Mobility of the giant protein nebulin in skeletal muscle sarcomeres using PALM and FRAP

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Abstract

To date, it is unknown how giant muscle proteins are integrated and replaced in continuously contracting muscles. A prime example is nebulin (800 kDa), which binds F-actin and spans the length of the sarcomeric thin filament, with its C-terminus located in the z-disc and the N-terminus near the thin filament pointed-end. To establish the in vivo localization and mobility of nebulin in adult sarcomeres, we designed a mouse with photoconvertible Dendra2 inserted at the nebulin N-terminus (Dendra2-KI).

The mobility of nebulin was studied in mature skeletal muscle fibers of Dendra2-KI mice using intra-vital (imaging window) or ex vivo FRAP microscopy. Dendra2 was converted from green to red fluorescent state and fluorescence was followed over time. Data indicate that, in both intra-vital and ex vivo mature muscle, nebulin turnover follows a two phase dynamic: a quick mobility (10±4% and 14±3%, respectively; mobile fraction) of green Dendra2 in the first day, followed by a slower recovery (~2.5%/day and ~1.7%/day, respectively; replacement of incorporated nebulin). After 19 days, converted Dendra2 was still clearly present in the intra-vital ROI. Using PALM the location of the Dendra2-tagged nebulin was determined with nanometer resolution. The results showed that 14±6% of the molecules were not incorporated into the sarcomere, supporting the mobile fraction measured with FRAP.

Other mouse lines with Dendra2 attached to sarcomeric proteins were engineered and recovery was followed ex vivo using FRAP microscopy. Data showed a much faster recovery of the small, thin filament associated, proteins Tmod4 (~69% in 15h) and Lmod3 (preliminary; >90% in 2 min) compared to nebulin (~28% in 9 days). Recovery of titin, another giant sarcomere protein (~3.7 MDa) showed similar recovery as nebulin (~29% in 9 days).

A mouse line resembling a patient mutation in nebulin (heterogeneous deletion of exon 55, Δex55) results in less organized sarcomeric structure, and a lower mobility in the first day (11±3%), followed by a similar recovery (~1.8%/day). Using PALM, we determined an unincorporated nebulin fraction of 11±4%, suggesting that, as in patients, less nebulin protein is produced.

Summarizing, our data indicate that the replacement kinetics of the giant proteins nebulin and titin in mature muscle are very slow. In Δex55 mice, mobility is lower due to a lower fraction of unincorporated nebulin protein, but replacement kinetics are similar to muscle of mice with non-mutated nebulin.
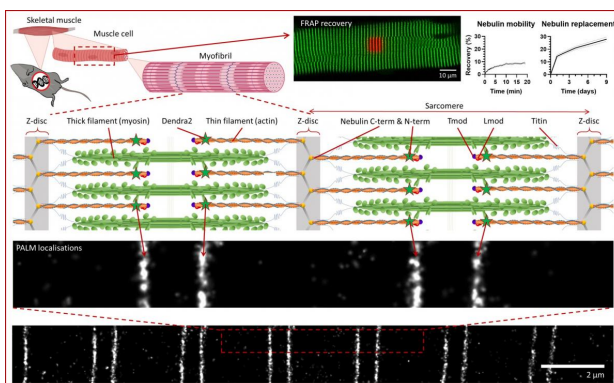
Fig 1. Schematic overview of a mouse muscle fiber (top left) and a photoconverted muscle fiber (top middle) used for FRAP analysis (top right), showing a mobile fraction of 9% within 20 minutes after photoconversion and 28% replacement of nebulin after 9 days. Schematic overview of the sarcomere in muscle (middle) with the thin filament associated proteins; actin, Dendra2-tagged nebulin, Tmod and Lmod and the thick filament associated proteins; myosin and titin. A reconstruction of the nebulin localisations in the sarcomere measured by PALM (bottom).