Session 2. Cardiac/smooth muscle ultrastructure and regulation

Lectures

L2.1

Regulation of the thin filament – novel paradigms revealed by cryo electron microscopy

Cristina M. Risi[1], Ian Pepper[1], Betty Belknap[1], Maicon Landim-Vieira[2], Howard D. White[1], Kelly Dryden[3], Jose R. Pinto[2], P. Bryant Chase[4], Vitold E. Galkin[1]

[1]Department of Physiological Sciences, Eastern Virginia Medical School, Norfolk, VA 23507, USA; [2]Department of Biomedical Sciences, Florida State University College of Medicine, Tallahassee, FL, USA; [3]Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA, USA; [4]Department of Biological Science, Florida State University, Tallahassee, FL, USA

Vitold E Galkin <galkinve@evms.edu>

Every heartbeat relies on cyclical interactions between myosin thick and actin thin filaments (TFs) orchestrated by rising and falling Ca^{2+} levels. The structural mechanics of the TF regulation by Ca^{2+} was mostly unknown until single particle approach to reconstruction of frozen hydrated thin filaments was developed. The structures of TF obtained at low and high Ca^{2+} levels revealed the transition between the Ca^{2+}-free and Ca^{2+}-bound states. We resolved the structure of the porcine TF at physiological (systolic) Ca^{2+} levels to show that the two strands of the thin filament are comprised of a mixture of regulatory unit structures, which may be Ca^{2+}-free, Ca^{2+}-bound, or mixed troponin (Tn) complexes. We traced Tn conformations along and across individual TFs to demonstrate that at systolic Ca^{2+} levels two TF strands are activated stochastically with short-range cooperativity on one of the two strands as well as across the strands. Interestingly, this is different from the cooperativity observed in murine cardiac TFs. Our novel data provide more insights into the structure of the TF junction region. Overall, our findings show the power of cryo-EM in revealing the mechanisms by which cardiac muscle is regulated by narrow range Ca^{2+} fluctuations.

L2.2

The interacting-heads motif of myosin: from smooth muscle to cardiac

C. A. Scarff[1], G. Carrington[1], M. Giralt-Pujol[1], B. Barua[2], D. Winkelmann[2], J. Trinick[1]

[1]Astbury Centre for Structural Molecular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK; [2]Department of Pathology and Laboratory Medicine, Robert Wood Johnson Medical School, Rutgers University, Piscataway, New Jersey 08854, USA

Charlotte Anne Scarff <c.a.scarff@leeds.ac.uk>

Myosin-2 forms a switched-off state, conserved across myosin-2 isoforms, in which ATPase activity is very low. In smooth and non-muscle myosin-2, in this state, termed shutdown, myosin filaments are disassembled into monomers, the filament-forming tail wraps around the two heads of the monomer, with the heads bent back and interacting with one another in the so-called interacting-heads motif (IHM). This shutdown state serves as a storage state and enables diffusion. In striated muscle, in the switched-off state, the tails remain polymerised into filaments but the heads on the filament surface adopt an IHM. The IHM has been associated with the super-relaxed state of muscle and has been proposed to be destabilised in the heart by mutations that cause hypertrophic cardiomyopathy (HCM), a common inherited heart disease.

Here, we investigate the structural relationship between the IHM of smooth muscle myosin, and that of beta-cardiac myosin by creating a homology model from our cryo-EM structure of shutdown myosin-2 (Scarff *et al.*, 2020, *Nature*). We examine the structure of the beta-cardiac myosin IHM and explore its interactions with cardiac myosin binding protein-C (cMyBP-C) using electron microscopy, mass spectrometry and modelling approaches. We show that HCM mutations can destabilise the beta-cardiac myosin IHM whilst cMyBP-C can stabilise it, providing experimental evidence of a link between IHM stability and HCM pathogenesis.

