Session 1. Breaking news on the skeletal muscle sarcomere

Lectures

L1.1

Cryo-ET reveals sarcomere structures at molecular resolution

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In my presentation, I will explain how we managed to obtain high-resolution structures of native sarcomeres using cryo-electron tomography (cryo-ET). Our cryo-ET reconstructions reveal molecular details of the three-dimensional organization and interaction of actin and myosin in the A-band, I-band and Z-disc and demonstrate that α-actinin cross-links antiparallel actin filaments by forming doublets with 6 nm spacing. Structures of myosin, tropomyosin and actin at ~10 Å further reveal two conformations of "double-headed" myosin, where the flexible orientation of the lever arm and light chains enable myosin not only to interact with the same actin filament, but also to split between two actin filaments. Our results provide unexpected insights into the fundamental organization of vertebrate skeletal muscle and serve as a strong foundation for future investigations of muscle diseases.

L1.2

The molecular elasticity of insect titin determines sarcomere and thick filament length

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Sarcomeres have a stereotypic architecture across animal species. In mammalian muscles, sarcomere length is controlled by the large sarcomere ruler titin spanning from the Z-disc to the M-band. Similarly, non-chordate species such as insects and worms do contain sarcomeres of defined length, with varying length depending on the muscle fiber-type. However, these species do not contain a large titin molecule spanning across a half sarcomere, hence a simple ruler model cannot generally apply. To investigate how sarcomere length is controlled in non-chordates, we have manipulated the length and elasticity of the *Drosophila* titin homolog Sallimus (Sls). In *Drosophila* larval muscles Sls contains a long PEVK spring domain and extends over 2 μm across the I-band linking the Z-disc to the I-band. Surprisingly, we find that genetically reducing the spring domain length of Sls not only changes the I-band length, as expected, but also reduces the A-band and hence myosin filament length. Hence, we hypothesize that a biomechanical feedback mechanism controls sarcomere length in non-chordate species. In support of this mechanism, we have quantified the mechanical forces across Sls in different *Drosophila* muscle types and find that the Sls forces are high in sarcomeres with long I-bands, and lower in sarcomeres with shorter I-bands.

L1.3

Order from disorder in the sarcomere: FATZ forms a fuzzy complex and phase-separated condensates with α-actinin

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In sarcomeres, α-actinin crosslinks actin filaments and anchors them to the Z-disk. FATZ proteins interact with α-actinin and five other core Z-disk proteins, contributing to myofibril assembly and maintenance as a protein interaction hub.

Here we report the first structure and its cellular validation of α-actinin-2 in complex with a Z-disk partner, FATZ-1, which is best described as a conformational ensemble. We show that FATZ-1 forms a tight fuzzy complex with α-actinin-2 and propose a molecular interaction mechanism via main molecular recognition elements and secondary binding sites. The obtained integrative model reveals a polar architecture of the complex which, in combination with FATZ-1 multivalent scaffold function, might organise interaction partners and stabilise α-actinin-2 preferential orientation in the Z-disk.

Finally, we uncover FATZ-1 ability to phase-separate and form biomolecular condensates with α-actinin-2, raising the intriguing question whether FATZ proteins can create an interaction hub for Z-disk proteins through membraneless compartmentalization during myofibrillogenesis.

Oral Presentations

O1.1

The molecular basis of the difference in slow-fast muscle efficiency

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The performance of mammalian skeletal muscle depends on the myosin heavy chain (MHC) isoform of the myosin II motor. With sarcomere-level mechanics in skinned Ca^{2+}-activated fibres of rabbit skeletal muscle (sarcomere length 2.4 μm, 12°C) we find that the power of slow muscle (soleus, containing the MHC-1 isoform) is twenty times smaller than that of fast muscle (psoas, containing the MHC-2X isoform), while the efficiency of slow muscle is reported to be larger especially at high loads (Smith *et al.*, 2005, *Prog Biophys Mol Biol* **88**: 1). In clear contradiction with a possible molecular explanation of the larger efficiency of slow muscle, we found that the stiffness and the force of the slow myosin isoform are three times smaller than those of the fast isoform (Percario *et al.*, 2018, *J Physiol* **596**: 1243). To clarify the molecular basis of the efficiency, the comparison of slow-fast performance has been extended to the isotonic velocity transient elicited by stepwise drops in force superimposed on the steady isometric force (T_0). At high load (0.8 T_0) the early rapid shortening that estimates the amplitude of the working stroke is smaller in the slow fibre (2 nm) than in the fast fibre (5 nm), while at low load it is similar (7-8 nm at 0.2 T_0). A reduced size of the working stroke of the slow isoform, together with the smaller force, exacerbates the question on the mechanism underlying the higher macroscopic efficiency of the slow muscle.

