ISSN 1990-519X, Cell and Tissue Biology, 2018, Vol. 12, No. 3, pp. 238–246. © Pleiades Publishing, Ltd., 2018.
Original Russian Text © N.A. Rysev, O.E. Karpicheva, V.V. Sirenko, A.O. Simonyan, C.S. Redwood, Y.S. Borovikov, 2017, published in Tsitologiya, 2017, Vol. 59, No. 12, pp. 888–896.

The Effect of the *Arg91Gly* and *Glu139del* Mutations in β-Tropomyosin Associated with Congenital Myopathy of Human Skeletal Muscles on Actin–Myosin Interaction

N. A. Rysev[a], O. E. Karpicheva[a], V. V. Sirenko[a], A. O. Simonyan[a, b], C. S. Redwood[c], and Y. S. Borovikov[a, *]

[a] *Institute of Cytology, Russian Academy of Sciences, St. Petersburg, 194064 Russia*
[b] *St. Petersburg State University, St. Petersburg, 199034 Russia*
[c] *University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, United Kingdom*
**e-mail: borovikov@incras.ru*

Received July 27, 2017

Abstract—The structural changes in proteins of the contractile apparatus of muscle fiber and the violation of their function due to point mutations in these proteins can be a cause of many hereditary diseases of human muscular tissue. Some such diseases are cap-myopathy and distal arthrogryposis, which may be connected with tropomyosin mutations. The deletion of glutamic-acid residue at position 139 of β-tropomyosin leads to the development of cap-myopathy, and the replacement of arginine at position 91 with glycine in this protein is linked to distal arthrogryposis. To understand how the *Arg91Gly* and *Glu139del* mutations disrupt the coordinated work of the contractile system of muscle fibers, recombinant wild-type and mutant β-tropomyosins were overexpressed and incorporated into thin filaments of ghost-muscle fiber. Fluorescent probes of 1,5-IAEDANS or FITC-phalloidin were specifically linked to the Cys707 of the myosin subfragment-1 and the three neighboring actin monomers, respectively. The polarized-microfluorimetry technique was used to study the spatial arrangements of actin and myosin in mimicking different stages of the ATPase cycle (in the presence of ADP or ATP and in the absence of a nucleotide) at low and high concentration of calcium ions. Both mutations were shown to change the conformational rearrangements of the myosin head and actin in the ATP hydrolysis cycle, which may be caused by abnormal behavior of the mutant tropomyosins during regulation. The altered work of the contractile system may be a cause of muscle weakness in congenital myopathies associated with these mutations.

Keywords: mutation in tropomyosin, hereditary myopathy, actin–myosin interaction, regulation of muscle contraction, muscle fiber, polarized fluorescence

DOI: 10.1134/S1990519X18030112

INTRODUCTION

Muscle contraction is generated by the interaction between actin and myosin coupled with hydrolysis of ATP. Tropomyosin and calcium-sensing protein troponin are involved in the regulation of this interaction. F-actin, together with tropomyosin and troponin, forms the thin filament of the sarcomere. It has been suggested that, at low concentrations of Ca^{2+}, troponin interacts with actin monomers and switches actin monomers off (Borovikov et al., 2009) and tropomyosin is shifted toward the outer actin domain and occupies a "blocked" position (Lehman et al., 2013). Switched-off actin monomers cannot activate ATP hydrolysis on myosin heads. Tropomyosin strands hide the specific myosin-binding sites on the actin filament that are involved in strong and weak forms of myosin binding to actin (McKillop and Geeves, 1993; Lehman, 2016). Switched-off actin monomers facilitate tropomyosin's occupation of "blocked" position on actin (Borovikov et al., 2017). Muscle-fiber contraction is activated by high Ca^{2+} in the sarcomere and Ca^{2+} binding by troponin. Troponin switches on actin monomers, which become capable of activating ATP hydrolysis in myosin; tropomyosin moves toward the inner domain of actin and assumes a "closed" position, allowing myosin to form a weak binding to actin (Galińska-Rakoczy et al., 2008; Lehman, 2016). The myosin cross bridges to actin generate force when actin monomers are almost fully switched on and tropomy-

Abbreviations: ATP—adenosine triphosphoric acid, ADP—adenosine diphosphoric acid, F-actin—fibrillar actin, FITC—fluorescein-5-isothiocyanate, EGTA—ethylene glycol tetraacetate, EDTA—ethylenediaminetetraacetic acid, 1,5-IAEDANS—N-(iodoacetaminoethyl)-1-naphthyl-amine-5-sulfonic acid, PMSF—phenylmethylsulfonyl fluoride, S1—myosin subfragment-1.

osin moves further to the inner domain of actin and assumes an "open" position (Borovikov et al., 2009, 2017; Lehman, 2016).

It has been shown that point mutations in the genes of muscle proteins can cause serious structural and functional disorders in a muscle cell and severe cardiac and skeletal muscle diseases in humans. Thus, at least 50 mutations have been identified so far in the tropomyosin genes that cause different congenital muscle disorders (Wallgren-Pettersson et al., 2011; Marttila et al., 2012). Cap-myopathy and distal arthrogryposis are some of these disorders. Cap-myopathy can be linked to deletion of glutamic-acid residue at position 139 in β-tropomyosin (the *TPM2* gene, the *Glu139del* or *E139X* mutation). Distal arthrogryposis can be associated with substitution of arginine at position 91 with glycine in β-tropomyosin (the *Arg91Gly* or *R91G* mutation).

Cap myopathy was first described in 1981 (Fidzianska et al., 1981). It is characterized by the accumulation of caplike structures containing disorganized contractile proteins and Z-disc proteins that are located under the sarcolemma. The characteristic clinical sign is slowly progressive muscle weakness. Distal arthrogryposis is a congenital malformation characterized by an underdeveloped nervous system and muscles with multiple contractures and joint deformities. The name "arthrogryposis" was coined by Stern in 1923 and is derived from Greek, literally meaning "curving of joints." The molecular mechanisms underlying these diseases remain unclear today. Therefore, their diagnosis and treatment are difficult (Marttila et al., 2012).

In the present work, we examined the effect of the *E139X* and *R91G* mutations on the regulation of actin–myosin interaction by the troponin-tropomyosin complex in a single muscle fiber in different states of the ATPase cycle using polarized microfluorimetry.