Oral Presentations

O2.1

In situ cryo-electron tomography reveals filamentous actin with the microtubule lumen

D. M. Paul[1], J. Mantell[2], U. Borucu[3], J. Coombs[2], K. Surridge[2], J. M. Squire[4,1], P. Verkade[2], M. P. Dodding[2]

[1]School of Physiology, Pharmacology and Neuroscience, Faculty of Life Sciences, University of Bristol, Bristol, United Kingdom; [2]School of Biochemistry, Faculty of Life Sciences, University of Bristol, Bristol, United Kingdom; [3]GW4 Facility for High-Resolution Electron Cryo-Microscopy, University of Bristol, Bristol, United Kingdom; [4]Department of Metabolism, Digestion and Reproduction, Imperial College, London, United Kingdom

Danielle Paul <danielle.paul@bristol.ac.uk>

Many cellular processes like migration and cell division depend on filamentous (F-) actin and microtubules. These dynamic filamentous proteins are major components of the cytoskeleton. Biophysical and cell biology experiments continue to reveal how the actin and microtubule cytoskeletons work together and are intimately linked. The complex interactions or crosstalk between these two structural scaffolds is thought to be regulated by proteins that interface between them. The role of many microtubule associated proteins (MAPs) is now becoming clearer, however less is known about the role of proteins that reside within the microtubule lumen. Microtubules assemble from tubulin dimers to form hollow tubes of protofilaments with a luminal width of ~15 nm, which limits access to antibody epitope or small-molecule binding sites, used in the detection of associated proteins. We have used cryo-electron tomography to demonstrate that the microtubule lumen can be occupied by extended segments of F-actin in small-molecule induced, microtubule-based cellular projections. This is the first observation of these two crucial protein scaffolds in such a conformation (Paul *et al*., 2020). We determined two classes of actin filament and evidence of an additional protein regularly labelling the actin in the class II filaments. We have uncovered an unexpected versatility in cytoskeletal form that may prompt a significant development of our current models of cellular architecture and offer a new experimental approach for the *in situ* study of microtubule structure and contents.

Refrences:

Paul DM, Mantell J, Borucu U, Coombs J, Surridge KJ, Squire JM, Verkade P, Dodding MP (2020) *In situ* cryo-electron tomography reveals filamentous actin within the microtubule lumen. *J Cell Biol* **219**(9): e201911154. doi: 10.1083/jcb.201911154.

O2.2

Phosphorylation-dependent interactions of myosin binding protein-C and troponin coordinate the myofilament response to PKA

I. R. Sevrieva[1], S. Ponnam[1], Z. Yan[2], M. Irving[1], T. Kampourakis[1], Y.-B. Sun[1]

[1]Randall Centre for Cell and Molecular Biophysics, and British Heart Foundation Centre of Research Excellence, King's College London, London, SE1 1UL, United Kingdom; [2]Centre for Craniofacial & Regenerative Biology, King's College London, London, SE1 9RT, United Kingdom

Ivanka Sevrieva <ivanka.r.sevrieva@kcl.ac.uk>

Protein kinase A (PKA) mediated phosphorylation of sarcomeric proteins improves heart muscle performance in response to β-adrenergic stimulation and is associated with enhanced relaxation and a steeper Frank-Starling relationship. At the cellular level, the latter translates to a larger dependence of Ca^{2+} sensitivity and maximum force on sarcomere length, i.e. enhanced length dependent activation (LDA). However, the mechanisms by which PKA phosphorylation of the most notable sarcomeric PKA targets, troponin I (cTnI) and myosin binding protein-C (cMyBP-C) lead to these effects, remain elusive. We altered the phosphorylation level of cTnI specifically, in heart muscle cells, and characterized the structural and functional effects at different levels of cMyBP-C phosphorylation. We found very different roles for cTnI phosphorylation at Ser22 and Ser23 than in previous reports. Mono-phosphorylation was correlated with lower Ca^{2+} sensitivity, also lower thin filament activation during stretch in relaxing conditions. In contrast, bis-phosphorylation *increased* Ca^{2+} sensitivity at longer sarcomere length and was indispensable for the enhanced shift in pCa_{50} associated with LDA, as was cMyBP-C phosphorylation by PKA. Taken together, the phosphorylation-dependent sensing of resting length changes by the thin filament and the high level of coordination between cTnI and cMyBP-C suggest a common mechanism. This was further supported by our finding that cardiac troponin (cTn) can directly interact with cMyBP-C *in vitro*, in a phosphorylation- and Ca^{2+}-dependent manner. These results suggest that the augmentation in LDA downstream of β-adrenergic signalling may be achieved by coordinated coupled changes in cTnI and cMyBP-C.

Acknowledgements of Financial Support: Supported by the British Heart Foundation and Wellcome Trust.