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O1.2

Graded titin cleavage reduces residual force enhancement and lengthens optimal sarcomere length in permeabilized skeletal fibers

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Titin is a sarcomeric protein that functions as a viscoelastic spring and is known as a major contributor to passive force, but its role during isometric and dynamic contraction is unresolved. Here, we define titin's role using a titin-cleavage mouse model to specifically cut titin springs. We conducted a series of isometric and stretch-hold contractions between 2.7 and 3.0 μm sarcomere length (SL), before and after titin cleavage. We report that 50% titin cleavage decreased isometric force at both SLs, but the effect was stronger at 2.7 μm SL, leading to the unusual finding of greater active force at 3.0 rather than 2.7 μm SL. At the longer SL, titins are stretched and provide more stabilization to the sarcomere, potentially limiting the decrease in contractile performance. During a dynamic stretch-hold contraction from 2.7 to 3.0 μm SL, active force is greater after stretch than compared to the isometric force at 3.0 μm SL. This phenomenon, called residual force enhancement (RFE), is hypothesized to be completely caused by the stretch of titin springs, however, 50% titin cleavage only reduced RFE by ~30%. Therefore, while the stretch of titin springs is a main contributor, other factors may be at play. The story is not yet complete, as titin springs affect many facets of contraction, from thick filament activation to force transmission, with studies underway to detail these properties and provide insight in titin function during isometric and dynamic contraction.

O1.3

The emerging role of titin's N2A element in muscle mechanical performance

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The role of titin's N2A element in active force generation in muscle is a standing subject of debate. Very recently, we have characterised the 3D-structure of the N2A region (spanning unique sequence and poly-Ig tandem) and investigated its interactions with the actin filament and CARP, a protein rapidly up-regulated in muscle in response to mechanical or toxic stress. We revealed the existence of an unidentified, small helical domain in N2A, which recruits CARP to titin, assisted by domain I81[1]. Using actin co-sedimentation, co-transfection in C2C12 cells and proteomics on heart lysates, we showed collaboratively that the CARP/N2A complex further associates with the actin filament. Further collaboration in CARP-soaked myofibrils revealed that the cross-linking of titin and actin increases sarcomere stiffness, possibly by effectively shortening the length of titin's spring regions[1]. This finding has been confirmed by others[2]. In the absence of CARP, titin N2A was not observed to interact with actin[3]. We found the results to be independent of calcium. We conclude that the recruitment of CARP to N2A is a fundamental mechanism of striated muscle stress response, directed to preserve mechanical performance upon overload stress. Whether this mechanism is synergistic with a potential basal N2A/actin association remains to be investigated.

References:

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Virtual Posters

P1.1

Salbutamol ameliorates the neuromuscular junction-related phenotype in *Col6a1*$^{-/-}$ mice

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Mutations in the human genes encoding for Collagen VI (ColVI) cause Bethlem myopathy (BM) and Ullrich congenital muscular dystrophy (UCMD), rare diseases for which no cure is available yet. Beside its role as a component of skeletal muscle basement membrane, ColVI was recently acknowledged as a key factor in the maintenance and stabilization of the neuromuscular junction (NMJ). Indeed, ColVI lack results in an extensive fragmentation of endplates, altered expression of NMJ-related proteins and impaired neurotransmission. Here, we show that salbutamol, an FDA-approved selective b2-adrenergic agonist, recently repurposed for the treatment of a variety of neuromuscular disorders, is beneficial for *Col6a1*$^{-/-}$ mice. Remarkably, 1-month systemic salbutamol administration in *Col6a1*$^{-/-}$ mice led to a dose-dependent expansion and stabilization of endplates, ultimately normalizing AChR clusters fragmentation. Moreover, remodeling of skeletal muscle was observed in terms of induced muscle hypertrophy, which was sustained by enhanced myofiber regeneration. In parallel to a mild increase in absolute muscle strength in *Col6a1*$^{-/-}$ mice, the treatment significantly ameliorated some of the altered electrophysiological parameters, including diaphragm EPP and mEPP. Our results provide for the first time a proof-of-concept for the NMJ as a novel therapeutic target in ColVI-related myopathies and highlight salbutamol as a promising candidate for future therapeutical strategies.