MATERIALS AND METHODS

Reagents: potassium chloride, $MgCl_2$, sodium chloride, $CaCl_2$, disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$), monobasic potassium-phosphate anhydrous (KH_2PO_4), sodium carbonate (Na_2CO_3) and sodium-hydrogen carbonate ($NaHCO_3$), potassium carbonate (K_2CO_3), sodium azide (NaN_3), EDTA, PMSF, EGTA, glycerol, acetic acid, hydrochloric acid, phenolphthalein, dithiothreitol, Tris, α-chymotrypsin, ATP, ADP, FITC-phalloidin (Sigma, United States), 1,5 IAEDANS (Invitrogen, United States).

Preparation of Glycerinated Muscle Fibers

Single rabbit skeletal-muscle fibers with reconstructed contractile and regulatory systems were used. Muscle fibers were separated from rabbit *m. psoas* according to the Szent-Gyorgyi method (Szent-Gyorgyi, 1949). A bundle of fibers 2 mm in diameter was separated from rabbit psoas muscle and tied to sticks at rest-length and placed into glycerinating solution cooled to 4°C for 24 h containing 50% glycerol, 100 mM KCl, 1 mM $MgCl_2$, and phosphate buffer (67 mM, pH 7.0). In 24 h, bundles of muscle fibers were transferred into a fresh portion of glycerinating solution for 24 h. The material was again transferred into fresh glycerinating solution and stored at −20°C for 3–4 months. Two hours before the experiment, single fibers were isolated from the bundles and rinsed in a solution cooled to 4°C containing 100 mM KCl, 1 mM $MgCl_2$, and phosphate buffer (67 mM, pH 7.0).

Preparation of Ghost-Muscle Fibers

Ghost-muscle fibers composed of more than 80% of actin were prepared from single glycerinated rabbit skeletal-muscle fibers by extraction of myosin, tropomyosin, and troponin. Fibers were placed into an extracting solution containing 800 mM KCl, 1 mM $MgCl_2$, 10 mM ATP, 67 mM phosphate buffer (pH 7.0) and incubated under constant slow stirring for 1.5 h at 25°C. Afterward, ghost-muscle fibers were fixed on glass slides and placed into a rinsing solution containing 100 mM KCl, 1 mM $MgCl_2$, 67 mM phosphate buffer (pH 7.0).

Preparation of Myosin and Myosin Subfragment-1

Myosin was obtained from rabbit skeletal muscles as described by Ivanov and Yur'ev (Ivanov and Yur'ev, 1961) with modifications. Myosin subfragment-1 (S1) without regulatory light chains was prepared by mild proteolytic cleavage of skeletal-muscle myosin with α-chymotrypsin (Okamoto and Sekine, 1985) in a buffer of 10 mM Tris-HCl (pH 6.8), 120 mM NaCl, 2 mM EDTA, and 1 mM NaN_3 with a myosin to chymotrypsin weight ratio of 300 : 1 at 25°C and constant stirring for 20 min. The cleavage reaction was stopped by addition of PMSF to a final concentration of 1 mM and cooled on ice. Mg^{2+} solution was added to a concentration of 3 mM and centrifuged at 10000 *g* for 15 min. The supernatant was mixed with two volumes of saturated ammonium sulfate solution (up to 70% saturation) and centrifuged at 10000 *g* for 15 min. The resulting S1 pellet fraction was dissolved in 1 mL of buffer containing 20 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.1 mM NaN_3, and dialyzed overnight against the same buffer.

Preparation of Recombinant β-Tropomyosin

Recombinant mutant β-tropomyosin was produced using site-directed mutagenesis and a bacterial expression system of *Escherichia coli* BL21(DE3) as

described previously (Karpicheva et al., 2016). The specimen was frozen on dry ice and stored at −70°C.

Labeling of Ghost Fibers with Fluorescent Probes

F-actin of the ghost fibers was conjugated with FITC-phalloidin in the muscle fiber. The muscle fiber was incubated in a rinsing solution (see above) containing 40 μM of dye for 20 min at 25°C (Galazkiewicz et al., 1987). According to published data (Oda et al., 2005), the bound phalloidin derivatives are located at the contact region of three actin monomers. Labeling of the most reactive sulfhydryl group of myosin subfragment-1 SH1 (the Cys707 residue) with 1,5-IAEDANS fluorescent dye was performed as described previously (Borejdo and Putnam, 1977). S1 in buffer containing 60 mM KCl, 0.1 mM dithiothreitol, 30 mM Tris-HCl (pH 7.5) was mixed with threefold excess of dye for 18 h at 4°C. The reaction was stopped with excess dithiothreitol. The unbound dye was removed by dialysis against a solution containing 10 mM KCl, 1 mM $MgCl_2$, 0.1 mM dithiothreitol, and 10 mM Tris-HCl (pH 6.8). The specimen was purified using a Sephadex G-25 column. The degree of labelling estimated using an absorption coefficient of 6100 $M^{-1}\,cm^{-1}$ at 336 nm was 0.90–0.95.

Troponin was extracted from rabbit skeletal muscles using procedures described in (Potter, 1982).

Measurement of S1 Actin-Activated ATPase Activity

The rate of the ATPase reaction was assessed in a solution containing 1 μM S1, 7 μM F-actin, 3 μM troponin, and 3 μM wild-type or mutant (the *E139X* and *R91G* mutations) tropomyosin in buffer containing 12 mM Tris-HCl (pH 7.9), 2.5 mM $MgCl_2$, 15 mM KCl, 20 mM NaCl, 0.2 mM dithiothreitol, and 2 mM ATP at 25°C. The reaction was performed with Ca^{2+} concentrations ranging from 10^{-9} to 10^{-4} M. The concentration of free Ca^{2+} was estimated in the presence of 2 mM EGTA using the Maxchelator software. The reaction was stopped in 10 min by addition of trichloroacetic acid to a final concentration of 5%. The amount of formed inorganic phosphate was measured using the Fiske and Subbarow method (Fiske and Subbarow, 1925). A total of three experiments were conducted to measure inorganic phosphorus. The data were statistically analyzed, the pCa_{50} was estimated, and graphs were constructed using the GraphPad Prism software.

