Tropomyosin movement is described by a quantitative high-resolution model of X-ray diffraction of contracting muscle

Koubassova, Natalia A.; Bershitsky, Sergey Y.; Ferenczi, Michael Alan; Narayanan, Theyencheri; Tsaturyan, Andrey K.

2016

Koubassova, N. A., Bershitsky, S. Y., Ferenczi, M. A., Narayanan, T., & Tsaturyan, A. K. (2017). Tropomyosin movement is described by a quantitative high-resolution model of X-ray diffraction of contracting muscle. *European Biophysics Journal*, 46(4), 335-342.

<https://hdl.handle.net/10356/82598>

<https://doi.org/10.1007/s00249-016-1174-6>

© 2016 European Biophysical Societies' Association. This is the author created version of a work that has been peer reviewed and accepted for publication in *European Biophysics Journal*, published by Springer Berlin Heidelberg on behalf of European Biophysical Societies' Association. It incorporates referee's comments but changes resulting from the publishing process, such as copyediting, structural formatting, may not be reflected in this document. The published version is available at:
[<http://dx.doi.org/10.1007/s00249-016-1174-6>].

Downloaded on 26 Apr 2023 19:22:40 SGT

Tropomyosin movement is described by a quantitative high resolution model of x-ray diffraction of contracting muscle

Pre-publication : not for distribution

Koubassova N.A.¹, Bershitsky S.Y.², Ferenczi M.A.³, Narayanan T.⁴, Tsaturyan A.K.¹

¹Institute of Mechanics, M.V. Lomonosov Moscow University, Moscow, Russia;

²Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russia;

³Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore

⁴European Synchrotron Radiation Facility, Grenoble, France

- Contact information:

Dr. Natalia Koubassova
Institute of Mechanics
Moscow University
1 Michurinsky prosp.
Moscow 119192 Russia

E-mail: natalia@imec.msu.ru

Abstract

Contraction of skeletal and cardiac muscle is controlled by Ca^{2+} ions via regulatory proteins, troponin (Tn) and tropomyosin (Tpm) associated with the thin actin filaments in sarcomeres. In the absence of Ca^{2+} , Tn-C binds actin and shifts the Tpm strand to a position where it blocks myosin binding to actin, keeping muscle relaxed. According to the 3-state model (McKillop, Geeves 1993), upon Ca^{2+} binding to Tn, Tpm rotates about the filament axis to a 'closed state' where some myosin heads can bind actin. Upon strong binding of myosin heads to actin, Tpm rotates further to an 'open' position where neighbouring actin monomers also become available for myosin binding. Azimuthal Tpm movement in contracting muscle is detected by low angle x-ray diffraction. Here we used high-resolution models of actin-Tpm filaments based on recent cryo-EM data for calculating changes in the intensities of x-ray diffraction reflections of muscle upon transitions between different states of the regulatory system. Calculated intensities of actin layer lines provide a much improved fit to the experimental data obtained from rabbit muscle fibres in relaxed and rigor states than previous lower resolution models. We show that the intensity of the second actin layer line at reciprocal radii from 0.15 nm^{-1} to 0.3 nm^{-1} quantitatively reports the transition between different states of the regulatory system independently of the number of myosin heads bound to actin.

Keywords: actin, tropomyosin, myosin heads, x-ray diffraction, modelling

INTRODUCTION

Actin-myosin interaction that powers contraction of skeletal and cardiac muscles is controlled by calcium ions via regulatory proteins, troponin (Tn) and tropomyosin (Tpm) (Gordon et al. 2000; Nevzorov and Levitsky 2011). Tpm is a $\sim 40 \text{ nm}$ -long coiled-coil dimer of α -helices. Tpm molecules bind each other in a tail-to-head manner forming two stiff continuous strands, which lie in two grooves of a pseudo-two-fold long helix on the surface of F-actin (Moore et al. 2016). Every Tpm molecule forms a complex with Tn which consists of Tn-C, Tn-I and Tn-T subunits. The steric blocking theory of muscle regulation was put forward more than 40 years ago (Haselgrove 1972, Huxley 1972, Spudich et al. 1972). It postulates that in the absence of Ca^{2+} regulatory proteins block myosin-binding sites on actin and keep muscle relaxed, while Ca^{2+} binding to Tn releases the block and allows muscle to contract. Modern versions of the theory, the 3-state model (McKillop, Geeves 1993) now supported by protein crystallography