O2.3

Cryo-electron tomography of intact cardiac muscle reveals myosin binding protein-C linking myosin and actin filaments

Xinrui Huang[1,2], Iratxe Torre[3], Michele Chiappi[3], Zhan Yin[3], Oliver Raschdorf[4], Morgan Beeby[5], Bonnie Chaban[5], Pieter de Tombe[6], Jun Liu[2], Edward Morris[7], Pradeep Luther[3]

[1]Department of Biochemistry and Biophysics, School of Basic Medical Sciences, Peking University, Beijing 100191, China; [2]Yale School of Medicine, Yale University, West Haven CT 06516, USA; [3]National Heart and Lung Institute, Imperial College London, London SW7 2AZ, UK; [4]Thermo Fisher Scientific, Eindhoven, North Brabant, Netherlands; [5]Department of Life Sciences, Imperial College London, London SW7 2AZ; [6]Loyola University Chicago Medical School, Maywood, IL60153, USA; [7]Institute of Cancer Research, London SW3 6JB, UK
Pradeep Luther <p.luther@imperial.ac.uk>

Myosin binding protein C (MyBP-C) is a component of the thick myosin filament in vertebrate striated muscle. Electron microscopy (em) of the sarcomere in these muscles shows MyBP-C located in each half A-band with a periodicity of 430Å forming 7-9 stripes in the so-called C-zone. It is a linear protein composed of 10 or 11 Ig/FNIII domains. There is great interest in the cardiac isoform since the discovery that mutations in the gene are a major cause of hypertrophic cardiomyopathy. Early biochemical evidence showed that MyBP-C regulates contractility in the heart by binding to myosin or actin. Support for the latter comes from recent high resolution cryo-em structures of MyBP-C N-terminal domains binding to isolated actin filaments. To understand how MyBP-C may regulate contractility, we need to understand its structure in intact cardiac muscle. Here we have used cryo-electron tomography and subtomogram averaging to study the 3D structure of MyBP-C in intact muscle. We used "Tokuyasu"-type cryosections of chemically fixed rat cardiac muscle which were thawed, rinsed and refrozen for cryo-em analysis. The cryosections are dimensionally stable, allowing identification of the different MyBP-C stripes. Subtomogram averaging of coarsely binned tomograms identified the main features of the A-band which was followed by fine averaging centred on each MyBP-C stripe. Within the resulting 40 Å resolution maps the structure of the myosin filaments agrees well with previous studies by Al-Khayat *et al.* (2013) and Zoghbi *et al.* (2008). Further subtomogram averaging over each of the 9 MyBP-C stripes revealed protein density running between the myosin and actin filaments consistent with a set of Ig domains characteristic of MyBP-C.

Virtual Posters

P2.1

Stearoyl-CoA desaturase deficiency promotes cardiac myocytes maturation

Volodymyr Balatskyi, Marcin Wolosiewicz, Pawel Dobrzyn

Laboratory of Molecular Medical Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland
Volodymyr Balatskyi <v.balatskyi@nencki.edu.pl>

Cardiomyocytes maturation during early postnatal period is recently recognized as the last step of heart muscle cells differentiation on their way to finally acquire the adult phenotype. The transition from the anaerobic to oxidative metabolism is the most prominent hallmark during this process. Stearoyl-CoA desaturase (SCD) is a known regulator of cellular metabolism, and in the present research we aimed to investigate the role of widely expressed SCD1 and the heart-specific SCD4 in cardiomyocytes maturation. To this end, we used primary cardiomyocytes isolated from one-day-old SCD1 and SCD4 knock-out (KO) mice. We found that the ablation of either SCD1 or SCD4 promotes cardiomyocytes maturation compared to wild-type (WT) cells. Proliferation of KO cardiomyocytes was reduced along with an unaffected viability. Moreover, expression of adult isoforms of light (*MLC2v*) and heavy (*a-MHC*) myosin chains were increased. Metabolic phenotype of KO cardiomyocytes was shifted to more oxidative compared to WT myocytes, evidenced by enhanced oxygen consumption rate in SCD1 and SCD4 KO cardiomyocytes as well as decreased extracellular acidification rate in SCD4 KO cells. KO cardiomyocytes exhibited increased rate of calcium reuptake in sarcoplasmic reticulum, indicating a functional maturation of those cells. In aggregate, our data show that ablation of SCD1 or SCD4 stimulates maturation of cardiomyocytes, possibly through the activation of oxidative metabolism.