Polarized Microfluorimetry

Fluorescence-polarization measurements from probes bound to actin and myosin were performed using a polarized microfluorimeter (Borovikov et al., 2004). The polarized fluorescence from 1,5-IAEDANS and FITC-phalloidin probes was excited at 407 ± 5 and 436 ± 5 nm, respectively. The fluorescence was recorded at 500–600 nm. Measurements were performed in a solution containing 10 mM KCl, 1 mM $MgCl_2$, 1 mM NaN_3, 6.7 mM Na-, K-phosphate buffer (pH 7.0), and 0.1 mM $CaCl_2$ or 2 mM EGTA in the absence or presence of 2.5 mM ADP or 5 mM ATP (Borovikov et al., 2009).

The intensities of four components of polarized fluorescence were detected for each fiber: ${}_{\parallel}I_{\parallel}$, ${}_{\parallel}I_{\perp}$, ${}_{\perp}I_{\perp}$, and ${}_{\perp}I_{\parallel}$. The subscripts "∥" and "⊥" designate the direction of polarization parallel and perpendicular to the fiber axis, the left one denoting the direction of polarization of the incident light and the right that of the emitted light. The mathematical model used to analyze experimental data is based on the following assumptions (Kaulin et al., 1968; Rozanov et al., 1971; Tregear and Mendelson, 1975; Yanagida and Oosawa, 1978; Wilson and Mendelson, 1983; Irving, 1996; Borovikov et al., 2004). A muscle fiber is assumed a cylindrically symmetrical system with the symmetry axis oriented along the fiber. It is assumed that the fiber has randomly distributed fluorophores (present in an amount of N) and the fluorophores are ordered in an spiral (present in an amount of 1–N). The fluorophores are immobile and do not interact with each other. Absorption and emission of light are carried out by linear, completely anisotropic oscillators of absorption (A) and emission (E) that are rigidly aligned with fluorophore molecules. The axes of oscillators of the ordered fluorophores are arranged in a spiral along the forming surface of the cone, the axis of which coincides with the axis of F-actin. The oscillators of absorption and emission form angles Φ_A and Φ_E, respectively, at the top of the cone (Fig. 1). Angle γ between the axes of absorption and emission oscillators is constant for fluorophores of each probe. The thin filament is assumed to be flexible with angle $\theta_{1/2}$ of its deviation from the fiber axis. The ratios of four intensities from fluorescence polarization ${}_{\parallel}I_{\perp}/{}_{\parallel}I_{\parallel}$, ${}_{\perp}I_{\perp}/{}_{\parallel}I_{\parallel}$, and ${}_{\perp}I_{\parallel}/{}_{\parallel}I_{\parallel}$ are functions of angles Φ_A, Φ_E, and $\theta_{1/2}$ and of the number of disordered fluorophores N (Kakol et al., 1987). In the model, the values of Φ_A and Φ_E indicate orientation of the fluorophores in the fiber. $\theta_{1/2}$ is reflective of the flexural rigidity of the thin filament. The value of N is directly proportional to the mobility of the labeled protein. The pattern of changes in Φ_A was similar to that of Φ_E. Therefore, the Φ_A values are not presented in this paper. The statistical significance of changes in fluorescence was estimated using Student's *t*-test, $P < 0.05$.

RESULTS AND DISCUSSION

The effect of the *E139X* and *R91G* mutations in β-tropomyosin on the regulation of actin–myosin interaction by the troponin–tropomyosin complex has been studied using polarized fluorimetry. Ghost-muscle fibers containing pure actin filaments were recon-

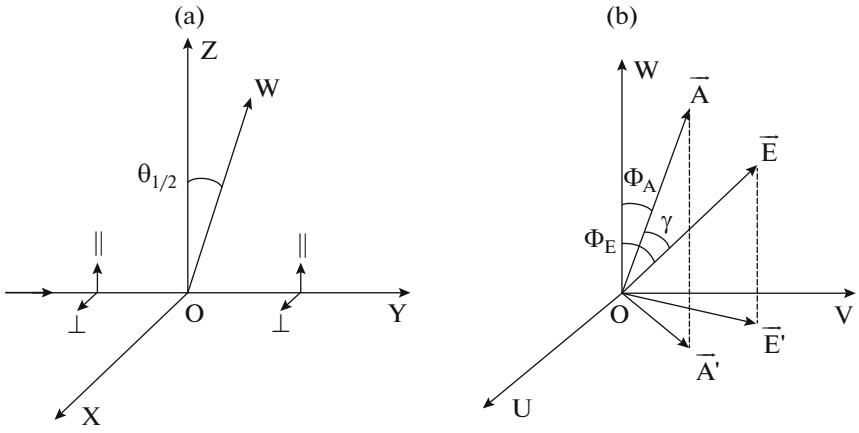

Fig. 1. Diagrams showing (a) angular coordinates of the thin filament and (b) oscillators of absorption (Φ_A) and emission (Φ_E) of the fluorophores. (a) The actin-filament axis (OW) deviates from the long fiber axis (OZ) by angle $\theta_{1/2}$; the subscripts "⊥" and "||" designate the orientation of the polarization plane parallel or perpendicular to the fiber axis. (b) Oscillators of absorption (A) and emission (E) are arranged along the forming surfaces of cones the axes of which coincide with the axis OW and form angles Φ_A and Φ_E, respectively, at the apex of the cones.

structed by incorporating contractile and regulatory systems. The reconstruction procedure involved the incubation of ghost-muscle fibers in solutions containing tropomyosin, troponin, and S1. To study the conformational states of S1, the fluorescent label 1,5-IAEDANS was covalently bound to the Cys707 residue of the SH1 helix of the motor domain of S1 (S1-AEDANS). Conformational rearrangements in actin were studied using FITC–phalloidin (actin–FITC–phalloidin), which is specifically bound to three adjacent actin monomers (Oda et al., 2005).

As illustrated in Fig. 2, the *E139X* mutation in tropomyosin has a significant effect on the conformation of actin and on the binding of myosin head to F-actin when mimicking different states of the ATP-hydrolysis cycle. Thus, at high Ca^{2+} (pCa 4), the Φ_E value for the actin–FITC–phalloidin complex bound to mutant tropomyosin was higher by 0.9° compared to wild-type tropomyosin (Fig. 2a). In contrast, at low Ca^{2+} (pCa 8), the Φ_E value was lower than that of the ghost fiber containing wild-type tropomyosin (control tropomyosin). The higher and lower Φ_E values at high and low Ca^{2+} concentrations, respectively, were also observed when mimicking most states of the ATPase cycle. Thus, when mimicking the strong binding of myosin to actin (in the presence of S1 and ADP), the Φ_E angle for the actin–FITC–phalloidin complex was higher by 0.4° at pCa 4 and lower by 0.5° at pCa 8. In the presence of ATP, the Φ_E angle was lower than or similar to the Φ_E value revealed in experiments with wild-type tropomyosin (Fig. 2a; all differences are significant, $P < 0.05$).

