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ABSTRACTS

# XXXVIth European Muscle Conference of the European Society for Muscle Research

## Stockholm, Sweden, September 8–12, 2007

**Organizing Committee**

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Prof. L. Larsson
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9:13 Raffaello, A., Sandri, M., Lecker, S. H., Lanfranchi, G., Goldberg, A. L. Over-expression of JunB in skeletal muscle causes growth and prevents atrophy inhibiting the expression of atrogin-1 and MuRF-1
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9:15 Gantelius, S., Hedström, Y., Pontén. E. Higher levels of the MyHC IIx is seen in wrist flexors than in wrist extensors in both healthy children and children with cerebral palsy
9:16 Betik, A. C., Thomas, M. T., Riel, C. D., Wright, K. J., Hepple, R. T. Treadmill training of late middle age rats into senescence does not preserve aerobic capacity
9:17 Lindström, M., Carlsson, L., Thornell, L.-E. Split fibers in human muscle
9:18 Calura, E., Laveder, P., Raffaello, A., Millino, C., Romualdi, C. Meta-analysis of gene expression data and regulatory networks reconstruction during skeletal muscle atrophy
9:19 Maricic, N., Gedrange, T., Harzer, W. Myogenic factors, MGF, myostatin and myosin heavy chain mRNA expression in the masseter muscle of patients after orthognathic surgery
9:20 Bean, C., Facchinello, N., Salamon, M., Faulkner, G., and Lanfranchi, G. The effects of Ankrd2 alteration suggest an important role in cell cycle regulation during muscle differentiation
9:21 Brocca, L., Desaphy, J. F., Pellegrino, M. A., Conte Camerino, D., Bottinelli, R. Proteome analysis of altered protein expression in soleus muscle of hindlimb suspended mice

9:22 Lunde, I. G., Ekmark, M., Rana, Z. A., Buonanno, A., Gundersen, K. PPAR$\delta$ expression differs in slow and fast skeletal muscle and is influenced by muscle activity
9:23 Fokina, N. M., Tavitova, M. G., Shenkman, B. S. Choline acetyltransferase content as a cellular marker of spinal motoneuron activity under conditions of rat hindlimb suspension
9:24 Tavitova, M. G., Fokina, N. M., Shenkman, B. S. Triglyceride and glycogen content as a cellular marker of muscle fiber activity under conditions of rat hindlimb suspension
9:25 Ramonatxo, C., Sirvent, P., Ricardelli, S., Galbes, O., Douillard, A., Py, G., Lionne, C., Chatonnet, A., Cazorla, O., Lacampagne, A., Candau, R. Effect of chronic clenbuterol treatment on isometric force, myosin ATPase activity and $Ca^{2+}$ transient in slow and fast skeletal muscles
9:26 Desaphy, J.-F., Pierno, S., Liantonio, A., Brocca, L., Giannuzzi, V., Cippone, V., Giotta, C., Bottinelli, R., Conte Camerino, D. Age-dependent effects of disuse on electrophysiogical parameters and calcium homeostasis in the soleus muscle of hindlimb-unloaded mice
9:27 Cacciani, N., Patruno, M., Sacchetto, R., Murgia, M., Reggiani, C. Effects of endurance training on HIF-1 transcriptional activity and HIF-1$\alpha$ expression in rat slow and fast muscles
9:28 Gjövaag, T. F., Dahl, H. A. Effect of training with different intensities and volumes on muscle fibre enzyme activity in the m. triceps brachii of untrained men and women

**Session X: Gene therapy in muscle dystrophy**

*Oral Presentations*

10:1 Sweeney, H. L. Premature stop codon suppression as a therapeutic strategy for a subset of patients with Duchenne muscular dystrophy
10:2 Peter, A. K., Crosbie, R. H. Sarcospan ameliorates muscular dystrophy by stabilizing the utrophin–glycoprotein complex
10:3 Clarke, N. F., Maugenre, S., Urtizberea, J. A., Gray, F., Bouchet, C., Seta, N., Mégarbané, A., Guicheney, P. MDC1D due to a large genomic insertion in LARGE that causes abnormal gene splicing
10:4 Peter, A. K., Walsh, K., Crosbie, R. H. Akt activation in dystrophin-deficient muscle improves pathology and prevents muscle degeneration

*Poster Presentations*

10:5 Clarke, N. F., Kolski, H., Dye, D., Lim, E., Smith, R. L. L., Patel, R., Fahey, M. C., Laing, N., North, K. N. TPM3 is a recurrent cause of congenital fibre type disproportion
10:6 Clarke, N. F., Smith, R. L. L., Kornberg, A., Shield, L., Manson, J., Beggs, A. H., North, K. N. Defining diagnostic boundaries for congenital fibre type disproportion

**Session XI: Muscle mechanics and energetics**

*Oral Presentations*

11:1 Kawai, M., Stanton, K. J., Lu, X., Wandling, M. W., Hitchcock-Degregori, S. E. Period 3 of tropomyosin (Tm) is essential to enhance isometric tension in the thin-filament reconstituted cardiac muscle fibers
11:2 Curtin, N. A., Bickham, D. C., Woledge, R. C., Barclay, C. J., Van der Laarse, W. J. Over-expression of uncoupling protein-3: Where does the energy go?
11:3 Cecchi, G., Nocella, M., Benelli, G., Colombini, B., Bagni, M. A. Crossbridge properties during force enhancement by stretch in activated frog muscle fibres

*Poster Presentations*

11:4 Bagni, M. A., Colombini, B., Cecchi, G., Bernstorff, S., Amenitsch, H., Griffiths, P. J. Intensity changes of equatorial 1.1 1.0 and meridional 14.3 nm X-ray reflections during slow stretching of activated frog muscle fibre bundles
11:5 Fredsted, A., Clausen, T., Overgaard, K. Effects of step exercise on muscle cell damage in young men and women
11:6 Dou, Y., Andersson-Lendahl, M., Arner, A. Structure and function of skeletal muscle in Zebrafish (*Danio Rerio*) larvae
11:7 Dou, Y., Boels, P. J. M., Arner, A. Blebbistatin inhibits actin-myosin interaction in skinned muscle preparations

**1:1**

### Titin expression, mechanics, and mechanosensor function in health and disease

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The section of the sarcomere that does not directly participate in actin-myosin interaction, the Z-disk/I-band region, has important structural and signalling functions and is centrally involved in the process of mechanotransduction. A mechanosensor function has been attributed to a ternary protein complex in the Z-disk consisting of the N-terminal titin Ig-like domains (Z1/Z2), telethonin (T-Cap), and muscle lim protein (MLP). Telethonin caps titin at the N-terminus and has been thought to be critical for the anchorage of titin filaments in the Z-disk and for titin assembly. However, in the sarcomeres of telethonin (–/–) knockout mice, Z-disk structure and N-terminal titin anchorage are preserved and titin isoform expression is normal. Alternative anchorage sites, such as interaction with the alpha-actinin-actin complex, firmly attach titin to the Z-disk. A role in mechanotransduction has also been suggested for the I-band titin springs, which interact with various proteins and whose main function is as a determinant of myofibrillar passive stiffness. Recent data have established the plasticity, mechanical role and regulation of the elastic titin springs during cardiac development and in human heart disease. Titin-isoform switching, as well as phosphorylation by protein kinases, affect titin elasticity in cardiac muscle, with important consequences for diastolic wall stiffness.

**1:3**

### Myomesin forms a two-chained, antiparallel filament across the M-band in muscle sarcomeres

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The protein myomesin has been suggested to play a critical role in linking major filament systems in cross-striated muscle cells. However, in the absence of available structural data it has remained unknown how this protein could connect filaments over more than 40 nm distance. The crystal structure of the two-domain fragment My12–My13 reveals that the myomesin C-terminus dimerizes *via* an antiparallel beta-sheet. The antiparallel orientation is propagated by rigid alpha-helical linkers, yielding a filamentous shape of the dimer. Using solution X-ray scattering of an extended construct My9–My13, we provide evidence that the C-terminal part of myomesin extends into a 37 nm long two-chained filament, concatenated by repetitive Ig domain/linker units. Our findings provide evidence for a rigid, filamentous structure of the myomesin dimer, allowing to connect the M4/M4′-lines across the central M1 line. Our structural observations are consistent with previous imaging data, and to the best of our knowledge provide the first experimental model for the connection of major filament systems by a filament-containing sarcomeric protein.

**1:4**

### Electron tomography reveals the structure of the C-zone in striated muscle

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The C-zone in vertebrate striated muscle is the region in each half A-band where myosin-binding protein C (MyBP-C) forms at least 7 bands of 43 nm spacing. Mutations in cardiac MyBP-C are a major cause of familial hypertrophic cardiomyopathy; hence there is great interest in understanding the function of MyBP-C. We show that the organisation of the C-zone is very similar in cardiac and skeletal muscles. We report on the 3D organisation of the C-zone by electron tomography of an exceptionally well-preserved frog skeletal muscle. Thin ~100 nm sections were imaged using an FEI CM300 electron microscope to obtain three dual-axis tomograms. An average image of the thick filament was calculated from extracted cylindrical volumes that included single filaments and surrounding six actin filaments. The average thick filament shows similar structure within each 43 nm band in the C-zone, which comprises three 14.3 nm layers of myosin head crowns with MyBP-C at Layer 1. We show the results of fitting myosin heads to the three levels. At Layer 1 we attribute MyBP-C to strong density sharply defined along a narrow axial zone. We show that MyBP-C projects beyond the heads, reaching the actin filaments. We show that MyBP-C may have a hand which allows contact with different sets of actin filaments. We discuss the implications of this finding for the role of MyBP-C in the sarcomere.

**1:5**

### The interaction of ponsin and paxillin in costameres

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The differentiation of cross-striated muscle coincides with the transition from motile myoblasts to sessile myotubes. The assembly of a highly regular contractile apparatus requires cytoskeletal remodelling and is coordinated with a transformation of focal adhesions into costameres. In this context we searched for specific binding partners of paxillin and identified an interaction with ponsin. We found that the second SH3 domain of ponsin binds to a N-terminal proline-rich motif in paxillin. To understand the molecular basis of this interaction, we determined the structure of this SH3 domain at 0.83 Å resolution, as well as its complex with the paxillin binding peptide at 1.63 Å resolution. The overexpression of ponsin resulted in altered muscle

cell–matrix contact morphology, suggesting its involvement in the establishment of mature costameres. Ponsin expression was found to be down-regulated in end-stage failing hearts, and that this effect was reverted upon mechanical unloading.

**1:6**

### Lasp-2 expression, localization, and ligand interactions: a new Z-disc scaffolding protein

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The nebulin family of actin-binding proteins plays an important role in actin filament dynamics in a variety of cells including striated muscle. We report here the identification of a new striated muscle Z-disc associated protein: lasp-2 (LIM and SH3 domain protein-2). Lasp-2 is the most recently identified member of the nebulin family and was found widely expressed in different tissues including brain, kidney, lung, placenta and pancreas. To evaluate a possible role of lasp-2 in striated muscle, lasp-2 gene expression and localization were studied in chick and mouse tissue, as well as in primary cultures of chick cardiac and skeletal myocytes. Lasp-2 mRNA was detected as early as chick embryonic stage 25 and lasp-2 protein was associated with developing premyofibril structures, Z-discs of mature myofibrils, and intercalated discs of cultured cardiomyocytes. Expression of GFP-tagged lasp-2 deletion constructs showed that the C-terminal region of lasp-2 is important for its localization in striated muscle cells. Lasp-2 organizes actin filaments into bundles and interacts directly with the Z-disc protein alpha-actinin. These results are consistent with a function of lasp-2 as a scaffolding and actin filament organizing protein within striated muscle Z-discs.

**1:7**

### Does the calcium-dependence of titin control the charge pattern throughout the sarcomere?

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In glycerinated rabbit psoas muscle [Coomber et al (1999) Cell Calcium 25:43–57; (2001) 30:297–300) used Donnan potential measurements to show that the electric charge on the actin-myosin matrix undergoes a sharp switch-like transition at $pCa_{50} = 6.8$. The potentials are 2 mV less negative at the lower $pCa^{2+}$ ($P < 0.001$; Z-test, unmatched samples). In the presence of ATP the muscle contracts and breaks the microelectrode so these were muscles in rigor. There is no reason to suppose *a priori*, however, that a similar voltage switch does not occur in contracting muscle.

This calcium dependence is still apparent in muscles stretched beyond overlap (sarcomere length = 4.2 μm). It is also seen in the gap filaments between the A- and I-band ends; further stretching (to sl > 4.3 μm) abolishes the dependence. The experiments suggest very strongly that the calcium dependence is controlled by the titin component of the sarcomere and is lost when titin filaments break. We suppose that the effect is mediated by the titin kinase domain in the M-line region (and S100?) and also involves the extensible PEVK region of the titin filaments.

There is now renewed interest in the electric charge on muscle proteins within the structural system; here we suggest how changes in these charges may control the calcium-activation process. We also suggest a simple experimental approach that would clarify the effects.

**1:8**

### Atomic force microscopy and spectroscopy of synthetic myosin thick filaments

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Thick filaments are supramolecular asssemblies of myosin II molecules that interact via their tail domains. To explore their structure and mechanical stability, here we imaged and manipulated individual synthetic thick filaments with atomic force microscopy (AFM). Thick filaments were polymerized from purified rabbit back muscle myosin by decreasing the KCl concentration. Filaments were adsorbed to mica and imaged with AFM under buffered aqueous conditions. We observed individual myosin heads branching from a central shaft, and the protofilamentous structure of thick filaments could be resolved. Morphological analysis showed that reducing ionic strength results in more compact global filament structure. In force spectroscopy experiments we stretched elastic structures away from the thick filament to a distance ~240 nm, which is longer than the myosin molecule (~150 nm), suggesting that the manipulated structure corresponds to either longitudinally associated and/or unfolded myosin molecules. The force spectra contained an extended relaxation plateau at ~24 pN, which may correspond to the re-zipping of myosin molecules from the thick filament shaft or to the cooperative unfolding of the coiled-coil tail domain.

**1:9**

### Interaction forces between F-Actin and titin PEVK domain

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Titin determines muscle elasticity and plays role in regulatory processes. Its PEVK domain interacts with F-actin, generating viscous forces of unknown magnitude. We measured, with optical tweezers, the forces necessary to dissociate F-actin from individual molecules of recombinant PEVK fragments rich either in polyE or PPAK motifs. The probability of PEVK-actin interaction was high (80%) at ionic strengths up to 150 mM, suggesting physiological significance. Dissociation forces at a stretch rate of 250 nm/s displayed wide, non-normal distribution, with a peak at ~8 pN in the case of both fragments. Dynamic force spectroscopy revealed low spontaneous off-rates that were increased even by low forces. The loading-rate dependence of dissociation force was biphasic for polyE in contrast with the monophasic response of PPAK. Analysis of the molecular lengths at which dissociation occurred indicated that there are numerous actin-binding regions along the fragments' contour, suggesting that the PEVK domain is a promiscuous actin-binding partner. PEVK-actin interaction may safeguard sarcomeric structural integrity in the relaxed state and modulate thixotropic behavior during contraction.

**1:10**

### Thyroid hormone promotes titin isoform transition in heart development

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During cardiac development titin isoform composition changes rapidly; in rat hearts from 100% N2BA (>3,200 kDa) shortly before birth to almost exclusively N2B (3,000 kDa) postnatally. To determine factors regulating the switching, titin-protein and mRNA expression was analyzed in primary cardiomyocyte (CM) cultures prepared from E18 rats. Under standard culture conditions the mean N2B-percentage increased from 14% on day 2 to 73% on day 9, resembling the in vivo transition. The titin-isoform switching was independent of myocyte hypertrophy and was not altered by pacing, increased contractility, contractile arrest, or cell-stretch, but was impaired by decreasing substrate rigidity. CMs grown after culture-day 2 in the absence of fetal calf serum expressed relatively less N2B, suggesting that serum components affect titin-isoform switching. Indeed, in CMs cultured in steroid hormone-free medium, the average N2B proportion was <50%, but addition of thyroid-hormone, triiodo-L-thyronine (T3), increased N2B to ~80%. This T3-effect was not prevented by bisphenol-A, a specific inhibitor of the classical genomic pathway of T3 action, but was reversed by the phosphatidylinositol-3-kinase (PI3K) inhibitor, LY294002. We conclude that T3 regulates perinatal titin-isoform switching in CMs via a non-classical pathway involving PI3K-dependent signaling.

**1:11**

### Zooming into the overall architecture of the giant muscle protein titin

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The giant muscle protein titin extends over one half of the muscle sarcomere. In its largest isoform titin comprises more than 38,000 residues and about 300 domains. Its structural complexity does not allow the application of classical structural biology methods to determine its overall architecture. Therefore, we have decided to chop the protein into smaller fragments and determine the high-resolution structures of representative parts. Within this endeavor, we have also become interested to consider known ligands involved in the titin "interactome" for structural/functional analysis. Over the last decade, we determined structures of the N-terminal assembly complex [Zou et al. 2003, 2006; Pinotsis et al. 2006], from the I-band [Mayans et al. 2001; Vega et al., unpublished] and from the A-band including the kinase domain and down-stream signaling complexes [Mayans et al. 1998; Müller et al. 2006, 2007, unpublished; Chen et al., unpublished). The available data allow modeling a large part of the titin "proteome" and to interpret available low-resolution data of the entire titin filament.

**1:12**

### Thin filament assembly depends on the interaction of a ruler, nebulin, with a capper, CapZ

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Contractile activity in striated muscle requires that the actin-containing thin filaments precisely assemble with the correct polarity and spacing, and maintain specific lengths. The barbed ends of actin filaments in striated muscle are anchored within the Z-disc and capped by CapZ, a protein that regulates actin dynamics in vitro. The mature lengths of the thin filament are likely specified by the giant "molecular ruler" nebulin. Here we report that CapZ specifically interacts with the C-terminus of nebulin within modules 160–164 by blot overlay, solid-phase binding, and SPOTs membrane assays. Binding of nebulin modules 160–164 to CapZ does not modulate CapZ's capping activity in actin polymerization assays. Knockdown of nebulin in chick skeletal myotubes using siRNA results in both a reduction of CapZ, and a loss of the uniform alignment of the barbed ends of the actin filaments. These data indicate that nebulin has a role in regulating the lengths of thin filaments within the Z-disc, perhaps via its interaction with CapZ. We propose a novel molecular model of Z-disc architecture in which nebulin interacts with CapZ of a thin filament of an adjacent sarcomere, thus providing a direct link between adjacent sarcomeres.

**1:13**

### Direct visualisation and kinetic analysis of normal and nemaline myopathy actin polymerisation using total internal reflectance microscopy

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Actin filaments were formed by elongation of pre-formed nuclei (short crosslinked actin-HMM complexes) that were attached to a microscope cover glass. By using TIRF illumination we could see actin filaments at high contrast despite the presence of 150 nM TRITC in the solution. Actin filaments showed rapid bending and translational movements due to Brownian motion but the presence of the methylcellulose polymer network constrained lateral movement away from the surface. Both the length and the number of filaments increased with time. Some filaments did not change length at all and some filaments joined up end-to end (annealing). We did not see any decrease in filament length or filament breakage. The rate of polymerisation increased with increasing actin monomer concentration. For quantitative analysis of polymerisation time course we measured the contour length of all the filaments in a frame at a series of time points and also tracked the length of individual filaments over time. Elongation rate was the same measured by both methods (0.56 μm/min at 0.1 μM actin) and was up to 10 times faster than previously published measurements. The annealed filament population reached 30% of the total after 40 min. Total polymerisation rate (length × number) increased

linearly with actin concentration and critical concentration was less than 20 nM. This technique was used in study of transgenic mouse actin (D286G) polymerisation. D286G actin appeared to elongate at a lower rate than non-transgenic actin.

This work is supported by the British Heart Foundation.

## 1:14

### Immunohistochemical analysis of myofibrillar lesions in HMERF

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Myofibrillar inclusions, which stained for phalloidin but where devoid of desmin staining, were the hallmarks of an unusual autosomal-dominant hereditary myopathy with early respiratory failure (HMERF) [Edstrom et al (1990) J Neurol Sci). At the ultrastructural level the inclusions were composed of an abundance of thin filaments, which expanded out from divided Z discs. HMERF is now known to be caused by a mutation in the titin kinase domain [Lange et al (2005) Science].

In the present study we have further elucidated using high-resolution immunochemistry, the composition of the Z disc and associated proteins. We show that the myofibrillar inclusions stained strongly for myotilin and filamin but lacked staining for alpha-actinin, FATZ, ZASP, telethonin, titin and nebulin. Interestingly increased staining for titin, nebulin, desmin and nestin, were on the other hand observed in the myofibrils of normal appearance in muscle fibres with inclusions.

Undoubtedly the mutation in the titin kinase affects the myofibrils in several ways. Even the normal appearing myofibrils had an altered composition, which might lead to the disarrangement and expansion of the Z disc core, composed of F-actin, myotilin and filamin. Further studies are needed to understand the pathogenesis of the muscle degeneration.

## 1:15

### New high-molecular bands in electrophoresis gels: intact titin forms or titin aggregates?

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To study titin isoform composition we have applied SDS-electrophoresis in (a) vertical agarose-strengthened 2–2.3% polyacrylamide gels and in (b) horizontal agarose-strengthened gel with 1–1.3% polyacrylamide. Skeletal and cardiac muscles of ground squirrel, rat, rabbit, mouse and human were tested. In addition to the bands corresponding to known N2A, N2B and N2BA-titin isoforms new slowly migrating bands of high-molecular weight were found in both electrophoresis systems used. Western blot analysis has confirmed titin origin of these bands. Nucleic acids did not contribute to these bands. A considerable difference in electrophoretic mobility of new titin forms was found in horizontal gel. Their M.w. averaged ~3,230 to 3,300 kDa in cardiac and ~3,380 to 3,730 kDa in skeletal muscles. M.w. of N2A, N2B and N2BA-titin bands averaged ~2,420 to 2,800 kDa. We assume that the high-molecular bands are an intact titin isoforms, while the titin bands designated on electrophoregrams as N2A, N2B and N2BA are their fragments. However the possibility that these band represent titin aggregates cannot be ruled out completely.

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## 1:16

### The age dependent changes in the viscous, visco-elastic and elastic properties of intact rat fast and slow skeletal muscle fibres

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In this study, the dynamic biophysical properties of resting intact mammalian muscle fibre bundles (mean cross-sectional area ~150 μm) isolated from the edl (fast) and soleus (slow) of rats aged between 1 and 90 days were examined at 20°C. The preparations were mounted horizontally between two stainless steel hooks, one attached to a force transducer and the other to a high-speed servo motor, in a flow through muscle chamber with a glass bottom. They were then subjected to small ramp stretches (amplitude ~2–3% resting muscle length, $L_o$) of different stretch speeds. As previously reported, the tension responses to a stretch could be resolved into three tension components; a viscous, a visco-elastic and an elastic tension. Examined at stretch speeds close to the maximum shortening velocities of the muscles (~2 $L_o\,s^{-1}$ in the soleus and 6 $L_o\,s^{-1}$ in the edl) the amplitudes of all three tension components increased with age and attained their adult values at ~48 days of age. Furthermore, from day 14 onwards, all three-tension components showed characteristic fast/slow fibre type differences that were absent in muscles isolated from younger animals. From these and other results we suggest that the biophysical properties of resting muscles, like active tension, differentiates within the first 3 weeks after birth and that these changes may play an important role in muscle contraction.

## 1:17

### Structural and functional roles of the intermediate filament syncoilin in striated muscle

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Syncoilin is an intermediate filament-like protein, specifically expressed in cardiac and skeletal muscle where it is localized at the sarcolemma, the neuromuscular junction, and the Z-line. Syncoilin interacts with α-dystrobrevin-1 and desmin and is thought to provide a link between the extracellular matrix via the dystrophin associated protein complex (DAPC) and the intermediate filament network. Syncoilin is upregulated in various forms of neuromuscular disease, suggesting that it might play an important role in muscle. To study the functional role of syncoilin in cardiac and skeletal muscle in vivo we have generated syncoilin deficient mice.

Syncoilin knockout mice are viable and born at Mendelian ratios. The mice appear normal and exhibit no ultrastructural abnormalities or changes in heart weight to body weight ratios However, functional studies in skeletal muscle revealed decreased isometric and passive stresses by 20% and 30%, respectively, while the response to injury following eccentric contractions was

unchanged compared to wildtype. We are currently analyzing the cardiac and skeletal muscle phenotype of these mice by gene and protein expression analyses as well as histological and physiological methods. This will provide further insights into the structural and functional roles of syncoilin in striated muscle.

### 1:18

### Correlation of M-band structure with myosin filament lattice order

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The M-band is suggested to crosslink the thick filaments in the sarcomere. Its main structural components are myomesin and M-protein, two closely related proteins consisting mainly of Ig- and Fn-like domains. EH-myomesin splice isoform contains additional segment, which has a disordered conformation.

Using quantitative PCR, immunohistochemistry and electron microscopy (EM) we analyzed sarcomere structure and M-band composition of extraocular muscle (EOM) and tibialis anterior muscle (TA) of rat. The EOM is subdivided into two functionally distinct layers, the orbital layer (OL) and global layer (GL). The majority of the OL fibers express a higher proportion of EH-myomesin and are completely devoid of M-protein. Accordingly, the OL sarcomeres appear fuzzy in longitudinal EM images, with unregistered A-bands and diffuse M-bands. Quantification of the transversal EM images reveals higher variance of inter-filament distances and pure rotational order of thick filaments in OL sarcomeres. TA fibers express the short myomesin isoform and a lot of M-protein and demonstrate a highly ordered myosin filament lattice. GL contains a mixture of both types of fibers.

These results show the correlation between M-band protein composition and organisation of myosin filament lattice. The fuzzy design of sarcomere in eye muscles might favour their stability in the eccentric contraction range.

### 1:19

### Contractility-dependent actin dynamics in cardiomyocyte sarcomeres

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The mechanisms of actin dynamics in non-muscle cells are relatively well established, whereas the regulation of actin dynamics in muscle cells is less well understood. The structure and dynamics of the actin cytoskeleton are both spatially and temporally regulated by an array of proteins that interact with actin filaments and/or monomeric actin. Among the most central actin-binding proteins are ADF/cofilins, which increase actin dynamics in non-muscle cells by depolimerizing filaments from their pointed ends. Interestingly, a specific ADF/cofilin isoform, cofilin-2, is strongly expressed in muscle tissues (especially in heart), suggesting that actin filaments in muscles may be more dynamic than previously expected. To examine the mechanisms of actin dynamics in muscles, we expressed GFP-actins in neonatal rat cardiomyocytes. Our analysis showed that the actin fusion proteins localize normally in myofibrils, indicating that these tools can be applied in studies concerning actin dynamics in myofibrils. Interestingly, our FRAP (Fluorescence-Recovery-After-Photobleaching) experiments revealed that actin filaments are relatively dynamic in myofibrils. Furthermore, our siRNA studies demonstrated that depletion of cofilin-2 from cardiomyocytes leads to severe defects in sarcomere assembly.