(Vinogradova et al. 2005) suggests that in the absence of Ca^{2+} , Tn holds Tpm in a blocked, or B-state, where it protects actin monomers from myosin binding. In the presence of Ca^{2+} and in the absence of myosin heads, Ca^{2+} binds Tn-C and causes conformational changes in other Tn subunits. This releases Tpm from the B-state and allows a transition to so called closed, or C-state. During the B- to C-state transition Tpm rotates azimuthally about the filament axis by $\sim 25^\circ$ (Vibert et al. 1997) and partially opens actin sites for myosin binding. When a myosin head binds actin in a strong, stereospecific manner, it causes the Tpm strand to move further by $\sim 10^\circ$ (Vibert et al., 1997) to the open, or O-state, where neighbouring actin sites are fully exposed for myosin binding.

The structural changes associated with Tpm movement are studied by electron microscopy (Vibert et al. 1997) or low-angle x-ray diffraction. The latter is the only method that allows the monitoring of Tpm movement in functioning contracting muscle, either intact (Kress et al. 1986) or chemically permeabilised (Poole et al. 2006). As F-actin with bound Tpm strands forms a near-helical structure with a pitch of ~ 36 nm, azimuthal Tpm movement can be monitored by measuring the intensity of the actin layer lines, especially the second actin layer line, A2, that has spacing of $\sim 1/(18 \text{ nm})$ (Haselgrove 1972, Huxley 1972). More recently, using time-resolved x-ray diffraction on a synchrotron radiation source and 1D detector positioned at a radial spacing of $\sim 0.23 \text{ nm}^{-1}$, Kress et al. (1986) observed changes in the intensity of the A2 layer line which reported Tpm movement during the onset of contraction of frog skeletal muscle. An increase in the A2 intensity precedes the tension rise and changes in the equatorial x-ray reflections showing that Tpm rotation precedes not only force generation but also the radial movement of myosin heads associated with their binding to actin (Kress et al. 1986). The data demonstrated that low angle x-ray diffraction reports Tpm transition from B- to C- and O-states. Monitoring the Tpm transition from B- to O-state and *vice versa* in fully activated muscle is more difficult as the expected changes in the x-ray intensities upon mechanical perturbations are smaller, and are accompanied by a change in the number of myosin heads bound to actin, which also contribute to the intensities of the actin layer lines. As phases of the x-ray reflections cannot be found experimentally, direct mathematical modelling (Koubassova et al. 2002, 2008) is required for quantitative interpretation of the changes in the x-ray diffraction intensities in terms of molecular movement. Early models based on low resolution EM data (Poole et al. 2006, Koubassova 2008) provided some useful insight into the problem. However, the quality of those models was insufficient for making convincing estimates and predictions. For example, the ratio of the intensities of generic A6 and A7 actin layer lines at $1/(5.9 \text{ nm})$ and $1/(5.1 \text{ nm})$, respectively, predicted by the models was twice that observed experimentally (Koubassova 2008).

Here we report the results of modelling of the intensities of the actin layer lines using new high-resolution near-atomic models of the actin-Tpm complex obtained in the presence (O-state, Behrmann et al. 2012) and in the absence (von der Ecken et al. 2015) of myosin heads strongly bound to actin. The results of simulations were compared to experimental x-ray intensities obtained at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) from thin bundles of permeabilised fibres from fast rabbit skeletal muscle in relaxed and rigor states. Our aims were to determine how the high-resolution models of thin filament quantitatively derive the intensities of the actin layer lines experimentally determined by x-ray diffraction, and to identify the x-ray reflections whose intensities provide a quantitative estimate of the transition between C- and O-states independently of the number of myosin heads strongly bound to actin.

