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博 士 論 文 の 要 約

博士の専攻分野の名称： 博　士（農学）　　氏名　　上仲（市村）　恵美

学 位 論 文 題 名

The mechanism of the myosin replacement in the thick filament of the skeletal muscle

(骨格筋筋原線維内の太いフィラメントにおけるミオシン分子の置換機構)

Myosin is one of the most abundant proteins in the myofibrils of skeletal muscle. Approximately 300 myosins form a bipolar thick filament with 1.6 μm in length in vivo. We previously have shown that about eGFP tagged myosin (eGFP-Myh3) is replaced in the myofibrils in 10 hours by fluorescence live imaging technique (Ojima et al., 2015). However, little is known about the mechanism underlying myosin replacement in the thick filament while maintaining muscle contraction and myofibril structure. To address this, the thesis consists of three chapters: (1) spatiotemporal observation of myosin replacement in the thick filament, (2) regulation of myosin replacement by muscle-specific ubiquitin ligase, and (3) potential involvement of post-transcriptional modification in myosin replacement.

Chapter 1: Spatiotemporal observation of myosin replacement in the thick filament

First, to investigate how rapidly myosin replacement occurred and whether the myosin exchange rate differed depending on the region of the thick filament, myosin release and insertion rates were measured in myotubes expressing a photoconvertible fluorescence protein-tagged

myosin over a short period of time. About 20% of myosins were replaced within 10 min, while 70% of myosins were exchanged in 10 h with symmetrically and biphasically alteration of myosin release and insertion rates. A fluorescence pulse-chase assay showed that newly synthesized myosin was located in the end rather than the center of the thick-filament in 7 min of pulse-chase labeling and was observed in the rest of the thick filament by 30 min. In addition, the thick filament-dissociated myosin was reused for myosin replacement in the thick filament. The recycled myosin also tended to be preferentially incorporated into the tip of the thick filament than the center of the filament. These results suggest that the myosin replacement rate differs depending on the regions of the thick filament.

Chapter 2: Regulation of myosin replacement by muscle-specific ubiquitin ligase

Next, to study the involvement of a protein degradation system in the myosin replacement process, I studied whether the muscle-specific ubiquitin ligase Ozz regulated the replacement rate of Myh3. Ozz overexpression significantly decreased the replacement rate of eGFP-Myh3 in the myofibrils, whereas it did not affect other myosin isoforms. It is likely that ectopic overexpression of Ozz promoted myosin degradation through increment of ubiquitinated myosin, and decreased myosin supply for replacement, thereby reducing myosin replacement rate. Intriguingly, treatment with a proteasome inhibitor MG132 also decreased myosin replacement rate, although MG132 enhanced the accumulation of ubiquitinated myosin in the cytosol where replaceable myosin was pooled, suggesting that ubiquitinated myosin is not replaced by myosin in the myofibril. Collectively, our findings showed that Myh3 replacement rate was reduced in the presence of overexpressed Ozz, probably through enhanced-ubiquitination and -degradation of Myh3 by Ozz.

Chapter 3: Potential involvement of post-transcriptional modification on myosin

molecule in myosin replacement

Finally, to identify the intracellular environment was involved in the myosin replacement, myosin replacement rate was compared between Myh1 and Myh7 in myotubes co-expressing Myh1 and Myh7. The Myh1 replacement rate was faster than the Myh7. This result showed the myosin replacement rate was differed between myosin isoforms in the same myotubes. Then, I focused on post-translational modifications that affect protein-protein interaction and localization. In vivo, the different muscle type-specific myosin methylation pattern was detected by western blotting, and the different methylation sites of myosin were identified between isoform and fraction by mass spectrometry. Since the methylation sites located on the myosin rod domain that is important for polymerization and thick filament formation were identified, I hypothesized that different methylation sites between isoforms or fractions might regulate the replacement rate of myosin by modifying the biochemical properties of myosin molecules.

In this thesis, I showed that thick filament associate-myosin was frequently and uniformly replaced in the thick filament by both newly synthesized and recycled myosin. The myosin replacement rate was controlled by Myh specific ubiquitin ligase. Moreover, the myosin replacement rate was different between myosin isoforms, which might be potentially regulated by the methylation pattern of myosin molecules.