### 1:20

### News about the FATZ family (calsarcin/myozenin)

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Members of the FATZ family of sarcomeric proteins, FATZ-1 (calsarcin-2, myozenin-1), FATZ-2 (calsarcin-1, myozenin-2) and FATZ-3 (calsarcin-3, myozenin-3), are localized in the Z-disc and bind to $\alpha$-actinin-2, $\gamma$-filamin, myotilin, telethonin, ZASP as well as calcineurin. The importance of ZASP and the FATZ family of proteins in muscle function has been shown; in fact ZASP as well as other muscle proteins such as myotilin, filamin C, desmin and alpha beta-crystallin have been shown to be associated with myofibrillar myopathy. However the pathway involved has still to be discovered. It is noteworthy that the FATZ family of proteins interact with three of these proteins namely ZASP, myotilin and filamin C. Recently mutations in FATZ-2 have been found to cosegregate with inheritance of hypertrophic cardiomyopathy and its gene (*MYOZ2*) has been proposed as a causal gene for this disease.

Here we will present work to show that the FATZ family and myotilin can bind ZASP and some other muscle and non-muscle PDZ containing proteins. Using in vitro and in vivo techniques we managed to study in more detail these interactions and how they are regulated. Since ZASP has a role in maintaining the structure of the Z-disc we speculate that the FATZ family may share this role through its interaction with ZASP. We were also able to identify new binding partners for FATZ-3.

### 1:21

### Determination of myosin compliance using steered molecular dynamics

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Detailed understanding of the force produced by the myosin-actin complex requires a first principles analysis of the molecular forces present. With the advent of protein pulling techniques such as Atomic Force Microscopy and Optical Tweezers we may now gain direct evidence of force-extension relationships at the molecular level. Most proteins that have been subject to such pulling experiments have behaved like worm-like chains and produced non-linear force-extension profiles driven by entropic forces (a property known as entropic elasticity) and there are no compelling reasons that myosin should behave any differently. Nevertheless most biophysical models of muscle assume a strictly linear force-extension relation for myosin,

requiring complex myosin-actin binding dynamics to account for non-linear muscle properties such as the hyperbolic force-velocity relationship. Steered Molecular Dynamics was used in the present work to investigate the molecular forces involved in bending a select region of the myosin neck region. It is concluded that the force-producing region of myosin is more than just a linear spring, and that entropic elasticity might play a role. Additionally, small unfolding events take place that reduce force in a manner similar to what would be required to explain the double-hyperbolic force-velocity relation. A mathematical model of muscle will be presented.

**1:22**

**Regulation of oscillatory contraction in insect flight muscle by troponin**

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Insect indirect flight muscle (IFM) is activated by sinusoidal length changes at constant [$Ca^{2+}$]. A rapid stretch produces a delayed rise in tension, and release results in transient deactivation; this enables the muscle to produce work in response to oscillatory length changes. Work is measured from the area of work loops in the tension vs. length diagram. At [$Ca^{2+}$] above 10 μM, IFM contracts isometrically (without stretching) and work loops are superimposed on a level of isometric tension that depends on [$Ca^{2+}$]. IFM has two TnC isoforms: F1 has a single $Ca^{2+}$ bound to site IV at the C-terminus, and F2 has $Ca^{2+}$ at the N-terminal site II and at site IV. Stretch-activation acts through F1 and isometric tension through F2. The ratio of F1 to F2 in myofibrils is 7:1. Native *Lethocerus* fibres performed maximal oscillatory work at pCa 6, when isometric tension was low. Fibres substituted with F1, produced work over a greater range of [$Ca^{2+}$] and frequency than native fibres. Fibres substituted with F2 contracted isometrically, but did not produce oscillatory work. Fibres substituted with F1 and F2 produced maximum work at F1:F2 100:1, and work was often greater than for native fibres. Yeast two-hybrid assays showed that the C-terminal lobe of both TnCs is sufficient to form a complex with TnI. The N-lobe of F1 may bind another ligand, enabling it to act as a stretch sensor.

**2:1**

**The myosin family of molecular motors**

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Myosins comprise one of the three major families of molecular motors that together are responsible for almost all movement within and by eucaryotic cells. The most familiar of the myosins is myosin II, which is the major motor protein of muscle (skeletal, cardiac and smooth muscle). However, the broader family of myosin motors is involved in a wide range of movement and transport processes (vesicle transport, phagacytosis, cell division). Yet other myosins are not involved in transport but generate and sense mechanical forces in the cell. In the last five years there has been huge progress in mapping the members of the myosin family (18–25 family groups depending on the definition), exploring the cellular function of the different myosins, unravelling the regulation of the myosins and defining the underlying molecular mechanism. We are now just at the point where the study of the dazzling variety of behaviours and functions of different myosins is beginning to allow the underlying principles to emerge of how a prototypical myosin can be adapted for a myriad of different functions. We will present recent data on myosins from fast and slow muscle as well as non-muscle myosins.

**2:2**

**Electron microscopic evidence for the cross-bridge preparatory stroke in living thick filaments**

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Using a gas environmental chamber, with which the image of living biomolecules can be recorded under an electron microscope [Sugi et al (1997) PNAS 94:4378], we have attempted to decide the amplitude and duration of ATP-induced cross-bridge movement in living bipolar thick filaments, consisting only of rabbit skeletal muscle myosin. Gold particles were attached to the cross-bridges to serve as position markers. In the absence of ATP, the cross-bridge position did not change appreciably with time, indicating that their time-averaged position remains unchanged despite their thermal motion. In response to iontophoretically applied ATP, the cross-bridges moved by about 7 nm, indicating that they can move without attaching to the thin filaments. In a few cases, we could record the ATP-induced cross-bridge movement at both sides of the thick filament bare region; the cross-bridges were found to move away from, but not towards, the bare region, suggesting the presence of cross-bridge preparatory stroke, having an amplitude identical with, and the direction opposite to, the cross-bridge powerstroke. We wish to thank Japan Electron Optics Laboratory for providing facilities to carry out the experiments.

**2:3**

**Structural and functional modelling of the N-terminus of the ELC of Type II myosin**

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The 3D structure of Type-II myosin comprises the motor and neck domains associated with an essential light chain (ELC) and a regulatory LC. A large part of the N-terminus of the ELC (46 amino acids) is missing in this model. The most N-terminus of the ELC (aa1–15) binds to the C-terminal (aa360–364) of actin. We modelled the missing 46 N-terminal aa to the ELC of the acto-myosin-S1. The missing 46aa revealed an elongated structure with a length of 9.1 nm, i.e. long enough to bridge the gap between the ELC and the actin filament. We generated transgenic rats which overexpressed N-terminal 15aa of human atrial or ventricular ELC in cardiomyocytes. Expression of N-terminal human ELC peptides in TGR (range:3–6 μM) was associated with significant ($P < 0.001$) improvements of the intrinsic contractile state of the isolated perfused heart. Using synthetic hVLC-1/1-15 as a TAT fusion peptide, we observed specific accumulation of the peptide

in the sarcomeres of intact adult cardiomyocytes as well as increased shortening amplitude at constant activating intracellular free $Ca^{2+}$ (maximal effect at 1 μM).

**2:4**

**Force generation is regulated via conformational changes of actin and myosin initiated by changes in position and mobility of troponin-tropomyosin on thin filaments**

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Force generation in striated muscle results from myosin cyclically interacting with actin, a $Ca^{2+}$-regulated process mediated by the actin-associated regulatory proteins, troponin (TN) and tropomyosin (TM), located on the thin filament. We have investigated the molecular mechanisms by which TN–TM regulates ATPase cycle using fluorescent probes specifically bound to Cys36 of recombinant $\beta$-TM, Cys707 of subfragment-1 (S1) or Cys374 of actin. The fluorescent-labeled proteins were incorporated into muscle ghost fibers containing TN and orientation and mobility of the fluorescent dipoles were studied in the absence or presence of $Ca^{2+}$, nucleotides and analogs of ATP. Simulation of various intermediate states of actomyosin has shown discrete changes in orientation and mobility of the fluorescent dipoles, which is the evidence for multistep changes in the structural state of S1, actin and TM during the ATPase cycle. TN modulates these changes in a $Ca^{2+}$-dependent manner. It is suggested that S1 interaction with actin at ATPase cycle results in nucleotide-dependent displacement of TM molecule and the change in its binding to actin. TN shifts equilibrium between weak and strong binding in actomyosin. Thus, regulation of the actomyosin ATPase activity is mediated by changes in TM position on the thin filament and in its interaction with actin. Supported by RFFR and BHF.

**2:5**

**Phylogenic and functional analysis of the myosin light chain amino terminal extensions**

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The myosin light chains of vertebrates and invertebrates have amino-terminal extensions that have similar amino acid motifs, including lysine clusters near the terminus that are involved in thin filament binding and repetitive sequences of proline and alanine in the extended arm. Parsimony analysis of amino acid sequences (obtained from the EST library in GenBank) indicates that certain branches of two major metazoan lineages, Protostomes and Deuterostomes, independently acquired the N-terminal extensions (NTE). Protostomes (arthropods, mollusks, and earthworms) acquired the NTE in the regulatory light chains (RLC), but in Deuterostomes (echinoderms, tunicates, vertebrates) only vertebrates acquired the NTE in the essential light chains (ELC). Mechanical studies of fly indirect flight muscle and mouse myocardial strips indicate the NTE modify the kinetics of power-producing actomyosin cross-bridges: In flies, genetic ablation of the RLC NTE reduces the optimum frequency of oscillatory power production, resulting in a loss of flight ability. In mice, genetic deletion of amino acids 5–14 of the ELC NTE reduces myosin attachment time to actin, resulting in slightly larger and less efficient hearts. Both phenotypes suggest the primary role of the NTE is to facilitate actomyosin cross-bridge formation.

**2:6**

**The effect of phosphate and temperature on isometric force generation of permeabilized fibres of rabbit and dogfish muscle**

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Previous experiments have shown that increased inorganic phosphate concentration [$P_i$] reduces isometric force production by skeletal muscle. However, most investigations of this effect on permeablized fibres have been performed at sub-physiological temperatures. The available data suggest that the inhibitory effects of $P_i$ are reduced as temperature is increased. The aim of this study was to investigate the effect of $P_i$ on isometric force (IF) production at physiological temperatures (PT) in Triton-permeablized white muscle of dogfish (PT = 12°C) and rabbit psoas fibres (PT = 39°C). Experiments were conducted by activating the fibres from a $Ca^{2+}$-rigor state (free $Ca^{2+}$ = 32 μmol/l with backup system) in the presence of 0.5, 5.5, 10.5, 20.5 mM $P_i$ in sequence of either increasing or decreasing [$P_i$]. Peak normalised tension (tension/tension with no added [$P_i$], i.e., 0.5 mM) versus log [$P_i$] showed that (1) for the dogfish there was no statistically significant ($P > 0.05$) effect of [$P_i$] on IF output; (2) for the rabbit at 20°C, [$P_i$] did reduce IF significantly ($P < 0.005$). However, at 35°C, the effect of [$P_i$] on IF was much smaller than at lower temperatures ($P > 0.05$). In conclusion, at physiological temperatures, the inhibitory effects of $P_i$ on force generation were reduced compared to those at lower temperatures for single fibres of dogfish white muscle and rabbit psoas.

**2:7**

**cMYBP-C modulates actomyosin interactions: evidence from cMYBP-C$^{-/-}$ mice**

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The precise role of cardiac myosin binding protein C (cMyBP-C) on actomyosin interaction (AMI) remains unknown. We hypothesized that the lack of cMyBP-C impaired cardiac AMI. Left ventricular papillary muscle experiments and in vitro motility assays were performed on 16-weeks old cMyBP-C$^{-/-}$ KO and WT ($n$ = 20/group). Using Huxley's equations, we found that the probability for myosin to be weakly bound to actin was higher in KO than in WT (8.6 ± 0.3 vs. 5.4 ± 0.2%, $P < 0.05$), whereas the number of

strongly bound, high-force generated state cross-bridges was lower in KO (6.4 ± 0.9 vs. 11.6 ± 1.0 × $10^9$/$mm^2$, $P < 0.001$). In addition, the unitary force per AIM was lower in KO than in WT ($P < 0.01$). Myosin-based velocities of actin were slower in KO than in WT (1.65 ± 0.01 vs. 1.98 ± 0.01 μm/s, $P < 0.01$). The minimum amount of α-actinin needed to completely arrest thin filament motility, an index of relative isometric force was significantly higher in WT than in KO (73.3 ± 1.1 vs. 29.1 ± 0.1 μg/l, $P < 0.001$). We conclude that cMyBP-C regulates AIM by limiting inefficient cross-bridge formation and by enhancing the power stroke step.

**2:8**

### CD97 knock-out mice show a disturbed structure of the sarcoplasmatic reticulum (SR) structure in skeletal muscles

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CD97 is a large heptahelical cell surface receptor expressed in leukocytes and muscle cells. To investigate the exact location and specific function of CD97 in skeletal muscle, we examined skeletal morphology in CD97 knock-out (ko) mice at the (ultra)structural level.

Immunostaining and Western blotting of muscular lysates revealed the presence of CD97 in wild-type (wt) but not in ko mice. Macroscopic and microscopic parameters of several skeletal muscles were not different between both mice. In addition, there were no differences in the distribution and percentage of slow and fast twitch fibers and in the metabolic activity within the fibers.

At the ultrastructural level, the myofibrils and mitochondria retained a normal structure in ko muscles. However, we found a dilated SR in ko fibers indicating the location of CD97 within this structure. Triade diameter, intertriade distance and distance between T-tubule and A–I band were significantly higher in ko compared to control fibers. Thus, the volume fraction (V) of the SR was increased whereas the V and the number of mitochondria was decreased in CD97-deficient compared to normal muscles.

In summary, the intracellular location and distribution pattern of CD97 indicates a function within the SR either in excitation–contraction coupling or in the maintenance of a precise SR or triad structure.

**2:9**

### Effect of ADP on the sliding velocity of actin filaments on fast and slow skeletal myosins

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We recently suggested that whereas unloaded shortening velocity (Vo) of slow skeletal myosin isoforms is mostly defined by the rate of ADP release (k-AD) from acto-myosin, Vo of fast isoforms might significantly depend also on the rate of acto-myosin dissociation by ATP, at least in some experimental conditions [Nytray et al (2006) J Mol Biol 355:432–442). To further understand the relative role of k-AD in defining Vo, this study aims to compare the effect of MgADP on the sliding velocity of actin (Vf) on slow and fast skeletal myosin isoforms from the rat. Vf was measured in the absence and in the presence of 2.00 mM MgADP, varying MgATP concentrations in the range 0.01 mM and 2.00 mM, at 25°C and 50 mM ionic strength. As expected, the presence of MgADP decreased Vf of rat fast myosin and shifted the substrate concentration dependence of the velocity toward higher MgATP concentrations. The analysis of the effect of MgADP on rat slow myosin is ongoing. Comparison of the effect of ADP on Vf of slow and fast isoforms of the rat s will be presented at the meeting.

**2:10**

### Limulus myosin III, a circadian clock regulated actin-binding protein

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Class III unconventional myosins are potentially both signaling and motor proteins as they have an N-terminal kinase and a C-terminal myosin motor domain. *Limulus* myosin III (LimIII) is particularly interesting because it is a phosphoprotein abundant in photoreceptors that becomes more heavily phosphorylated at night in response to signals from an endogenous circadian clock. LimIII phosphorylation correlates with dramatic changes in the actin-rich photoreceptor structure. We previously localized circadian input-dependent phosphorylation sites within loop 2 of the LimIII motor domain. Loop 2 is known to be an important actin interaction surface in other myosins we therefore hypothesize that LimIII phosphorylation will affect its actin affinity and the structure of the photoreceptor. Interestingly, we found that LimIII does not possess any ATPase activity due to amino acid changes in the otherwise conserved ATP-binding sequence of the active site. However, LimIII does bind actin ($K_d$ = 0.1 μM). To assess whether this binding is phosphorylation dependent, we engineered loop 2 mutants mimicking constant phosphorylated and unphosphorylated states of the myosin motor domain. Our ongoing actin binding experiments on these mutants will allow us to explain how LimIII affects photoreceptor function in the *Limulus* eye.

**2:11**

### Lysenin-induced structural changes in actin filaments

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Lysenin is a toxin causing a prolonged smooth muscle contraction. We have found that $10^{-2}$ to $10^{-9}$ μM lysenin induces an increase of the actomyosin ATPase activity in the presence of caldesmon (0.6 μM) and EGTA. Pelleting of reconstituted smooth muscle actomyosin system with or without lysenin revealed that lysenin did not induce dissociation of the filament constituents. Thus, the abolishment of caldesmon inhibitory properties might be related to the suppression of caldesmon effect on the actin filament structure.

Fluorescence studies using acrylodan- and AEDANS-labeled actin have shown that lysenin induces G-actin polymerization, while addition of lysenin to F-actin leads to the changes in the actin filament structure. Polymerization of G-actin has been confirmed by electron microscopic studies. The data suggest that lysenin impact on actin filaments can ensue from the changes in the parameters of the actin double-stranded helix. The modified helix favors the increase of the actomyosin ATPase activity while restricting at the same time its increase beyond the certain limit. The filaments affected by lysenin tend to form networks and/or bundles. Supported by grant 2 P04A 046 26 from Polish State Committee for Scientific Research.

**2:12**

**O-N-Acetylglycosaminylation is involved in the contractile activity of skeletal muscle**

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O-linked-*N*-acetylglucosaminylation (*O*-GlcNAc) is a regulatory post-translational modification of nucleo-cytoplasmic proteins corresponding to the attachment of *N*-acetylglucosamine (GlcNAc) to Ser or Thr residues. We have previously reported that numerous proteins were *O*-GlcNAc modified in skeletal muscle and that *O*-GlcNAc variations could be implicated in the control of protein homeostasis and muscular atrophy. Our data demonstrated that key contractile proteins, myosin heavy and light chains, actin and tropomyosins are modified by *O*-GlcNAc. The role of this post-translational modification in the contractile properties was studied by establishing T/pCa curves in presence of GlcNAc to inhibit *O*-GlcNAc dependent interactions. We demonstrated a decrease in calcium sensivity and affinity of muscle fibers. A similar effect was measured in presence of Wheat Germ Agglutinin, a lectin specific of *O*-GlcNAc. This effect was abolished after denaturation of WGA. Thus, *O*-GlcNAc moeties not involved in protein–protein interactions might play also a role in the contractile properties. Numerous *O*-GlcNAc sites were localized using the BEMAD approach and mass spectrometry on MHC, actin and tropomyosin. These sites could be involved in polymerization processes or in contractile protein interactions. Finally, *O*-GlcNAc seems to be involved in the contractile physiology of skeletal muscle by modulating contractile force as well as the structure of the contractile filament.

**3:1**

**Phosphorylation of cardiac myosin binding protein C accelerates myocardial contraction kinetics in vitro and in vivo**

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Cardiac output increases with increased sympathetic tone, partly due to phosphorylation of cTnI and cMyBP-C; however, little is known about the contributions of these proteins to increased contractility. Using mice lacking cMyBP-C (cMyBP-C$^{-/-}$) or expressing a non-PKA phosphorylatable cTnI (cTnI$_{ala2}$), or both (cMyBP-C$^{-/-}$/cTnI$_{ala2}$), we studied the roles of cTnI and cMyBP-C phosphorylation in myocardium [Stelzer et al. (in press)]. PKA accelerated stretch activation in WT and cTnI$_{ala2}$ skinned myocardium such that the response was indistinguishable from that in cMyBP-C$^{-/-}$ or cMyBP-C$^{-/-}$/cTnI$_{ala2}$ myocardium; however, PKA had no effect on cMyBP-C$^{-/-}$ or cMyBP-C$^{-/-}$/cTnI$_{ala2}$ myocardium. These results indicate that the acceleration of stretch activation in WT and cTnI$_{ala2}$ myocardium is due to phosphorylation of cMyBP-C and not cTnI and also predict that phosphorylation of cMyBP-C in living myocardium contributes to accelerated force development in systole. To test this idea, we have subsequently generated mouse lines expressing cMyBP-C that cannot be phosphorylated by PKA. Consistent with our predictions, these mice exhibited kinetics of myocardial stretch activation similar to WT, but stretch activation was not accelerated by PKA. The mutant mice also exhibited significantly smaller increases in ejection fraction following in vivo administration of beta agonists than did wild-type.

**3:2**

**Phosphorylation of sarcomeric proteins and cardiac decompensation**

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Our studies test the hypothesis that the relative phosphorylation of sites on cardiac sarcomeric proteins is a major determinant of cardiac dynamics and ejection fraction. We have evidence that phosphorylation of some sites induces an increase in cross-bridge kinetics, whereas phosphorylation of other sites induces a decrease. A corollary to our hypothesis is that the balance of these phosphorylations is essential to cardiac homeostasis and that a maladaptive shift in this balance leads to cardiac dysfunction. In the case of cardiac troponin I (cTnI) several lines of evidence indicate phosphorylation of sites in its unique N-terminal region by either PKA or PKC promotes the kinetics of cross-bridge cycling, whereas phosphorylation of sites in a near N-terminal region by PKC depresses cross-bridge kinetics. Major questions we address in this presentation are: Do these changes occur in heart failure? And, what are potential maladaptive mechanisms, which shift the balance between the levels of phosphorylation of these sites and therefore reduce cardiac power?

**3:3**

**Blebbistatin: use as myofilament uncoupling agent**

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Blebbistatin (BLEB) is a recently discovered compound that specifically inhibits myosin-II ATPase activity. If specific, this agent would be useful to uncouple myofilament contractility

from electrical events that lead to cytosolic $Ca^{2+}$ release in the cardiac myocyte (uncoupling agent). We investigated the actions of BLEB in skinned rat cardiac trabeculae and intact rat myocytes. BLEB incubation of trabeculae (>2 h) reduced maximum force ($EC_{50}$ = 0.4 μM). BLEB did not affect cross-bridge cycling kinetics indexed by tension cost (TC; dTC = +1.2%), while at a similar reduction of force (–70%), BDM increased TC 218%. >2 h 0.1 μM BLEB blocked the contraction of most myocytes. BLEB is light sensitive; we used two-photon microscope to measure calcium transients (Fluo4-AM); unlike BDM, BLEB did not significantly affect the $Ca^{2+}$ transient. We conclude that BLEB specifically uncouples cardiac myofilament activation from $Ca^{2+}$ activation without affecting cross-bridge cycling or EC-coupling. However, the compound is very sensitive to light, a property that severely limits its application to mechanistic physiological studies.

### 3:4

### Proteolytic modification of troponin T and troponin I in functional adaptation

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A shared structural feature of cardiac troponin T (cTnT) and cardiac troponin I (cTnI) is their modulatory N-terminal region that conveys functional regulations. The N-terminal variable region of cTnT and the N-terminal extension of cTnI both can be proteolytically removed under stress conditions, conferring rapid post-translational regulations of myocardial contractility. The N-terminal truncated cTnT (cTnT-ND) is produced during ischemia reperfusion. Transgenic mouse hearts over-expressing cTnT-ND had higher cardiac output than the control due to a longer time of rapid ejection, especially when the afterload was high, suggesting a compensatory adaptation to energetic crises. A deletion of the cTnI N-terminal extension that contains protein kinase A phosphorylation sites under $\beta$-adrenergic regulation is up-regulated during cardiac adaptation to decreased circulatory volume. Transgenic mouse hearts over-expressing the N-terminal truncated cTnI (cTnI-ND) showed enhanced relaxation, mimicking $\beta$-adrenergic effect. The cTnI-ND hearts had less reduction of cardiac output than the control when the preload was decreased, indicating a result of utilizing the Frank-Starling mechanism through facilitated ventricular filling. The data demonstrate that the N-terminal truncations of cTnT and cTnI provide novel mechanisms for physiological and pathological adaptations of striated muscle.

### 3:5

### Cardiac troponin I is a potential novel substrate for AMP-activated protein kinase

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AMP-activated protein kinase (AMPK) is critical in the regulation of energy balance and in signalling. Mutations affecting the regulatory $\gamma$2 subunit have been shown to cause a cardiac phenotype of hypertrophy and conduction disease, thus suggesting a specific role for this subunit in the heart. The $\gamma$ isoforms are highly conserved at their C-terminus but very different at their N-terminus; hence we investigated whether the unique N-terminus of $\gamma$2 could be involved in conferring substrate specificity or in determining intracellular localization. We undertook a *GAL4*-based yeast two-hybrid assay to screen a human heart cDNA library using the N-terminal 273 residues of $\gamma$2 as bait. Five proteins were identified as true interactors in the yeast assay, one of them being cardiac troponin I (cTnI). In vitro studies showed that cTnI (isolated or reconstituted in the troponin complex) is a good substrate for AMPK. Studies using site-specific cTnI mutants and mass spectrometry identified Ser-150 as the principal residue phosphorylated by AMPK. Actomyosin ATPase assays showed that phosphorylation at this residue resulted in increased $Ca^{2+}$ sensitivity of contractile regulation. We hypothesize that cTnI is phosphorylated at Ser-150 by AMPK in vivo, potentially representing a novel mechanism of regulation of cardiac function.

Supported by the British Heart Foundation.

### 3:6

### Studies of the E361G mutation in cardiac muscle actin

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We have generated transgenic mice expressing the DCM-causing E361G mutation in ACTC at 50%. Synthetic thin filaments were studied by in vitro motility assay. Actin-tropomyosin-troponin filament motility at 3.9 μM $Ca^{2+}$ and $Ca^{2+}$-sensitivity were the same for TG and NTG. When we used dephosphorylated troponin the E361G-containing thin filaments had a higher $Ca^{2+}$-sensitivity than NTG (ratio of EC50 1:2.6) and when we compared thin filaments containing non-failing (phosphorylated, 2.1 mol Pi/mol) troponin with dephosphorylated troponin, motility and $Ca^{2+}$-sensitivity were indistinguishable in contrast to NTG actin where $EC_{50}$ was 3.1 times higher. We conclude that the major functional change induced by the E361G mutation in cardiac actin is the abolition of the response to troponin I phosphorylation.