P1.2

Tissue specific loss of B-type lamin affects muscle development and morphology in *Drosophila melanogaster*

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Lamins are type V intermediate filament proteins. Together with associated proteins, they are a structural part of the nuclear lamina underlining the inner nuclear envelope. Lamins and nuclear membrane proteins provides mechanical stability, regulates chromatin positioning and many others. Mutations in those and related genes are known for causing genetic disorders. This group is called laminopathies and among them are several muscular dystrophies such as EDMD1 and EDMD2. Fly genome encodes single gene for lamin B-type – lamin Dm and single for lamin A type – lamin C. In contrast to the most popular research on A-type lamins, we decided to focus our interest at equally important B-type lamins and investigate their involvement in the formation and development of mesodermal origin tissue-muscles. For the purposes of this project we use the *D. melanogaster* model system with the availability of tissue selective silencing of B-type lamin *via* GAL4 driver system, and screening crosses for properties *in vivo*. We use Dmef2 "driver" and lamDmRNAi "responder". We have chosen 3rd instar larvae bodywall muscles for analyses. By western blot and immunofluorescence staining we confirmed at least 50% knockdown of lamin Dm in larval muscles. Preliminary data showed easy detectable changes within the examined tissues. We noticed disorganized contractile apparatus (abnormal distribution of Z-line and M-line, high level of actin). Moreover, we observed atypical nuclear positioning and aberrant tendons formation. These results might suggest that B-type lamin is an essential protein for proper organization of muscles at least in the fly system. Also, there could be interplay between A and B-type lamins, what may contribute to the muscle phenotype in EDMD.

P1.3

Effects of different *Ttn* deletions (*Ttn* $^{\Delta 112\text{-}158}$ and *mdm*) on residual force enhancement (RFE) in permeabilized EDL fiber bundles

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RFE is defined as the increase in steady-state force after active stretch compared to the isometric force at the same final length. Recent studies suggest a pivotal role for titin in RFE via an activation-dependent increase in titin stiffness. The *Ttn*$^{\Delta 112\text{-}158}$ transgenic mouse expresses a titin protein with a large deletion in the PEVK region that greatly increases titin-based passive tension. *Ttn*$^{\Delta 112\text{-}158}$ muscles shift their operating length towards shorter sarcomere lengths, but their active isometric force is unaffected. The impact of the deletion on RFE is unknown. In comparison, the muscular dystrophy with myositis (*mdm*) mouse caries a small deletion in N2A titin that appears to impair N2A-actin interactions, which reduces activation-dependent titin stiffness and RFE. We investigated how RFE varies among permeabilized EDL fibers from Ttn$^{\Delta 112\text{-}158}$, *mdm*, and wild type (WT) mice. We predicted an increase in RFE in *Ttn*$^{\Delta 112\text{-}158}$ fibers compared to WT and *mdm* fibers based on previous research that showed *Ttn*$^{\Delta 112\text{-}158}$ has higher passive tension and similar active tension compared to WT fibers. *Ttn*$^{\Delta 112\text{-}158}$ fibers (n = 25, 36 ±2 .1 mN/mm^2) produced significantly (ANOVA, $P<0.0001$) more RFE than *mdm* fiber bundles (n = 19, 23.6 ± 2.1), but both *Ttn*$^{\Delta 112\text{-}158}$ and *mdm* fibers produced significantly less (ANOVA, $P<0.0001$) RFE than wild type fibers (n = 20, 69.1 ± 2.1). This result conflicts with the prediction that *Ttn*$^{\Delta 112\text{-}158}$ fibers would have increased RFE compared to WT and *mdm* fibers. Surprisingly, the large PEVK deletion in *Ttn*$^{\Delta 112\text{-}158}$ reduced RFE. The decrease in RFE in *Ttn*$^{\Delta 112\text{-}158}$ EDL muscles compared to WT suggests that the process of muscle activation may alter muscle passive tension.

P1.4

Chemotherapeutic drugs trigger cachexia in skeletal muscles by altering SUMO isopeptidase dependent epigenetic cascade

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Cachexia is a debilitating wasting disorder associated with significant loss of muscle mass in people suffering from diseases, including cancer. Chemotherapy is the first line of treatment for cancer patients. However, chemotherapeutic agents induces muscle loss and exacerbates pre-existing conditions in cancer patients, leading to profound loss of muscle mass and function. Molecular mechanisms underlying the chemotherapy-induced cachexia remained elusive. Here, we found that specific classes of chemotherapeutic drugs (daunorubicin and etoposide) disrupt sarcomere organization and, thereby, the contractile ability of skeletal muscle cells. The sarcomere disorganization is accompanied by reduced levels of myosin heavy chain, MyHC-II, the main force-generating molecular motor protein. Notably, the drugs destabilize the SUMO isopeptidase enzyme SENP3 in muscle cells. The reduction in SENP3 protein level is the nucleation event to deregulation of epigenetic regulators, SETD7-p300 function. Mechanistically, drugs impede SENP3 mediated chromatin targeting of SETD7 histone methyltransferase and perturbs association and synergetic function of SETD7 with p300 histone acetyltransferase. The reduced histone acetylation exerts cascading effect on further transcriptional changes, leading to declined *MyHC-II* transcription. We established a unique epigenetic mechanism promoting chemotherapy-induced cachexia. We propose a pharmacological mediation into SUMO pathway as a possible tool to ameliorate chemotherapy-induced cachexia.