The increase in the Φ_E angle for the actin–FITC–phalloidin complex can be interpreted as the raised number of the switched-on actin monomers in the thin filaments, whereas a decrease in the Φ_E value indicates the reduction of the fraction of the switched-on actin monomers (Borovikov et al., 2009). This suggests that the *E139X*-mutant tropomyosin at high Ca^{2+} switched the actin monomers on in the thin filament (i.e., the actin monomers with the ability to activate the strong binding of myosin to actin), while at low Ca^{2+} it switches them off. Therefore, in the presence of mutant tropomyosin with the E139 deletion troponin retains its ability to perform Ca^{2+}-dependent regulation of actin–myosin interaction.

It was shown previously that the *E139X* mutation causes abnormally high sensitivity of the thin filaments to Ca^{2+} (Marston et al., 2013; Marttila et al., 2014). However, the molecular mechanisms underlying the high Ca^{2+}-sensitivity remain unclear up to now. Our data indicate that it is more likely that abnormally high Ca^{2+}-sensitivity caused by the *E139X* mutation is not associated with disturbed ability of troponin to switch the actin monomers off at low Ca^{2+}, since troponin retains its regulatory function—the ability to switch actin monomers on and off (Fig. 2a).

In the presence of the *R91G* mutation in tropomyosin, troponin also retains its ability of Ca^{2+}-dependent regulation. The data in Fig. 2a show that at a low concentration of Ca^{2+} (in the absence of S1) the Φ_E value for the actin–FITC–phalloidin complex becomes lower than even the values observed in the presence of control tropomyosin. Therefore, troponin increases the fraction of switched-off actin monomers; i.e., it retains the ability to switch actin monomers off in thin filaments at low Ca^{2+}. It is likely that the abnormally high Ca^{2+}-sensitivity shown in the presence of the *R91G* tropomyosin mutation (Marston

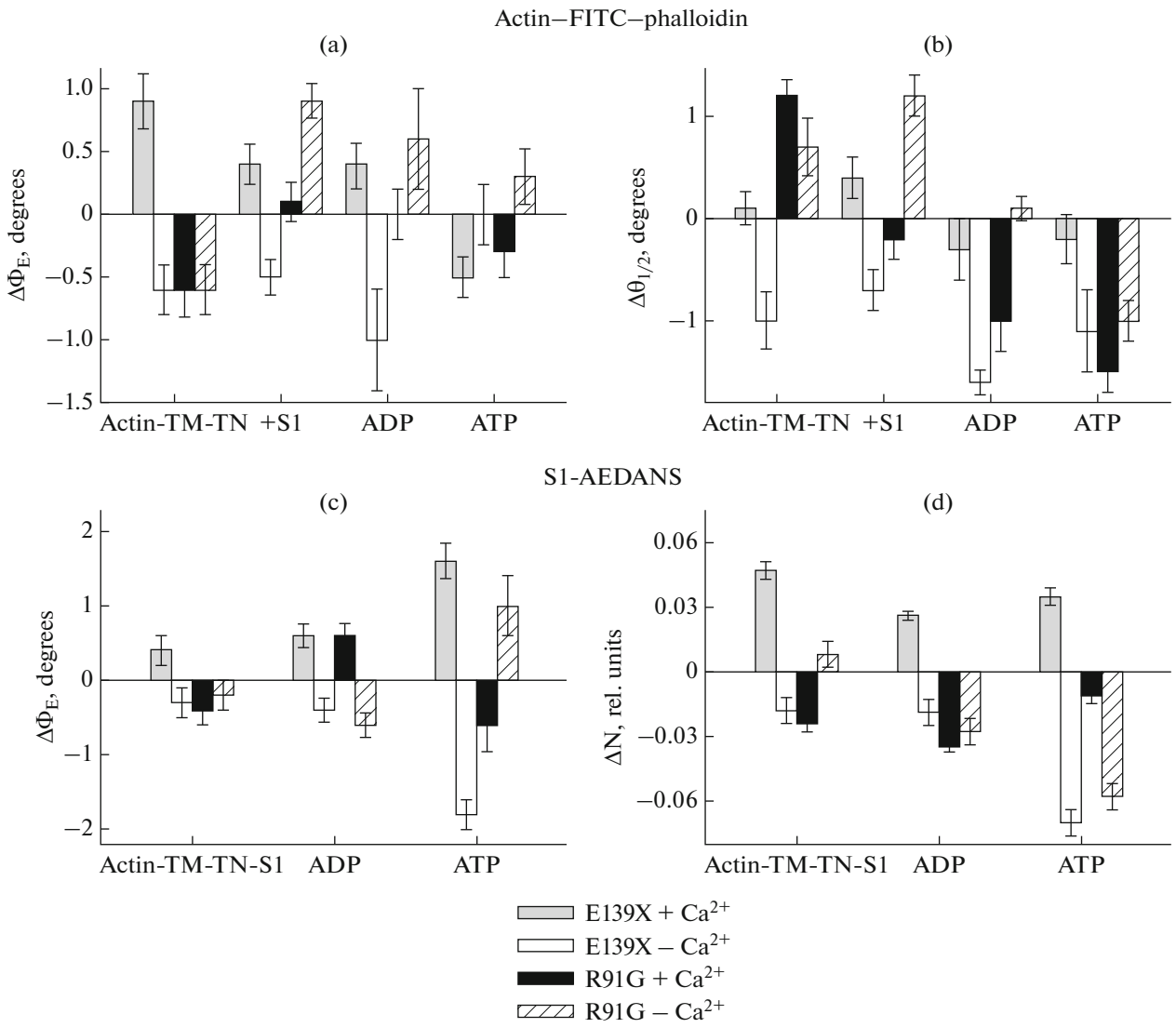

Fig. 2. Changes in values (a) Φ_E and (b) $\theta_{1/2}$ for the actin–FITC–phalloidin complex and (c) Φ_E and (d) N for S1-AEDANS in the presence of the *E139X*- and *R91G*-mutant tropomyosins compared to the corresponding values in the presence of control protein (wild-type) in mimicking different intermediate states of the ATPase cycle in ghost-muscle fiber. Φ_E, the angle between the thin filament axis and the emission dipole of the fluorophores; $\theta_{1/2}$, the angle of deviation of the thin filament from the fiber axis that indicates the actin filament flexibility; N, the number of randomly distributed fluorophores; $+Ca^{2+}$ and $-Ca^{2+}$, the data for high and low concentration of calcium ions, respectively; TN, troponin; and TM, tropomyosin. All changes are significant ($P < 0.05$). Vertical segments show standard deviation error.

et al., 2013) is not associated with inhibition of the troponin ability to switch off the thin filaments of the muscle fiber.