Cardiac myocyte contractility was investigated. Myocytes from TG mice did not show any difference in shortening speed, relaxing speed or in contraction amplitude than those from NTG mice.

Cine-MRI studies showed similar end systolic and diastolic volume and ejection fraction of mutant mice ($n$ = 7) and their littermates ($n$ = 6).

Supported by the British Heart Foundation.

### 3:7

### $Ca^{2+}$-independent myofilament-based enhancement of striated muscle contraction

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Myofilament-based approaches can potentially improve striated muscle contraction without altering intracellular $Ca^{2+}$ release, offering therapeutic benefits for several cardiac and skeletal muscle diseases. Multiple troponin C mutants with increased or decreased $Ca^{2+}$ binding affinity were produced to directly alter the thin filament response to $Ca^{2+}$. These mutants increased or decreased,

respectively, the $Ca^{2+}$ sensitivity of steady-state force and the rate of force development in skinned muscle cell preparations. Thick filament contribution can also be altered to provide a greater level of activation for a given $Ca^{2+}$ concentration. Specifically, the ATP analog, 2-deoxy-ATP (dATP), increased the level and rate of force development at all $Ca^{2+}$ levels in cardiac muscle and with submaximal $Ca^{2+}$ in skeletal muscle. Cultured neonatal cardiomyocytes were transduced with an adenoviral vector to increase expression of (1) a cardiac TnC mutant (L48Q) with increased $Ca^{2+}$ binding affinity or (2) to increase cellular production of dATP. Both of these strategies increased the extent and rate of cardiomyocyte shortening by more than 50%, without affecting the intrinsic frequency of contraction.

### 3:8

### Titin phosphorylation by protein kinases A and G in normal and failing human heart and consequences for myocardial passive stiffness

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Left-ventricular (LV) function can be altered via increased PKA activity following $\beta$-adrenoceptor stimulation or by promoting the cGMP-dependent signalling pathway, which accelerates relaxation and reduces diastolic tone through activation of PKG. PKA was shown to phosphorylate the giant protein titin in human LV tissue and decrease passive stiffness of myocardium from donor hearts. Whether titin phosphorylation and titin stiffness can be regulated by PKG had been unknown. We used 2% SDS-PAGE and autoradiography to study PKA and PKG mediated titin phosphorylation in skinned LV samples of control donor and end-stage failing human DCM hearts and we also measured the passive force-sarcomere length (SL) relationships. Both kinases phosphorylated the two main cardiac titin isoforms, N2B and N2BA; PKG produced relatively stronger signals. PKA and PKG reduced the passive stiffness to a similar degree, by 10–20% at physiological SLs, in non-failing and DCM hearts. De-phosphorylation/back-phosphorylation assays showed high inherent PKA-dependent titin phosphorylation, but low levels of inherent PKG-dependent titin phosphorylation, in both DCM and donor hearts. Altered inherent titin-phosphorylation levels could partly account for the alterations in passive stiffness observed in failing hearts. PKG-mediated reduction in titin stiffness may be useful to improve diastolic heart function.

### 3:9

### Post-translational modification of TnT, TnI, MyBP-C and MLC-2 in HOCM human heart muscle

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The Pro-Q Diamond phosphoprotein stain was used to measure calibrated phosphorylation levels of purified proteins from human non-failing (NF) donor hearts or from hypertrophic obstructive cardiomyopathy (HOCM) heart samples taken from biopsies obtained during septal myectomy. Despite significantly lower TnI and TnT phosphorylation levels in HOCM heart, no significant difference was observed between maximally activated sliding speed or $Ca^{2+}$ sensitivity of thin filaments reconstituted with troponin isolated from either HOCM or NF heart in the in vitro motility assay. Since dephosphorylation of NF troponin by acid phosphatase resulted in increased $Ca^{2+}$ sensitivity in IVMA, while dephosphorylation of HOCM troponin caused no functional change, it is likely that other forms of post-translational modification play a role in HOCM. Both MyBP-C and MLC-2 phosphorylation levels were found to be low in the HOCM heart samples: $2.36 \pm 0.42$ and $5.45 \pm 0.69$, $P = 0.002$ and $0.24 \pm 0.029$ and $0.33 \pm 0.029$, $P = 0.039$ mol Pi/mol, in HOCM and NF heart respectively. Other post-translational modifications (nitrosylation, glycosylation, oxidation) are also currently under investigation by modification specific antibodies and mass spectroscopy.

### 3:10

### MyBP-C mutation, expression and phosphorylation in non-failing, failing and HOCM human heart muscle

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Using antibody to the N-terminus of MyBP-C, normalised to actin, we found that myofibrils from 3 non-failing (NF) and 15 myectomy samples from HOCM patients (M) contained equal quantities of MyBP-C. 6 of the myectomy samples were identified with mutations in MyBP-C (three of them were truncations) but no truncated peptide or reduced expression was observed. We conclude that MyBP-C mutations do not cause haplo-insufficiency, nor do they act as poison peptides. No truncated peptide was observed in whole tissue homogenates from the samples with truncation mutations.

MyBP-C phosphorylation was measured with partially purified MyBP-C using Pro-Q Diamond. Phosphorylation level was calibrated against C0-C2 fragment phosphorylated with $^{g32}$P ATP using PKA. Mean value in NF samples was 5.6 mol Pi/mol. 16 NF samples from 5 different muscles were compared with 10 samples from 4 Failing heart muscles and showed a 42% lower phosphorylation level ($P < 0.001$). 9 NF from 4 muscles were compared with 21 Myectomy samples from 12 HOCM hearts. All the HOCM muscle samples were less phosphorylated than NF: M/NF = 0.45, $P < 0.0001$. There was no significant difference between the level of phosphorylation of myectomy samples with MyBP-C mutations and samples without MyBP-C mutations. We confirm that phosphorylation of MyBP-C is reduced in human heart failure, is further reduced in hypertrophic obstructive cardiomyopathy and is not related to the disease-causing mutation.

### 3:11

### A novel interaction between the N-terminus of cardiac myosin binding protein-C and light meromyosin

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It is known that the N-terminal region of Myosin Binding Protein-C (MyBP-C) binds to myosin via interaction with the Subfragment-2 (S2) portion of the rod. However, we have demonstrated via cosedimentation that a recombinant N-terminal fragment of cardiac MyBP-C (cMyBP-C) spanning domains C0–C2 (C0C2) binds

to the myosin rod with $K_d$ 2.42 μM and stoichiometry of 1.98:1, unexpectedly suggesting two binding sites per myosin. Further, C0C2 bound to the light meromyosin (LMM) portion of myosin (lacking S2), with $K_d$ 1.93 μM and a stoichiometry of 1.06:1. Our data suggest that there are two binding sites on the myosin rod for the N-terminus of cMyBP-C, one in the LMM region and one in the S2 region. It is known that MyBP-C also binds the LMM via its C-terminal C10 domain at a site located between residues 1,554 and 1,581 of β-myosin heavy chain (β-MyHC). We have localised the C0C2 binding site on LMM to within residues 1,582 and 1,806 of the β-MyHC sequence and confirmed, with a competition assay, that N-terminal and C-terminal fragments of cMyBP-C occupy independent binding within a 252 residue-long region of the myosin rod. This novel association must be considered in the developing models of MyBP-C arrangement on the thick filament and its role in physiology and disease.

### 3:12

### Actin-interaction of the C-terminal region of human smooth muscle caldesmon

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Caldesmon is a thin-filament protein implicated in smooth muscle contractile regulation. Phosphorylation of S759 and S789 in the C-terminal domain 4b may relieve caldesmon's inhibition of actomyosin ATPase. We have investigated the actin binding properties of recombinant and synthetic peptides of domain 4b by $^1$H and 2D HSQC NMR. Distinct actin interaction by sites B, 743–752 and B′, 775–782, was observed. Concurrent binding of B and B′ by an intact 4b fragment disrupted actin interaction of a peptide corresponding to the 50k/20k loop of the myosin head. Such displacement was not reproduced by simultaneous binding of two peptides corresponding to the individual sites, suggesting the importance of a structured linker between sites B and B′ in tethering an inhibitory conformation of domain 4b. Mutation of B′ residues 775–780 resulted in destabilisation of the conformational preference of the peptide backbone and weak actin interaction of 4b. Little change in the backbone conformation of domain 4b occurred upon mutation of the far C-terminal residues 787–793, indicating high tail end flexibility. Despite such flexibility, tail-end residues 784–788 interacted with actin, a contact that was destabilised by S789 phosphorylation. Phosphorylation of S789 may therefore modify the actin binding equilibrium of site B′ and the inhibitory properties of domain 4b.

### 3:13

### Length-dependence of $Ca^{2+}$ sensitivity in cardiac muscle

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We studied '$Ca^{2+}$-force' relationship in our mathematical model of myocardium mechanical activity [Katsnelson (2004) JTB]. The data simulated at different sarcomere lengths agreed completely with published experimental results [Konhilas (2002) J Physiol]. The conformity concerns both the slopes of the curves and their shifts along the pCa axis. Authors of the mentioned experiments drew attention to the notable feature of the results: a change in the muscle length led to both significant shift of the curve (i.e. of the $Ca^{2+}$ sensitivity) and quite small difference in the curve slopes (i.e. in the Hill coefficients of cooperativity). This might promote to hypothesize that length-dependence of $Ca^{2+}$-TnC kinetics is not determined by the cooperativity mechanisms. However, in the framework of our model just cooperativity of contractile and regulatory proteins underlies the length-dependence of $Ca^{2+}$-TnC kinetics. As the model reproduces the discussed experimental data successfully we deduce the following inference. Though the Hill coefficient does not change with the length, cooperativity does underlie the observed length-dependence of the $Ca^{2+}$ sensitivity.

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### 3:14

### Mechanical characteristics of different rabbit cardiac isomyosins obtained in an in vitro motility assay with regulated thin filaments

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An in vitro motility assay method with regulated thin filaments was applied to find concentrations of alpha-actinin (actin-binding protein), which stop thin filament movement for different [$Ca^{2+}$] in the flow cell. These concentrations of alpha-actinin were used to assess isometric force developed by myosin crossbridges at given $Ca^{2+}$ concentrations [Janson (1992) Cell Motil Cytoskel]. 'pCa-alpha-actinin' (i.e. 'pCa-force') relationships were separately plotted for rabbit cardiac isomyosins V1 and V3. Two various concentrations (200 and 300 μg/ml) of both V1 and V3 were tested. Hill cooperativity coefficients for the 'pCa-force' curves proved to be certainly higher for V3 than for V1: 4.6 (for V3 concentration equal to 200 μg/ml) versus 3.5 (for the same V1 concentration); and 2.53 versus 2.17 (for both concentrations equal to 300 μg/ml).

We also estimated 'force–velocity' relationships (plotting alpha-actinin concentrations versus respective movement velocities) for V1 and V3. Several concentrations of $Ca^{2+}$ were used.

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### 3:15

### The interaction of the N-terminal of cardiac troponin I with F-actin may contribute to the modulation of calcium sensitivity by phosphorylation

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Phosphorylation at the N-terminus of cardiac troponin I (cTnI) has been shown to regulate contractile activity in the myocardium with $Ca^{2+}$ sensitivity decreasing upon mono- and bisphosphorylation.

The molecular role of the N-terminal region in the regulatory function of cTnI is however still unclear. Our studies of human cTnI using proton magnetic resonance and fluorescence spectroscopy to localise regions of interaction have identified the association of the N-terminal of cTnI with F-actin. The use of defined fragments of human cTnI (hcTnI) showed that interaction involves residues 16–52 that contain the phosphorylation sites (Ser-22 and 23). Experiments carried out with overlapping sequences demonstrated that residues 16–29 play a significant role in F-actin interaction. We further observed that complex formation between the N-terminus of hcTnI and F-actin inhibited the association of F-actin with human cardiac $\beta$-myosin residues 398–414 and 623–660, myosin loop interactions correlated with actomyosin activity. Since cTnI in resting heart is predominantly monophosphorylated and dual phosphorylation upon $\beta$-adrenergic stimulation is associated with an increase in heart rate, we investigated how phosphorylated hcTnI peptides affect these actin-myosin contacts. Our results suggest that myocardial $Ca^{2+}$ sensitivity may be modulated by cTnI interaction with actin.

### 3:16

### Functional investigations into the contractility of human ventricular muscle from patients with hypertrophic cardiomyopathy

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Studies investigating the contractility of human ventricular muscle from patients undergoing surgery for hypertrophic cardiomyopathy (HCM) and controls from human ventricular tissue are currently under way in our laboratory. Two HCM samples have so far been examined, one with an identified truncation mutation in cardiac myosin-binding protein-C (cMyBP-C) and the other with no mutation as yet detected. Multicell ventricular preparations were dissected and permeabilised, and $Ca^{2+}$-dependent force development measured using a piezoelectric force transducer. The preparations containing the cMyBP-C mutant produced significantly higher maximum tension at pCa 4.0 ($21.6 \pm 2.6$ kN/m$^2$, $n = 11$; $P < 0.001$) compared to control preparations ($12.7 \pm 1.1$ kN/m$^2$, $n = 31$). A non-significant rightward shift in $pCa_{50}$ was seen, $pCa_{50} = 6.48$ compared to 6.52, in the yet to be genotyped HCM sample compared to controls. Experiments were performed at 20°C with solutions of 150 mM ionic strength. We are continuing to genotype and analyse HCM samples with the aim of identifying characteristic changes in contractility that define this phenotype at the whole tissue level.

### 3:17

### Impact of troponin phosphorylation on myofilament function in human myocardium

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Cardiac muscle contraction is regulated by intracellular calcium through the troponin complex (cTn). Protein kinase A (PKA) phosphorylates ser23/24 of cTnI. This results in a decrease in $Ca^{2+}$ sensitivity (pCa50) of force production, but the relation between pCa50 and the cTnI phosphorylation in human tissue are unclear. To address this, a selective exchange procedure was used in which 50% of the endogenous cTn in permeabilised human donor cardiomyocytes was exchanged with recombinant unphosphorylated human cTn (cTn-U) and with cTn, pre-treated with PKA to fully saturate ser23/24 of cTnI (cTn-P). First results show a significant reduction in $Ca^{2+}$ sensitivity for cardiomyocytes exchanged with Tn-P complex. The pCa50 values are $5.55 \pm 0.02$ for cTn-U and $5.45 \pm 0.05$ for cTn-P. In both groups a further decrease in $Ca^{2+}$ sensitivity to a similar level was observed after treatment of the exchanged myocytes with PKA. Additional experiments will be performed at higher levels of exchange to assess the relation on pCa50 and TnI phosphorylation. Moreover, in failing cardiomyocytes, in which phosphorylation levels of several proteins are significantly decreased, the impact of an altered phosphorylation background will be studied. {1415}

### 3:18

### The role of titin in the cardiac length-tension relationship

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The increase in systolic contraction force when ventricles are stretched due to greater end-diastolic volume is a fundamental property of the heart and is known as the Frank-Starling relationship. This mechanistic basis for the Frank-Starling law is not fully understood, but length-dependent variations in lattice spacing of the thick and thin filaments have been implicated. The giant protein titin, which spans the sarcomere from the Z-line to the M-line, has been proposed as a molecular modulator of lattice spacing, and thus changes in calcium sensitivity with sarcomere length. The current study investigates the impact of mutated titin, which is 30% larger than traditional titin, on mechanical function, and ultimately titin's role in the sarcomere. There was a dramatic increase in the resting force of myocardium expressing the normal isoform of titin at sarcomere lengths of 2.1–2.5 μm, but not in myocardium expressing mutated titin. In fact, resting tension equivalent to normal tissue was not reached in the titin mutants until sarcomere lengths of 2.8 μm were reached. Experiments are currently being performed to establish effects of mutated titin on other mechanical properties (calcium sensitivity, cross-bridge cycling kinetics, etc.) and ultimately how titin influences the Frank-Starling mechanism in myocardium.

### 3:19

### The intercalated disc in dilated cardiomyopathy

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Membranes of the intercalated disc (ID) that connect the extremities of cardiac muscle cells assume a complicated folded form that is structurally altered in dilated cardiomyopathy (DCM). In particular, the width of the ID (amplitude of the folds) is greater [Ehler et al (2001) J Cell Biol 153:763–772]. We investigated these changes in a transgenic mouse model of DCM in

which muscle LIM protein (MLP) is missing. We find that, whereas in control muscle the ID width remains constant throughout adult life (2–9 months), in MLP–/– muscle the ID width in left ventricle and papillary muscle increases steadily with age. The right ventricle is less affected. Also noted previously in these muscles is the disordered distribution of mitochondria. In particular, regions devoid of mitochondria have been identified. We find that these regions are near the ID. One explanation for this is that the myocytes elongate by adding sarcomeres at their extremities, near the ID, and that in the MLP–/– hearts, cell extension occurs without insertion of mitochondria. This creates a contractile band, close to the ID, lacking in energy supply (ATP) and likely to cause sarcomere disruption. This would explain why the sarcomeres are often badly organised near the ID in DCM and why cells of these hearts display overall disorder.

### 3:20

### Altered $Ca^{2+}$ handling and reduced ROS production in mTERF3–/– cardiomyocytes, a model of mitochondrial cardiomyopathy

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We have studied cytosolic $Ca^{2+}$ ($[Ca^{2+}]_i$) handling and mitochondrial ROS production in mTERF3–/– mouse cardiomyocytes. mTERF3 is a negative regulator of mtDNA transcription initiation. Ablating mTERF3 in heart causes respiratory chain dysfunction, increased mitochondrial mass and sudden early death. We used freshly isolated cardiac cells loaded with the $Ca^{2+}$ sensitive fluorescent indicator Fluo-3. The cells were electrically paced at 1 Hz and $[Ca^{2+}]_i$ was measured in a confocal microscope. In the mTERF3–/– cells, $[Ca^{2+}]_i$ transient amplitude was increased by 58% (WT: 2.6 ± 0.2 vs. KO: 3.9 ± 0.2) and the time constant of $[Ca^{2+}]_i$ transient decay was shorter (WT: 175 ± 10 ms vs. KO: 114 ± 9.6 ms). The SR $Ca^{2+}$ load was increased by 48% in the mTERF3–/– (amplitude WT: 4.3 ± 0.3 vs. KO: 6.4 ± 0.4).

Mitochondrial superoxide production (measured with the fluorescent indicator MitoSOX) was decreased in mTERF3–/–. SR $Ca^{2+}$ overload is associated with arrhythmia induced by spontaneous releases of SR $Ca^{2+}$. Spontaneous $Ca^{2+}$ events were observed in 13% of mTERF3 cells paced at 0.5 Hz, whereas no WT cells had such events. When we applied a $\beta$-adrenergic agonist, 43% of the mTERF3–/– cells had spontaneous $Ca^{2+}$ events, compared to 18% of the WT cells.

In conclusion, mTERF3 cardiomyocytes display an increased SR $Ca^{2+}$ load, which results in an increased frequency of spontaneous $Ca^{2+}$ events that may trigger arrhythmias.

### 3:21

### Regulation of hALC-1 promoter activity by vasopressin and sex hormones

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The promoter of the human atrial ligth chain (hALC-1) contains two transcriptional start sites, a proximal and a distal. To analyse their functional importance, we generated stably transfected H9c2 cardiomyoblasts either with the full-length promoter or a deleted promoter. We demonstrated that the deleted promoter and the full-length promoter have equal basal activities. However, both transcriptional start sites are required for vasopressin (5 μM) stimulation ($P < 0.001$). The treatment with estrogen (5 μM) and testosterone (5 μM) alone did not influence the hALC-1 promoter activity. However estrogen (5 μM) ($P < 0.01$) and testosterone (5 μM) ($P < 0.01$) significantly upregulates the vasopressin stimulated hALC-1 promoter activity. Thus the full-length promoter is required for hypertrohic activation of the hALC-1 gene.

### 4:1

### Load sensitivity of the acto-myosin working stroke

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Optical tweezers have been used to study the load dependency of biochemical steps within the acto-myosin ATPase cycle using a variety of different myosin types [1–4]. These single molecule mechanical studies enable controlled loads to be applied to the acto-myosin complex either during intermittent or processive interactions with actin. Applied loads act either to shorten or to extend the attached lifetime, depending upon both the polarity of the load and the biochemical step in the cycle that is being probed. Strain dependency of the kinetics gives rise to a number of physiologically important properties such as the force-velocity relation and overall efficiency of muscle contraction and the processivity of motors like myosin V and myosin VI. We will discuss the strain dependency of smooth muscle myosin II and myosin V and relate those findings to the geometry of the acto-myosin complex known from crystal structures.

1. Purcell et al (2005) Proc Natl Acad Sci USA 102:13873–13878
2. Veigel et al (2005) Nat Cell Biol 7:861–869
3. Veigel et al (2003) Nat Cell Biol 5:980–986
4. Rief et al (2000) Proc Natl Acad Sci USA 97:9482–9486

### 4:2

### Structural basis for the regulation of Drosophila myosin 7a

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We have been studying the regulation of enzymatic activity of Drosophila myosin 7a. Previous results from our lab have indicated that S1 from this myosin has a Vmax of around 1 $s^{-1}$ and a $K_{ATPase}$ of around 1 μM. Full length Drosophila myosin 7a (FLdM7a) is monomeric despite the presence of a short predicted coiled-coil forming domain in its tail. While its extrapolated Vmax is similar to that of S1, its $K_{ATPase}$ is 30–50 μM. This means that at low actin concentrations (~5 μM) the MgATPase activity of S1 is full activiated, whereas that of FLdM7a is still very low. Negatively-stained processed images of FLdM7a in the presence of ATP show that the molecule is monomeric and that a portion of the tail of the molecule is tightly folded back against the motor domain, obscuring the easily recognized features of the motor domain. Imaging FLdM7a at either high ionic strength or in the

absence of ATP results in an unfolding of the molecule revealing a clearly recognizable motor domain, the lever arm and some features of the tail region. Carboxyl terminal truncations of the tail were made to determine which portions of the multidomained tail were necessary for the regulation. Removal of the last 99 amino acids, which are highly conserved in all myosin 7 molecules, is sufficient to prevent formation of the folded moleculeand its $K_{ATPase}$ is similar to that of the S1 fragment. Other, larger carboxyl terminal truncations have similar effects. We are now searching for binding proteins or post translation modifications such as phosphorylations that might regulate the enzymatic activity of FLdM7a.

**4:3**

**Effects of surface adsorption on catalytic activity of heavy meromyosin—studies using a fluorescent ATP-analogue**

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Biochemical studies with molecular motors adsorbed to different surface chemistries are important for the elucidation of motor function. However, there is limited understanding of how surface adsorption affects motor properties, e.g. catalytic activity. Here we used a fluorescent ATP analogue (Alexa-fluor647-ATP; Alexa-ATP) to address this issue for heavy meromoysin (HMM). The mechanism of Alexa-ATP hydrolysis by HMM was similar to that for ATP and the analogue was also an effective fuel for HMM-propelled actin filament sliding. The effects of surface-adsorption were studied using both pure $SiO_2$ surfaces (with no HMM induced actin sliding) and trimethylchlorosilane (TMCS) derivatized $SiO_2$ that exhibited good actomyosin motility. Alexa-ATP turnover by surface-adsorbed HMM was studied using total internal reflection fluorescence (TIRF) spectroscopy. These studies indicate that a vast majority of the HMM molecules were catalytically active on both $SiO_2$ and TMCS. However, appreciable fractions of the HMM molecules on both surfaces exhibited a reduced ATPase activity compared to the situation in solution.

**4:4**

**Load-dependent mechanism of non-muscle myosin 2 enables highly efficient functioning**

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Mechanical effects are important in the regulation and coordination of molecular motors such as non-muscle myosin 2 isoforms, which play vital roles in cytokinesis and cell differentiation. To measure load dependence of nucleotide binding and dissociation, we exploited the intramolecular strain arising in myosin 2 molecules bound to actin via both heads. We found that human non-muscle myosins 2A and 2B show marked load-dependent changes in ADP release but not in ATP and ADP binding kinetics. These changes are brought about by affecting the equilibria between actomyosin-ADP states with different myosin lever orientations. Loads will thus markedly influence the duty ratio (fraction of cycle attached to actin) and ATPase cycle time of these myosins. This property provides a basis for energy-efficient tension maintenance by non-muscle myosin 2 without obstructing cellular contractility driven by faster motors such as smooth muscle myosin. While forward load accelerates the cycle of interaction with actin, resistive load increases duty ratio to favor tension maintenance by two-headed attachment.

**4:5**

**Phosphorylation of the regulatory light chains of skeletal myosin affects the conformation of myosin**

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The role of phosphorylation of the myosin regulatory light chains (RLC) is well established in smooth muscle contraction, but in striated (skeletal and cardiac) muscle its role is still controversial. We used an in vitro motility assay to elucidate the functional significance of light chain–heavy chain interactions upon phosphorylation of RLC of fast skeletal heavy meromyosin (HMM). The effect on actin filament sliding velocity, produced by phosphorylation of the RLC and $Ca^{2+}$ binding to the RLC, was studied using glass surfaces with different contact angles (different hydrophobicity). In accordance with previous studies we found no motility of actin filaments over non-phosphorylated HMM adsorbed to a glass surface with low contact angle (10 degrees, hydrophilic surface). However, HMM with phosphorylated RLC, propelled actin filaments with a velocity of about 2.5 μm/s under these conditions. The data can be as evidence that phosphorylation of the RLC has altered its interaction with the heavy chain and induced conformational changes in the lever-arm, sufficient to switch on the ability of HMM to propel actin filaments.

**4:6**

**A myosin motor that selects bundled actin for motility**

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Eukaryotic cells organize their contents through trafficking along cytoskeletal filaments. It is currently unclear how motor proteins identify the few filaments that lead to the correct destination, when presented with many similar alternatives from the limited set of filament types (actin and tubulin). To address this issue, we examined the motility of myosin X, an actin-based motor that is targeted to the distal tips of filopodia. Expressed myosin X moves single actin filaments poorly in in vitro motility assays. Many filaments form plectonemes (supercoils), suggesting that myosin X is attempting to walk along the helical arrangement of actin monomers, unlike processive myosins. At least two myosin X molecules are required to move single actin filaments. However, single molecules of myosin X move robustly and processively along fascin-actin bundles, which form the core of the filopodium. These fascin-actin bundles are necessary for in vivo localization as well. In fascin-depleted HeLa cells, overexpression of myosin X does not rescue filopodial growth, and myosin X remains in a

diffuse cytoplasmic pool. We present a model where myosin X straddles adjacent actin filaments in the fascin bundle while stepping.