P1.5

Analysis of selected lncRNAs during myogenic differentiation of pluripotent stem cells

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Proper skeletal muscle functioning requires precise space-time cooperation of multiple molecules. Recently, long non-coding RNAs (lncRNAs) have been identified as an important part of the cellular machinery. LncRNAs contain over 200 nucleotides in length and may be localized both in the nucleus and the cytoplasm where they control the expression of various genes. In recent years, many newly discovered lncRNAs have been characterized as potential factors engaged in skeletal muscle development, growth, and regeneration. The knowledge about their potential participation in the myogenic differentiation of pluripotent stem cells (PSCs) is scarce. This issue is important as PSCs serve both as a valuable research model as well as a potential and universal source of cells for therapies.

In the current project, we analyzed several lncRNAs which have been described as involved in myogenesis. Their expression profile has been determined in undifferentiated and differentiating mouse embryonic stem cells (ESCs) of two lines (H2B-GFP and 7AC5-YFP), cultured either in a control or in differentiating medium (enriched with retinoic acid, insulin, transferrin, and selenium - RA/ITS medium) for 7 and 21 days. As additional samples proliferating C2C12 myoblasts, differentiated C2C12 at the myotube stage and a 13-day-old mouse embryo were analyzed. All analyzed genes were detected in the mouse embryo, C2C12 myotubes/myoblasts, and ESCs, however, at a different, mostly low level. The expression of SRA and H19 was specifically detected, at a significant level, in ESC cultured in RA/ITS medium for 7 and 21 days. Thus, these two molecules could serve as a potentially good marker to follow the progression of PSC myogenic differentiation.

P1.6

Ca^{2+} homeostasis and contractile parameters are modified in skeletal muscle fibers of Obscurin knockout mice

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Obscurin (Obscn) is a giant sarcomeric protein preferentially localized at the M-band, where it interacts with: i) Titin and Myomesin, providing stabilization of the M-band during contraction; ii) sAnk1.5, contributing to tether the SR to the contractile apparatus; iii) Ankyrin B, participating in the organization of dystrophin at costameres. Accordingly, Obscn KO fibers display: i) M-band instability and/or disassembly following intense exercise; ii) aberrant longitudinal SR; iii) loss of localization of Ankyrin B at the M-band, leading to altered organization of the subsarcolemmal microtubule network and reduced distribution of dystrophin at costameres.

Here we report a further characterization of Obscn KO mice. Voltage clamp experiments on FDB fibers showed that Ca^{2+} homeostasis and Ca^{2+} release kinetics were impaired in Obscn KO mice. Accordingly, in these mice force parameters such as time to peak and half relaxation time were altered in diaphragm, EDL and soleus muscles. In addition, differences in force-velocity relationship and fatigability, but not in the recovery kinetics, were also observed in Obscn KO diaphragm with respect to WT control muscle. Surprisingly, analysis of mice running ability revealed that, at variance with what we previously reported, there was no difference between Obscn KO and WT mice, even though sarcomere contractures and M-band damage were still observed in KO mice following intense exercise on treadmill.

P1.7

Involvement of Septin-7 in the migration of myogenic cell line

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Septins are considered the fourth component of the cytoskeleton, but little is known about their contribution to myogenesis and muscle regeneration. Septin-7 is structurally unique among the septins, furthermore, it is an essential component of hetero-oligomeric septin complexes. Cytoskeletal proteins have also been shown to regulate appropriate cell migration.

We aimed to determine the role of Septin-7 in migration using mouse originated C2C12 cells (CTRL). Septin-7 expression was modified with an shRNA gene silencing technique, the generated scrambled (SCR) and knock-down (KD) cell lines and their migration was followed with Cytosmart Lonza system. Fluorescently-tagged Septin-7 fusion proteins were also established to analyze real time structural changes of septin filaments using AiryScan 880 laser scanning confocal microscope.