METHODS

Structural models of thin filament, intensity calculations. Two recently published atomic structures of the actin-tropomyosin complex were used for model calculations (Behrmann et al. 2012; von der Ecken et al. 2015). As actin monomers in these structures do not show a preserved helical periodicity, an ideal 13/6 helical model with axial shift of 2.75 nm and rotation angle of $12\pi/13$ between neighbour monomers was built using an actin monomer and an adjacent 5.5 nm-long segment of Tpm dimer as a repeating unit of the actin helix. Additional calculations have shown that the choice of a particular actin monomer from a PDB file (PDB code 4AF7 or 3J8A) exerted a negligible effect on the calculated layer line intensities. The helical repeat contained 13 actin monomers and tropomyosin segments in 6 turns of the left-handed helix with a pitch of 35.75 nm. The rotation angle was assumed to be 25° for the B- to C-state transition (Vibert et al. 1997) and 10° or 16° for the transition between C- and O-states. The smaller figure was reported by Vibert et al. (1997), the higher figure is the difference in the position of the Tpm coiled-coil in the cryo-EM models obtained in the presence (Behrmann et al. 2012) and in the absence (von der Ecken et al. 2015) of myosin heads bound to actin. The first model corresponds to the O-state; the second one presumably corresponds to the C-state. Besides the rotation, the two structures differ in the axial position of Tpm with respect to actin and/or twist of the Tpm coiled-coil. Because of the difference, we used both high-resolution models 4A7F and 3J8A for calculation of the intensity of the actin layer lines on the x-ray diffraction pattern and compared the difference.

The layer line intensities were calculated according to Klug et al. (1958) and Vanstein (1966) as described in Koubassova (2008), with the diffracting power of an amino acid assumed to be proportional to the number of electrons in it. Transitions between different states of the thin

filaments were modelled by Tpm rotation about the filament axis without any radial or axial shift or twist with respect to the axis of the Tpm coiled-coil.

Model of the filament lattice in a sarcomere: intensity calculation. Calculations of the x-ray diffraction intensities were performed as described earlier (Koubassova, Tsaturyan 2002, Koubassova et al. 2008) with some modifications. The unit cell contained 6 thin filaments (see above) and 3 myosin filaments; full axial period was 214.5 nm, that is equal to 6 repeats of the thin filament helix with a pitch of 35.75 nm and 5 periods of the myosin helix with a pitch of 42.9 nm. Myosin heads were attached to the energetically preferable actin monomers as described (Koubassova et al. 2002) so that the myosin-based ~43 nm repeat was present in the binding pattern. This repeat is essential as the modulation of myosin binding to actin by myosin-based repeat affects the intensities of some actin layer lines (Tsaturyan 2002).

The layer line intensities were calculated as described in Koubassova, Tsaturyan (2002). An atomic model of the rigor structure of two actin-bound heads of a myosin molecule was taken from the model obtained from electron microscopy of insect flight muscle (Chen et al. 2002, PDB code 1M8Q). The structure was fitted to the position of two neighbour actin-bound catalytic domains of myosin I in the actin-Tpm-S1 structural model (PDB code 4A7F; Behrmann et al. 2012) using a free version of ICM Browser (Molsoft, CA, USA).

Experimental data. Bundles of permeabilised fibres from *m. psoas* from the rabbit were prepared as described (Tsaturyan et al. 2005). X-ray diffraction data were collected on the beamline ID02 at ESRF (Grenoble, France) using a set-up and experimental protocols described earlier (Tsaturyan et al. 2005) at a camera length of 2.4 m. X-ray diffraction patterns in low tension rigor and in the relaxed state were collected from 17 bundles of 3 permeabilised fibres. The total exposure was 6.7 s in each state. The patterns were corrected for the detector response and the camera background and mirrored to improve the signal-to-noise ratio as described (Tsaturyan et al. 2005, Koubassova et al. 2008). Layer line intensities were extracted from the 2D pattern as described (Tsaturyan et al. 2005, Koubassova et al. 2008). As the camera length was short, we were unable to separate the intensities of the A1 and A2 actin layer lines from the myosin layer lines M1 and M2 as has been done with a longer camera (Tsaturyan et al. 2011). For this reason, we do not show the A1 intensity and consider that the inner part of A2 intensity shown here is possibly contaminated by a contribution from the M2 intensity. A single scaling factor was chosen to fit the experimental intensities of layer lines A2, A6, and A7 from relaxed and rigor muscles with intensities of the model. We used the same data set and the diffraction patterns in relaxed and rigor states as those shown in Fig. 1 in Tsaturyan et al. (2005).