**4:7**

**Relative expression of human myosin heavy chain isoforms by Real Time PCR in different muscles**

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In the present work we designed and tested different human specific primers and TaqMan probes for Real Time PCR in order to carry out a relative expression analysis of MHC isoforms in human skeletal muscle. In particular, we analyzed muscles with different composition in fibre types: skeletal muscles as gluteus, vastus lateralis, masticatory muscle as masseter and laryngeal muscles as tyroaritenoideus (ventricolaris and vocalis portion), interarytenoideus, cricothyroideus and the posterior portion of cricoarytenoideus. TaqMan probes provided reliable results in isoform-specificity for MHC 2A, 2X, 1/$\beta$-slow, $\alpha$-cardiac, and perinatal (or neonatal) isoforms. This was confirmed by the expression of type 1, 2A and 2X MHC isoforms in the "control" muscle, vastus lateralis. Real Time PCR revealed high expression of $\alpha$-cardiac MHC in masseter muscle, confirmed by protein electrophoresis, and a weak expression of the same isoform in laryngeal muscles. The specificity of perinatal MHC probe was confirmed by its expression in masseter only, where the perinatal MHC isoform was already described.

**4:8**

**Actomyosin motor activity produces surface gradient of biomolecules**

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We describe production of microscale biomolecular gradients on surfaces based on heavy meromyosin (HMM) propelled actin filament sliding. Actin filaments, biotinylated at lysine residues, were locally deposited on the HMM coated surface using a glass capillary micropipette. Gradient development was initiated by addition of ATP resulting in "motor-driven diffusion of the actin filaments". In analogy with the Einstein-Stokes relation the diffusion coefficient was given by the ratio of an energy term (motor force times actin filament persistence length) and motor friction. Biotinylation of actin (0.6 biotins per monomer) altered neither the sliding velocity nor the fraction of motile filaments. The development of the actin gradient was interrupted by infusion of ATP-free solution. A secondary gradient was then produced by streptavidin-mediated attachment to actin of biotinylated fibronectin. Interestingly, the actin filaments with attached fibronectin could also be transported by HMM.

**4:9**

**Conformation of myosin essential light chain in skeletal muscle fibres investigated by fluorescence lifetime imaging microscopy**

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Fluorescence lifetime imaging microscopy was applied to investigate the conformation of myosin essential light chain (ELC) in muscle fibres. Human myosin ELC was used to generate a number of mutants with single cysteine residues in the C-terminal lobe and in the linker region of ELC (Cys127, Cys142, Cys160 and Cys178). The ELCs were expressed in *E. coli*, isolated by nickel-agarose affinity chromatography and labelled with 7-diethylamino-3-((((2-iodoacetamido) ethyl)amino)carbonyl) coumarin. We demonstrated by confocal microscopy that coumarin-labelled ELC can be exchanged for the native light chain in rabbit *psoas* muscle fibres with high specificity, and isometric force measurements showed only mild reduction (10–20%). Single exponential fluorescence decay of free coumarin (0.37 ns lifetime) changed to double exponential when it was covalently bound to Cys178 of ELC, with 2.21 ns and 0.54 ns lifetime components. When introduced into muscle fibres, both components increased to 2.61 ns and 0.63 ns in rigor, and to 2.93 ns and 0.95 ns in relaxed state.

Supported by BBSRC and IRC in Bionanotechnology UK.

**4:10**

**Myosin VI is expressed in skeletal muscles where localizes mainly to fiber peripheries**

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Myosins, the actin-based molecular motors are ubiquitously expressed in all eukaryotic cells and are involved in a panoply of cellular functions, including cell migration, all the processes employing intracellular trafficking such as endo- and exocytosis, cytokinesis, and possibly in gene transcription. Several unconventional myosins (i.e. not resembling muscle isoforms) have been detected in myogenic cells and/or adult muscle where they seem to play important roles in muscle functioning and/or differentiation. We have recently detected using polyclonal anti-porcine myosin VI antibody that myosin VI, an unique myosin motor that translocates towards minus end of actin filament, is expressed in significant amounts in rat leg skeletal muscles. Localization studies performed on muscle cross-sections revealed that it concentrates mainly at the muscle fiber peripheries and in circular structures across the fiber. Interestingly, myosin VI has been found within the nuclei, in the regions lacking chromatin. It was earlier reported that a point mutation within the myosin VI gene leads—besides deafness—to a cardiac dysfunction (1). However, further studies are needed to elucidate the role of this myosin motor in skeletal muscle.

1. Mohiddin SA, Ahmed, ZM, Griffith AJ, Tripodi D, Friedman TB, Fananapazir L, Morell RJ (2004) Novel association of hypertrophic cardiomyopathy, sensineural deafness, and a mutation in unconventional myosin VI (MYO6). J Med Genet 41:309–314

**5:1**

### FOXO3 coordinates ubiquitin-proteasome and autophagy-lysosome systems during muscle atrophy

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The size of skeletal muscle is determined by a balance between protein synthesis and protein degradation. In mammalian cells half-life of proteins is controlled by two proteolytic systems the ubiquitin-proteasome and the autophagy-lysosome. Autophagy is an evolutionarily conserved mechanism that allows cell survival during starvation through the bulk degradation of proteins and organelles by lysosomal enzymes. However, the mechanisms responsible for the induction and regulation of the autophagy program are poorly understood. Here we show that FoxO transcription factors are required for the induction of autophagy in skeletal muscle. Suppression of FoxO3 activity prevents autophagosome formation induced by starvation. Akt/PKB activation blocks FoxO activation and autophagy, and this effect is not prevented by TORC1 inhibition. FoxO3 is able to induce autophagosome formation and up-regulation of the autophagy genes and Bnip3. Bnip3 appears to be a major mediator of FoxO3, as FoxO3-dependent autophagy is markedly reduced by knockdown of Bnip3. FoxO3 activates the proteasomal system by inducing the ubiquitin ligases atrogin-1 and MuRF1, however FoxO3-dependent autophagy is not affected by loss of atrogin-1 and MuRF1 or by blockade of proteasome. Thus FoxO3 controls independently the two major proteolytic systems in skeletal muscle, the ubiquitin-proteasome and autophagy-lysosome.

**5:2**

### Hereditary myosin myopathies

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Hereditary myosin myopathies have emerged as a new group of muscle diseases with highly variable clinical features and onset during fetal development, childhood or adulthood. They are caused by mutations in skeletal muscle myosin heavy chain (MyHC) genes. Mutations have been reported in two of the three MyHC isoforms expressed in adult limb skeletal muscle: type I (slow/$\beta$-cardiac MyHC; *MYH7*) and type IIa (*MYH2*). Several mutations in two different parts of the slow/$\beta$-cardiac MyHC rod region are associated with two distinct skeletal myopathies without cardiomyopathy: Laing early-onset distal myopathy and myosin storage myopathy (MSM). However, early-onset distal myopathy and MSM caused by *MYH7* mutations may also occur together with cardiomyopathy. MSM affects proximal or scapuloperoneal muscles whereas Laing distal myopathy primarily affects the dorsiflexor muscles of the toes and ankles. A myopathy associated with a specific mutation in *MYH2* is associated with congenital joint contractures and external ophthalmoplegia. The disease is mild in childhood but may be progressive in adulthood, with proximal muscle weakness affecting ambulation. Mutations in embryonic MyHC (*MYH3*) and perinatal MyHC (*MYH8*), which are myosin isoforms expressed during muscle development, are associated with distal arthrogryposis syndromes with no or minor muscle weakness.

**5:3**

### The human myofibrillar Z disc, molecular structure, composition and normal and pathological reactions

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The sarcomere is the smallest contractile unit of the myofibril extending between two successive Z discs. The main structures of the Z disc are overlapping thin filaments and interlinking bridges. The thin filaments are composed of F-actin of opposing polarity and the bridges of alpha-actinin. The width of the Z disc varies with fiber types. Irregular Z discs and broadened Z discs and smearing of Z discs are common features in exercised muscles and in muscle pathology. Another feature—Z disc rods are the hallmark of nemaline myopathy which can be caused by gene mutations in at least five different myofibrillar proteins. In the last decade an increasing number of new Z disc components have been identified and been related to specific myopathies. So far no comprehensive model of the human Z disc exists nor have nemaline rods caused by the different genetic defects revealed to vary in structure.

Using high resolution immunohistochemistry we have resolved the normal Z disc composition and variations related to Z disc smearing and streaming. Furthermore we show a heterogeneous composition of nemaline rods. From negatively stained ultrathin cryosections we can resolve a basic striated pattern of the Z disc and nemaline rods which differ among the patients likely reflecting different genetic backgrounds.

**5:4**

### Effects of an E41K $\beta$-tropomyosin mutation on the regulation of muscle contraction

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A novel E41K $\beta$-tropomyosin ($\beta$-Tm) mutation, associated with slowly progressive muscle weakness and cap disease was recently identified in a woman and her daughter. The intent of the present study was to explore the mechanisms underlying the impaired muscle function in the two patients with the E41K $\beta$-Tm mutation. Fibre cross-sectional area (CSA), maximum force normalized to CSA (specific force, SF), maximum velocity of unloaded shortening ($V_0$), apparent rate constant of force redevelopment ($k_{tr}$) and force-pCa relationship were evaluated in single chemically skinned muscle fibres from the two patients carrying the mutation and healthy control subjects. Results showed that, in fibres expressing the $\beta$/slow (type I) myosin heavy chain isoform, CSA was dramatically smaller ($P < 0.001$), but SF was maintained. However, a significant difference in shortening speeds were observed, such as a lower $k_{tr}$ ($P < 0.001$) and $V_0$ ($P < 0.001$). The force-pCa relationship also

differed between patient and control fibres, as attested by the smaller $Ca^{2+}$-sensitivity ($pCa_{50}$, $P < 0.001$) and larger Hill coefficient ($n$H, $P < 0.001$). Collectively, these results suggest slower cross-bridge attachment and detachment rates caused by the E41K $\beta$-Tm mutation, but this may not affect the maximal force-generating capacity of the cross-bridges. Nevertheless, the E41K $\beta$-Tm mutation may directly disrupt the sub-maximal force-generating capacity of the cross-bridges, altering their $Ca^{2+}$-activation. The functional alteration together with the muscle fibre atrophy result in overall muscle weakness.

### 5:5

### A Drosophila model for the oculopharyngeal muscular dystrophy (OPMD): study and development of therapeutic strategies

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Oculopharyngeal muscular dystrophy (OPMD) is a late onset disease characterized by eyelid dropping, swallowing difficulties and limb weakness. Alanine expansions in the coding region of poly(A) binding protein N1 (PABPN1) resulting from GCG trinucleotide repeat extensions lead to the dominant autosomal inherance of OPMD. In skeletal muscle fibers of OPMD patients, mutant PABPN1 protein aggregates as fibrillar nuclear inclusions, which are the hallmark of the disease.

We have generated a Drosophila model that recapitulates muscular phenotypes with similarities to those of OPMD: (i) muscle degeneration (ii) fibrillar nuclear inclusions containing PABPN1 in muscle nuclei [Chartier et al (2006) EMBO J]. Molecular analysis of this model showed that muscle disorder in Drosophila is induced by an intrinsic toxicity of PABPN1 brought by the RNA binding domain and enhanced by the alanine expansions. We are carrying out a genetic approach to identify cellular pathways that could suppress muscular phenotypes observed in our Drosophila OPMD model. In parallel, we investigated the potential of single-chain intracellular antibody (intrabody) as therapeutic agent for OPMD. We could induce muscle expression of several intrabodies against PABPN1 [Verhensen et al (2006) HMG], in the Drosophila OPMD model and we found that several of them can prevent PABPN1 toxicity and muscular phenotypes.

### 5:6

### Nemaline myopathy-causing mutations E117K and Q147P of $\beta$-tropomyosin impairs its function

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Nemaline myopathy (NM) is a rare congenital disease characterized by muscle weakness and by the formation of nemaline bodies in skeletal muscle fibers. Two missense NM-causing mutations E117K and Q147P have been identified in $\beta$-tropomyosin (TM). Wild type (WT) and mutant TMs were expressed in *E. coli* to analyze the mutation effect on TM function in vitro. The Q147P mutation dramatically decreased tropomyosin affinity to actin by cosedimentation assay. Despite this, the thermal stability is surprisingly slightly increased compared with WT TM. E117K TM bound to actin as well as WT TM and circular dichroism measurements reveal no mutation effect on TM secondary structure. However, the mutation significantly lowers maximum activation of actomyosin ATPase and also reduces $Ca^{2+}$-sensitivity of activation. To understand the molecular mechanism of altered ATPase regulation, Cys-36 of WT and mutant TMs was covalently labeled by 5-Iodoacetimido-fluorescein and the TMs were incorporated into ghost muscle fibers. The orientation and mobility of the fluorescent label were measured in the presence of the ATP analogs by polarized fluorescence techniques. Both mutations inhibited the changes in the orientation and mobility of the fluorophores during the ATPase cycle. We proposed that the mutations shift tropomyosin on the thin filament to ''OFF' position thus leading to tropomyosin insufficiency.

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### 5:7

### Distinct ganglioside composition at the neuromuscular junctions reveals the molecular basis for Miller Fisher syndrome

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Millier Fisher syndrome (MFS), a clinical variant form of Guillain-Barré syndrome, is an acute post-infectious paralytic illness and is characterized by an onset of ophthalmoplegia, ataxia and areflexia. Auto-antibodies against self-gangliosides are present in patients with MFS, in particular IgG antibodies to ganglioside GQ1b but also GD3 and GD1b. It has been shown that anti-ganglioside antibody binding at neuromuscular junctions (NMJs) leads to motor nerve terminal injury. The purpose of this study was to explore differences in ganglioside composition in human extraocular muscles (EOMs), limb muscles and muscle spindles. We showed that ganglioside GQ1b, GT1a, GD3 and GD1b epitopes are present in NMJs in EOMs and in the intrafusal fibers in muscle spindles. In contrast, no such epitopes were found in NMJs of limb muscles. Our study revealed distinct patterns of distribution of the relevant GQ1b ganglioside epitopes in NMJs of human EOMs, muscle spindles and limb muscles, supporting the hypothesis that NMJs in EOMs and muscle spindles are the targets of anti-ganglioside antibodies. These findings provide a molecular basis for the selective involvement of the EOMs and for the ataxia seen in MFS.

### 5:8

### Altered cellular localization and trafficking of TRPC3 cation channels in mdx mouse muscle fibres

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The transient receptor potential (TRP) channel TRPC3 is a non-voltage gated $Ca^{2+}$-permeable cation channel that is expressed in mouse skeletal muscle. In other cell types TRPC3 is known to contribute to $Ca^{2+}$ influx and to the activation of a calcineurin-dependent signalling pathway. Since an altered $Ca^{2+}$ homeostasis

seems to underlie the mdx muscle pathology we studied the localization of TRPC3 in muscle fibres from mdx and control mice. Immunofluorescent staining of isolated interosseus fibres with an anti-TRPC3 antibody revealed a cross striation pattern near the sarcolemma and a faint cytoplasmic fluorescence. Double labelling experiments showed co-localization of TRPC3 with vinculin, but not with dystrophin, ryanodine receptor 1 or the dihydropyridine receptor. While the latter results were confirmed for both genotypes, mdx fibres showed a more prominent cytoplasmic TRPC3 staining than controls. The unexpected cytoplasmic staining could be reverted by incubation of mdx fibres with $Gd^{3+}$ (50 μM), nifedipine (50 μM) or a $Ca^{2+}$-free solution for 20 min. Our data suggest a costameric localization of TRPC3 in skeletal muscle and a $Ca^{2+}$-dependent trafficking of the channel from cytoplasmic pools to the sarcolemma, that is alleviated in dystrophin-deficient mdx fibres.

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**5:9**

### Skeletal muscle and heart investigation of a KI-Lmna mouse model of Emery-Dreifuss muscular dystrophy and effect of the *N*-acetyl-L-cysteine treatment

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Autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD), affecting heart and skeletal muscles, is caused by mutations in the LMNA gene encoding the nuclear lamins A/C. The KI LmnaH222P/H222P mouse model reproduces a mutation identified in a family with typical EDMD and presents striated muscle defects. In 6-month old LmnaH222P/H222P mice, mechanical measurements of contraction and histological analysis indicate that in the whole skeletal muscle, type-1 fibres are specifically and progressively atrophied by the disease. Myofibrils isolated from the soleus muscle, which expresses 50% of type-1 fibres, showed no abnormalities. The chymotrypsine-like activity of the proteasome was not increased in striated muscles and is therefore unlikely to account for the type-1 fibre atrophy. A genome-wide expression profile (Affymetrix) in hearts at the incipience of the clinical signs revealed that activation of mitogen-activated protein kinases is a primary event in the time course of the cardiac pathology. At the end-stage of the disease, left ventricular dilation and hypokinesia associated with fibrosis, oxidative stress, glutathione depletion and increase in soluble tumor necrosis factor alpha are stabilized or normalized by treatment with the glutathione precursor *N*-acetyl-L-cysteine (NAC, 1.4 mg/10 g/day). These results suggest a potential benefit of NAC for EDMD patients.

**5:10**

### Amino acid supplementation counteracts metabolic damage and sarcopenia in skeletal muscle of STZ-diabetic mice

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We analyzed whether amino acid (Aa) supplements counteract the metabolic and structural changes induced by type-1 diabetes (Dia) in soleus muscle of the mouse. C57Bl6 mice were divided into three groups and treated for 15 days: C controls; D Dia (65 mg/kg streptozotocin STZ i.p.); DA Dia + Aa (1 g/kg/die). During treatment glycemia and insulinemia levels were measured in all groups. Changes in components of the antioxidant defense system and components of the oxidative and glycolytic enzyme systems were investigated by bi-dimensional electrophoresis (2D-SDS PAGE). Myosin Heavy Chain (MHC) composition was analyzed by SDS-PAGE and fibre cross sectional area (CSA) was measured on haematoxylin-eosin stained sections. Dia was associated with slow-to-fast transition in MHC composition, reduced fibres CSA, overall reduction of oxidative antioxidant enzymes expression. Aa administration counteracted the effects of Dia and was associated with increased fibre CSA and increased expression of several antioxidant and oxidative enzymes whereas non change in MHC composition was observed. Our data suggest a role for Aa supplements on myofibrillar and mitochondrial synthesis in STZ-diabetic mice independently from insulin.

**5:11**

### Taking obscurity out of the obscurin protein family

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A variety of structural, signalling and scaffolding proteins participate in the formation of the sarcomere, the minimal building block of the myofibrils in striated muscle cells. While the main function of the sarcomere is to generate contraction by the action of actin and myosin, a large amount of additional proteins are vital for the assembly, support, maintenance and adaptation of the muscle.

In a screen for binding partners to the M-band proteins myomesin and titin, we were able to identify obscurin-like 1 (Obsl1) and obscurin as potential interaction partners. Obscurin-like 1, a novel M-band protein displays high structural and functional homology to the obscurin N-terminus. Cell biological analysis of the interaction showed that the M-band localisation of obscurin and obscurin-like 1 relies solely on their association with myomesin and titin. The binding and subcellular localisation of the obscurin protein family is disturbed by mutations in titin that are known to cause muscular dystrophy.

Deciphering of the complex protein-interaction network using molecular biological, cell biological and genetic approaches will provide further insights into the function and role of obscurin and obscurin like-1 during muscle development, maintenance and disease.

**5:12**

### Radiation-induced arterial hypertension: possible involvement of $BK_{Ca}$ and PKC

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Ionizing radiation evokes arterial hypertension, but cellular mechanisms of this effect yet unknown.

The aim of this study was to evaluate the role of protein kinase C (PKC) in activation in large conductance $Ca^{2+}$-activated $K^+$ channels ($BK_{Ca}$) functional state in hypertension following $\gamma$-radiation.

Experiments were made using patch-clamp technique on freshly isolated rat aorta smooth muscle cells (SMC).

It was shown, that $BK_{Ca}$ current in SMC obtained from animals on 9 day after whole body $\gamma$-irradiation (6 Gy) was significantly decreased as compared with SMC from control animals. This effect persisted or even increased over 30-day experiment period. Paxilline ($5 \times 10^{-7}$), selective inhibitor of $BK_{Ca}$, reduced $BK_{Ca}$ current in SMC obtained from control rat but was without effect in irradiated SMC (30th day), and slightly decreased $BK_{Ca}$ currents in SMS on 9 day of post-irradiation. Blockade of PKC (chelerythrine, $10^{-6}$) caused significant increase $BK_{Ca}$ current from irradiated SMC up to level of $BK_{Ca}$ current that was seen in control aorta.

The data obtained suggest that $\gamma$-radiation suppresses functional activity of $BK_{Ca}$ channels and PKC activity. This may contribute to radiation-induced hypertension.

**5:13**

### Functional and biochemical characterization of skeletal muscles of FRG1 over-expressing transgenic mice, model of FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is a hereditary neuromuscular disorder characterized by progressive weakness and atrophy of the facial, shoulder, abdominal and pelvic girdle muscles. Evidence indicates the over-expression of genes mapped at distal long arm of chromosome 4 (4q35) as responsible for FSHD.

Transgenic mice over-expressing FRG1 (FSHD Region Gene 1) at low, medium and high level were utilized to confirm this hypothesis. Indeed FRG1 transgenics develop a progressive muscular dystrophy whose degree of severity correlates with the level of expression of the transgene. Contractile properties of soleus, extensor digitorum longus and biceps muscles were studied in vitro at 30°C. Calcium-sensitivity and specific tension of single fibres from soleus, vastus lateralis (VL) and biceps muscles were also analyzed. Functional properties were correlated to the expression of myosin heavy chains (MyHC), troponin C (TnC) and troponin T (TnT) isoforms.

All muscles showed a progressive loss of twitch and tetanic tensions, confirmed by the lower specific tension recorded in single fibres. The pCa-tension relationship of VL and biceps fibres was significantly shifted to the right, particularly in mice expressing FRG1 at the highest level. SDS-PAGE and WB analyses of dystrophic muscles show a general shift toward a slow phenotype of both MyHC and TnC isoforms and an altered expression of fast TnT isoform.

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**5:14**

### Cardiac remodeling on the model of daunorubicin-induced cardiomyopathy in rabbit

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Matrix metalloproteinases (MMPs) play a role during the cardiac remodeling. In the present study we aimed to assess MMPs in experimental induced cardiomyopathy (3 mg/kg i.v., weekly,10 weeks) in rabbits. The MMP-activity was observed only in the 70-kDa region in the gelatine (MMP-2) while no MMPs activities were detectable either in the casein or collagen containing zymograms. Significant decrease in MMP-2 activity occurred during the 5th week (76.5 ± 5.1%) and was accompanied with the higher plasma levels of cardiac troponin T and increased production of collagen. However, MMP-2 activity at the end of experiment was upregulated by ≈16%. The morphological picture demonstrated that following the administration of daunorubicin, the diffuse cytolysis of cardiomyocytes developed, followed by reparative changes resulting in interstitial fibrosis. To sum up, MMP are involved in the development of daunorubicin-induced cardiomyopathy and the temporal pattern of MMPs activation may be unique to the type of pathological stimulus.

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**5:15**

### Cardiac troponins as markers of drug-induced cardiotoxicity in vitro and in vivo

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Cardiac troponins are becoming acknowledged as useful markers of drug-induced cardiotoxicity. In this study we examined the release kinetics of cTnT and cTnI using an in vitro model of NVCM (0.1–3 μM of daunorubicin, 72 h) and compared it with data from rabbit model of chronic daunorubicin-induced cardiomyopathy in vivo (3 mg/kg, weekly, 10 weeks). In vitro, the cTnI and cTnT concentrations were negatively exponentially related to viability. With 3-μM daunorubicin, the relative increase of AUC of cTnT and cTnI was 2.4 and 5.3-fold higher than the increase of LDH activity, respectively. In rabbits, although the correlation between cTnT and cTnI (AUCs) was found ($R = 0.81$; $P < 0.01$) and both cardiac troponins corresponded well with systolic dysfunction, the first significant increase in cTnI levels was observed earlier. In conclusion, our study has confirmed cTnT and cTnI as very sensitive markers of anthracycline-induced cardiotoxicity.

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**5:16**

### Oxidative modifications of cysteine residues in muscle proteins caused by X-ray radiation

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Oxidative stress caused by reactive oxygen species (ROS) has been implicated in the pathogenesis of a number of diseases accompanied by muscle wasting, such as different forms of muscle dystrophies, rheumatoid arthritis, and diabetes. One cause might be oxidation of abundant proteins by ROS in the cells. Many proteins contain cysteines that stabilize the protein structure and thereby determine the protein functions by intra- or intermolecular disulfide bridges or are part of the active sites. Such thiols can undergo a number of oxidative modifications. Reversible formation of disulfides plays an important role in redox regulation of proteins.

Here the formation of disulfide bonds in muscular proteins as a result of oxidative stress was investigated in an in vivo model using rats irradiated by 5-Gy dose of X-rays. Proteins were isolated from skeletal muscle tissues 0, 3, 9 or 24 h after irradiation. The protein mixtures were separated by a diagonal gel electrophoresis and the protein bands were visualized with silver nitrate. Proteins of interest were identified by MALDI-TOF/TOF mass spectrometry. Intermolecular disulfide bonds in myosin light chain 1 protein were identified in all samples obtained after irradiation with increased spot intensities for protein oligomers over time. Similar alterations of the disulfide-contents were also obtained for other metabolic enzymes and contractile proteins.

**5:17**

### Cyclosporin A reduces calcium-dependent cell death and IP3R-1 expression through calcineurin pathway in dystrophin deficient cells

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Evidence for the involvement of the IP3 pathway has been demonstrated in dystrophin-deficient cell lines (SolC1(–)), when calcium is released at rest or during stimulation. These calcium signals are significantly reduced in SolC1(–) myotubes with the exposure to IP3R inhibitors (2-APB). Similar results were observed with cyclosporin A (CsA) known to, among other effects, reduce IP3R-1 expression through calcineurin pathway. Cell survival assays, performed with MTT test, revealed a protective effect of both 2-APB and CsA against natural cell death occurring in mature dystrophin-deficient myotubes. Moreover, CsA reduced IP3-R1 mRNA levels in SolC1(–), leading to levels measured in mini-dystrophin transfected myotubes (SolD(+)) in control conditions. These results underline a strong involvement of IP3 pathway in calcium-dependant cell death in dystrophin deficiency.