P2.2

The effect of estrogen deficiency on the mechanical function of rat atrial and ventricular cardiomyocytes

A. Kochurova[1], T. Myachina[1], X. Butova, R. Simonova[1], V. Berg[1], D. Kuznetsov[1], G. Kopylova[1], D. Shchepkin[1], A. Khokhlova[1,2]

[1]Institute of Immunology and Physiology, Russian Academy of Sciences, Yekaterinburg, Russia; [2]Ural Federal University, Yekaterinburg, Russia
Valentina Berg <valeoupi@gmail.com>

Estrogen deficiency has a significant impact on the excitation-contraction coupling in the ventricular myocardium, but its effect on the atrial contractile function is still poorly understood. We compared the parameters of sarcomere shortening, cytosolic Ca^{2+} transients in single cardiomyocytes, and actin-myosin interaction in the myocardium from the left atrium (LA) and left ventricle (LV) between ovariectomized (OVX) and sham-operated female Wistar rats. Six weeks after surgery, single cardiomyocytes were obtained as described previously (Butova *et al.*, 2021). The characteristics of actin-myosin interaction were studied in an *in vitro* motility assay.

We found that in LA myocytes, OVX increased end-diastolic sarcomere length and sarcomere shortening amplitudes but had no significant effect on the parameters of Ca^{2+} transients. In LV cells, OVX increased Ca^{2+} transient amplitudes and decreased time to peak sarcomere shortening, time to 50% relaxation, and time to 50% Ca^{2+} transient decay. We observed differences in OVX-induced changes in phosphorylation and functioning of sarcomere proteins between LA and LV. In LA, OVX decreased Tpm and TnI phosphorylation. In LV, OVX decreased TnI phosphorylation but increased cMyBP-C and Tpm phosphorylation. OVX reduced the Ca^{2+} sensitivity of actin-myosin interaction in LV only.

Thus, estrogen deficiency affects differently the contractile function of atrial and ventricular cardiomyocytes.

Acknowledgements of Financial Support: by the RFBR # 20-315-70006.

P2.3

Heart disease on the molecular level as revealed by Cryo-EM of thin filaments

Marston Bradshaw[1], Ed Morris[2], Giulia Bigotti[3], Danielle Paul[1]

[1]School of Physiology, Pharmacology and Neuroscience, University of Bristol, UK; [2]Division of Structural Biology at The Institute of Cancer Research, London, UK; [3]School of Biochemistry, University of Bristol, UK
Marston Bradshaw <mb16907@bristol.ac.uk>

The main components of cardiac thin filaments are actin and the regulatory proteins troponin and tropomyosin which confer calcium sensitivity. All these proteins can carry mutations related to heart disease (Marques and de Oliveira, 2016). To understand the process of muscle regulation and the effects of mutations at a molecular level we need to determine high resolution structures of this macromolecular complex and Cryo-EM has proved a powerful technique in muscle research (Bradshaw, 2019).

Hypertrophic and dilated cardiomyopathy (HCM and DCM), are primary cardiac muscle diseases. HCM is also the most common cause of sudden cardiovascular death in young adults and athletes. It was thought to affect more than 1 in 500 people in the general population (Mozaffarian *et al.*, 2016). All the proteins in the thin filament are known to have mutations that lead to HCM, mutations in troponin T alone account for 15~30% of all cases of HCM (Watkins *et al.*, 1995).

It is essential that we understand the changes to the protein-protein interactions that these mutations cause to understand these complex diseases of the sarcomere.

We have successfully isolated zebrafish cardiac thin filaments, imaged them in cryo-EM and used *in silico* classification to sort the data into two populations consisting of bare actin and regulatory protein decorated filaments. Using the bare actin subset we have generated a 3.7 Å map of actin using single particle analysis. The decorated filament subset have allowed us to employ our novel image processing procedures (Paul *et al.*, 2010, 2017) to calculate 3D reconstructions without imposing any helical symmetry, generating an intermediate resolution native thin filament reconstruction.