RESULTS

The data of Behrmann et al. (2012) suggest that upon binding of myosin heads to actin, Tpm rotates on the surface of F-actin without twist or axial shift. We therefore used this assumption in our modelling. The azimuthal rotation of Tpm associated with the transition from O-state to C-state was assumed to be 10° and the transition from the C-state to B-state that is induced by Ca^{2+} removal from the regulated thin filaments was assumed to correspond to further 25° rotation (Vibert et al. 1997). A near-atomic model (Behrmann et al. 2012) with PDB code 4A7F was chosen as the starting structure that corresponds to O-state. Models of the C- and B-states were obtained by rotation of the Tpm strand by 10° and 35° with respect to the O-state. The original and modified structures are shown in Fig. 1a.

The results of simulation of the effects of Tpm rotation on F-actin on the intensities of the A1, A2, A6, and A7 actin layer lines are shown in Fig. 2.

Tpm mainly contributes to the I_{A1} and I_{A2} while I_{A6} and I_{A7} do not change significantly when we removed it from the model. In the B-state Tpm increases I_{A1} and decreases I_{A2} diffracted by the F-actin model while in the C-state and, especially in the O-state, it increases I_{A2} and depresses I_{A1} (Fig. 2).

Tpm rotation about the F-actin axis induced significant oppositely directed changes in I_{A1} and I_{A2} but not in I_{A6} and I_{A7} (Fig. 2). Tpm movement from the B- to the C-state and further to the O-state causes a decrease in I_{A1} and a parallel increase in I_{A2} . It is known that the A1 layer line corresponds to diffraction on a ~ 36 nm long 2-fold actin helix. When the Tpm strands slide into the groove on the F-actin surface, the whole thin filament transforms into a 4-strand helix with a *ca.* 18 nm period. Such Tpm movement is accompanied by an increase in I_{A2} that corresponds to a 4-strand helix. When the Tpm strand was allowed to undergo Brownian random rotation with respect to F-actin axis from its average position in C-state with standard deviation of Gaussian distribution of 5° , the calculated I_{A2} decreased while the intensities of A1, A6, and A7 remained unchanged (Fig. 1). The difference is probably caused by the fact that a major contribution to I_{A2} comes from the J_2 Bessel function, while A1, A6, and A7 are mainly determined by the J_1 function (Haselgrove 1972, Huxley 1972). Changes in I_{A6} and I_{A7} upon the transitions between B-, C-, and O-states were small (Fig. 2).

The recent near-atomic structure of the F-actin-Tpm complex obtained from cryo-EM data in the absence of myosin heads (von der Ecken et al. 2015) is thought to correspond to C-state of thin filaments. The azimuthal position of the axis of the coiled-coil Tpm differs from that in the O-

state model (Behrmann et al. 2012) by 16° . The difference is somewhat higher than that estimated in earlier EM studies (Vibert et al. 1997). Besides, the two α -helices of Tpm in this myosin-free model structure (PDB code 3J8A) are rotated by *ca* 90° with respect to their position in O-state (PDB code 4A7F) in contrast to the conclusion made previously (Behrmann et al. 2012) that the C- to O-state transition is accompanied by Tpm sliding without twisting.

The original 3J8A and modified structures are shown in Fig. 1b. Changes in intensities of the actin layer lines, which one would expect to occur upon transition from the O- to the C-state and *vice versa*, were obtained by calculating the actin layer line intensities caused by Tpm rotation of 10° or 16° from the O-state (Fig. 3) or C-state (Fig. 3) model.

There was a significant difference between I_{A1} and I_{A2} in the C-state models calculated for Tpm rotation of 10° or 16° (Fig. 3a, b). However even the model obtained with 16° Tpm rotation from the O-state underestimated both I_{A1} and I_{A2} obtained from the C-state models suggested by von der Ecken et al. (2015). Only small changes in the A6 and A7 intensities were found upon Tpm rotation by either 10° or 16° .

