Replacement of myosin molecules in thick filament of cultured skeletal muscle

E. Ichimura1, Y. Yasukawa1, K. Ojima2, J. Wakamatsu1, T. Nishimura1;

1, Muscle Biology and Meat Science Laboratory, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan, 2, Animal Products Research Division, NARO, Institute of Livestock and Grassland Science, Tsukuba, Japan

Myosin is one of the major myofibrillar proteins of skeletal muscle. More than 300 myosin molecules assemble into a bipolar thick filament in the sarcomere. We have previously shown that myosin molecules in thick filaments are vigorously exchangeable in cultured myotubes using fluorescence recovery after photobleaching (FRAP) technique. However, it is still unclear how myosin molecules in thick filaments are replaced. In this study, we investigated the replacement of myosin molecules in myotubes, when protein biosynthesis was inhibited. Exogenously expressed eGFP-tagged myosin heavy chain 3 (eGFP-Myh3) were precisely incorporated into the A-band of the sarcomeres.

We carried out FRAP experiments in the presence of cycloheximide (CX) to inhibit protein biosynthesis. The fluorescence recovery of eGFP-Myh3 in myotubes treated with CX for an hour was not different from that of control without CX-treatment. However, the fluorescence of eGFP-Myh3 in myotubes treated with CX for ten hours recovered to a less extent compared with the control group. This result suggests that myosin molecules in thick filaments were exchanged largely by myosin molecules which might exist in the cytosol adjacent to myofibrils. To address this, we examined myosin molecules replacement in myotubes treated with streptolysin O (SLO) which makes pores in the cell membrane. The fluorescence recovery of eGFP-Myh3 in myotubes treated with SLO was suppressed compared to the control without SLO-treatment. This might be caused by the decrease in the amount of myosin molecules in the cytosol due to the outflow of myosin molecules that were not incorporated into thick filaments from myotubes.

Our data suggest that myosin molecules in the thick filament are actively replaced with myosin molecules that exist in the cytosol of skeletal muscle cells.

Key words: myosin, myofibril, turnover, FRAP

E-mail address: eichi@anim.agr.hokudai.ac.jp