**5:18**

### Fullerene $C_{60}$ HyFn destroys amyloid fibrils of sarcomeric cytoskeletal X-protein and increases of cell viability

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It is not clear whether amyloids induce cell death via specific mechanisms or it results from amyloid mass accumulation and mechanical damage. Earlier we have demonstrated by electron, polarization fluorescence microscopy and spectral methods that sarcomeric cytoskeletal X-protein is capable to form in vitro amyloid fibrils and hydrated fullerene $C_{60}$ HyFn destroys these fibrils and prevents the formation of mature fibrils. Here we show that amyloid aggregates of sarcomeric cytoskeletal X-protein induce cell death of polymorphonuclear leukocytes (PMNL) of tumor-bearing animals. The cell viability was accessed by the trypan blue exclusion assay. The destruction of X-fibrils by $C_{60}$ HyFn led to the increase of cell viability. The $C_{60}$ HyFn itself did not show a toxic effect. Our results evidence the potential of this approach for evaluating the effectiveness of drugs preventing or treating amyloidoses. This work was supported by grant of Ministry of Russian Federation education No. 1.0.06 and Program of the Presidium RAS "Fundamental sciences for medicine".

**5:19**

### Effects of rotator cuff ruptures on the cellular and intracellular composition of the human supraspinatus muscle

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The rotator cuff of the human shoulder is that group of muscles and their tendons that stabilize this joint. Consequences of rotator cuff ruptures are muscle fibre atrophy and loss of muscle flexibility. There is also fatty degeneration of the muscle and fibrosis, both of which are detectable by MRI and thus widely used as a main diagnostic feature of ruptures. The present study uses muscle biopsy samples of the M. supraspinatus from elderly patients to examine the rupture-induced muscle change at both the cellular and the fine structural level. The results will help to establish the true spectrum of supraspinatus muscle damage in humans induced by tendon rupture and improve the interpretation of the MRI sequences that are routinely taken for pre-surgery diagnose and post-surgery control. Amounts of fatty tissue, connective tissue and muscle were assessed by light microscopy-based morphometry and stereology. Stereology of electron micrographs was employed to determine volume densities of muscle fibre mitochondria, myofibrils and intracellular lipid. Results indicate that the supraspinatus muscles of patients with a rupture contain significantly higher amounts of fatty tissue and of muscle fibre intracellular lipid than those of control subjects. Patients with an advanced stage rupture also exhibit a major decrease in relative amounts of myofibrils.

**5:20**

**CAPN3 loss of function effects in LGMD2A patients**

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Autosomal recessive limb-girdle muscular dystrophies (LGMD2s) are a clinically and genetically heterogeneous group of disorders including at least 10 different genetic entities. The calpainopathies (LGMD2A), a subgroup of LGMD2s, are the most common forms of LGMD2 in the population. They are usually characterized by symmetrical and selective atrophy of pelvic, scapular and trunk muscles and a moderate to gross elevation of serum CK. LGMD2A is associated to mutations in the gene coding for the muscle calpain-3 protein (CAPN3). We studied the gene expression in muscle samples from 13 LGMD2A patients using microarray and qRT-PCR techniques. We evidenced an impairment condition in mitochondrion energy mechanism and up regulation for genes involved in the muscle response to inflammation. These traits are common for muscles affected by other dystrophic syndromes while specific traits that we have evidenced in LGMD2A muscles are:

(1) the down regulation of genes involved in protein ubiquitination, evidencing a process preventing protein degradation and leading the formation of stress fibers. This was confirmed by the up-regulation of genes involved in the actin filament stabilization;
(2) the up regulation of titin, a protein to which calpain-3 is known to be linked in Z-, N2A- and M-regions of the sarcomere;
(3) the up regulation of mRNAs for proteins involved in sarcomere assembly and remodeling such as nebulin, nebulette, alpha-actinin-2-associated LIM protein, and FHL1.

These data suggests a complex role for calpain-3 in the assembly of sarcomeric ordered structure.

**5:21**

**Regulation of $Ca^{2+}$-activation in human single skeletal muscle fibres with a preferential myosin loss**

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The preferential loss of the motor protein myosin, observed in patients with acute quadriplegic myopathy (AQM) and cancer cachexia, cause a decreased force production at the muscle fibre level, generalized muscle wasting and weakness. It remains unclear, however, whether this myosin loss influences other important features of contractility, such as $Ca^{2+}$-activation of the contractile proteins. To address this question, we have studied $Ca^{2+}$-activation at the single muscle fibre level with specific reference to (a) the force-pCa relationship and (b) the force development ($k_{tr}$)-pCa relationship. Chemically skinned single skeletal muscle fibres have been studied from three patients with AQM and one patient with cancer cachexia with a preferential loss of myosin; and six healthy control individuals. Results show that muscle fibres with a preferential myosin loss have an impaired $Ca^{2+}$-activation with a rightward shift of the force-pCa curve in fibres expressing the type I MyHC isoform and an upward shift of the $k_{tr}$-pCa relationship at low pCa levels in fibres expressing the type I and IIa MyHC isoforms. These results suggest that the myosin loss reduces the cross-bridge cooperativity at low pCa levels by limiting the spread of activation, reducing the force production and accelerating the force development. The altered $Ca^{2+}$-activation of the contractile apparatus in patients with a preferential myosin loss is forwarded as a factor contributing to the general muscle weakness observed in these patients.

**5:22**

**Macromolecular complex between TRPC1/TRPC4 and alpha1-syntrophin/dystrophin: regulation of Capacitative Calcium Entries (CCEs) by PLC and DAG pathway**

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Drastic calcium disorder in Duchenne dystrophic muscle suggested that dystrophin, a cytoskeleton protein, might regulate calcium influx pathway. Abnormal calcium entries leading to an increase in the free calcium concentration under the sarcolemma, imply uncontrolled store-operated $Ca^{2+}$ currents supported by a heterotetrameric channel: TRPC1/TRPC4. This study is the first to show in muscle cells by co-immunoprecipitation a stable complex between TRPC1, TRPC4, dystrophin and alpha1-syntrophin through its PDZ domain. On the other hand, the activity of PLC and the production of $IP_3$ are increased in dystrophic muscles, and TRPC channels could be activated by DAG. We have thus studied the regulation of CCEs by OAG (an analogue of DAG) and U73122 (an inhibitor of PLC) in normal and dystrophic muscle cells. The results have demonstrated by quenching of fura-2 by manganese, a calcium influx dependent on DAG and PLC pathway in skeletal muscle cells. We suggest that the dystrophin/alpha1-syntrophin complex anchoring TRPC1/TRPC4 tetramers could also regulate the activity of PLC and modulate the production of DAG in proximity of channels. This, could participate in the normal regulation of calcium entries through the non-selective TRPC1/TRPC4 channels, which would be altered when dystrophin is absent in dystrophic muscles.

**5:23**

**Calcium handling in skeletal muscle from the trained and untrained knee extensor of heart failure (HF) patients and controls**

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We have previously reported reduced fatigue resistance in slow twitch skeletal muscle from rats with post infarction HF [(2002) J Physiol 540:571]. However, intracellular calcium release was not related to fatigue development in these HF rats [(2006) Circ Res 98:1514]. We therefore hypothesized that training might affect intracellular calcium cycling differently in muscles from patients with HF as compared to controls. Muscle biopsies were taken from vastus lateralis before and after 6 weeks of one-legged endurance

training. Biopsies were analyzed for calcium handling proteins and the capacity of sarcoplasmic reticulum (SR) for calcium release and uptake. Maximal sustainable workload was higher in the trained leg compared to the untrained leg in both HF patients and controls (16% and 8%, respectively). The content of the fast calcium pump (SERCA1) was significantly reduced (21%) in the trained leg as compared to the untrained leg from the HF patients, in contrast to what was observed in the control group. However, the expression of the slow calcium pump (SERCA2) tended to be higher in the trained leg in both groups. We conclude that in HF patients endurance training causes a reciprocal shift of the expression of the two isoforms of SERCA towards a slower phenotype.

### 5:24

### M-band alterations during cardiomyopathy

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The M-band is a prominent part of the sarcomeric cytoskeleton believed to stabilise the thick filament lattice during contraction. The main structural components of the M-band are three modular proteins from the myomesin family: the well-known myomesin and M-protein, as well as the recently discovered myomesin 3. Each muscle is characterised by its unique M-band protein composition, depending on the contractile parameters of a particular fiber.

To investigate the role of the M-band in pathological situations, we analysed the cardiac muscle of mouse during development of cardiomyopathies. The severity of the disease process was assessed by echocardiography. In several mouse models for heart disease we characterised specific alterations of the M-band protein expression pattern that are particular to dilated cardiomyopathy (DCM). These alterations are cell-specific, with some cells switching completely to an embryonic phenotype and resulting in a strong heterogeneity of M-band pattern through the cardiac wall.

The alterations of the M-band protein composition might be an adaptation of the sarcomeric cytoskeleton to unfavorable working conditions in the failing heart. We suggest that specific changes in the M-band might serve as a convenient marker for DCM and help to understand the mechanisms of the pathology development.

### 5:25

### Drosophila indirect flight muscles: a model for human nemaline myopathy

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Nemaline myopathies are dominant, often lethal, genetic disorders of human skeletal muscle, caused by mutations of thin filament proteins. Biopsies reveal aberrant muscle structure and nemaline bodies, consisting of Z-disc proteins. More than 110 α-skeletal actin (ACTA1) mutations cause the disease with varying severity. Using the *Drosophila* indirect flight muscle specific *Act88F* actin gene, as a model to study this human disease we have created flies expressing the human mutant actin homologues: G15R, D154N, D292V and R372H that exhibit different aspects of the disease. As wild type heterozygotes, all exhibit distinct phenotypes with aberrant myofibrillar structure. D154N mutants produce a novel "Z-ring" phenotype. Atypically, flies heterozygous for the R372H *Act88F* mutation complete normal IFM myogenesis and young flies can fly, but become flightless and by day 7 show the *Drosophila* equivalent of the human nemaline phenotype. Using immunofluorescence and EM we have followed the progressive loss of muscle structure. From the ultrastructure, the phenotypic requirement for muscle usage and the known α-actinin binding sites on the actin monomer we argue that the R372H mutation reduces the strength of F-actin/α-actinin binding, leading to muscle damage during use. These studies of *Act88F* mutants show that *Drosophila* IFM are a useful model for understanding this myopathy.

### 5:26

### Sleep disorders aggravate muscle abnormalities in patients receiving hemodialysis therapy

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Hemodialysis patients manifest muscle disturbances collectively termed uremic myopathy. Half of this population also suffers by various sleep disorders significantly affecting their quality of life. We have previously shown that sleep disorders add to the muscle disturbances; however the type of muscle studied may have confounded our results. Here we examined whether dialysis patients with sleep disorders would manifest additional alterations in muscle size, composition and functional capacity when compared to matched counterparts with normal sleep. Locomotory (thigh) and non-locomotory (rectus abdominis) muscles were examined accounting for physical inactivity, an important contributor to atrophy. Based on their Apnea Hypopnea Index score, 20 hemodialysis patients (10F, 56 ± 11 years) were divided in two matched groups: 10 with profound (AHI = 29.4 ± 22.2) and 10 with no signs (AHI = 1.8 ± 1.4) of sleep disorder. We assessed group differences in muscle performance and in composition and size of locomotory and non-locomotory skeletal muscles (by CT). Functional capacity was lower in patients with sleep disorders ($P < 0.05$). No muscle size differences in either locomotory or non-locomotory skeletal muscles were found between groups. However, muscle composition was altered favoring fat deposition in both types of muscle in patients with sleep disorders compared to patients with normal sleep ($P < 0.05$).

### 5:27

### Molecular studies on the titinopathies TMD/LGMD2J

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Tibial muscular dystrophy (TMD) is a dominant late onset distal myopathy caused by mutations in M-line titin. In the few homozygotes known, the mutations lead to the different, more severe limb-girdle muscular dystrophy LGMD2J. Most of the TMD/LGMD2J mutations, including the Finnish FINmaj, are located in M10, the most C-terminal of titin domains.

The molecular pathways behind TMD/LGMD2J are unknown, but muscle selectivity and normal sarcomere ultrastructure suggest a signalling or regulatory defect rather than a structural one. Loss of protein interactions of C-terminal titin is likely, caused by direct disruption of the binding or by cleavage of the entire titin C-terminus. Our aim is to elucidate the molecular pathomechanism of TMD/LGMD2J by identifying the protein interactions disrupted and by determining the effect of the mutations on the stability of titin.

In a yeast two-hybrid (Y2H) screen, myospryn (CMYA5) and phosphoglucomutase 1 (PGM1) among others were identified as potential ligands of the M10 domain. At least the interaction with myospryn seems genuine, as it is disrupted in the Y2H system by the FINmaj mutation, and supported by coimmunoprecipitation experiments.

Several lines of evidence also suggest that proteolytic processing of C-terminal titin is altered in TMD/LGMD2J, potentially extending the effect of the disease mutations to a larger region in M-line titin.

**5:28**

### Muscle paralysis and myosin loss in a patient with cancer cachexia

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Cancer cachexia has a significant negative effect on quality of life, survival and the response to treatment. Recent studies have shown that myosin may be the primary target of the muscle wasting associated with cancer cachexia. In this study, we have extended these analyses to detailed studies of regulation of myofibrillar protein synthesis at the gene, myofibrillar protein expression and regulation of muscle contraction at the muscle cell level in a in a 63-year-old man with a newly diagnosed small cell lung cancer and a rapidly progressing lower extremity muscle wasting and paralysis. A significant preferential loss of the motor protein myosin together with a downregulation of protein synthesis at the transcriptional level was observed in the patient with cancer cachexia. This had a significant negative impact on muscle fiber size as well as maximum force normalized to muscle fiber cross-sectional area.

**5:29**

### Single muscle fibres from myopathic Tfam-I-mice display alterations in $Ca^{2+}$-handling

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Mitochondrial transcription factor A (Tfam) is essential for maintenance of mitochondrial DNA by playing critical roles in transcription, replication, damage sensing and repair. Skeletal muscle tissue specific ablation of Tfam leads to development of a myopathy characterized by ragged-red fibers, accumulation of abnormally functioning mitochondria, together with a progressively deteriorating respiratory chain function. It has been proposed that the increase in mitochondrial mass may be a compensatory effect to counteract the respiratory deficiency, and a previous study showed that muscles from mice devoid of Tfam (Tfam–/–) do not fatigue more rapidly than their wildtype counterparts. The absolute forces in Tfam–/– were however lower, and more so at lower frequencies. This reduction was believed to be caused by a deficient in the force generating cross bridges and/or a changed $Ca^{2+}$ homeostasis. We have studied the mechanisms underlying the reduction in force using single, intact muscle fibers. As in whole muscles, Tfam–/– single fibers did not fatigue more rapidly and displayed lower absolute forces when compared to controls. (Tfam–/– 288 ± 13 kPa, WT: 355 ± 13 kPa, $p < 0.05$ at 70 Hz). We also found that tetanic $Ca^{2+}$ was lower in Tfam–/– compared to WT (0.9 ± 0.05 μM vs. 1.6 ± 0.2 μM, $p < 0.05$ at 70 Hz). Tetanic $Ca^{2+}$ in the presence of 5 mM caffeine, which reflects the SR $Ca^{2+}$ load, was also lower in Tfam–/– (3 ± 0.5 μM, vs. 7 ± 1.5 μM, $p < 0.05$). Furthermore, quantitative PCR showed a reduced mRNA-expression of the SR-calcium buffering protein calsequestrin-1 in Tfam–/– muscle. In conclusion, the force decrease in Tfam–/– muscle is due to decreased SR-$Ca^{2+}$. This finding indicates an intricate SR-mitochondria interplay in the control genes encoding for $Ca^{2+}$ handling proteins.

**5:30**

### Regulation of capacitative calcium entries by syntrophin and its PDZ domain: Macromolecular association between TRPC1/TRPC4 and the syntrophin dystrophin complex

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Calcium mishandling in Duchenne dystrophic muscle suggested that dystrophin, a cytoskeleton protein, might regulate calcium influx pathways. Higher calcium entries in dystrophic muscles involve uncontrolled store-operated $Ca^{2+}$ currents supported by TRPC1 and TRPC4 channels. This study is the first to show the regulation of capacitative calcium entries (CCEs) by the syntrophin-dystrophin complex. Transfected α1-syntrophin restored normal CCEs in dystrophic muscle cells, as well as expression of mini-dystrophin. Transfection of α1-syntrophin deleted of its PDZ domain, failed to restore normal CCEs in dystrophic myotubes, which suggests that this domain is necessary for this regulation. Transfected α1-syntrophin formed a complex with TRPC1 channels and GST pull-down assays showed that TRPC1 binds to the α1-syntrophin PDZ domain. Co-immunoprecipitation assays showed the association of TRPC1 with dystrophin and α1-syntrophin in muscle tissue. The complex also contained TRPC4, suggesting that these channels are formed of heterotetramers. It is proposed that normal regulation of CCEs in skeletal muscle is dependent on the association between TRPC1/TRPC4 channels and α1-syntrophin, which may anchor the store-operated channels to the dystrophin-associated complex. The loss of this molecular association could participate in the calcium alterations observed in dystrophic muscles.

**5:31**

**Impact of exercise training on the global and cellular contractile properties in rats with heart failure**

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In the healthy heart, sub-endocardial cells (ENDO) contract more than sub-epicardial cells (EPI). This gradient of contractility disappears in the failing heart by affecting the ENDO cells. Our study tested the effect of exercise on the global and cellular contractility across the left ventricular (LV) wall in post-myocardial infracted rats (PMI).

Rats were exercised 15 weeks after infarction for 5 weeks on treadmill. Global cardiac function was analyzed by echocardiography. Excitation–contraction coupling ($Ca^{2+}$ transient, shortening) of intact cells isolated from EPI and ENDO LV layers and the stretch-induced sensitisation of $Ca^{2+}$ activation of the myofilaments on skinned cells ($Ca^{2+}$ sensitivity of the contractile machinery at 1.9 and 2.3 μm sarcomere length (SL)) were analyzed.

Echocardiography shows a gradient of shortening velocity from EPI to ENDO altered during pathology and partially restored after exercise. At the cellular level, cell shortening, and $Ca^{2+}$ transient were reduced in PMI in particular in ENDO cells. $Ca^{2+}$ sensitivity of the contractile machinery was reduced only in ENDO PMI at 2.3 μm SL reducing the transmural stretch sensitization. Exercise increased Endo PMI cell shortening by improving both $Ca^{2+}$ transient and $Ca^{2+}$ sensitivity of the myofilaments. Thus exercise is able to restore par of the gradient of contractility of the failing heart.

**6:2**

**Skeletal muscle fatigue following dynamic contractions**

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Skeletal muscle fatigue following repeated contractions in experimental studies is almost exclusively studied using isometric contractions. However, most muscles shorten during normal use and we propose that the fatigue mechanism will be different as compared with static contractions. Fatigue was induced in rat soleus muscles in situ by isotonic shortening contractions. Muscles were stimulated repeatedly for 1 s at 30 Hz with 1 s rest in-between for a total of 15 min. The muscles were allowed to shorten isotonically at 1/3 of maximal isometric force. Between contractions the muscles were restretched to initial length. Maximal isometric force (Fmax) was reduced after 100 s but returned to almost initial values at the end of the stimulation protocol. Maximal unloaded shortening velocity (V0) and relaxation rate after the 1 s contraction followed the same pattern. Likewise ATP and CrP were reduced and lactate and ADP was increased after 100 s, but recovered to initial values after 5 min. In contrast, the ability to shorten isotonically and the rate of rise of force and shortening did not recover during the remainder of the stimulation protocol. MLC2 was dephosphorylated after 100 s and did not recover. While metabolic changes seem to account for the change of Fmax, dF/dt and V0, dephosphorylation of MLC2 may be involved in the decreased muscle shortening and the slower contraction velocities.

**6:3**

**The scorpion toxin maurocalcine; a tool to study the excitation–contraction coupling in skeletal muscle**

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Maurocalcine (MCa) is a 33 amino acid residue peptide toxin. MCa binds directly to the type 1 RyR on the same binding site as the domain A of the II-III loop of the α1 subunit of the dihydropyridine receptor (DHPR). MCa induces a large increase in ryanodine binding on RyR1, and the appearance of long-lasting channel openings in a mode of smaller conductance. We used MCa to study the coupling of DHPR and RyR in mammalian skeletal cells. External application of MCa to cultured myotubes produces $Ca^{2+}$ release from intracellular stores without changing depolarisation induced $Ca^{2+}$ release. In adult cells, MCa does not induce $Ca^{2+}$ release by itself but strongly modifies the frequency and amplitude of calcium release events without modifying their spatial distribution. We propose that functional coupling of RyR and DHPR in $Ca^{2+}$ release unit prevents MCa from either reaching its binding site or from being able to modify the RyR gating.

**6:4**

**Assembly and dynamics of sarcoplasmic reticulum domains in skeletal muscle cells**

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The sarcoplasmic reticulum (SR) is a specialized form of smooth endoplasmic reticulum present in muscle cells and dedicated to $Ca^{2+}$ storage and release. The SR consists of a network of tubules and cisternae where functionally distinct sub-domains (i.e. longitudinal reticulum and junctional region) are observed. How these sub-domains assemble is largely unknown. The timing and the efficiency of SR proteins to selectively segregate, following differentiation, to specific SR sub-domains suggest the existence of dedicated retention mechanisms in the assembly of these proteins within developing SR sub-domains. To better characterise this process, we generated GFP-fusion proteins of representative proteins of SR. The assembly and dynamics of these proteins were analysed by FRAP experiments. The results revealed that all GFP-proteins were apparently freely moving in non-differentiated cells, while a reduced mobility was observed for most proteins in differentiated myotubes. Interestingly, the most dramatic changes were observed amongst proteins of the junctional SR. These results are strongly suggestive that specific retention mechanisms, including interaction with cytoskeletal proteins, may be involved in the localization of SR proteins and that the organization the $Ca^{2+}$ release machinery at the junctional region of the SR requires the assembly of a large multi-protein complex.

**6:5**

### Cardiac and skeletal cholinergic receptors modulation by *Buthus occitanus tunetanus* non-toxic venom fraction

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The action of acetylcholine (ACh) is mediated by two receptor types: mAChRs and nAChRs. They are present on cardiac and skeletal muscle cells respectively. Scorpion venoms contained number of known toxins, however they also contain compounds of which effects remain to be investigated. The aim of this work was to study the effects of a scorpion non-toxic venom fraction, M1, on cardiac properties and on calcium activity in cardiomyocytes and myotubes. M1 significantly decreased the amplitude and rate of contractile activity of isolated heart. On cardiomyocytes, patch-clamp studies showed that M1 reduced L-type calcium current with a negative shift of its steady-state availability-voltage relationship. These effects were reversibly blocked by the specific mAChRs antagonist atropine and prevented in cells pretreated with Pertussis toxin. On myotubes, M1 induced a transient increase of $[Ca^{2+}]_i$ prevented by a prior application of $\alpha$-bungarotoxin. So, at least one component from M1 interacts with cardiac mAchRs and skeletal nAChRs.

**6:6**

### Analysis of the complex between calcium channel beta subunit and ahnak

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The carboxyterminal (CT) of ahnak (1,002 a.a.) has been implicated in Cav1.2 current modulation by interaction with the regulatory $\beta 2$ subunit. Potential binding modules are a leucin zipper and a SH3-interacting PxxP motif located within the proximal and distal ahnak CT, respectively. In the proximal ahnak CT, binding studies defined two populations of $\beta 2$ subunit binding sites without engagement of the leucine zipper. The PxxP-core motif, however, was critical for $\beta 2$ subunit binding. Mutation of both proline residues to alanine converted the two-site, high affinity interaction (Kd1 ~ 60 nM; Kd2 ~ 250 nM) into a single set of low affinity binding sites (Kd ~ 1 μM). Nonetheless, the mutation did not abolish the functional effects of ahnak CT on Cav1.2 current as assessed on left ventricular adult rat cardiomyocytes under whole cell patch clamp conditions. Intracellular perfusion with AxxA-mutated ahnak-CT slowed the channel inactivation to a similar extent as the PxxP-containing wild-type ahnak CT. Thus, low affinity $\beta 2$ subunit binding to ahnak CT, outside of the PxxP-motif, are necessary and sufficient to modulate Cav1.2 current inactivation.

**6:7**

### The delayed force recovery after fatigue in skeletal muscle depends on reactive oxygen species metabolism

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We focus on the role of reactive oxygen species (ROS) in a delayed force recovery after fatiguing stimulation in skeletal muscle. Intact, single muscle fibres were dissected from flexor digitorum brevis muscles of rats and mice (wildtype and SOD2 overexpressing). Force and myoplasmic free $[Ca^{2+}]$ ($[Ca^{2+}]_i$) were measured. Fibres were stimulated at different frequencies before and 30 min after fatigue induced by repeated tetani. The results show a marked force decrease at low stimulation frequencies 30 min after fatiguing stimulation in all fibres. This decrease was associated with reduced tetanic $[Ca^{2+}]_i$ in wildtype mouse fibres but decreased myofibrillar $Ca^{2+}$ sensitivity in rat fibres and mouse SOD2 overexpressing fibres. The decreased $Ca^{2+}$ sensitivity in rat fibres could be partially reversed by application of the reducing agent dithiothreitol, whereas the decrease in tetanic $[Ca^{2+}]_i$ in wildtype mouse fibres was not affected by dithiothreitol. In conclusion, delayed force recovery can have different underlying mechanisms depending on the ROS metabolism. These findings may have clinical implications since ROS-mediated impairments in myofibrillar function can be counteracted by reductants and antioxidants, whereas changes in SR $Ca^{2+}$ handling appear more robust.

**6:8**

### Altered excitation–contraction coupling in muscle fibers of a transgenic mouse expressing malignant hyperthermia mutation Y522S

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Malignant hyperthermia (MH) is a life-threatening hyper-metabolic state originating from excessive calcium release in skeletal muscle triggered by certain pharmaceuticals. Susceptible individuals often show point mutations in the ryanodine receptor (RyR1). Transgenic mice heterozygous for one such mutation (Y522S) have recently been introduced [Chelu et al (2006) FASEB J 20:329]. The aim of this study was to search for functional changes induced by this mutation under non-triggering conditions. Recording from voltage-clamped muscle fibers of adult mice we found that calcium release responded more sensitive to depolarization than in controls whereas the activation of calcium inward current was unchanged. In addition, the voltage dependence of steady state inactivation was shifted by about 10 mV to more negative potentials for both $Ca^{2+}$ release and $Ca^{2+}$ current. These results point to an antagonistic effect of the inactivation mechanism that compensates for the selective alteration of $Ca^{2+}$ release activation and possibly originates from a modulation of the dihydropyridine receptor.

