IN VIVO IMAGING OF SARCOMERE DYNAMICS DURING MUSCLE CONTRACTION IN MICE

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SUMMARY
Sarcomeres are contractile units of muscle and their lengths provide the degree of availability of myosin motors interacting with actin filaments. Therefore, sarcomere lengths in myofibrils and muscle fibers are regarded as an important mechanical parameter to understand the mechanics of muscle contraction. However, there is a gap in understanding of muscle contractile mechanics between molecular (e.g., myosins and actins) and higher levels of muscle structure. Thus, in order to fill this gap, we have developed the in vivo measurement system on dynamics of individual sarcomeres and targeted contractile protein molecules by the combination of fluorescence microscopy and protein engineering technique. The striation patterns of sarcomeres in mouse tibialis anterior (TA) muscle were fluorescently labeled by either expressing GFP-actinin or staining DNA by SYTO 16 and were successfully observed during isometric contractions. Interestingly, different dynamic behaviors of sarcomere contractions between adjacent fibers during isometric contractions were also revealed. In the near future, the TA forces will be measured by the force transducer implanted on the TA tendon. From these results, we will discuss the variability of sarcomere length within same fibers and between adjacent fibers at different isometric force levels.

INTRODUCTION
Muscle contraction is achieved through the formation of cross-bridges between the myosin and actin filaments sliding past each other. The patterns of striation arise from these contractile structures are characterized by the highly organized arrangement of contractile units, sarcomeres, containing thousands of myosin and actin molecules. Since the length of sarcomeres potentially provide the availability of myosin motors interacting with actin filaments [1] and so, they have been extensively investigated as a indicator of muscle contractile ability and dynamics of myosin motors in single myofibrils or muscle fibers [2,3].

In the last two decades, the single molecule techniques have been astonishingly improved to detect dynamics and kinetics of individual molecular motors [4, 5]. Recently, we have shed light on how collectively skeletal myosins can generate the force in an assembly of motors without molecular interference [6]. However, there is a gap in understanding of muscle contractile mechanics between molecular and higher levels of muscle structure, such as myofibrils and fibers, because of complexity of muscle hierarchy structures. Thus, in vivo observations on dynamics of sarcomeres and individual contractile proteins in living animal muscles are important to fill a gap in understanding of contractile dynamics between micro and macro structure levels [7]. Furthermore, none of the previous studies investigated whether the adjacent muscle fibers contract homogenously, that is typically assumed in the commonly-used muscle models. Thus, we have currently developed the in vivo measurement system on dynamics of individual sarcomeres and targeted contractile protein molecules in mouse muscles by the combination of the fluorescence microscopy and genetic engineering techniques. Here, we focused on i) the development of the experimental system to observe the fluorescently labeled sarcomeres and protein molecules in in vivo mouse muscle with a high temporal resolution (~1-2 ms), ii) the investigation on dynamics of individual sarcomere within single fibers or between fibers during isometric contractions at different muscle lengths.

METHODS
The striation patterns of sarcomere structure were visualized by either the expression of GFP-fused actinin (GFP-actinin) or the green-fluorescent nucleic acid stain (SYTO 16, Invitrogen) in mouse tibialis anterior (TA). The plasmid DNA of GFP-actinin mixed with LipofectamineTM LTX (Invitrogen) was transfected into mouse TA of anesthetized mice by syringe injection and incubated for at least 48 hours of daily activities. SYTO 16 was injected into TA and incubated for 15-30 minutes before observations. After the incubation period, mice were anesthetized by isoflurane inhalation, and skin was opened to expose the muscle belly of TA. The exposed TA muscle was faced downward and mounted on the top of objective lens (60x / 1.33 NA, oil). In order to minimize motion artifacts induced by the respiratory movements, the lower limb was only fixed on the microscope stage and other parts of body were mounted on the separated rack without any contact on the stage (Figure 1). Fluorescence images were obtained through the confocal microscope (CSU22, Yokokawa) and projected onto the electron multiplying charge coupled device (EM-CCD) camera (IXON, Andor) at the frame rate of 30-500 fps. For isometric contractions, TA was electrically stimulated using a custom-made electrodes (1 mm diameter of stainless wire) connected to a function generator (DF1906, DF corporation).

RESULTS AND DISCUSSION
The striation patterns of individual sarcomeres in in vivo mouse TA muscle were successfully visualized by using both GFP-actinin and SYTO 16. Actinins are known to
localize along the Z-discs so that the fluorescence images of actinin can be used to measure sarcomere lengths during muscle contractions. The striation patterns made by SYTO 16 (Figure 2) is much brighter than those by GFP-actinin and thus, they can be visualized with a time resolution of 2 ms. The potential reason why the fluorescence intensity of GFP-actin was lower than that of SYTO 16 is attributed to low efficiency of the transfection of DNA and/or the expression of GFP-actinin in \textit{in vivo} mouse TA. Therefore, we need to optimize the condition to enhance the expression level of GFP-actinin.

We also frequently observed the different dynamic behaviors of sarcomere contractions between adjacent fibers during isometric contractions. The lengths of sarcomeres in one fiber remained constant, while those in the adjacent fiber oscillate. These unique dynamics of sarcomere can be observed only by the \textit{in vivo} situation. In the near future, the lengths of sarcomere will be quantified by image processing analyses on the fluorescence images, and the TA forces will be measured by the force transducer implanted on the TA tendon. From these results, we will discuss the variability of sarcomere length within same fibers and between adjacent fibers at different isometric force levels.

CONCLUSIONS
By the combination of confocal microscopy and the protein engineering technique to fluorescently label sarcomere structure, the \textit{in vivo} imaging of individual sarcomeres were successfully obtained during the isometric contraction in mouse TA. These \textit{in vivo} observations also revealed the different dynamic behaviors of sarcomeres between adjacent muscle fibers.

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REFERENCES