus, the binding of Ca^{2+} released from the sarcoplasmic reticulum to troponin C is thought to cause a lateral shift of the inhibitory troponin complex and increase mobility of tropomyosin (TM) molecules on the surface of the actin filaments between positions that either block or expose myosin head attachment sites on actin. Strong attachment of myosin heads to exposed binding sites on the actin filament stabilises the TM in an 'open' position, uncovering more sites for cross-bridge formation. Here we show evidence of qualitative differences between the mechanisms of myosin head attachment in slow and fast vertebrate skeletal muscle fibres, where the term "attachment" refers to the binding of the myosin head to the actin filament with subsequent force generation. This is contrary to the belief that dynamic contractile differences between fibre types relate mainly to differences in the speed of cross-bridge pulling cycles.

In this study, force-generating attachments of myosin heads were investigated by applying small perturbations of myosin head pulling cycles in stepwise stretch experiments on segments of mechanically skinned single skeletal muscle fibres of different type activated to different levels. Fibres were obtained from freshly dissected muscles from the female clawed frog *Xenopus laevis* (105-230 g) and the rat (Fisher 344 strain, 3-6 months old) after the animals were killed in accordance with animal ethics procedures approved at the University of Salzburg, where the experiments were conducted. The frogs were cooled to 0-2°C and killed by decapitation and double pithing and the rats were anaesthetized with sodium pentobarbitone and killed by bleeding. Single fibres were mechanically skinned, attached to a force transducer and activated in solutions of different $[Ca^{2+}]$ and $[Sr^{2+}]$ as previously described (Andruchova *et al.,* 2006; Bortolotto *et al.,* 2000). After reaching a stable activation level, fibres were subjected to small stepwise stretches to induce a small perturbation in the interaction of myosin heads with actin. The rapid stretches result in characteristic force transients which include a simultaneous rise in force with the stretch (phase 1), a rapid decay (phase 2) and a subsequent delayed force rise (phase 3). The delayed force rise (characterized by the time to peak, **t3**) is tightly correlated with the MHC isoform composition of a fibre (see Andruchova *et al.,* 2006) and can be used as a measure of kinetics of myosin head attachment. After the mechanical experiments, the fibres were collected for analysis of the MHC isoform content. This was carried out using a refined SDS-PAGE protocol described earlier (see Andruchova *et al.,* 2006; Bortolotto *et al.,* 2000).

Slow fibres (frog tonic and rat slow-twitch) exhibited only one 'slow-type' of myosin head attachment over the entire activation range (10 – 100% maximum Ca^{2+}-activated force (Tmax)) which was characterized by a gradual decrease in **t3** as the level of activation increased. In contrast, the fast fibres from both frog and rat displayed two types of myosin head attachment: a 'slow-type' of myosin head attachment at low levels of activation (< 25% Tmax), which was not dissimilar from that observed in the slow fibres, and an up to 30-times faster type at high levels of activation (>75% Tmax). Importantly, at intermediate levels of activation (25 – 75% Tmax) the force traces for fast fibres typically displayed both the 'slow-type' and the 'fast-type' of stretch-induced delayed force rises.

These results indicate that there are two qualitatively different types of myosin head attachment in fast fibers, but only one type in slow fibres, demonstrating that the dynamic contractile properties of different fibre types differ not only with respect to the speed of myosin-head pulling cycles, but also with respect to molecular mechanisms of myosin head attachment.

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