**6:9**

### Calcium release and changes in T-system morphology during hyperosmotic stimulation of mammalian skeletal muscle

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Exposure to a hypertonic solution containing elevated $Ca^{2+}$ (50 mM) has been shown to induce $Ca^{2+}$ spark activity in adult mammalian skeletal muscle fibers [Wang et al. (2005) Nat Cell Biol7:525]. It was proposed that structural changes induced by the hypertonic conditions may remove an inhibitory effect of the dihydropyridine receptor (DHPR) on the ryanodine receptor (RyR1). Using confocal microsopy, we observed a substantial swelling of transverse tubules, which may be involved in triggering

the local $Ca^{2+}$ release events. We pursued the role of the DHPR by comparing dysgenic myotubes derived from the GLT cell line lacking the DHPR-alpha1-subunit with control myotubes (derived from the C2C12 cell line). Almost all of the C2C12 myotubes subjected to hyperosmotic stimulation with high extracellular $Ca^{2+}$ responded with $Ca^{2+}$ release activity both local (sparks) or global (often in the form of large oscillations). GLT myotubes responded qualitatively in a similar way. When sucrose replaced 95% of the $Ca^{2+}$, muscle fibers and C2C12 myotubes showed only a modest decrease in their response to the hyperosmotic challenge. In contrast, oscillations and spark activity almost disappeared in GLT myotubes. In conclusion, $Ca^{2+}$ release activity during hyperosmotic challenge does not require intact DHPRs. However they appear to make the response independent of extracellular $Ca^{2+}$.

### 6:10

### Impaired calcium release and reduced calcium transients in single muscle fibres of mouse with inactivation of calsequestrin-1 gene

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Calsequestrin (CS) is the major $Ca^{2+}$-binding protein in the sarcoplasmic reticulum (SR) with a dual role in excitation–contraction coupling: to buffer free $Ca^{2+}$, thus increasing SR capacity, and to modulate the activity of the $Ca^{2+}$ release channels. A mouse carrying a null mutation for the skeletal muscle isoform, or CS1 was generated. CS1-*null* mice were viable and fertile and their skeletal muscles are slightly atrophic but without signs of change in fibre type distribution. The maximal amount of calcium release was measured in single muscle fibres dissected from the of tibialis anterior and permeabilized with saponin and found to be reduced to less than 50% in CS1-null compared to WT animals. Calcium transients were recorded after loading fibres with fura-2. The amplitude of the transient induced by electrical stimulation was significantly lower in CS1-null compared to WT animals. Together the results indicate that CS-1 is important for the ability of the SR to accumulate and release suitable amounts of calcium.

### 6:11

### The role of serca2 in skeletal muscle function

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Sarcoplasmic reticulum (SR) $Ca^{2+}$ ATPases (SERCA) plays a central role in $Ca^{2+}$ homeostasis and contractile function in skeletal muscle. We hypothesized that skeletal muscle-specific loss of SERCA2 would affect slow-twitch muscle relaxation by slower removal of $Ca^{2+}$ from the cytosol and reduced force generation due to reduced $Ca^{2+}$ transient size. To study the role of SERCA2 in skeletal muscle function, we have a new mouse model, the $Serca2^{flox/flox}MLC\text{-}1f^{wt/cre}$ (MLC1f-SERCA2KO) mice, in which the Serca2 gene is specifically deleted in slow twitch muscle fibres. The *Serca2* gene was excised in skeletal muscle but not in the heart or in non-muscle tissue, confirming the activity of the MLC-1f gene promoter. The MLC1f-SERCA2KO mice appeared overall normal. In soleus muscle, *Serca2* mRNA and SERCA2 protein were reduced to 6 ± 2% (SEM) and 6 ± 2% of control values, respectively. Serca1 and Serca3 mRNAs or proteins were not regulated. The mRNA levels of other $Ca^{2+}$ transporting proteins or myofilament components were unaltered. Preliminary in situ contractile measurements show no difference in the maximum tetanic force or relaxation rate between the MLC1f-SERCA2KO soleus and the control mice. Our preliminary conclusions are that MLC1f-SERCA2KO soleus muscle show normal contractile properties in spite of near loss of SERCA2. Possible compensatory mechanisms such as the expression of other SERCA ATPases or augmented expression of other central $Ca^{2+}$ handling proteins were not observed.

### 6:12

### In situ structural studies of Troponin I—Illuminating molecular movements in myocardial regulation with Rhodamine

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Myocardial contraction is initiated by $Ca^{2+}$ binding to the trimeric Troponin complex (Tn) on the thin filament. The $Ca^{2+}$ binding induces a series of structural reorganisations within the complex, which lift their inhibitory actions on the actomyosin crossbridges in the sarcomere. The inhibitory subunit Troponin I (TnI) serves a key regulatory function in this process. Presently the dynamic and allosteric process of the $Ca^{2+}$-induced conformational changes is poorly characterised at the detailed molecular level. Understanding the conformational changes of TnI would illuminate the physiological regulatory actions and give insight into pathological disruptions of regulation. Structural changes induced by $Ca^{2+}$ binding will be studied in situ in trabeculae by measuring fluorescence anisotropy of bifunctional rhodamine (BR) probes anchored at different sites on the TnI protein. Recombinant TnIs have been produced with different labelling sites. The tropomyosin binding subunit (TnT) and $Ca^{2+}$ binding subunit (TnC) of Tn have also been expressed in order to reassemble Tn complexes containing these modified TnIs which will be introduced into trabeculae. Fluorescence anisotropy will reveal information on the molecular movement of individual helices of TnI in relation to the actin filament axis during contraction, and its dependence on $Ca^{2+}$ binding and myosin head binding.

**Acknowledgments** British Heart Foundation

**Key words** Troponin I · Myocardial regulation · Fluorescence anisotropy · Bifunctional rhodamine

### 6:13

### TRPC3 and GLUT4 are co-localized and interact in insulin-mediated glucose uptake in adult skeletal muscle

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We examined the role of the $Ca^{2+}$ permeable canonical transient receptor potential channel 3 (TRPC3) in insulin-mediated glucose

uptake in adult skeletal mouse muscle, using a novel transfection technique involving carbon nanotubes to introduce small interfering RNA (siRNA) targeted against TRPC3. We previously showed that insulin causes an influx of $Ca^{2+}$ in insulin-stimulated muscle fibers (Bruton et al. 1999) and that maneuvers that increased $Ca^{2+}$ influx in the presence of insulin was associated with increased insulin-mediated glucose uptake in muscles from both normal and insulin-resistant obese *ob*/*ob* mice (Lanner et al. 2006). Here we show that reduction of TRPC3 expression with siRNA by ~40% in isolated mouse toe fibers resulted in ~80% decrease in insulin-mediated glucose uptake. TRPC3 can be directly activated by diacylglycerol (DAG) and knock down of TRPC3 expression inhibited DAG-induced $Ca^{2+}$ influx. Moreover, TRPC3 was detected in GLUT4 immunoprecipitates and immunofluorescence staining showed a clear overlap between TRPC3 and GLUT4 in the proximity of the transverse tubular (t-tubular) system. In conclusion, TRPC3 appears to functionally and physically interact with GLUT4 and the two co-localize in the t-tubular system, which is considered to be the predominant site for insulin-mediated glucose transport in skeletal muscle.

### References

Bruton JD, Katz A, Westerblad, H. (1999). Insulin increases near-membrane but not global $Ca^{2+}$ in isolated skeletal muscle. Proc Natl Acad Sci USA 96:3281–3286

Lanner JT, Katz A, Tavi P, Sandstrom ME, Zhang SJ, Wretman C, James S, Fauconnier J, Lannergren J, Bruton JD, Westerblad H (2006) The role of $Ca^{2+}$ influx for insulin-mediated glucose uptake in skeletal muscle. Diabetes 55:2077–2083

### 7:1

### Genome-wide view of cell fate specification: *ladybird* acts at multiple levels during diversification of muscle and heart precursors

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Correct diversification of cell types during development ensures the formation of functional organs. The evolutionarily conserved homeobox genes from *ladybird*/*Lbx* family were found to act as cell identity genes in a number of embryonic tissues. A prior genetic analysis showed that during *Drosophila* muscle and heart development *ladybird* is required for the specification of a subset of muscular and cardiac precursors. To learn how *ladybird* genes exert their cell identity functions we performed muscle and heart-targeted genome-wide transcriptional profiling and a ChIP-on-chip search for direct Ladybird targets. Our data reveal that *ladybird* not only contributes to the combinatorial code of transcription factors specifying the identity of muscle and cardiac precursors but also regulates a large number of genes involved in setting cell shape, adhesion and motility. Among direct *ladybird* targets, we identified *Bric-a-brac 2* gene as a new component of identity code and *inflated* encoding αPS2-integrin playing a pivotal role in cell–cell interactions. Unexpectedly, *ladybird* also contributes to the regulation of terminal differentiation genes encoding structural muscle proteins or contributing to muscle contractility.

Thus the identity gene-governed diversification of cell types is a multistep process involving the transcriptional control of genes determining both morphological and functional properties of cells.

### 7:2

### A molecular analysis of the morphogenesis of skeletal muscles in vertebrates

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Using electroporation, real time confocal analyses of cell movements and the quail-chick grafting technique, we have recently analyzed the morphogenesis and the growth of skeletal muscles in bird. Our analyses have revealed that myogenesis follows two distinct stages. In a first stage of myotome morphogenesis, the epithelial borders of the dermomyotome (i.e. the dorsal-most compartment of somites) generate postmitotic myocytes that elongate along the antero-posterior axis of the embryo to form a structure named the primary myotome. The second stage of myotome growth is dependent upon the emergence of a population of muscle progenitors within the primary myotome, triggered by the EMT of the dermomyotome. We have examined the tissue and molecular interactions that regulate the spatial organization of the primary myotome. Using the in vivo electroporation of cDNA constructs encoding for molecules that interfere with the Wnt non-canonical (i.e. PCP) pathway, we show that this pathway regulates the correct orientation of myocytes in the AP axis of the embryo. Interestingly, Wnt11, expressed at the border of the dermomyotome acts an instructive cue on which myocytes orient. These data identify the PCP pathway as essential for the morphogenesis of skeletal muscles in vertebrates.

### 7:3

### A systematic genome-wide analysis of muscle morphogenesis and function in *Drosophila*

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The strategy of utilising a muscle network to execute deliberate movements is found in all higher animals. It is crucial for the success of this strategy that all muscles in the body form at the correct position, grow to the right size and can produce enough force to match all requirements in life. A systematic genetic study addressing how muscles form, grow and function in vivo is lacking. We used a genome-wide inducible RNAi library to systematically screen for genes required for muscle morphogenesis and function in *Drosophila*. Cell autonomy of transgenic RNAi in *Drosophila* restricts gene knock-down to muscle cells only. We screened about 85% of the genes for a role in muscles and identified about 2000 to be essential at some stage of muscle morphogenesis or vital for muscle function as assessed by lethality or flight test. Knock-down of about 300 genes leads to viable but completely flightless animals. We are in the process of classifying these primary hits into functional groups using different secondary assays to resolve sarcomeric structure and organisation and to specifically analyse adult myogenesis, in particular heart and flight muscle morphogenesis and function with muscle subtype specific gene knock-down techniques. This may allow us to assign the *Drosophila* genes into groups required for a particular step in formation and function of the fly's muscular system.

7:4

### Transcriptional control of the ubiquitin pathway by the muscle regulatory factor MyoD1

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A major focus of our laboratory is the dissection of the complex networks of transcription factors, which progressively orchestrate the differentiation of skeletal muscle. We employ genomic and biochemical approaches to provide a detailed and mechanistic model of transcriptional regulation during muscle differentiation and maintenance of muscle mass. In that effort, we discovered that pharmacological inhibition of the Ubiquitin (Ub)-proteosomal pathway results in a major defect in muscle differentiation. Exploring this observation in detail, we have identified transcriptional targets of the known myogenic regulator MyoD that are involved in the atrophic skeletal muscle response. These data reveal possible transcriptional mechanisms, downstream of MyoD, which may lead to the defects in myogenesis that are observed when the Ub-proteasomal degradation pathway is perturbed. Intriguingly, through ChIP-on-chip analysis and RNAi ablation, we have found that MyoD acts to directly govern processes related to muscle differentiation and atrophy through regulation of E3 ligases in the Ub-proteasomal degradation pathway. Thus, these data, by illuminating the downstream effectors of MyoD, provide crucial insights into potential therapeutic strategies for several disease conditions in which skeletal muscle atrophy is a major component of morbidity and mortality, for example sepsis, cancer and AIDS.

7:5

### Re-expression of cytokinesis-regulating proteins during cardiac hypertrophy

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We investigated whether the inability of postnatal cardiomyocytes to undergo cell division is caused by a downregulation of proteins involved in the regulation of cytokinesis. Analysis of the expression pattern of Septins and small GTPases like RhoA along with their downstream effectors like ROCK I, ROCK II and Citron Kinase in the embryonic, early postnatal and adult heart showed a developmental stage specific expression, with high levels being expressed in cardiomyocytes only at stages when cytokinesis still occurs (i.e. embryonic and perinatal). This suggests that downregulation of many regulatory and cytoskeletal components involved in the formation of the actomyosin ring may be responsible for the uncoupling of cytokinesis from karyokinesis in rodent cardiomyocytes after birth.

Interestingly, we could detect a re-expression of cytokinesis-regulating proteins in cardiomyocytes from different models of pathological hypertrophy. Nevertheless the adult cardiomyocytes do not appear to divide. We suggest that the inability to undergo complete division could be due to the presence of stable, highly ordered and functional sarcomeres in the adult myocardium or could be caused by the inefficiency of degradation pathways, which facilitate the division of differentiated embryonic cardiomyocytes by myofibril disintegration.

7:6

### Effects of early treadmill exercise on the regenerative process after muscle contusion

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In severe skeletal muscle injuries, full functional recovery is often limited by the development of fibrosis. The purpose of this work was to analyze the effect of early treadmill exercise on the development of fibrosis after muscle contusion.

Ten wild type mice were separated into 2 groups: contusion (C) and contusion + treadmill (C-TM). Mice were put in a supine position, the hindlimb was extended, and 18 g stainless steel ball was dropped through an impactor from a height of 100 cm onto the tibialis anterior (TA) muscle. One hour after contusion, the mice in C-TM group started a treadmill exercise protocol. Running speed and duration were gradually increased to 15 mpm for 1 h. All mice were sacrificed on 8th day, and TAs muscles were harvested, and cryosectioned. Masson trichrome staining was done to identify the fibrotic tissue, and the total fibrotic area was quantified. Statistical analysis was performed with students *t*-test, and significance was set to $P < 0.05$.

Results showed a significantly less fibrotic area in the C-TM group (39.5% ± 19.7), when compared with the C group (65.2% ± 14.8) ($P = 0.04$). These results suggest that early treadmill exercise may decrease the formation of fibrotic tissue after muscle contusion allowing better muscle regeneration and functional recovery.

7:7

### Lms, a new muscle identity gene required for lateral transverse muscle development in Drosophila embryos

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The four lateral transversal muscles (LT1–LT4) are thought to be specified by the combinatorial activity of the *Kruppel* (*Kr*), *apterus* (*ap*), and *muscle specific homeobox* (*msh*) genes whilst the activity of the *ladybird* (*lb*) genes is required for proper formation of the segmental border muscle (SBM) in the same lateral area. We report here expression pattern of a new gene (CG13424), we named *lateral muscle specific* (*lms*) and its function in myogenesis. It encodes a product with potential transcription factor activity. *lms* starts to be expressed in early stage 11 embryos in somatic LT muscle progenitors and is seen until stage 16 in the resulting LT muscle fibers. Knock down of *lms* induced by ds RNA injection into Myosin Heavy Chain (MHC)-GFP embryos leads to the partial loss of LT muscles, indicating that *lms* plays a key role in LT muscle identity. Our data indicate that *lms* represents a new component of the combinatorial genetic code specifying lateral muscles.

7:8

### Differential expression analysis in slow and fast mouse muscle

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Vertebrate skeletal muscles consist of fibers with fast and slow contractile properties; several fast- and slow-specific genes have been known for many years, but an overall picture of gene expression in these two types of fibers is still unclear.

To investigate this problem we performed microarray gene expression experiments on three mouse skeletal muscles composed of slow (soleus), fast (tibialis) and mixed fibers (gastrocnemius) and on the cardiac muscle. Microarray analysis identified tissue specific clusters of genes up- and down-regulated and confirmed previous reports that some genes encoding structural proteins, calcium channels, metabolic enzymes were expressed at significantly different levels in slow, fast and cardiac fibers. Some novel transcripts, not yet known as fiber type specific, have been identified in this study, and a few of these were confirmed by real time PCR.

As expected, when we analysed differentially expressed genes, we found some particularly enriched Gene Ontology categories like muscle contraction, regulation, development and fibers differentiation.

Moreover, we are analyzing the putative promoter regions of differentially expressed genes to discover possible regulatory elements on the basis of their overrepresentation as upstream motifs (Caselle et al. 2002).

**7:9**

**The position and function of obscurin in *Drosophila* muscle**

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Obscurin is a modular protein of ~800 kDa containing immunoglobulin and signalling domains. In vertebrate skeletal and cardiac muscle, obscurin is involved in sarcomere assembly and muscle turnover. The *Drosophila* homologue is ~420 kDa and there are several isoforms. Obscurin is expressed at late embryonic stage 16 and is present in the M-line during all larval stages. In early pupal stages, obscurin is in the developing M-line of flight muscle and may be needed for sarcomere assembly. *Drosophila* obscurin has two kinase domains in the C-terminal region and sequence of a Rho guanidine nucleotide exchange factor (a signalling domain) in the N-terminal region. The kinase domains are conserved in 11 *Drosophila* species and show some divergence from vertebrate and nematode sequences. A conserved aspartic acid residue in the catalytic loop of eukaryotic kinase domains, which participates in phosphorylation, is missing in all *Drosophila* species. We aim to determine the structure of *Drosophila* obscurin kinases and their function in signal transduction.

**7:10**

**Elucidation of the signalling targets of β2-adrenergic receptor agonists in skeletal muscle**

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The understanding of the growth and development of skeletal muscle is one of the most important goals in both clinical and production science. β2-Adrenergic receptor agonists (β2-agonists) such as clenbuterol are known to be potent agents in inducing skeletal muscle hypertrophy and fibre-type switching from slow/I to fast fibres. However, specific mechanisms that lead to these effects are not well understood. The objective of this study was to document the time course effects of the β2 agonist clenbuterol on muscle cell proliferation, differentiation, fibre size (hypertrophy) and fibre type on murine C2C12 myoblasts, an immortalised, well established muscle satellite cell line.

The Affymetrix GeneChip Mouse Exon 1.0 ST Array platform has been utilised in order to identify differential gene expression in C2C12 cells at various time points after administration of the β2-agonist in comparison to untreated controls. This full-genome Microarray allows identification of novel genes that are differentially expressed in response to clenbuterol treatment and will aid in the elucidation of the physiological pathways involved in skeletal muscle growth and phenotype determination.

**7:11**

**Expression of *Pax*-3 gene in progenitor muscle cells during myotomal myogenesis in *Coregonus lavaretus* (Teleostei: Coregonidae)**

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In *Coregonus lavaretus* two classes of cells in unsegmented mesoderm, besed on their position, morphologies and expression of MyoD and slow–MHC can be distinguished. First class of cells adjacent to the notochord and neural tube called adaxial cells, they are the first cells to express MyoD and slow–MHC protein. During the later stages of myogenesis these cells form slow muscle fibres, which are segregated into wedge-shaped region of the myotome at the lateral end of the horizontal septum. The second class of unsegmented mesoderm cells (non-adaxial cells) are smaller and irregularly shaped and do not express Myod and MHC–slow proteins, these cells differentiate into fast muscle fibres. During somitogenesis *Pax-3* transcripts come be expressed in a single layer of cells superfacial to the myotome (between primary myotome and epidermis). Their expression profile resembles the "exteranl cells" (revealed in many teleosts species) or dermomyotome described in amniota. External cells in *C. lavaretus* form structures similar to dorsomedial and ventrolateral lips of the amniote dermomyotome. TEM analysis revealed that external cells are spindle-shaped and extremely flattened. Their cytoplasm contains rough endoplasmic reticulum. These cells are characterized by nuclei with electron-dense chromatin beneath the nuclear envelope. They do not show any characteristics of muscles fibres, such myofibrils or clusters of filaments. After the hatching, mononucleated mesenchymal cells were for the first time observed in intermyotomal spaces, and subsequently, in myotomes between the myotubes. These cells are mitotic active. In nuclei of these cells immunoreaction for PCNA (marker of S-phase of cell cycle) was observed. During this stage of myogenesis *Pax-3* gene is expressed in cells in intermyotomal space and then subsequently in myotomes between myotubes. These cells are tightly associated with already existing myotubes. Mesenchymal (*Pax-3* positive) cells, which have migrated into the myotomes between myotubes, differentiate into secondary myoblasts and form secondary muscle fibres. These muscle fibres are formed in discrete germinal zones at the lateral margins of the myotome, a prosess termed "stratified" hyperplasia. Secondary muscle fibres have considerably smaller diameter, and are located in the neighbourhood of fibres of the larger diameter with well developed myofibrils.

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**8:1**

**Angiotensin II, focal adhesion kinase, and PRX1 enhance smooth muscle expression of lipoma preferred partner and its newly identified binding partner palladin to promote cell migration**

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LPP is a proline rich LIM domain family protein highly expressed at plasma membrane dense bodies and focal adhesions in smooth muscle cells with its newly identified partner, palladin. Both LPP and palladin enhanced cell migration and spreading. LPP and palladin expression was decreased, in contrast to vinculin or paxillin, in migration defective focal adhesion kinase (FAK) null cells, but was restored by expression of the paired-related homeobox gene-1 protein. In FAK null cells, expression of FAK, upregulated expression of LPP and palladin as well as the paired-related homeobox gene-1 protein Prx1. The expression of both LPP and palladin, like smooth muscle α-actin, was increased by angiotensin II, regulated by actin dynamics, upregulated by myocardin and expressed in the neointima of injured aorta. Overall, we suggest that the function of LPP and palladin is context dependent, that they play a critical role in cytoskeletal remodeling, respond to signals induced by vascular injury as well as signals that induce smooth muscle cell hypertrophy, such as angiotensin II.

**8:2**

**Regulation of nonmuscle myosin regulatory light chain phosphorylation in smooth muscle**

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Although there are several reports consistent with nonmuscle myosin contributing to force regulation in smooth muscle, the participation of nonmuscle myosin in force maintenance is controversial. To participate in force maintenance, nonmuscle regulatory light chain phosphorylation must be regulated in smooth muscle. To investigate this question, the regulatory light chains of smooth muscle myosin ($MLC_{20}$) and nonmuscle myosin (RLC) were cloned, expressed and purified. The purified proteins could be resolved on silver stained two-dimensional gels. Intact mouse aortic smooth muscle strips were stimulated with either KCl depolarization or angiotensin II, and the 4 distinct spots were resolved on the silver stained 2-D gels. Using mass spectroscopy, these spots were identified as four distinct proteins; phosphorylated RLC, non-phosphorylated RLC, phosphorylated $MLC_{20}$ and non-phosphorylated $MLC_{20}$. $MLC_{20}$ phosphorylation, but not RLC phosphorylation, increased during KCl depolarization. However, angiotensin II stimulation increased both $MLC_{20}$ and RLC phosphorylation. These data suggest that in smooth muscle, nonmuscle myosin is not regulated by $Ca^{2+}$-calmodulin activated MLCK, but rather by a G-protein mediated signaling pathway, possibly phosphorylation of the RLC by Rho kinase and/or PKC.

**8:3**

**Urocortin induced cAMP-dependent $Ca^{2+}$-desensitization of vascular smooth muscle contraction: role of PKA-dependent and EPAC-dependent signalling**

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Urocortin-1 (UCN-1), a vasodilatory peptide belonging to the corticotropin releasing factor (CRF) peptide family, relaxes submaximally (pCa 6.1) activated α-toxin permeabilized mouse tail arteries and dephosphorylates the regulatory light chains of myosin in a cAMP-dependent manner. UCN-1 and the PKA activator, bnz-cAMP decrease the concentration of active Rho as well as the inhibitory phosphorylation of MYPT1 at Thr696 and increase PKA-dependent phosphorylation of MYPT1-Ser695. An activator of EPACs (exchange protein directly activated by cAMP), 8-pCPT-cAMP also decreases $Ca^{2+}$-sensitivity and MYPT-Thr696 phosphorylation but has no effect on MYPT1-Ser695 phosphorylation. Our results suggest that UCN-1 decreases $Ca^{2+}$-sensitivity of contraction by activation of MLCP which is mediated by a PKA-dependent phosphorylation of MYPT1-Ser695 and inactivation of Rho. Both pathways will decrease MYPT1-Thr696 phosphorylation. Since EPACs are expressed in tail arteries the possibility remains that in addition, MLCP may be activated by UCN-1 in a PKA-independent manner.

**8:4**

**The catch state of mollusc smooth muscle is inducible despite myosin head blockage**

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The catch state is a passive holding state of muscle. It is maintained by unknown linkages connecting the myofilaments. These catch linkages seem to be formed during active contraction and persist afterwards. Their formation is initiated by dephosphorylation of the thick filament protein twitchin due to a $Ca^{2+}$-stimulated phosphatase. Their detachment is caused by phosphorylation of twitchin due to a cAMP-dependent protein kinase stimulated by serotonin. There is a decade-long controversy about the question if catch linkages are represented by myosin heads which are persistently attached to actin filaments or by alternative linkage structures. To elucidate this fundamental problem we blocked the myosin heads and tested if a catch state was still inducible by examining stretch resistance. All experiments were carried out on intact anterior byssus retractor muscles (ABRM) of the mussel Mytilus treated with blebbistatin. In these preparations the excitatory neurotransmitter acetylcholine was not able to initiate force any more. However, stretch resistance was markedly increased. This increase was reversed by serotonin suggesting that the additional stretch resistance was due to catch linkages. Thus, it seems that a catch state can still be induced, when myosin heads are

blocked. Therefore, maybe not the myosin heads but alternative linkages are responsible for the catch state.