It is noted that mutations affect differently the ability of troponin to switch actin monomers on in thin filaments at high Ca^{2+} (Fig. 2a). Thus, the presence of the *E139X*-mutant tropomyosin increases the ability of troponin to switch on actin monomers, and, vice versa, the presence of the *R91G*-mutant tropomyosin reduces this ability. In fact, at high Ca^{2+}, the presence of the *R91G*-mutant tropomyosin led to the lower values of angle Φ_E and the presence of *E139X*-mutant tropomyosin was associated with higher Φ_E values compared to wild-type tropomyosin (Fig. 2a). Therefore, in the absence of S1, the *E139X* mutation increases the proportion of switched-on actin monomers and the *R91G* mutation reduces the number of these monomers under similar experimental conditions.

In the presence of S1 and nucleotides, the *E139X*-mutant tropomyosin increases the number of switched-on actin monomers at high Ca^{2+} in almost all mimicked states of the ATP-hydrolysis cycle and decreases the fraction of switched-on actin monomers at low Ca^{2+}. In the presence of the *R91G* mutation, the fraction of switched-on actin monomers also

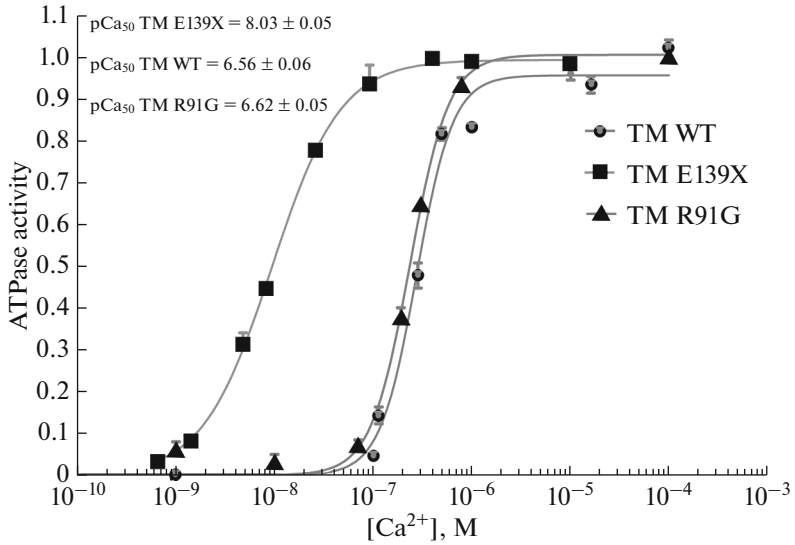

Fig. 3. The Ca^{2+}-dependent ATPase activity of myosin subfragment-1 bound to actin in the presence of troponin and wild-type and mutant tropomyosins (*E139X* and *R91G*). The ATPase activity was assessed using the method of Fiske and Subbarow (Fiske and Subbarow, 1925). TM, tropomyosin; TM WT, wild-type tropomyosin, and pCa_{50}, the value when half of the maximum of the ATPase activity is achieved (calcium sensitivity). Vertical segments show standard deviation error.

decreases at low Ca^{2+} during ATP hydrolysis; however, at high Ca^{2+} the proportion of switched-on actin monomers insignificantly differs from the fraction in experiments with wild-type tropomyosin (Fig. 2a). Thus, the *R91G* and *E139X* tropomyosin mutations allow troponin to switch on and off actin monomers in the thin filament at high and low Ca^{2+} during the ATPase cycle; however, the *E139X* mutation activates this ability while the *R91G* mutation inhibits it (Fig. 2a).

Both mutations affect the flexibility of F-actin in the thin filament. Thus, in the absence of S1, the E139 deletion increases the $\theta_{1/2}$ value by 0.1° ($P < 0.01$) at high Ca^{2+} and decreases the $\theta_{1/2}$ value by 1.0° at low Ca^{2+} concentration (Fig. 2b). The *R91G* mutation causes an increase in $\theta_{1/2}$ by 1.0° and 0.7° at high and low Ca^{2+}, respectively (Fig. 2b). An increase in the $\theta_{1/2}$ value indicates increased flexibility of the actin filament (Borovikov et al., 2009). Therefore, in the absence of S1, the E139 deletion increases filament flexibility at high Ca^{2+} and significantly decreases it at low concentration. Meanwhile, the *R91G* mutation raises the flexibility of the actin filament both at high and low Ca^{2+}.

Since an increased flexibility of the thin filament can facilitate binding of the myosin head to actin, it is suggested that the increased flexibility observed at low Ca^{2+} for the *R91G* mutation can facilitate the binding of myosin to actin and raise the sensitivity of actomyosin to Ca^{2+}. This conclusion well agrees with our data indicating that the *R91G*-mutant tropomyosin increases Ca^{2+}-sensitivity of actomyosin (Fig. 3). As shown in Fig. 3, Ca^{2+}-sensitivity of the activating system consisting of actin, myosin subfragment-1, troponin and the *R91G*-mutant β-tropomyosin is noticeably higher than the sensitivity of the system with wild-type tropomyosin. A significantly higher Ca^{2+}-sensitivity (more than one order) was revealed for the *E139X*-mutant tropomyosin (Fig. 3), which can be accounted for by a decreased affinity of the mutant tropomyosin for actin. It is interesting to note that the authors in the literature references failed to study the calcium-dependent movement of fluorescent-labelled actin filaments in an artificial mobility system because the interaction between the *E139X*-mutant tropomyosin and actin was very weak (Marttila et al., 2012). It is likely that glutamic acid at position 139 is important for the binding of tropomyosin to actin. In fact, it was shown that glutamic acid at position *f* of the heptapeptide repeat forms a bond with actin (Brown et al., 2005). At the same time, the E139 deletion only insignificantly impairs coiled coil of tropomyosin at the region between amino acids 138–154 (Lehtokari et al., 2007) indicating that the disrupted structure of the coiled coil is not the main cause of the disease.