The A1 intensity calculated for Tpm rotation of 16° from the myosin-free model (von der Ecken et al. 2015, 3J8A) is very similar to that calculated for the O-state 4A7F model (Behrmann et al. 2012), while the Tpm rotation of 10° gives a different intensity profile (Fig. 4a). The difference in I_{A2} calculated for the Tpm rotation of 10° or 16° is less significant: both models predict ~ 2 -fold change upon the transition between C- and O-states although the effect of rotation by 16° is slightly more pronounced and predicted I_{A2} that is even higher than that obtained from the C-state model (Behrmann et al. 2012). Only small changes in the calculated A6 and A7 intensities were found for the Tpm rotation from the O-state model (Fig. 3) and practically no changes were found for the C-state model (Fig. 4) with different orientation or axial position of the Tpm coiled coil with respect to actin. The data show that despite the difference in Tpm position and orientation both high-resolution models predict similar changes in I_{A2} upon transition between C- and O-states.

To estimate the effect of actin-bound myosin heads on the intensities of the actin layer lines, an additional set of calculations was performed and compared to experimental ones. We modified the O-state model obtained at full saturation of actin monomers with bound heads of myosin 1 (Behrmann et al. 2012) to simulate the structure of regulated thin filament in rigor where all

myosin heads are bound to actin and compared the calculated layer line intensities to experimental ones, obtained from rabbit skeletal muscles (Fig. 5). We also calculated the layer line intensities for a model of B-state obtained by rotating the Tpm strands (Fig. 2) and compared them with experimental intensities obtained from relaxed muscle fibres (Fig. 5).

A single scaling factor was used for simultaneous fitting of all six experimental intensity profiles: three actin layer lines A2, A6, and A7 in two states, relaxed and rigor (Fig. 5). The scaling factor was chosen to minimise the root mean square deviation between five of six experimental and calculated intensity profiles, except I_{A2} in rigor as the experimental I_{A2} in this state contained an unquantified contribution from the myosin layer line M2. The contribution from M2 was mainly seen at low reciprocal radii where the contribution of myosin heads is essential (see Methods for details). There was a broad spread of A2 intensity at reciprocal radii of $0.15 \text{ nm}^{-1} - 0.275 \text{ nm}^{-1}$ due to an axial misalignment of the thin filaments and variation in the helical angle of F-actin. To include the whole A2 intensity in I_{A2} we used a rather wide meridional integration range which included some M2 intensity that was mainly present at low reciprocal radii below 0.1 nm^{-1} . The O-state model fits quite well the outer part of I_{A2} at the reciprocal radii $>0.15 \text{ nm}^{-1}$ and the whole profiles of I_{A6} , I_{A7} in rigor muscle fibres (Fig. 5). The only difference was a slight shift in the high-angle peak position in rigor from $\sim 0.24 \text{ nm}^{-1}$ towards the smaller reciprocal radii of $\sim 0.22 \text{ nm}^{-1}$ in calculated I_{A2} . Models of B-state obtained from EM-derived models by Tpm rotation also fit the intensities of these layer lines in relaxed muscle (Fig. 4). Actin-bound myosin heads contribute to the low angle part of A2, but do not affect its intensity at reciprocal radii of $0.15 \text{ nm}^{-1} - 0.275 \text{ nm}^{-1}$ (Fig. 5).

DISCUSSION

Tropomyosin movement and x-ray diffraction. Two Tpm strands wind along the F-actin helix with a pitch of ~ 36 nm. For this reason, the main contribution of the x-ray scattering of Tpm strands is expected to be on the actin layer line with the **reciprocal** spacing multiple to $1/(36 \text{ nm})$. In contracting muscles, the brightest actin layer lines with measurable intensities are A1, A2, A6, and A7, while lines A3, A4, and A5 are weak and difficult to measure, except A5 in rigor. Besides, the overlap of two neighbour Tpm molecules and, more importantly, the Tn complexes bound to each Tpm molecule, have axial spacing of ~ 38.5 nm, equal to the length of a 14 monomer-long segment of F-actin and therefore gives rise to meridional x-ray reflections Tn1, Tn2, etc. with spacing multiple of $1/(38.5 \text{ nm})$. The intensities of meridional Bragg reflections depend critically on the degree of crystallinity of the filament lattice (Bershitsky et al. 2009) and for this reason it is difficult to use them for quantitative interpretation in terms of molecular movement. Among all actin layer lines, the first two, A1 and A2, at higher reciprocal radii are the most informative for detection of Tpm movement. This is because their shift into the groove in the long actin helix transforms a near two-strand helix with pitch of ~ 36 nm into a near four strand one with pitch of ~ 18 nm (Kress et al., 1986).