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**8:5**

**Twitchin from molluscan catch muscles is a new potent thin filament regulator**

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Twitchin regulates catch state of molluscan muscles in a phosphorylation-dependent manner conceivably by linking thin and thick filaments [Shelud'ko et al. (2004) Arch Biochem Biophys 432:269–277]. In vitro interaction of twitchin with mussel thin filaments inhibits
the actin-activated Mg-ATPase activity of rabbit skeletal myosin in the presence of Ca2+. The polarized fluorescence from 1,5-IAEDANS attached to Cys-374 of actin or to Cys-707 of skeletal myosin subfragment-1 incorporated into ghost muscle fibers in the absence and presence of MgADP, MgAMP-PNP, MgATPyS or MgATP was studied. The changes in orientation and mobility of the probes were observed depending on the nucleotide or ATP analog present; this suggests the multistep change of the orientation and mobility of myosin heads and subdomain-1 of actin monomers during ATP hydrolysis cycle. When twitchin was present in the fibers, the unidirectional changes in the orientation of actin, subdomain-1 of actin, and S1 were uncoupled. It is suggested that twitchin ''freezes'' the actin filament structure in the ''OFF'' conformation and thereby limits the tilting and mobility of myosin heads during ATPase cycle, in a manner analogous to that of caldesmon in vertebrate smooth muscle [Borovikov et al. (2006) Biochim Biophys Acta 1764:1054–1062].

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**8:6**

**Artery elasticity following stomach-bypass operation in rats**

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Obesity is often treated by a stomach-bypass operation. Effects of this operation are not entirely known. The aim of our study was to look at the effect of a stomach bypass on arterial elasticity in non-hypertensive, normal-weight rats. Sprague Dawley rats were either sham-operated (controls) or stomach-bypass operated, linking the oesophagus to the duodenum; $n = 5$ in each group. Rats were killed at 57 weeks of age. Pieces of aorta were dissected out, immersed in a 37°C PBS-Ringer bath pH 7.4, and attached to a fixed hook at one end and an FTO3 transducer (Grass Instruments, RI) at the other. The transducer was connected via an amplifier to an 8S MacLab A/D Converter (AD Instruments, UK). The aorta piece was tensioned in step-wise increases. Pressure was calculated applying LaPlace's equation. The tension applied was equivalent to 1.9% of normal pressure. The elasticity was measured as the recoil (N $ms^{-1}$ mg wet $wt^{-1}$). The bypass-operated rats showed a clear reduction in elasticity compared to the control group, 1.9 ± 0.5 $10^{-7}$ and 4.9 ± 2,7 $10^{-7}$ N $ms^{-1}$ mg wet $wt^{-1}$, respectively. The change in elasticity may be due to a change in protein turn-over following the bypass operation.

**8:7**

**A possible role of the cholinergic and purinergic receptor interaction in the regulation of the urinary bladder function**

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During micturition the shortening of the upper (dome) and lower (base) parts of the human (h) urinary bladder (UB) with outlet obstruction are different. Namely, during voiding the base does not show strong contraction. This phenomenon is also present in mature rat (r) UB. We studied how can different parts of the UB have opposite response to the same stimulation pattern and why this is important.

Electrical field stimulation (EFS, 2 Hz, 600 shocks) evoked rUB muscle strip contractions were measured. The phasic part of the EFS was mostly acethyl-choline and the tonic part was mostly ATP mediated response. The parasympathetic agonist carbachol (Cch) significantly increased the phasic, but reduced the tonic part of the contractions on base strips.

In other experiments cultured smooth muscle cells (SMC) were used. When ATP was administered together with Cch or nicotine (nic), Cch or nic prevented the ATP-evoked $Ca^{2+}$ transients in the hSMC from the base of the UB with outlet obstruction. This cross-inhibition was not found in healthy hUB. This Cch-ATP-evoked cross interaction was also found in rSMC of the base of the UB. However, the nic-ATP-evoked cross interaction was not present in rSMC. Immunocytology revealed alfa3 in hUB and alfa7 nic receptors in rUB.

This fine tuning role of ATP may be the underlying reason which prevents the lower part of UB from contraction during voiding.

**8:8**

**Dissociation between MLC20-dephosphorylation and cyclic nucleotide induced relaxation in murine gastric fundus**

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Endothelin-1 (ET-1) precontracted gastric fundus strips were treated with electrical field stimulation (EFS, 30 V, 10 Hz, 0.5 ms) for up to 30 s. EFS induces relaxation by the activation of NANC neurons which release nitric oxide (NO) and vasointestinal peptide (VIP) as transmitters that activate cGMP- and cAMP-dependent pathways. The cGMP content rises to a steady state level after 5 s and amounts to (in pmol cGMP/mg protein) 0.07 in ET-1 contracted controls (ECC), 0.2 after 2 s, 0.35 after 5 s and 0.34 after 30 s of EFS. The relaxation after 2, 5 and 30 s of EFS was 3.8 ± 0.5%, 31.2 ± 2.3% and 84.74 ± 0.74%, respectively, compared to ET-1 induced contraction. As expected, the extent of myosin light chain (MLC20)-phosphorylation declines by approx. 50% from 35.1 ± 0.64% in ECC to 18.3 ± 1.1% after 2 s of EFS. Thereafter, phosphorylation returns to its initial control

value (30 s: 33 ± 0,7%) despite the fact that the muscle is still relaxed. Our data imply that the cAMP/cGMP-mediated gastric relaxation is controlled initially in a MLC220- phosphorylation-dependent, and later on in a phosphorylation-independent manner due to other regulatory systems (e.g. heat shock proteins, caldesmon). We currently investigate the phosphorylation profile of proteins regulating MLC20-phosphorylation and proteins involved in regulating contraction independent of MLC20-phosphorylation.

**8:9**

**Twitchin: a catch-maintaining link in invertebrate smooth muscle**

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Catch is a state of force maintenance with very low ~P usage at submaximal $[Ca^{+2}]_i$ in invertebrate smooth muscles (ABRM of *Mytilus edulis*). Phosphorylation of twitchin (Tw·P) relaxes catch force. Catch force is zero at saturating $[Ca^{+2}]$, and increases to a maximum as $[Ca^{+2}]$ is lowered to pCa > 8, where Tw·P causes full relaxation. Results suggest that twitchin serves as a catch force-maintaining link between thin and thick filaments: (1) when myosin crossbridges are in the weak-binding state (Pi, BDM, TFP or blebbistatin) total force decreases and catch force *increases* at all $[Ca^{+2}]$ except pCa > 8; (2) the strain dependence of myosin-bound ADP turnover is nil at pCa > 8, increases markedly at high $[Ca^{+2}]$ where catch force is small, and at intermediate $[Ca^{+2}]$ increases only when twitchin is Tw·P. The catch-maintaining link shows no strain-dependent ADP turnover, but provides a mechanical constraint that alters the strain dependence of $Ca^{+2}$-bound, cycling myosin crossbridges. Myosin crossbridges in the high force state may interact with and cause detachment of this link, and myosin crossbridges in the low force state may allow its attachment if twitchin is unphosphorylated.

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**8:10**

**ACE inhibition results in preservation of the $LZ^+$ MYPT1 isoform of myosin light chain phosphatase via suppression of MAPK pathways**

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We have demonstrated that the decrease in sensitivity to nitric oxide (NO) in CHF lies at least partially at the level of the smooth muscle and is due to a reduction in the relative expression of the leucine zipper positive ($LZ^+$) isoform of the myosin targeting subunit (MYPT1) of myosin light chain phosphatase. Since the attenuated vasodilatory response to NO in CHF has been shown to be secondary to increased activity of the renin-angiotensin system, ACE inhibition could maintain $LZ^+$ MYPT1 isoform expression. To test this hypothesis following LAD ligation, rats were divided into control, captopril-, or prazosin-treated groups. Captopril and prazosin attenuated the fall in LVF following LAD ligation. In the control aorta and iliac artery, the expression of the $LZ^+$ MYPT1 isoform fell between 2 and 4 weeks post-MI, whereas in rats treated with captopril, but not prazosin, MYPT1 isoform expression did not change. A decrease in the sensitivity to cGMP mediated relaxation mirrored that of $LZ^+$ MYPT1 expression. Gene microarray expression and real-time PCR analysis confirmed that captopril treatment suppressed key mediators in the mitogen-activated protein kinase (MAPK) pathways. These data suggest that in CHF, the molecular switch from the $LZ^+$ to $LZ^-$ MYPT1 isoform likely involves MAPK signaling pathways downstream from angiotensin II mediated G-protein coupled receptor activation.

**8:11**

**Different inflammatory responses in mouse tracheal smooth muscle between C57BL/6 and BALB/c mice**

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Asthma is characterized by chronic inflammation in the airways and increased airway responsiveness. OVA-sensibilization of mice mimics these reactions in vivo but differences between strains have been shown and the functional outcome has been suggested to be due to differences in accumulation of inflammatory cells. The objective for this study was to investigate the responses to inflammatory mediators in the airway smooth muscle in vitro from C57BL/6 and BALB/c mice. Tracheal ring segments were cultured 4 days with TNFα, LPS or polyIC (a Toll-like receptor 3 ligand) and the contractile responses to carbachol, serotonin, bradykinin and U46619 (a thromboxane $A_2$ analogue) were subsequently analyzed. The treatment with inflammatory mediators did not affect the maximum contraction or the potency to carbachol. The contractions to serotonin and bradykinin were equal in C57BL/6 and BALB/c cultured control segments. However, all three inflammatory mediators up-regulated the contractions of serotonin and bradykinin responses to a larger extent in BALB/c mice compared to C57BL/6 mice. A trend to an increase of the U46619 contractions was seen after treatment with TNFα and LPS in both strains. This study indicates that the airway smooth muscle has the capacity to directly respond to inflammatory stimuli but that it differs between mice strains.

**8:12**

**Increased Rho-kinase mediated $Ca^{2+}$-sensitization and protein kinase C response in hypertrophic urinary bladder smooth muscle of the mouse**

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We examined the role of Rho-kinase and protein kinase C (PKC) signaling during smooth muscle contraction in normal and obstructed mice bladders. Upon electrical field stimulation, obstructed bladders showed increased cholinergic and decreased purinergic involvement in bladder contraction. Obstructed bladders showed increased sensitivity and enhanced sustained contractile phase to carbachol. The Rho-kinase inhibitor, Y27632, and the PKC-inhibitor, GF109203X, alone or together, decreased the peak and sustained contractile response to carbachol in both obstructed and control bladders. Direct PKC activation (PDBu)

resulted in a pronounced contraction in obstructed bladders. Sensitivity to $Ca^{2+}$ in depolarized tissues was significantly increased in obstructed bladders. This $Ca^{2+}$-senstitivity was abolished by Y27632. Obstructed bladders showed increased expression of RhoA and CPI-17, and decreased expression of MYPT1. In conclusion, this study shows that obstructed bladders have increased $Ca^{2+}$-sensitivity linked to the Rho-kinase pathway which in turn correlates with an increased expression of RhoA and CPI-17, and decreased MYPT1 expression. The PKC-elicited contraction in obstructed bladders might also be related to an increase of CPI-17, and a decrease in MYPT1.

**8:13**

**Phenylephrine contracts male but not female mouse urethra-segments**

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The circumferential smooth muscle reactivity of mouse urethra was studied under in vitro isometric conditions. We obtained male (♂) and female (♀) urethral ring-sized (~1 mm length) segments from adult C57Bl6 mice, which were mounted in a small vessel-type myograph and stretched to optimal circumference for isometric force. In view of the documented longitudinal cellular heterogeneity of the urethra, proximal (posterior to trigone, prx) and distal (anterior to meatus, dis) segments were also compared. All segments reacted to high $K^+$ (isotonic replacement of $Na^+$, 125 mmol/l), the contractions being larger in prx than in dis and larger in ♂ than in ♀. Arginine-Vasopressin ($ED_{50}$ ~ 10 nmol/l) contracted all segments concentration-dependently with amplitudes larger in prx than in dis (only ♀) and larger in ♂ than in ♀. Phenylephrine contracted only ♂ segments with maximal amplitudes larger than $K^+$ = 125 mmol/l and with $ED_{50}$ (~1 μmol/l) larger in prx than in dis. Acetylcholine was without noticeable effects when applied under unstimulated conditions but relaxed AVP-precontracted segments. From these investigations, we conclude that (i) smooth muscle of urethral segments can react vigorously to autocoidal substances, depending on gender and location of the segment studied but the teleology of these differences remains to be explained; (ii) contrasting its well-documented constrictor effect in bladder smooth muscle, cholinergic stimulation relaxes urethral smooth muscle.

**8:14**

**Postnatal development alters signal transduction pathways of muscarinic receptor mediated activation in the mouse urinary bladder**

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$M_2$ and $M_3$ muscarinic receptor activation, source of calcium and role of PKC signalling in muscarinic contractions were examined in newborn (0–2 days) urinary bladders. Comparisons were made with adult (8–12 weeks). Concentration-response relations for carbachol in the presence of 4-DAMP ($M_3$ receptor antagonist) and methoctramine ($M_2$ receptor antagonist) showed that $M_3$ receptors are the main activating pathway in newborn. Inhibition of sarcoplasmic reticulum calcium release with thapsigargin or ryanodine did not influence muscarinic responses of newborn or adult bladders. Carbachol-induced contractions were not effected by the NO synthase inhibitor L-NAME or the guanylate cyclase inhibitor ODQ. Inhibition of gap-junctions influenced carbachol-induced contractions in newborn and adult tissue similary, showing that cell–cell interactions are not different. Apamine and charybdotoxin slightly increased the amplitude of the contractile responses in both newborn and adult, suggesting that SK and BK potassium channels attenuate contraction. Blockade of PKC with GF109203X inhibited carbachol contractions to a larger extent in newborn showing that the PKC pathway is more active at this stage. However, maximal activation of PKC with PDBu abolished carbachol responses of newborn suggesting the presence of a PKC target involved in the coupling between $M_3$ receptors and calcium influx.

**9:1**

**$Ca^{2+}$-dependent regulation of muscle fiber type specification via NFAT**

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Using in vivo transfection techniques and gain- and loss-of-function approaches, we have previously shown that a calcineurin-NFAT pathway is involved in the induction of the slow phenotype in regenerating skeletal muscle and in the maintenance of the slow phenotype in adult muscle (Serrano et al. 2001; McCullagh et al. 2004). We have now addressed two open issues, concerning (i) the origin of the $Ca^{2+}$ signal responsible for the activation of the calcineurin-NFAT pathway, and (ii) the role of the different NFAT isoforms (NFATc1, -c2, -c3, -c4) on fiber type properties. To identify the origin of the $Ca^{2+}$ signal, we used single fibers from adult mouse FDB muscle transfected with NFATc1-GFP. Nuclear translocation of NFATc1 was found to be dependent on $Ca^{2+}$ released from ryanodine receptors but not from IP3 receptors. An RNAi in vivo approach has been applied to determine the role of different NFAT isoforms. We have found that activity patterns control NFATc1 but not NFATc4 nuclear translocation and transcriptional activity. Preliminary studies indicate that type 2A, -2X and -2B myosin heavy chain promoters are differentially regulated by NFAT isofoms.

**9:2**

**Activity-dependent differentiation of regenerating muscle fibers in fast and slow muscles of adult rats**

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Satellite cells (SCs) in soleus (SOL) and ext. dig. long. (EDL) muscles of adult rats appear intrinsically different [Kalhovde et al. (2005) J Physiol 562:847]. Here we confirm and extend this study. First, SOL and EDL were denervated and injected with myotoxic bupivacain to induce muscle necrosis, activation of SCs, and regeneration. Chronic muscle stimulation started immediately or 7 days later. Either way, myosin heavy chain (MyHC) expression patterns were markedly different in SOL and EDL despite

identical stimulation, suggesting that SCs are already committed to diverse developmental fates when activation occurs. Second, during regeneration and slow-pattern chronic stimulation, original type 2B and 2X fibers in EDL failed to express type 1 MyHC, in contrast to original type 1 and 2A fibers. Here, single fibers, regenerating in one end but not in the other, were followed along their length for identification. Thus, SCs in different fiber types within a muscle also appear intrinsically different. Third, assessing slow SOL muscle plasticity by the expression of fast MyHC genes during chronic fast pattern stimulation, we show that the plasticity, substantial at early stages of regeneration, gradually decreases and is lower than normal after 90 days. Finally, we show that chronic stimulations of regenerating SOL and EDL muscles induce corresponding changes in MyHC expression, isotonic shortening velocities, and isometric contraction times.

**9:3**

**MLC2 expression and post-translational modifications in human soleus**

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This study investigated the effects of a 60-day bedrest with or without countermeasures on muscular phenotype and post-translational modifications of the regulatory MLC2 protein. Soleus muscle biopsies were obtained on female subjects before and after bedrest. While control (BR) subjects were assigned to bedrest-only group, BR + Ex subjects were submitted to combined aerobic and resistive exercises, and BR + Nut to nutrional leucine and valine diet. We assessed MLC2 composition of whole muscles using SDS-PAGE. MLC2 phosphorylation was measured on 2D gels and *O*-N-Acetyl Glucosaminylation (*O*-GlcNAc) level of MLC2 was determined using an anti-*O*-GlcNAc antibody. Our results showed a slow-to-fast shift of MLC2 isoforms in BR, less marked in BR + Nut, and no change in MLC2 composition in BR + Ex conditions. After BR, the phosphorylation state of MLC2 was increased while the global level of MLC2 glycosylation was decreased. Exercises prevented the variations of phosphorylation and glycosylation observed after BR whereas nutrition had no effects. These results are consistent with the idea of an interplay between phosphorylation and glycosylation of MLC2 during muscle plasticity, which could contribute to the regulation of muscle atrophy and modulate muscle contraction.

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**9:4**

**Analysis of skeletal muscle fibers in three dimensional images**

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Knowledge of the detailed three dimensional organization of nuclei in skeletal muscle fibers is of fundamental importance for the understanding of the basic mechanisms involved in muscle wasting associated with for example neuromuscular disorders and aging. An ongoing interdisciplinary collaboration between the Centre for Image Analysis (CBA), and the Muscle Research Group (MRG), both at Uppsala University, addresses the issue of spatial distribution of myonuclei using confocal microscopic techniques together with advanced methods for computerized image analysis. Performing quantitative analysis on true three dimensional volume images captured by confocal microscopy gives us the option to perform in-depth statistical analysis of the relationship between neighboring myonuclei. The three dimensional representation enables extraction of a number of features for individual myonuclei, e.g., size and shape of a nucleus, and the myonuclear domain (in which each myonucleus control the gene products). This project investigates data sets from single muscle fibers sampled from mouse, rat, pig, human, horse and rhino to determine the myonuclei arrangement between species with a 100,000 fold difference in body weight. The appropriate image analysis tools needed for gaining the understanding of organization in three dimensional volume images are developed within the project to facilitate the analysis of similarities between species, and unique features within a species. The accumulated understanding of the spatial organization of myonuclei, and the effect of individual myonuclei size, will lead to an increased knowledge of basic mechanisms underlying muscle wasting in various neuromuscular disorders. This knowledge will hopefully lead to new therapeutic strategies that can be evaluated in experimental animal models prior to clinical testing trials in patients.

**9:5**

**Comparison of protein expression in human extraocular muscle (EOM) and psoas muscle, using two-dimensional fluorescence difference gel electrophoresis (2D-DIGE)**

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*Introduction* The extraocular muscles (EOMs) have unique functional properties and cellular organization and are considered a separate muscle allotype. 2D-DIGE enables simultaneous comparison and parallel identification of over 2000 proteins in two different muscle samples. *Methods* Human EOM and psoas muscle samples were homogenized and solubilised. The proteins were separated, quantified and analysed by 2D-DIGE and identified by mass spectroscopy.

*Results* From 300 protein spots differentially expressed in human EOM compared with psoas muscle, 44 distinct proteins were identified. Proteins associated with oxidative metabolism were enhanced in EOM, while the expression of glycolytic proteins was lower in EOM. Differences in expression of proteins modulating contraction as well as proteins involved in transport and oxidative stress were also seen.

*Discussion* At the protein level, the EOMs differed from skeletal muscle in key metabolic enzymes and structural proteins fitting their unique properties of fast contraction and lack of fatigability.

Most of the differentially detected proteins were metabolic enzymes and contractile proteins, which fit previous data on the properties of the EOMs. They are also the most abundant proteins in the muscles and further 2D-DIGE analysis of separate fractions is therefore needed for detecting additional proteins.

**9:6**

### Muscle cytoskeletal proteins and HSP27 under eccentric exercise and hindlimb suspension (HS) of rats: Effects of calcium L-type channels blockade

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It is known that during eccentric exercise and HS the calcium accumulation and degradation of cytoskeletal proteins takes place. This study was aimed to investigate the role of baseline calcium accumulation in cytoskeletal degradation and the way of calcium inflow into skeletal muscle under conditions of eccentric exercise and hindlimb suspension. Wister rats were divided into control (C), eccentric exercise (EE) (one set of downhill treadmill running (–16 degrees) at a speed of 20 m $min^{-1}$ for 40 min) and eccentric exercise plus nifedipine administration (EEN) groups (with a daily supplementation of 6,25 mg/kg nifedipine in drinking water during 2 days). After 24 h post-exercise dystrophin layer integrity and desmin level in m.soleus were declined in EE and didn't change in EEN gr. vs. C ($P < 0.05$). HSP27 were decreased in EEN in comparison with EE gr. ($P < 0.05$). Titin and nebullin were not changed after exercise. In experiment with HS calcium level was increased after 14 days in HS but not changed in HS plus nifedipine (HSN) vs. C gr. In HSN slow-to-fast fibers shift and atrophy of ST fibers after 2 weeks were ameliorated (vs. C) in contrary to HS gr. Conclusion: calcium L-type channel blocker attenuates, or reduces the contraction-induced damage of cytoskeletal proteins and ameliorates of fiber atrophy and slow-to-fast shift duringHS.

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**9:7**

### Stretch induced anabolic pathways in C2 C12 myotubes are ERK mediated

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Mechanical strain is a potent stimulus for skeletal muscle hypertrophy. We examined the role of intracellular signalling pathways in stretch induced anabolism. The inhibitor of mTor, Rapamycin (Rap) and the MEK/ERK inhibitor PD98059 (PD) both caused a reduction in stretch induced elevation of protein synthesis in C2C12 myotubes. We examined the effects of static stretch upon the pathways that regulate protein synthesis. Stretch had no effect upon the levels of activated Akt or mTor but ERK1/2 and p70S6K, a regulator of translation, were rapidly activated. Another key step in translational control occurs at the level of the eukaryotic initiation factor 4F (eIF4F) a major regulatory part of which eIF4E was shown to be activated by static stretch. eIF4E function is regulated in part by its association with the repressor protein 4E-BP1. This factor was activated in response to stretch, as was Mnk1 an activator of the eIF4F complex. We have also carried out studies on the effects of Rap and PD upon stretch-induced activation of the components of the pathways regulating protein synthesis. These data suggest that the ERK pathway is a major regulator of protein synthesis in response to stretch.

**9:8**

### Post-natal fast myosin heavy chain genes are early targets of calcineurin signalling in oxidative fibre type conversion

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In skeletal muscle, calcineurin is crucial for myocyte differentiation and in the determination of slow oxidative fibre phenotype; both processes are important determinants of muscle performance, metabolic health and meat animal production. Fibre type is defined by the isoform identity of the skeletal myosin heavy chain (MyHC). This study examined the responses of the major *MyHC* genes to calcineurin signalling over a time course period of fibre formation of muscle C2C12 cells. We found that calcineurin signalled to up-regulate the fast-oxidative *MyHC2a*, and down-regulate the faster *MyHC2x* and *MyHC2b* genes in a manner that appeared NFAT-independent. Contrary to expectation, the up-regulation of *MyHCslow* by calcineurin appeared time-dependent and was only detected after the initial differential expression of the post-natal fast *MyHC* genes had been established. The simultaneous elevated expression of *MyHC2a*, and the repression of *MyHC2x* and *2b* expression indicate that both processes (elevation and repression) were actively coordinated in the process of oxidative fibre conversion. We further discovered that muscle LIM protein (MLP), a calcineurin-binding Z-line co-factor, was induced by calcineurin, and its co-expression with calcineurin had an additive effect on *MyHCslow* expression. Hence, this study demonstrates that post-natal fast *MyHCs* are important early effector targets of calcineurin, and that *MyHCslow* up-regulation is mediated in part by calcineurin-induced MLP.

**9:9**

### Proteomics of skeletal muscle after exercise

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*Introduction* The molecular mechanisms and the specific proteins involved in human skeletal muscle adaptation to physical exercise are to some extent unknown. By using a proteomic approach several hundred, or in some cases thousands, of known and unknown proteins can be differentiated, quantified and identified at one time.

*Methods* Human skeletal muscle biopsies were obtained from the muscles before and after exercise. Three different exercise modes were used, eccentric, endurance and strength training. Muscle tissue samples were homogenized and quantified and analysed by two-dimensional difference gel electrophoresis (DIGE). Protein was identified by mass spectroscopy.

*Results* Approximately 2,300 protein spots were detected in each muscle sample. Significant changes in protein profile depended on exercise mode: Eccentric exercise changed 32 protein spots, endurance training changed 25 protein spots and strength training changed 16 protein spots.

*Conclusions* By using high sensitive fluorescence dyes and an internal standard, changes in protein expression of $< 15\%$ can be detected in human skeletal muscle by DIGE. Comparisons between exercise modes are complex. In general, oxidative metabolism appears up-regulated while glycolytic metabolism is down-regulated, independent of exercise mode.

### 9:10

### Intraoperative measurement of muscle properties reveal a relationship between muscle remodeling and contracture formation

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*Material and methods* During upper limb surgery of children cerebral palsy sarcomer lengths of the flexor carpi ulnaris (FCU) were measured using laser diffraction in vivo ($n = 17$ children). Measurements from the FCUs of radial nerve injury patients were used as "control" values to represent normally innervated muscle.

*Results* Intraoperative $L_s$ were extremely long in spastic FCU muscles compared to normal FCUs from radial nerve injury patients ($4.6 \pm 0.3$ μm vs. $2.9 \pm 0.2$ μm). Importantly, there was a highly significant correlation between the degree of contracture and the intraoperative sarcomere length ($r = 0.70$, $P < 0.005$). Specifically, the greater the contracture, the longer the measured $L_s$. The relationship between degree of contracture (in degrees) and intraoperative $L_s$ was: $y\ (°) = 15.5\ L_s\ (°/\mu m) + 130°$.