According to Fig. 2, the *R91G* mutation decreases actin flexibility in the thin filament at high Ca^{2+} during mimicking of all forms of S1 binding to actin. At low Ca^{2+} this mutation raises F-actin flexibility during mimicking of the strong binding and decreases F-actin's flexibility during mimicking of the weak binding of myosin to actin (Fig. 2b). In contrast, the *E139X* mutation decreases the rigidity of the thin filament in almost all forms of binding at both high and low concentrations of Ca^{2+}, except for the strong bind-

ing in the absence of nucleotides at high Ca^{2+}. The *E139X* mutation also increased Ca^{2+}-sensitivity (Robinson et al., 2007; Marttila et al., 2012; Marston et al., 2013). However, increased Ca^{2+}-sensitivity did not correlate with an increased flexibility of actin (Fig. 2b).

It has been shown previously that during the transition of the muscle fiber from relaxed to rigor state, an increase in the fraction of switched-on actin monomers in the thin filaments was accompanied by the shift of tropomyosin to the inner domain of actin and an increase in the fraction of myosin heads that form the strong binding with actin that is essential for force generation (Borovikov et al., 2009). This behavior was also observed in the presence of the wild-type tropomyosin and troponin (Borovikov et al., 2009). The data in Fig. 2c indicate that the *R91G* and *E139X* mutations in tropomyosin can impair this behavior. It was found that, in the presence of these mutations, an increase in the fraction of switched-on actin monomers was not always accompanied by an increase in the fraction of myosin heads with the strong binding to actin. Thus, the deletion of glutamic acid at position 139 increases the Φ_E value for S1-IAEDANS at high Ca^{2+} and decreases this value at low Ca^{2+} (Fig. 2c) during mimicking of most states of the ATPase cycle. An increase in the Φ_E value for S1-IAEDANS is interpreted as a decrease in the fraction of myosin heads that form the strong binding with actin (Borovikov et al., 2009). Therefore, the fraction of S1 with strong binding is decreased at high Ca^{2+} alongside an increase in the fraction of switched-on actin monomers. At low Ca^{2+}, the fraction of S1 with the strong binding to actin increased and the fraction of switched-on actin monomers decreased (Figs. 2a, 2c). Hence, this mutation in tropomyosin impairs the correlation, which is characteristic of wild-type tropomyosin, between switching actin monomers on and the formation of the strong binding of myosin to actin. Therefore, our data indicate impaired regulation of the actin–myosin interaction with the *E139X*-mutant tropomyosin. Despite that troponin retains the ability to switch on and off actin monomers in the thin filament, tropomyosin lost its ability to prevent the strong binding of myosin to actin at low Ca^{2+} and weakens the ability of troponin to activate the strong binding of myosin to actin at high Ca^{2+} (Figs. 2a, 2b). The appearance of strongly bound myosin heads at low Ca^{2+} indicates that the *E139X* mutation in tropomyosin raises the sensitivity of actomyosin to Ca^{2+}. This conclusion agrees with the data in Fig. 3 showing that the *E139X* mutation markedly increases the Ca^{2+}-sensitivity of actomyosin.

Note that this mutation increases the N value at high concentration of Ca^{2+} (Fig. 2d) and is interpreted as an increase in the mobility of myosin heads. This may be associated with the decreased affinity of myosin to actin (Borovikov et al., 2009). At low Ca^{2+} the deletion increases the mobility of S1 in the absence of nucleotide and decreases it in the presence of ATP. In parallel experiments, the fraction of switched-on actin monomers becomes increased and decreased at high and low Ca^{2+}, respectively (Fig. 2a). Therefore, concerted rearrangements of actin and myosin were also impaired in these experiments.

The *R91G* mutation at high Ca^{2+} increases the fraction of myosin heads involved in the strong binding in the absence of nucleotide and in the presence of ATP and decreases their number in the presence of ADP (Fig. 2d). At low Ca^{2+} this mutation increases the number of myosin heads that are rigidly bound with actin at mimicking the strong binding and decreases the number of myosin heads at mimicking the weak form of binding (in the presence of ATP). These changes agree with the pattern of changes in the behavior of actin in the thin filament. An increase in the fraction of switched-on actin monomers correlates with an increase in the fraction of myosin-head molecules involved in the strong binding to actin. Therefore, the *R91G* mutation does not impair concerted conformational changes in actin and myosin. At low Ca^{2+}, during mimicking of most states of the ATPase cycle, there was an increase in the fraction of switched-on actin monomers and myosin heads involved in the strong binding to actin essential for force generation. This can enhance the contractile function of the muscle tissue, which is typical for distal arthrogryposis associated with the *R91G* mutation (Marttila et al., 2012).

The clinical symptoms described in patients with the *R91G* and *E139X* mutations slightly differ from one another. Thus, the *R91G* mutation causes muscle contractures (Sung et al., 2003), whereas such muscle abnormalities were not revealed in the presence of the *E139X*-mutant tropomyosin (Marttila et al., 2012). In addition, according to our data, these mutations demonstrate some differences at the molecular level. The *E139X* mutation increases the fraction of myosin heads strongly bound to actin at low Ca^{2+} when mimicking different states of the ATPase cycle. The appearance of rigor myosin cross bridges was revealed even during mimicking of relaxed muscle fiber (Fig. 2c). It is clear that the appearance of these cross bridges during fiber relaxation and an increase in the fraction of myosin heads strongly bound to actin during ATP hydrolysis can both weaken contractile function and cause muscle fiber ruptures. Probably, the appearance of these myosin cross bridges is one of the causes in the formation of cap-structures common for myopathy associated with the *E139X* mutation in tropomyosin (Marttila et al., 2012). In contrast, the *R91G*-mutant tropomyosin was not associated with marked appearance of rigor myosin cross bridges at mimicking the relaxed muscle fiber (Fig. 2c); however, this mutation also increased the fraction of myo-

sin heads strongly bound to actin that can weaken the contractile function of the muscle fiber containing the *R91G*-mutant tropomyosin.