Comparison with earlier models and x-ray data. Both recent high-resolution models of the actin-Tpm complex provide much better fit of the intensities of A6 and A7 actin layer lines than earlier models (Pirani et al. 2006, Poole et al. 2006, Koubassova 2008) confirming the validity of the models (Behrmann et al. 2012, von der Ecken et al. 2015) used here for F-actin in muscle. The ratio of the A6 and A7 intensities calculated for Tpm decorated F-actin for both models was 3.5-4 for both relaxed muscle and rigor muscle fibres (Fig. 4; Kraft et al. 2002) while it was ~ 2.5 for previous models based on low-resolution data (Koubassova, 2008; Koubassova et al. 2008). Tpm only slightly contributes to I_{A6} and I_{A7} intensities while it affects the I_{A1} and I_{A2} intensities at higher reciprocal radii. As for the effects of Tpm rotation that corresponds to transitions between B-, C- and O-states of the regulated thin filaments on the intensities of the actin layer lines, new and earlier models give similar results. Ca^{2+} activation in the absence of myosin heads (B- to C-transition) leads to a three-fold decrease in I_{A1} intensity and to a more than 5-fold increase in I_{A2} . Calculated profiles of I_{A2} , I_{A6} and I_{A7} in relaxed muscle were close to those found experimentally (Fig. 5) except for the position of the peak on A2 which was slightly shifted compared to experimental data. The positions of the peaks on A6 and A7 as well as the shapes of the intensity profiles on these layer lines were quite well reproduced by the model. This means that sample

preparation for cryo-EM and data collection and analysis procedures in (Behrmann et al. 2012, von der Ecken et al. 2015) did not introduce a systematic error in the radius and general shape of the thin filaments and its components. Similarly, I_{A2} , I_{A6} and I_{A7} in rigor were fitted quite well with a modified model of Behrmann et al. (2012) which was supplemented by a proper model of rigor myosin heads (Fig. 5).

C-state to O-state transition. Less pronounced while also significant and measurable changes in I_{A1} and I_{A2} were found upon simulation of Tpm rotation that corresponds to the transition between C- to O-states. A rotational Brownian fluctuation in the Tpm position in C-state (Fig. 2) and an uncertainty in the amount of Tpm rotation (Figs. 3, 4) set a range for the amount of decrease in I_{A2} intensity that one would expect to take place during the transition. Despite the scatter, the results of our calculation show a significant and measurable decrease of 35-58% in the A2 intensity in the range of reciprocal radii of 0.15 nm^{-1} to 0.0275 nm^{-1} . Our calculation showed that the contribution of myosin heads to I_{A2} in this range of reciprocal radii is negligible (Fig. 5). Therefore, I_{A2} intensity at high angle is a convenient measure of the C- to O-state transition in contracting muscle even when the fraction of myosin heads bound to actin can vary significantly.

Based on simulations of the x-ray intensities using high-resolution cryo EM data (Behrmann et al. 2012, von der Ecken et al. 2015), we show that I_{A2} at the higher angle measures Tmp movement in contracting muscle and detects the C- to O-state transition as was suggested a long time ago by Kress et al. (1986) for the B- to O-state transition.

ACKNOWLEDGEMENTS

The work was supported by grants from the Russian Foundation for Basic Research 15-04-02174 (to NK) and 16-04-00693 (to AT) and by ESRF. Authors thank Mr J. Gorini for excellent technical support.