P2.4

Quantifying the contribution of titin and actin filaments to cardiomyocyte passive stiffness

J. K. Freundt, C. M. Loescher, W. A. Linke

University of Muenster, Institute of Physiology II, Muenster, Germany
Johanna K. Freundt <Johanna.Freundt@ukmuenster.de>

Question. The giant sarcomere protein titin bears passive load in cardiomyocytes (CMs), and increased titin-based stiffness occurs in HF. Here, we aimed to quantify the contribution of titin and actin filaments to CM passive stiffness, using a new genetic mouse model that allows the specific and acute cleavage of the titin springs.
Methods & Results. In the mouse model, a tobacco etch virus (TEV) protease-recognition site and a HaloTag were cloned into elastic titin (HaloTag-TEV knock-in (KI)). This cassette allows for specific in-situ cleavage of titin and visualization of titin cleavage with fluorophore-conjugated HaloLigand labeling. Recombinant TEV protease caused the rapid, specific, and complete cleavage of cardiac titin in homozygous KI heart samples, and ~50% cleavage in heterozygous KI. Single CMs isolated from homozygous KI hearts were stretched and the resulting force recorded before/after cleavage of titin. The specific titin cleavage resulted in a 61±5% (n=10) reduction in elastic force. Actin filaments were severed using a Ca^{2+}-independent gelsolin fragment, which reduced the elastic force of single CMs by 30±6% (n=7). Cleavage of titin first and actin second decreased the total force by 67±4% (n=10) while cleavage of actin first and titin second reduced the total force by 63±7% (n=7).
Conclusions. The HaloTag-TEV mouse allows the direct and reliable quantitation of the titin contribution to CM stiffness. Our findings show that the intact titin springs are responsible for the majority of the elastic forces of the mouse CM. Actin filaments contribute much less to CM elastic force than titin. The order of cleavage of these cytoskeletal structures is important, suggesting the presence of a cellular tensegrity architecture.

P2.5

Is there force depression in cardiac myofibrils?

Seong-won Han, Torri Heiser, Venus Joumaa, Walter Herzog

Faculty of Kinesiology, University of Calgary, Calgary, Canada
Seong-won Han <seongwon.han@ucalgary.ca>

History-dependent properties, such as residual force depression (i.e. the decrease in steady-state isometric force of a muscle, following active shortening, compared to the corresponding force of a purely isometric contraction) have been consistently observed in skeletal muscles. However, the corresponding work on force depression in cardiac muscle is still limited. The purpose of this study was to examine residual force depression in cardiac muscle. Because of the structural similarities between cardiac and skeletal muscle sarcomeres, we expected force depression to be present in cardiac tissue as it is in skeletal muscle.
Myofibrils (n=4) isolated from the left ventricle of rabbits were held at an average sarcomere length (SL) of 2.2 μm. Myofibrils were then activated, and actively shortened to an average SL of 1.8 μm, held constant at this length until the force reached a steady-state. Then, myofibrils were deactivated and allowed to recover for 5 mins. Finally, myofibrils were activated at an average SL of 1.8 μm, to measure the purely isometric force at this length.
All four myofibrils tested to date exhibited force depression, averaging 33.7% (±8.9%) of the purely isometric reference force. This result suggests that force depression occurs in cardiac muscle within a physiologically relevant range of function and that a mechanism for force depression is at the origin of force depression in skeletal and cardiac muscles.

P2.6

Cold exposure for 3 days mediates mitochondria-dependent cardioprotection

Petr Kasik[1], Aneta Benesova[1], Andrea Zbirkova[1], Barbara Elsnicova[1], Frantisek Galatik[1], Pavel Vebr[1], D. Hornikova[1], Veronika Tibenska[1], Jiří Novotny[1], Frantisek Kolar[2], Olga Novakova[1,2], Jitka M. Zurmanova[1*]

[1]Department of Physiology, Faculty of Science, Charles University, Prague, Czech Republic; [2]Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic
Petr Kašík <kasikpet@natur.cuni.cz>