It is considered that the main cause of muscular dysfunction associated with tropomyosin mutations at positions 91 and 139 of amino acid sequence consists in an increased sensitivity of the thin filaments to calcium ions, which was first shown in an in vitro *motility assay* (Robinson et al., 2007; Marttila et al., 2012; Marston et al., 2013). Our data do not contradict these viewpoints. Indeed, the data in Figs. 2 and 3 indicate that, at low amount of Ca^{2+} myosin, cross bridges can form the strong binding to actin essential for force generation.

It was shown previously that in the absence of troponin the *E139X* and *R91G* mutations cause shifting of tropomyosin to the inner domain of actin resulting in a decrease and an increase in the fraction of myosin heads, respectively, strongly bound to actin during the ATPase cycle. In parallel studies, the *E139X* and *R91G* mutations caused an increase or decrease in the fraction of switched-on actin monomers, respectively (Borovikov et al., 2015). Therefore, in the absence of troponin, the data on the effects of mutations on conformational rearrangements of actomyosin are close to our data observed in the presence of troponin at high Ca^{2+} (Figs. 2a, 2c). Since troponin retains the ability to perform Ca^{2+}-dependent regulation of actin–myosin interaction in the presence of these mutant tropomyosins, it is suggested that mutations cause these conformational changes in tropomyosin, resulting in an altered response of the myosin heads and actin during the ATPase cycle while troponin retains its regulatory functions.

Therefore, the abnormal behavior of mutant tropomyosins in the ATPase cycle and impaired myosin–actin interaction are likely some of the causes of impaired contractile function described in the literature for muscle tissue containing the *E139X*- and *R91G*-mutant tropomyosins (Muthuchamy et al., 1999; Sung et al., 2003; Kremneva et al., 2004; Ochala et al., 2008; Marston et al., 2013).

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research (project no. 16-34-00865, "Study of Conformational Rearrangements of Actin and S1 in Mimicking Different States of the ATPase Cycle in the Presence of Troponin and Mutant Tropomyosins") and the Russian Science Foundation (project no. 17-14-01224, "Preparation of Recombinant Wild-Type and Mutant Tropomyosins and Evaluation of Functional Properties of the Studied Proteins").

REFERENCES

Borejdo, J. and Putnam, S., Polarization of flourescence from single skinned glycerinated rabbit psoas fibres in rigor and relaxation, *Biochim. Biophys. Acta*, 1977, vol. 459, pp. 578–595.

Borovikov, Y.S., Dedova, I.V., dos Remedios, C.G., Vikhoreva, N.N., Vikhorev, P.G., Avrova, S.V., Hazlett, T.L., and Van Der Meer, B.W., Fluorescence depolarization of actin filaments in reconstructed myofibers: the effect of S1 or pPDM-S1 on movements of distinct areas of actin, *Biophys. J.*, 2004, vol. 86, pp. 3020–3029.

Borovikov, Y.S., Karpicheva, O.E., Avrova, S.V., Robinson, P., and Redwood, C.S., The effect of the dilated cardiomyopathy-causing mutation Glu54Lys of alpha-tropomyosin on actin-myosin interactions during the ATPase cycle, *Arch. Biochem. Biophys.*, 2009, vol. 489, pp. 20–24.

Borovikov, Y.S., Avrova, S.V., Rysev, N.A., Sirenko, V.V., Simonyan, A.O., Chernev, A.A., Karpicheva, O.E., Piers, A., and Redwood, C.S., Aberrant movement of β-tropomyosin associated with congenital myopathy causes defective response of myosin heads and actin during the ATPase cycle, *Arch. Biochem. Biophys.*, 2015, vol. 577–578, pp. 11–23.

Borovikov, Y.S., Rysev, N.A., Avrova, S.V., Karpicheva, O.E., Borys, D., and Moraczewska, J., Molecular mechanisms of deregulation of the thin filament associated with the R167H and K168E substitutions in tropomyosin Tpm 1, *Arch. Biochem. Biophys.*, 2017, vol. 614, pp. 28–40.

Brown, J.H., Zhou, Z., Reshetnikova, L., Robinson, H., Yammani, R.D., Tobacman, L.S., and Cohen, C., Structure of the mid-region of tropomyosin: bending and binding sites for actin, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, vol. 102, pp. 18878–18883.

Fidzianska, A., Baburska, B., Ryniewicz, B., and Dembek, I., "Cap disease": new congenital myopathy, *Neurology*, 1981, vol. 31, pp. 1113–1120.

Fiske, C.H. and Subbarow, Y., Determination of inorganic phosphate, *J. Biol. Chem.*, 1925, vol. 66, pp. 375–400.

Gałazkiewicz, B., Borovikov, Y.S., and Dabrowska, R., The effect of caldesmon on actin–myosin interaction in skeletal muscle fibers, *Biochim. Biophys. Acta*, 1987, vol. 916, pp. 368–375.

Galińska-Rakoczy, A., Engel, P., Xu, C., Jung, H., Craig, R., Tobacman, L.S., and Lehman, W., Structural basis for the regulation of muscle contraction by troponin and tropomyosin, *J. Mol. Biol.*, 2008, vol. 79, pp. 929–935.

Irving, M., Steady-state polarization from cylindrically symmetric fluorophores undergoing rapid restricted motion, *Biophys. J.*, 1996, vol. 70, pp. 1830–1835.

Ivanov, I.I. and Yur'ev, V.A., *Biokhimiya i patobiokhimiya myshts* (Biochemistry and Pathobiochemistry of Muscles), Leningrad: Medgiz, 1961.

Kakol, I., Borovikov, Y.S., Szczesna, D., Kirillina, V.P., and Levitsky, D.I., Conformational changes of F-actin in myosin-free ghost fibre induced by phosphorylated or dephosphorylated heavy meromyosin, *Biochim. Biophys. Acta*, 1987, vol. 913, pp. 1–9.

Karpicheva, O.E., Simonyan, A.O., Kuleva, N.V., Redwood, C.S., and Borovikov, Y.S., Myopathy-causing Q147P TPM2 mutation shifts tropomyosin strands further towards the open position and increases the proportion of

strong-binding cross-bridges during the ATPase cycle, *Biochim. Biophys. Acta*, 2016, vol. 1864, pp. 260–267.

Kaulin, A.B., Polarized fluorescence of acridine orange in muscle fibers in normal and damaged, *Tsitologiia*, 1968, vol. 10, no. 1, pp. 123–125.