REFERENCES

1. Bershtitsky SY, Ferenczi MA, Koubassova NA, Tsaturyan AK (2009) Insight into the actin-myosin motor from x-ray diffraction on muscle. *Front Biosci* 14:3188-213 doi:10.2741/3444
2. Behrmann E, Muller M, Penczek PA, Mannherz HG, Manstein DJ, Raunser S (2012) Structure of the rigor actin-tropomyosin-myosin complex. *Cell* 150:327-338 doi: 10.1016/j.cell.2012.05.037

3. Chen LF, Winkler H, Reedy MK, Reedy MC, Taylor KA (2002) Molecular modeling of averaged rigor crossbridges from tomograms of insect flight muscle. *J Struct Biol* 138: 92-104
4. Gordon AM, Homsher E, Regnier M (2000) Regulation of contraction in striated muscle. *Physiol Rev* 80:853-924
5. Haselgrove JC (1972) X-ray evidence for a conformational change in the actin-containing filaments of vertebrate striated muscle. *The Mechanism of Muscle Contraction*. Cold Spring Harbor Laboratory; Cold Spring Harbor, NY, pp 341–352
6. Huxley HE (1972) Structural changes in the actin- and myosin-containing filaments during contraction; *The Mechanism of Muscle Contraction*. Cold Spring Harbor Laboratory; Cold Spring Harbor, NY, pp 361–368
7. Klug A, Crick FHC, Wyckoff HW (1958) Diffraction by helical structures. *Acta Cryst* 11: 199-213
8. Koubassova NA (2008) A comparison of the models of a thin filament in the muscle with low-angle X-ray diffraction data obtained for the relaxed rabbit muscle. *Biofizika* 53: 936-942
9. Koubassova NA, Bershtitsky SY, Ferenczi MA, Tsaturyan AK (2008) Direct modeling of X-ray diffraction pattern from contracting skeletal muscle. *Biophys J* 95:2880-2894
10. Koubassova NA, Tsaturyan AK (2002) Direct modeling of x-ray diffraction pattern from skeletal muscle in rigor. *Biophys J* 83:1082-1097
11. Kraft T, Mattei T, Radocaj A, Piep B, Nocula C, Furch M, Brenner B (2002) Structural features of cross-bridges in isometrically contracting skeletal muscle. *Biophys J* 82:2536-2547
12. Kress M, Huxley HE, Faruqi AR, Hendrix J (1986) Structural changes during activation of frog muscle studied by time-resolved X-ray diffraction. *J Mol Biol* 188:325-342
13. McKillop DF, Geeves MA (1993) Regulation of the interaction between actin and myosin subfragment 1: evidence for three states of the thin filament. *Biophys J* 65:693-701
14. Moore JR, Campbell SG, Lehman W (2016) Structural determinants of muscle thin filament cooperativity. *Arch Biochem Biophys* 594:8-17
15. Nevzorov IA, Levitsky DI (2011) Tropomyosin: double helix from the protein world. *Biochemistry (Mosc)*. 76:1507-1527
16. Pirani A, Vinogradova MV, Curmi PM, King WA, Fletterick RJ, Craig R, Tobacman LS, Xu C, Hatch V, Lehman W (2006) An atomic model of the thin filament in the relaxed and Ca²⁺-activated states. *J Mol Biol* 357:707-717

17. Poole KJ, Lorenz M, Evans G, Rosenbaum G, Pirani A, Craig R, Tobacman LS, Lehman W, Holmes KC (2006) A comparison of muscle thin filament models obtained from electron microscopy reconstructions and low-angle X-ray fibre diagrams from non-overlap muscle. *J Struct Biol* 155:273-284
18. Spudich JA, Huxley HE, Finch JT (1972) Regulation of skeletal muscle contraction. II. Structural studies of the interaction of the tropomyosin-troponin complex with actin. *J Mol Biol* 72:619-632
19. Tsaturyan AK (2002) Diffraction by partially occupied helices. *Acta Crystallogr A* 58: 292-294
20. Tsaturyan AK, Koubassova N, Ferenczi MA, Narayanan T, Roessle M, Bershitsky SY (2005) Strong binding of myosin heads stretches and twists the actin helix. *Biophys J* 88:1902-1910
21. Tsaturyan AK, Bershitsky SY, Koubassova NA, Fernandez M, Narayanan T, Ferenczi MA. (2011) The fraction of myosin motors that participate in isometric contraction of rabbit muscle fibers at near-physiological temperature. *Biophys J* 101:404-410
22. Vainstein BK (1966) Diffraction of X-rays by chain molecules. Elsevier, Amsterdam.
23. Vibert P, Craig R, Lehman W (1997) Steric-model for activation of muscle thin filaments. *J Mol Biol* 266:8-14
24. Vinogradova MV, Stone DB, Malanina GG, Karatzaferi C, Cooke R, Mendelson RA, Fletterick RJ (2005) Ca(2+)-regulated structural changes in troponin. *Proc Natl Acad Sci U S A*. 102:5038-5043
25. Von der Ecken J, Mülle M, Lehman W, Manstein DJ, Penczek PA, Raunser S (2015) Structure of the F-actin-tropomyosin complex. *Nature* 519:114-117

