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Isoform Diversity of Giant Proteins in Relation to Passive and Active Contractile Properties of Rabbit Skeletal Muscles

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Abstract

The active and passive contractile performance of skeletal-muscle fibers largely depends on the myosin-heavy-chain (MHC) isoform and the stiffness of the titin spring, respectively. Open questions concern the relationship between titin-based stiffness and active contractile parameters, and titin's importance for total passive-muscle stiffness. Here, a large set of adult rabbit muscles (n=37) was studied for titin-size diversity, passive mechanical properties, and possible correlations with the fiber/MHC composition. Titin-protein analysis showed isoform sizes between 3300 and 3710kDa; 31 muscles contained a single isoform, six muscles coexpressed two isoforms. Psoas muscle coexpressed two isoforms at a ratio of 30:70 and all fibers contained a similar isoform-ratio. Gel-electrophoresis and Western-blotting of two other giant muscle proteins, nebulin and obscurin, demonstrated muscle-type-dependent size differences of ≤70kDa. Single-fiber and single-myofibril mechanics performed on a subset of muscles showed an inverse relationship between titin size and titin-based passive tension. Force measurements on muscle-fiber bundles suggested that high titin-borne stiffness does not necessarily imply high total passive stiffness, as a large contribution to stiffness comes from extramyofibrillar structures, particularly collagen. Titin's contribution to total stiffness was higher in Psoas (~57%) than in Soleus (~24%), but total passive stiffness of Soleus exceeded that of Psoas muscle. Plots of titin size versus percentage of fiber-type or MHC-isoform (I-IIB-IIA-ID) determined by myofibrillar ATPase staining and gel-electrophoresis revealed modest correlations with the type-I-fiber and MHC-I proportions. No relationships were found with the proportions of the different type-II-fiber/MHC-II subtypes. Thus, a low correlation exists between the active and passive mechanical properties of skeletal-muscle fibers. Whereas slow muscles usually express long titin(s), predominantly fast muscles can express either short or long titin(s), giving rise to low titin-based passive stiffness in slow muscles and highly variable stiffness in fast muscles. Titin contributes substantially to total passive stiffness, but this contribution varies greatly among muscles.
Key words: titin – nebulin – obscurin – passive tension – fiber types