After 3 hours of migration the total covered distance and the average speed were similar in SCR and KD cells, while the angle of movement was significantly lower in KD cultures. In migrating cells, more pronounced appearance of Septin-7 filaments was observed, in addition its co-localization with actin was more profound, especially in the projections. More dedicated movement revealed on KD cultures could be the consequence of altered cell morphology with less projections as found in previous experiments.

These results indicate the functional relevance of Septin-7 in the migration of myoblasts further suggesting its contribution to muscle regeneration.

P1.8

Brain, skeletal muscle and plasma BDNF levels from exercised rats

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Backround. Exercise triggers activity at a molecular level, by releasing neurotrophins, e.g. BDNF, in an activity-dependent fashion. Therefore, the aim was to investigate whether an aerobic exercise protocol induced a BDNF upregulation in the brain, skeletal muscle and plasma of exercised rats; and to investigate whether the upregulation of circulating BDNF levels is a result of BDNF produced and released from the brain (cerebral circulation) or, otherwise, from peripheral organs (peripheral circulation).

Methods. 12 Wistar rats were equally divided into an exercise group, subjected to a 4-week exercise protocol, and a sedentary control group. Plasma samples from the jugular and tail vein were collected before [T0], during, at week 2 [T1], and following the protocol [T2]; homogenized muscle and brain tissues were assessed.

Results. Exercise appears to considerably decrease the BDNF concentration levels found in the hippocampus and in the cortex; concerning the concentration levels analyzed in the gastrocnemius and the soleus, exercise seems to significantly increase BDNF values found in both muscles. Our results suggest that the type of muscle fiber may play an important role in BDNF production, i.e., the soleus, predominantly composed of type I muscle fibers, appears to produce greater amounts of BDNF than the gastrocnemius, composed of a greater percentage of type II muscle fibers; between T0 and T1, a significant rise of BDNF was observed in the plasma from the cerebral circulation of the exercised rats; conversely, from T1 to T2, a return to baseline levels was stated within the exercise group, with no statistical differences being established between groups at T2. Considering the plasma collected via peripheral circulation, no differences were observed between groups.

Conclusion. Biochemical activity from the brain of the exercised rats appears to trigger the production of BDNF that is promptly used for neuronal function; however, due to a greater production than consumption of BDNF by the brain, the BDNF seems to be drained directly into the cerebral circulation. BDNF appears to acumulate in the muscle, suggesting the existance of an autocrine BDNF-loop. Muscle fiber type also seems to correlate with the variation of BDNF production within the muscle.

P1.9

MARP1 "locks" titin to the thin filament and is a passive force regulator

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Muscle ankyrin repeat protein 1 (MARP1) is frequently upregulated in stressed muscle. In the heart MARP1 has been shown to regulate pathologic remodeling, however its effect on skeletal muscle function is poorly understood. Here, we focused on its interaction with the titin-N2A element, found in titin's I-band spring region. We show that MARP1 binds to F-actin, and that this interaction is stronger when MARP1 forms a complex with titin-N2A. Mechanics and super-resolution microscopy on myofibrils isolated from MARP triple KO mice and human diaphragm biopsies, exposed to GST-MARP1 proteins, revealed that MARP1 "locks" titin-N2A to the thin filament. This locking mechanism causes increased extension of titin's elastic PEVK element and, importantly, increased passive force. In support of this mechanism, removal of thin filaments abolished the effect of MARP1 on passive force. The clinical relevance of this mechanism was established in diaphragm myofibers of mechanically ventilated rats and of critically ill patients, which both show increased levels of MARP1 by western blot and increased passive force in mechanics experiments. Thus, MARP1 tunes passive force by locking titin to the thin filament. We propose that this mechanism protects the sarcomere from mechanical damage during conditions associated with muscle stress.

P1.10

The molecular basis for sarcomere organization in vertebrate skeletal muscle

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Sarcomeres are force-generating and load-bearing devices of muscles. A precise molecular understanding of how sarcomeres are built underpins understanding their role in health and disease. Here, we determine the molecular architecture of native vertebrate skeletal sarcomeres by electron cryo-tomography. Our reconstruction reveals unprecedented details of the three-dimensional organization and interaction of actin and myosin in the A-band, I-band and Z-disc and demonstrates that α-actinin cross-links antiparallel actin filaments by forming doublets with 6 nm spacing. Structures of myosin, tropomyosin and actin at ~10 Å further reveal two conformations of "double-headed" myosin, where the flexible orientation of the lever arm and light chains enable myosin not only to interact with the same actin filament, but also to split between two actin filaments. Our results provide unexpected insights into the fundamental organization of vertebrate skeletal muscle and serve as a strong foundation for future investigations of muscle diseases.