FIGURE LEGENDS

Fig. 1. a: cryo-EM structure (Behrmann et al. 2012, PDB code 4A7F) of the actin (light gray) complex with Tpm (green ribbon) in the O-state, two models of the C-state obtained by Tpm rotation by 10° and 16° (light and dark red ribbons, respectively) and of B-state (35° Tpm rotation, black ribbon); **b:** cryo-EM structure (von der Ecken et al. 2015, 3J8A) of the actin (light gray) complex with Tpm (red ribbon) in the C-state, two models of the O-state obtained by Tpm rotation by 10° and 16° (light and dark green ribbons, respectively).

Fig. 2. a-d: the intensities I_{A1} , I_{A2} , I_{A6} , I_{A7} of the actin layer lines A1, A2, A6, A7 calculated using the cryo-EM structure of the actin-Tpm-myosin complex (Behrmann et al. 2012, PDB code 4A7F). Black lines correspond to B-state, red lines correspond to C-state and green lines correspond to O-state. C- and B-states were obtained by Tpm rotation from the O-state by 10° and 35°, respectively. Red dash-dot lines represent the intensity profiles calculated for the Gaussian distribution of the Tpm azimuthal position with a standard deviation of 5° from its average position in the C-state. Note that dash-dot red line appears different from the continuous red one only on A2 (**b**). Purple lines show the intensities calculated from the F-actin model without Tpm.

Fig. 3. a-d: the intensities I_{A1} , I_{A2} , I_{A6} , I_{A7} calculated for the O-state model (Behrmann et al. 2012, 4A7F, bold green lines), and for the same model with Tpm azimuthal rotation about the F-actin axis without axial shift or twist about the Tpm coiled-coil axis. Intensity profiles calculated with Tpm rotation by 10° (dotted red lines) and 16° (dashed red lines) are presented as alternative model of C-state. Note these profiles are seen only in **a** and **b** while in **c** and **d** they are hidden behind the green lines. The intensities calculated for the C-state model of von der Ecken et al. (2015; 3J8A) are shown by bold red lines.

Fig. 4. a-d: the intensities I_{A1} , I_{A2} , I_{A6} , I_{A7} calculated for the C-state model (von der Ecken et al. 2015, 3J8A, bold red lines), and for the same model with Tpm azimuthal rotation about F-actin axis without an axial shift or twist about the coiled-coil axis. Intensity profiles calculated with Tpm rotation by 10° (dotted green lines) and 16° (dashed green lines) are shown for alternative O-state models. Note these profiles are seen only in **a** and **b** while in **c** and **d** they are hidden behind the red lines. The intensities calculated for the O-state model (Behrmann et al. 2012, 4A7F) are shown by bold green lines.

Fig. 5. a-c: calculated (continuous lines) and experimental (dashed lines) I_{A2} , I_{A6} and I_{A7} . **Blue lines** correspond to rigor muscle bundles or O-state model (Behrmann et al. 2012) with rigor myosin heads bound to actin (for details see Methods), black lines correspond to relaxed muscles or B-state model obtained as shown in Fig. 1. **Green lines** show the intensities calculated from the O-state model without myosin heads.

Fig. 1

Fig 2

Fig 3

Fig 4

Fig 5