Recently, we developed a unique cardioprotective model of chronic mild cold acclimation (8±1ºC, 5 weeks) that decreases the extent of myocardial infarction and improves mitochondrial resistance to Ca^{2+}-overload in rats. This protocol preserves the adenylyl cyclase signalling pathway without any apparent side effects such as hypertension and hypertrophy. In the present study, we aimed to establish the minimum duration of mild cold exposure sufficient for an improvement of cardiac tolerance to acute ischemia/reperfusion injury for further therapeutical use. A further goal was to reveal potential cardioprotective mechanisms triggering the cold-elicited cardioprotective phenotype. Male Wistar rats (350-400g) were exposed to mild cold (9±1ºC) for 1 or 3 days or control animals were maintained at the temperature of 24ºC and then subjected to 20-min ischemia and 3-hours reperfusion. The left ventricles of parallel groups were used for our analyses. Our data revealed that 3 days, but not 1day of cold significantly reduced the extent of myocardial infarction, whereas mitochondrial resistance to Ca^{2+} overload was increased in both groups. Moreover, western blot and quantitative immunofluorescence analyses revealed an increased association of hexokinase II with mitochondria after 3 days of cold. Furthermore, b3-adrenergic receptors (b3-ARs) and the downstream protein kinase G (PKG) showed increased association with the T-tubule system and phospholamban, respectively. Administration of the specific b3-ARs inhibitor (SR 59230A) prior to ischemia abolished the infarct size limiting effect. We conclude that cardioprotection elicited by acute cold exposure for 3 days is mitochondria dependent and acts via the b3-ARs/PKG pathway.

Acknowledgements of Financial Support: This work was supported by Charles University Grant Agency (GAUK No. 375221).

P2.7

Cardiac stiffness is tuned through unfolded domain oxidation (UnDOx) and in-register aggregation in the distal I-band region of titin

C. M. Loescher[1], M. Breitkreuz[2], Y. Li[1], A. Unger[1], B. Mohamed[3], K. Toischer[3], L. Leichert[4], N. Hamdani[2], W. A. Linke[1]

[1]Institute of Physiology II, University of Muenster, Muenster, Germany; [2]Institute of Physiology, Ruhr University, Bochum, Germany; [3]Department of Cardiology and Pneumology, University Medicine Goettingen, Goettingen, Germany; [4]Institute for Biochemistry and Pathobiochemistry, Ruhr University, Bochum, Germany
Christine Margaret Loescher <loescher@uni-muenster.de>

Question: Titin stiffness, a determinant of myocardial stiffness, is thought to be modulated by oxidative stress. *In vitro*, titin oxidation is promoted by unfolding of titin immunoglobulin-like (Ig) domains. However, it is unknown if the required unfolding can be detected *in vivo* through the differential oxidation of sarcomeric I-band (extensible) and A-band (inextensible) titin. Further, how oxidative stress alters titin-based passive tension is still unclear.
Methods & Results: Titin oxidation was studied in *ex vivo* perfused (Langendorff) mouse hearts treated with H_2O_2 and *in vivo* in aorto-caval shunt mouse heart, a chronic volume overload model developing oxidative stress. Titin oxidation was quantified by *isotope-coded affinity tag* labelling of heart tissue followed by mass spectrometry. Hundreds of cysteines in titin became more oxidized under oxidative stress in both models. However, in shunt hearts, I-band titin was more oxidized than A-band titin. The preferred sites of titin oxidation change were in the distal Ig-domain region. In skinned human cardiomyocytes, we determined that when oxidized under stretched conditions, passive force decreased after S-glutathionylation but increased after disulfide bonding. Finally, recombinantly expressed I-band Ig domains were thermally unfolded then S-glutathionylated, which resulted in increased aggregation.
Conclusion: Elastic titin becomes more oxidized than A-band titin following stretch *in vivo* through unfolded domain oxidation (UnDOx). UnDOx modulates passive stiffness through oxidation type-specific modification of titin and promotes aggregation of unfolded titin domains. We propose a mechanism whereby UnDOx enables the controlled homotypic interactions within the distal titin spring region to modulate passive stiffness of the heart.

P2.8

Molecular dynamics studies of the effects of phosphorylation and mutation on cardiac troponin dynamics

Z. Yang, I. Gould, S. Marston

Imperial College London
Steven B Marston <s.marston@imperial.ac.uk>

Cardiac troponin function is modulated physiologically by phosphorylation of Ser 22 and 23 of cTnI and is altered by mutations that cause cardiomyopathy. To understand the structural basis of this modulation we used molecular dynamics simulations (MD) of the Ca^{2+}-bound troponin core. 1500ns simulations within a 140Å cube indicate there are no stable changes of the key mobile elements (cTnI 1-30, cTnI 134-149, cTnT 270-288) on phosphorylation. We therefore investigated the dynamic effects of phosphorylation.

