Peritubular Myoid Cells from Rat Seminiferous Tubules Contain Actin and Myosin Filaments Distributed in Two Independent Layers.

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Supplementary Materials and Methods.

Low-Temperature Treatment of ST Segments and Assay of Assembled PM Cell Myosin

ST were incubated in PBS at 4°C or 37°C for 10 min respectively (they will be mentioned as 4°C or 37°C treatment). ST of 4°C and 37°C treatment were fixed with 4 % paraformaldehyde in PBS at 4°C and 37°C respectively, for 20 min. ST segments were isolated and analyzed by confocal microscopy.

For the analysis of assembled PM cells myosin, we used a procedure described previously (Fernandez et al. 2008 [6]). Briefly, 0.5 g of ST from 4°C and 37°C treatments were processed at 4°C and 37°C, respectively, in the following way: Homogenized in 1 ml PMEE buffer (PIPES 35 mM, MgSO4 5mM, EGTA 1 mM and EDTA 0.5 mM) and centrifuged at 14 000 g for 20 min. The supernatants (S1) containing the fraction of soluble myosin were collected and the pellets (P1) containing the fraction of MyF were resuspended with 0.6 M KCl in PMEE buffer, to disassemble MyF, and centrifuged at 4°C to obtain supernatants (S2) and pellets (P2). S1 and S2 supernatants were separated by 7.5% SDS-PAGE, transferred to nitrocellulose and myosin was detected with myosin Ab and anti mouse IgG conjugated with horse radish peroxidase (Jackson) and developed with Immuno-Blot assay kit (Thermo Scientific) according to the manufacturer’s directions. The total amount of myosin in S1 and S2 was estimated by quantification of the optical density of S1 and S2 bands using Image J software.