Kremneva, E., Boussouf, S., Nikolaeva, O., Maytum, R., Geeves, M.A., and Levitsky, D.I., Effects of two familial hypertrophic cardiomyopathy mutations in alpha-tropomyosin, Asp175Asn and ArgGlu180Gly, on the thermal unfolding of actin-bound tropomyosin, *Biophys. J.*, 2004, vol. 87, pp. 3922–3933.

Lehman, W., Thin filament structure and the steric blocking model, *Compr. Phisiol.*, 2016, vol. 6, pp. 1043–1069.

Lehman, W., Orzechowski, M., Li, X.E., Fischer, S., and Raunser, S., Gestalt-binding of tropomyosin on actin during thin filament activation, *J. Muscle Res. Cell Motil.*, 2013, vol. 34, pp. 155–163.

Lehtokari, V.L., Ceuterick-de Groote, C., de Jonghe, P., Marttila, M., Laing, N.G., Pelin, K., and Wallgren-Pettersson, C., Cap disease caused by heterozygous deletion of the beta-tropomyosin gene *TPM2, Neuromuscul. Disord.*, 2007, vol. 17, pp. 433–442.

Marston, S., Memo, M., Messer, A., Papadaki, M., Nowak, K., McNamara, E., Ong, R., El-Mezgueldi, M., Li, X., and Lehman, W., Mutations in repeating structural motifs of tropomyosin cause gain of function in skeletal muscle myopathy patients, *Hum. Mol. Genet.*, 2013, vol. 22, pp. 4978–4987.

Marttila, M., Lemola, E., Wallefeld, W., Memo, M., Donner, K., Laing, N.G., Marston, S., Gronholm, M., and Wallgren-Pettersson, C., Abnormal actin binding of aberrant beta-tropomyosins is a molecular cause of muscle weakness in TPM2-related namaline and cap myopathy, *Biochem. J.*, 2012, vol. 442, pp. 231–239.

Martilla, M., Lehtokari, V.L., Marston, S., Nyman, T.A., Barnerias, C., Beggs, A.H., Bertini, E., Ceyhan-Birsoy, O., Cintas, P., Gerard, M., Gilbert-Dussardier, B., Hogue, J.S., Longman, C., Eymard, B., Frydman, M., Kang, P.B., Klinge, L., Kolski, H., Lochmüller, H., Magy, L., Manel, V., Mayer, M., Mercuri, E., North, K.N., Peudenier-Robert, S., Pihko, H., Probst, F.J., Reisin, R., Stewart, W., Taratuto, A.L., de Visser, M., Wilichowski, E., Winer, J., Nowak, K., Laing, N.G., Winder, T.L., Monnier, N., Clarke, N.F., Pelin, K., Grönholm, M., and Wallgren-Pettersson, C., Mutation update and genotype-phenotype correlations of novel and previously described mutations in TPM2 and TPM3 causing congenital myopathies, *Hum. Mutat.*, 2014, vol. 35, pp. 779–790.

McKillop, D.F. and Geeves, M.A., Regulation of the interaction between actin and myosin S1: evidence for three states of the thin filament, *Biophys. J.*, 1993, vol. 65, pp. 693–701.

Muthuchamy, M., Pieples, K., Rethinasamy, P., Hoit, B., Grupp, I.L., Boivin, G.P., Wolska, B., Evans, C., Solaro, R.J., and Wieczorek, D.F., Mouse model of a familial hypertrophic cardiomyopathy mutation in alpha-tropomyosin manifests cardiac dysfunction, *Circ. Res.*, 1999, vol. 85, pp. 47–56.

Ochala, J., Li, M., Ohlsson, M., Oldfors, A., and Larsson, L., Defective regulation of contractile function in muscle fibres carrying an E41K beta-tropomyosin mutation, *J. Physiol.*, 2008, vol. 586, pp. 2993–3004.

Oda, T., Namba, K., and Maeda, Y., Position and orientation of phalloidin in F-actin determined by X-ray fiber diffraction analysis, *Biophys. J.*, 2005, vol. 88, pp. 2727–2736.

Okamoto, Y. and Sekine, T., A streamlined method of subfragment one preparation from myosin, *J. Biol. Chem.*, 1985, vol. 98, pp. 1143–1145.

Potter, J.D., Preparation of troponin and its subunits, *Methods Enzymol.*, 1982, vol. 85, pp. 241–263.

Robinson, P., Lipscomb, S., Preston, L.C., Altin, E., Watkins, H., Ashley, C.C., and Redwood, C.S., Mutations in fast skeletal troponin I, troponin T, and beta-tropomyosin that cause distal arthrogryposis all increase contractile function, *FASEB J.*, 2007, vol. 21, pp. 896–905.

Rozanov, Y.M., Chernogryadskaya, N.A., Barskii, I.Y., Borovikov, Y.S., and Shudel, M.S., Polarized ultraviolet fluorescence of muscle fibers and some other cytological anisotropic objects, *Tsitologiia*, 1971, vol. 13, no. 2, pp. 190–200.

Sung, S.S., Brassington, A.M., Grannatt, K., Rutherford, A., Whitby, F.G., Krakowiak, P.A., Jorde, L.B., Carey, J.C., and Bamshad, M., Mutations in genes encoding fast-twitch contractile proteins cause distal arthrogryposis syndromes, *Am. J. Hum. Genet.*, 2003, vol. 72, pp. 681–690.

Szent-Gyorgyi, A.G., Free-energy relations, contraction of actomyosin, *Biol. Bull.*, 1949, vol. 96, pp. 140–161.

Tregear, R.T. and Mendelson, R.A., Polarization from a helix of fluorophores and its relation to that obtained from muscle, *Biophys. J.*, 1975, vol. 15, pp. 455–467.

Wallgren-Pettersson, C., Sewry, C.A, Nowak, K.J., and Laing, N.G., Nemaline myopathies, *Semin. Pediatr. Neurol.*, 2011, vol. 18, pp. 230–238.

Wilson, M.G.A. and Mendelson, R.A., A comparison of order and orientation of cross-bridges in rigor and relaxed muscle fibres using fluorescence polarization, *J. Muscle Res. Cell Motil.*, 1983, vol. 4, pp. 671–693.

Yanagida, T. and Oosawa, F., Polarized fluorescence from e-ADP incorporated into F-actin in a myosin-free single fibre: conformation of F-action and changes induced in it by heavy meromyosin, *J. Mol. Biol.*, 1978, vol. 126, pp. 507–524.

Translated by M. Novikova