Using the angle between A and B helices as a metric, we found phosphorylation affected the angle distribution with an increase from 82% to 99% being within the range defined as the 'open' state that binds TnI switch peptide. The principle motion in troponin is hinge motion between the NcTnC and CcTnC/cTnI/cTnT domains; the average angle of the hinge increases on phosphorylation. Energetics calculations show that the interaction of NcTnC with the TnI switch peptide is enhanced by 3.4 kCal/mol on phosphorylation.

The TnC G159D mutation causes DCM since Ca^{2+}-sensitivity is not modulated by TnI phosphorylation. In MD simulations of uP G159D all the metrics were different from WT. Moreover phosphorylation changed the metrics of G159D in the opposite direction to WT, consistent with its abnormal function.

Calculation of ionic interaction probabilities residue-by-residue (Arpeggio) has enabled us to describe the local changes of dynamics at the atomistic level that underly the functional effects of phosphorylation and mutations.

P2.9

Self-assembly of smooth muscle myosin filament. Adaptations of the filament length by telokin and Mg·ATP

Apolinary Sobieszek

Retired from: Austrian Academy of Sciences; Dr. Ignaz Seipel-Platz 2; A-1010 Wien; Austria
Apolinary Sobieszek <apolinary@sobieszek.at>

The contractile apparatus of smooth muscle is malleable in order to accommodate stress and strain exerted on the muscle cell and to maintain optimal contractility. Structural labiality of smooth muscle myosin filaments is believed to play an important role in the cell's malleability. However, the mechanism and regulation of myosin filament formation is still poorly understood. In the present in vitro study, using a static light scattering method, length distributions were obtained from suspensions of short myosin filaments (SFs) formed by rapid dilution or long ones (LFs) formed by slow dialysis. The distributions indicated the presence of dynamic equilibriums between soluble myosin and the SFs; i.e.: trimers, hexamers and mini-filaments, covering the range up to 0.75 μm. The LFs were more stable, exhibiting favorable sizes at about 1.25, 2.4 and 4.5 μm. More distinct distributions were obtained from filaments adsorbed to glass surface, by evanescent wave scattering and local electric field enhancement. Addition of telokin (TL) to the suspensions of unphosphorylated SFs resulted in widening of the soluble range, while in the case of the LFs this shift was larger, and accompanied by reduced contribution of the soluble myosin species. Such changes were largely absent in the case of phosphorylated myosin. In contrast, the presence of Mg·ATP resulted in elongation of the filaments and clear separation of filaments from soluble myosin species. Thus, TL and Mg·ATP appeared to modify the distribution of myosin filament lengths, i.e., increasing the lengths in preparing for phosphorylation, or reducing it to aid dephosphorylation.

P2.10

Myosin assembly of smooth muscle. From ribbons and side polarity to a row polar helical model

I. J. Sobieszek, A. Sobieszek

Austrian Academy of Sciences, Dr.-Ignaz-Seipel-Platz 2, 1010 Vienna, Austria
Isabel Joanna Sobieszek <isabel.sobieszek@meduniwien.ac.at>

After decades of debate over the structure of smooth muscle myosin filaments, it is still unclear whether they are helical, as in all other muscle types, or square in shape. In both cases bipolar building units are proposed, but the deduced cross-bridge arrangements are fundamentally different. The opposite polarity of the adjusting longitudinal rows is proposed for the former, while in the latter the two faces of a ribbon are appositively polarized. Analysis of light meromyosin (LMM) paracrystals, myosin rod assemblies and the filament itself indicated that the rods were assembled with a 6-7° tilt angle from the rods' longitudinal axis, in contrast to the lack of tilt in LMM, both exhibiting a 14.3 nm myosin periodicity. Optical diffraction analysis of EM images of the rod assemblies and those of intact myosin confirmed their helical architecture characterized by 28 nm residue translations, 172 nm repeats and 516 nm pitch. A detailed helical model of these filaments was elucidated with bipolar tetramer building units made of two trimers. The filaments elongate at their two ends in a head-to-head manner, enabling targeted cross-bridge polarity of the adjacent rows, in the form of a unique Boerdijk-Coxeter type helix, similar to that of collagen or desmin fibers, with the covalent links replaced by a head-to-head clasp.