*Conclusion* There is a progressive remodeling of the muscle-tendon unit during contracture formation. These data suggest that the increase in sarcomere length may be due to progressive loss of serial sarcomeres during contracture formation. This would have the functional effect of pulling the wrist into flexion as sarcomeres are lost. The underlying mechanism for the muscular changes that occur secondary to upper motorneuron lesion is not known.

### 9:11

### Cellular mechanisms involved in the soleus fiber alterations during gravitational unloading

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The unloading-induced decreases of contractile properties and fiber size are known to be dependent on decreased neuromuscular activity or muscle strain. But the mechanisms mediated these influences on the fiber characteristics are still unknown. The hindlimb unloading in rats induced the significant decrease of the resting membrane potential (from 68 to 60 mV) and sufficient (nifedipine-dependent) increase of resting $Ca^{2+}$ concentration in myoplasm. This drop of RMP could activate some DHPR channels and lead to calcium inflow, which in-turn could induce cytoskeletal degradation. We supposed that maintaining of muscle properties during unloading via passive stretching could be associated with satellite cell recruiting and mTOR activation. The pre-unloading $\gamma$ irradiation inactivated satellite cells but had no effect on myonuclear pool and fiber size after passive stretching of unloaded soleus. The rapamycin administration ameliorated hypertrophic stretch effect on unloaded fiber size by reducing it only to the cage control level but not to the unloaded soleus level. Thus the signaling mechanisms, responsible for the fiber size maintaining in antigravity soleus muscle should be different from those involved in work hypertrophy.

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### 9:12

### Effects of combined strength and sprint training on regulation of muscle contraction at the whole-muscle and single fiber level in elite master sprinters

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This study examined the effects of high-intensity progressive strength training on regulation of contraction at the whole-muscle and single fiber levels in older sprint-trained athletes. Eleven men (52–78 years) were randomized to either a training (EX, $N = 7$) or a control (CTRL, $N = 4$) group. The EX participated in a 20-week program that combined sprint training with heavy and explosive strength exercises, while the CTRL maintained their usual sprint training schedules. The EX improved their maximal isometric and dynamic leg strength, explosive jump performances and force development in running-. Single fiber contractile properties, myosin heavy chain (MyHC), and histochemical data were analyzed from vastus lateralis biopsies. Specific tension and maximum shortening velocity at the single muscle fiber level were not significantly changed during the 20-week period in either the EX or CTRL groups. Fiber type proportions and MyHC isoform expression were not changed in the two groups. The cross-sectional area of type II and IIA fibers increased in EX and was confirmed at the single fiber level. An increase in the integrated EMG of the leg extensors during jump performance was observed in the EX compared with the CTRL. The strength-training stimulus improved maximal, explosive and sport-specific force production by mainly hypertrophic mechanisms.

### 9:13

### Over-expression of JunB in skeletal muscle causes growth and prevents atrophy inhibiting the expression of atrogin-1 and MuRF-1

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The mass of skeletal muscle is controlled by a network of pathways regulating transcriptional and post-transcriptional

processes. There have been important progresses in the understanding the signaling cascades involved in this process, although no effective therapies are available for the loss of muscle mass. Dr. Goldberg's laboratory has established that muscle atrophy caused by inactivity occurs by similar mechanisms with systemic diseases. This "atrophy program" has been studied and a subset of genes called "atrogenes" has been identified whose transcription rises or falls similarly in various types of atrophy. Among these genes was JunB, a member of the Jun family of transcription factors, whose expression fell sharply during all types of muscle atrophy. Over-expression of JunB in mouse muscle fibers appears to stimulate muscle growth of normal muscles, and to prevent the muscle mass loss during atrophy. Moreover, the over-expression of JunB inhibits the activation of the critical atrophy related genes atrogin-1 and MuRF-1. These data are the first to implicate JunB in control of muscle growth. Understanding the mechanisms responsible for the dramatic effects mediated by JunB on muscle could lead to the identification of new targets for drug development or improved exercise protocols to prevent atrophy of skeletal muscle and promote recovery of debilitated individuals.

**9:14**

**Functional alterations of single muscle fibers of m. soleus at the early stage of unloading**

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Real or simulated microgravity is known to cause significant structural and functional alterations in mammalian muscle fibers. It is known that functional unloading leads to accumulation of calcium ions performing triggering role in myoplasm. $Ca^{2+}$-dependent destruction or modification of cytoskeletal signal proteins is supposed to be the key mechanism, which changes protein expression in postural muscle fibers, and initiates the new expression pattern leading to atonia and atrophy development. Subsequently, the aim of the work was to find out whether calcium accumulation and hence the decrease in contractile activity of muscle fibers took place at the early stage of functional unloading. The experiments were carried out on the 3-days hindlimb suspension model with male Wistar rats. We found out that after 3-day unloading peak tension of single skinned m.soleus fibers of suspended rats decreased noticeably (by 22.6%). Moreover, $Ca^{2+}$ concentration in fibers of suspended rats at the early period of unloading profoundly increased. The fluorescence intensity of suspended rat fibers loaded with FLUO-4 calcium probe was 5.1 times higher then in control group ($p < 0.02$). These data are important for development of ways for prevention postural muscle atrophy and for understanding of the signal mechanisms underlying this process.

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**9:15**

**Higher levels of the MyHC IIx is seen in wrist flexors than in wrist extensors in both healthy children and children with cerebral palsy**

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*Introduction* Cerebral palsy (CP) is defined as a motor impairment due to a brain injury before two years of age. In the upper extremity a flexion contracture of the wrist often develops, even though the stretch reflex often is increased in both flexors and extensors

*Materials and methods* Muscle biopsies were taken from wrist flexors (FCU, FCR) and wrist extensors (ECRB, ECU) during tendon transfer surgery in children with CP ($n = 9$). Control biopsies were taken from the same muscles in healthy children (HC) ($n = 7$) during open reduction of forearm fractures. The expression of the type I, IIa and IIx myosin heavy chain (MyHC) isoforms in the muscle biopsies (two 10 μm muscle biopsy cross-sections) were determined on silver-stained 6% SDS-PAGE.

*Results* Significantly higher levels of the MyHC IIx were seen in wrist flexors than in wrist extensors in both HC (16% vs. 3%, $P = 0.01$) and children with CP (40% vs. 20%, $P = 0.01$). Children with CP had a significantly higher level of the MyHC IIx in both wrist flexors (40% vs. 16%, $P = 0.001$) and wrist extensors (20% vs. 3%, $P = 0.05$) compared to HC.

*Discussion* The higher expression of the fast MyHC IIx isoform in flexors than extensors of the wrist probably reflects a different use of the muscles during grasp and grip, although both are wrist stabilizers. Decreased use of the hand could in CP account for the increased occurrence of the IIx MyHC isoform.

**9:16**

**Treadmill training of late middle age rats into senescence does not preserve aerobic capacity**

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One of the classic consequences of aging is a reduction of muscle mass (sarcopenia) and, as we have shown, a reduction in muscle mass specific aerobic capacity. While it is well known that skeletal muscle oxidative capacity is very plastic in response to muscle activation and training, the degree of this plasticity and preservation of muscle mass beyond late middle age is not well known. To determine the extent of aerobic capacity plasticity and preservation of skeletal muscle mass and aerobic capacity in aged muscle, we subjected 28 mo old F344xBN rats to 60 min of treadmill running, 4 times/week, for 5 months. Compared to an age matched control group, the exercise trained rats had a lower body weight (464 ± 14 vs. 524 ± 20 g), primarily as a result of lower body fat percentage (16 ± 2 vs. 25 ± 1) and a longer endurance time in an increased intensity running protocol (7.0 ± 0.4 vs. 5.1 ± 0.2 min). However, the trained animals did not have a significantly higher *in-situ* aerobic capacity (307 ± 45 vs. 319 ± 30 $\mu mol \times min^{-1} \times 100$ g mass) as measured during hindlimb pump-perfusion experiments in which muscle blood flow was normalized to muscle mass so that the control and experimental groups received the same relative blood flow. Furthermore, there was no evidence of a preservation of muscle mass from endurance training since there was no difference in the mass of 10 lower limb muscles. This study shows that aerobic training into senescence may not be sufficient enough to preserve skeletal muscle mass and aerobic function.

**9:17**

**Split fibers in human muscle**

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The terms split fibers and muscle-fiber splitting are widely used and refer to muscle fibers that seem to be divided or fragmented into two or more smaller daughter fibers. This feature is most common in different myopathies, but is also typical for hypertrophic muscles as in power lifters. The mechanism for how muscle fibers become divided or fragmented is still not settled. We recently proposed that fiber splitting is due to defect regeneration [1].

In order to further elucidate the pathogenesis of muscle fiber splitting and whether the sequences of events of degeneration and regeneration, which we observed and detected with immunological methods in samples of power lifters, are also present in other muscles known to contain an abundance of fiber splitting. We have now examined biopsies from patients with Duchenne's muscular dystrophy, polymyositis and amyotrophic lateral sclerosis.

Using multiple labelling with up to four markers, staining for quiescent and activated satellite cells, myoblasts and extracellular matrix proteins, we can verify that muscle fibre splitting and occurrence of internal nuclei also in these conditions were due to a defective regeneration. We propose that the phenomenon of fiber splitting is due to overuse of the muscles.

1. Eriksson A, Lindstrom M, Carlsson L, Thornell LE (2006) Hypertrophic muscle fibers with fissures in power-lifters; fiber splitting or defect regeneration? Histochem Cell Biol 126: 409–417

**9:18**

**Meta-analysis of gene expression data and regulatory networks reconstruction during skeletal muscle atrophy**

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Public availability of expression datasets have opened up new challenges for the integration of different types of genomic data with the purpose of obtaining new insights on biological processes. To identify regulatory pathways involved in muscle atrophy we performed meta-analysis using expression data produced both with cDNA microarrays or DNA chip. Different atrophy models were analyzed, namely denervation, fasting, systemic diseases, disuse, ageing. Regulatory processes and differentially expressed genes have been investigated as possible novel markers. Genomic information at different cellular levels (expression, gene regulation, proteins interaction) has been integrated into a regulatory network. Not surprisingly, our results emphasize a general down regulation of energy production pathways during muscle atrophy, while several catabolic processes are activated. Regulatory networks point to a small set of genes/proteins (JUN, SMAD4, STAT3) that seem to be relevant for the atrophic process. Interestingly, we identify a number of enriched transcription factors, overexpressed in most atrophy models. In several instances (ARNT, MAX-Myc, E4BP4, SP1), there is a good correlation between overexpression of that factor and enrichment of the corresponding binding sites in many genes found differentially expressed in our study.

**9:19**

**Myogenic factors, MGF, myostatin and myosin heavy chain mRNA expression in the masseter muscle of patients after orthognathic surgery**

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In a previous study we found an adaptation in expression of Myosin heavy chain (MHC) isoforms in patients after orthognatic surgery from MHC-I to MHC-IIa. The aim of this study was to analyse the expression of myogenic factors (myogenin, MyoD, Myf5, MRF4), MGF and myostatin in the human masseter muscle of patients with prognathia or retrognathia of the mandible before and after orthognathic surgery. *Methods* 13 patients were involved in the study. Four tissue samples were taken from the anterior and posterior part of the left and right masseter muscle before (T1) and six months after surgery (T2). Isolated RNA was reverse transscripted and relative quantification of expression of myogenic factors, MGF and myostatin was performed with real time PCR. EF-2 was the house keeping gene. Gene regulations were analysed with the relative expression software tool. *Results* The gene regulations of the masseter muscle increased from T1 to T2 for MGF 3.0 ($P < 0.01$), for myogenin 1.7, for Myf5 1.1 fold and decreased for myostatin 0.9, MyoD 0.8 and MRF4 0.6 fold. *Conclusion* MGF, myogenin and MRF4 show more changing than the regulation of MyoD, Myf5 and myostatin in the adaptation process after orthognathic surgery. The hypothesis that a decrease in myogenin:MyoD expression ratio determined a slow to fast fiber shift could not be confirmed. Together with this fibre shift we found an increased myogenin:MyoD ratio of 2.1:1.

**9:20**

**The effects of Ankrd2 alteration suggest an important role in cell cycle regulation during muscle differentiation**

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Ankrd2 is a member of the Muscle Ankyrin Repeat Protein family (MARPs), consisting of sarcomere-associated proteins that can also localize in the nucleus where they likely influence muscle gene expression towards changes in the structure or function of the contractile machinery. Despite evidences suggesting that Ankrd2 is important for the structure and function of skeletal muscle, the precise role that it plays remains unclear. Recent findings highlight the influence of Ankrd2 in muscle regeneration. To investigate this regulatory role we examined by overexpression and silencing experiments how nuclear Ankrd2 influences muscle differentiation. A variety of traits of differentiating myocytes, where Ankrd2 was up- or down-regulated, were investigated. Transcriptional profiling revealed that the molecular pathways perturbed by changes of Ankrd2 protein level are congruent with the morphophysiological and biochemical data obtained in Ankrd2-modified myoblasts. Our results suggest that Ankrd2 plays a key role in the mechanism by which proliferation and apoptosis are coordinated during myogenic differentiation in vitro. Remarkably, our findings suggest that Ankrd2 has a different role, regulatory and structural, respectively in proliferating compared to differentiating myoblasts.

**9:21**

### Proteome analysis of altered protein expression in soleus muscle of hindlimb suspended mice

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In order to understand the adaptations to disuse induced atrophy we performed proteome analysis of soleus muscles of control mice and mice subjected to 14 days of Hindlimb Unloading (HU). Soleus muscle was chosen because preferentially involved in HU muscle atrophy. MHCs isoform distribution analysis, determined by SDS-PAGE, showed a marked shift towards fast MHC isoforms. The proteome map of soleus muscles of control and HU mice was defined and a differential proteome analysis was performed. About 1,000 protein spots on each gel were detected by fluorescent staining. Only a small percentage of detected spots were differentially expressed in HU mice in comparison with control animals. Most of the differentially distributed proteins were implicated in oxidative stress (Hsp, SOD1, PRDX6, CAH III). Data showed an upregulation of SOD1 and a downregulation of other defence systems: Hsp, PRDX6, CAH III, suggesting the presence of oxidative stress in hindlimb suspended mice and a damage of defence system against oxidative stress. Supported by the Italian Space Agency (project OSMA).

**9:22**

### PPAR$\delta$ expression differs in slow and fast skeletal muscle and is influenced by muscle activity

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The effects of exercise on skeletal muscle are mediated by a coupling between muscle electrical activity and gene expression. Activity correlates such as $Ca^{2+}$, hypoxia and metabolites like free fatty acids (FFAs) might initiate these signaling pathways regulating fibre-type-specific genes. FFAs can be sensed by lipid-dependent transcription factors of the peroxisome proliferator-activated receptor (PPAR) family. Our objective was to investigate if the predominant muscle isoform, PPAR$\delta$, is regulated by muscle activity. In this study, PPAR$\delta$ mRNA and protein were measured in the slow/oxidative soleus and the fast/glycolytic extensor digitorum longus (EDL) muscles of adult rats. mRNA was also quantified after electrical stimulation with a ''fast'' pattern mimicking IIb motor units and a ''slow'' pattern mimicking type I motor units (Hennig and Lømo 1985), delivered via the nerve to soleus and EDL, respectively. Endogenous mRNA level of PPAR$\delta$ was found to be three-fold higher in soleus compared to EDL and on histological sections, the most oxidative fibres displayed the highest levels of PPAR$\delta$ protein. After electrical stimulation with ''mismatch'' patterns, the PPAR$\delta$ mRNA level in soleus was reduced to less than half within 24 h, while increased 3-fold in EDL. Our data show that PPAR$\delta$ transcription is influenced by muscle activity and thus suggest that PPAR$\delta$ can mediate activity effects regulating muscle phenotype.

**Reference**

Hennig R, Lømo T (1985) Firing patterns of motor units in normal rats. Nature 314:164–166

**9:23**

### Choline acetyltransferase content as a cellular marker of spinal motoneuron activity under conditions of rat hindlimb suspension

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The main objective of the study was to evaluate the workability of choline acetyltransferase as the cellular marker of spinal motoneuron activity under conditions of simulated microgravity. In experiment performed on Wistar rats muscle unloading was simulated by means of hindlimb suspension for 7 days. Both in control and hindlimb suspended groups a retrograde labeling of soleus and tibialis anterior motoneurons was carried out by using a neuronal tracer—carbocyanin (De-Doncker et al. 2006). Transverse sections of spinal cord were immunohistochemically stained with monoclonal antibodies against choline acetyltransferase. So we had an opportunity to choose for study only corresponding motoneurons, which innervate soleus or tibialis anterior muscle. We counted the total number of such motoneurons and measured the soma size and mean intensity of fluorescence of choline acetyltransferase in order to estimate the motoneurons functional activity. So we for the first time analyzed choline acetyltransferase content in the soleus and tibialis anterior motoneurons under normal conditions and unloading.

**9:24**

### Triglyceride and glycogen content as a cellular marker of muscle fiber activity under conditions of rat hindlimb suspension

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We supposed that the energy substrates might be used as a cellular marker of muscle fiber activity and for the first time analyzed the triglyceride and glycogen content in the fast- and slow-twitch muscle fibers of m. soleus and m. tibialis anterior under conditions of 7-day rat hindlimb suspension. Although the hindlimb suspension led to decrease of triglyceride content in the fast-twitch fibers of m. soleus and slow-twitch fibers of m. tibialis anterior, these differences were not significant. In spite of this the obtained data do not contradict our initial hypothesis. To conclude whether the triglyceride and glycogen content may be used as a cellular marker of muscle fiber activity it is necessary to evaluate the energy substrate content on the other durations of unloading.

**9:25**

### Effect of chronic clenbuterol treatment on isometric force, myosin ATPase activity and $Ca^{2+}$ transient in slow and fast skeletal muscles

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Three weeks of at 4 mg clenbuterol $kg^{-1}$ $day^{-1}$ induced anabolic effects especially in fast muscle ($P < 0.05$) and increased isometric

force in both fast and slow ($P < 0.001$) twitch muscles (EDL and *soleus*, respectively) compared with control groups. Nevertheless, when normalised to muscle cross-sectional area, maximal tetanic force was unchanged and even depressed in *soleus* muscle ($P < 0.001$). Myofibrillar ATPase activities increased both in relaxed and activated conditions ($P < 0.001$) in *soleus* muscle suggesting that the depressed specific tension was not due the contractile machinery itself. The alteration of $Ca^{2+}$ release at each contraction (decreased global $Ca^{2+}$ transients amplitude $-P < 0.01$, associated with a reduced *sarcoplasmic reticulum* $Ca^{2+}$ load $-P < 0.01$) could explain at least in part the reduced tetanic tension. In EDL muscle, normalised tension and myosin ATPase activity were unchanged. In addition, unloaded shortening duration of fast myofibrils was unchanged suggesting a lack of effect on maximal shortening velocity. Finally, reduction of fatigue resistance with clenbuterol treatment was observed in EDL ($P < 0.05$) associated with large myosin heavy chain shift to fast and more fatigable isoforms. In conclusion, chronic clenbuterol treatment induced anabolic effects but reduced the contractile efficiency and increased muscle fatigability.

**9:26**

**Age-dependent effects of disuse on electrophysiogical parameters and calcium homeostasis in the soleus muscle of hindlimb-unloaded mice**

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After disuse induced by hindlimb unloading, the slow-twitch soleus muscle of adult rats undergoes a severe atrophy and a slow-to-fast fiber type transition. We also observed an increase of the sarcolemma chloride conductance at rest (gCl) together with a reduction of the resting cytosolic calcium concentration (restCa) (Fraysse et al. 2003; Desaphy et al. 2005). With the availability of transgenic strains, it is however important to characterize the effects of disuse on mouse soleus muscle in order to design future experiments. Three-months old and 6-months old C57BL mice were tail-suspended for 14 days (HU14), using the same procedure as for rats, and compared to age-matched controls. In both age groups, atrophy of the plantarflexor muscles, soleus and gastrocnemius, was observed after HU14 as a reduction of muscle weight. Atrophy was however more pronounced in the older mice. The antagonist dorsiflexors, EDL and *tibialis anterior*, showed no atrophy. In older mice, the gCl was increased by 30–50% after HU3–HU14 and the restCa was decreased in a manner consistent to rats. In contrast to rats and older mice, no gCl change was found after HU3–HU14 in the younger mice, while the restCa was increased after HU14. These results highlight the age-dependence of disuse effects on soleus muscle in mice.

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**9:27**

**Effects of endurance training on HIF-1 transcriptional activity and HIF-1α expression in rat slow and fast muscles**

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The effects of endurance swimming on Hypoxia Inducible Factor 1 (HIF-1) transcriptional activity and HIF-1α mRNA expression was assessed on rat soleus (slow) and EDL (fast) muscles. The swimmig protocol lasted for 4 weeks, 5 days/week; in the first week the rats swam 60 min/day, and, during the second week, 90 min/day with an external weight corresponding to 1.5% of the animal's body mass; during the third and fourth week the extra-weight was increased to 3% and 6%, respectively. HIF-1 transcriptional activity was assessed by a dual Luciferase assay after in vivo transfection of a plasmid carrying Luciferase under the control of a HRE concatamer and by quantitative RT-PCR on two HIF-1 target genes (VEGF and Aldolase A); HIF-1α mRNA expression was measured by quantitative RT-PCR.

Our results show that at the end of the training period: (i) Luciferase activity decreased significantly in the soleus muscle; (ii) VEGF mRNA levels increased in EDL; (iii) Aldolase mRNA levels increased significantly in the soleus muscle; (iv) HIF-1 mRNA expression decreased not significantly in both muscles.

**9:28**

**Effect of training with different intensities and volumes on muscle fibre enzyme activity in the m. triceps brachii of untrained men and women**

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The effect of intermittent or continuous training and different training volumes on muscle enzyme activity is not resolved in man. Forty subjects were randomized to one of four groups performing intermittent or continuous elbow extensions in a training apparatus. The subjects performed intermittent training sessions at 60% of triceps 1RM, 3 times week$^{-1}$ until they had lifted ~100 tons (low volume) or ~180 tons (high volume). Continuous training sessions were performed at 30% of triceps 1RM 3 times week$^{-1}$, until a matching low or high volume was achieved. Typically, 5 or 8 weeks were needed to complete a low or high volume. SDH, 3-HbDH and α-GPDH activity of type 1 and type 2 muscle fibres were subsequently analysed in biopsies from the triceps muscle.

SDH and 3-HbDH activity in type 1 muscle fibres increased to similar levels regardless of type of training, while the α-GPDH activity in type 1 fibres was larger following intermittent training compared to continuous training (59 vs. 6%, respectively), $P < 0.001$. In type 2 muscle fibres, SDH and α-GPDH activity increased more after intermittent training (90 and 22%, respectively) compared to continuous training (40 and –5%, respectively), both differences; $P < 0.05$. There was no effect of training volume or gender on enzyme activities. Detraining for 8 weeks reduced all enzyme activities to baseline, $P < 0.01$ for all.

**10:1**

**Premature Stop codon suppression as a therapeutic strategy for a subset of patients with Duchenne muscular dystrophy**

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Premature stop codons (nonsense mutations) promote premature translational termination and cause approximately 15% of the individual cases of Duchenne muscular dystrophy (DMD). To address the need for a drug capable of suppressing premature termination, we identified PTC124, an orally bioavailable new chemical entity that selectively induces ribosomal readthrough of premature but not normal termination codons. PTC124 activity, optimized using nonsense-containing reporters, promoted dystrophin production in primary muscle cells from humans and *mdx* mice expressing dystrophin nonsense alleles and rescued striated muscle function in *mdx* mice within 2–8 weeks of drug exposure. PTC124 was well tolerated in animals at plasma exposures substantially in excess of those required for nonsense suppression. The selectivity of PTC124 for premature termination codons, its well-characterized activity profile, oral bioavailability, and pharmacological properties suggest that this drug may have broad clinical potential for the treatment of a large group of genetic disorders with limited or no therapeutic options. It currently is in Phase 2 trials for DMD.

**10:2**

**Sarcospan ameliorates muscular dystrophy by stabilizing the utrophin-glycoprotein complex**

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Mutations in the dystrophin gene cause Duchenne muscular dystrophy and result in loss of dystrophin and the entire dystrophin–glycoprotein complex from the sarcolemma. Utrophin replaces dystrophin to form a functionally analogous protein complex that is restricted to the neuromuscular junction. We show that sarcospan, a unique tetraspanin-like component of the dystrophin- and utrophin–glycoprotein complexes, ameliorates muscular dystrophy in dystrophin-deficient *mdx* mice. The mechanism of rescue occurs by restoration of the utrophin–glycoprotein complex, which provides a functional linkage between the extracellular matrix and the intracellular actin cytoskeleton. Thus, sarcospan packages and addresses the utrophin–glycoprotein complex to the extra-synaptic sarcolemma thereby rescuing muscular dystrophy. Our data provide novel and unexpected insights into the function of sarcospan in membrane targeting of this important complex. In addition, our findings have direct relevance to understanding the mechanical properties of the dystrophin–glycoprotein complex and will impact the future design of therapeutics for muscular dystrophies.

**10:3**

**MDC1D due to a large genomic insertion in LARGE that causes abnormal gene splicing**

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We report a consanguineous Lebanese family with two children who had typical features of an alpha-dystroglycanopathy, including absent a-DG on muscle immunohistochemistry, moderate mental retardation, hypoplasia of the pons and cerebellum and persistent white matter hyperintensities on MRI. A genome-wide microsatellite study showed linkage to LARGE but gene sequencing from genomic DNA (gDNA) was normal. Sequencing LARGE from cDNA derived from patient lymphocytes showed an abnormal sequence inserted between exons 10 and 11. Most mRNA transcripts contained a 197 bp insertion but low levels of a 171 bp insertion and a normally spliced transcript were also seen. The abnormal sequences mapped to a spliced EST (DA935254) of unknown function, normally located 100 kb centromeric to LARGE on chromosome 22q. Both abnormal sequences are predicted to introduce a premature stop codon. Quantitative PCR (qPCR) analysis of the EST sequence showed twice the copy number in patient gDNA compared to controls, consistent with a duplication of this sequence. qPCR analysis for regions 5′ and 3′ of the EST showed the duplication involves between 30 and 57 kb of genomic DNA. We have strong evidence for a cryptic duplication/insertion involving intron 10 of LARGE that causes an EST to be abnormally spliced into most LARGE mRNA transcripts, likely resulting in very low expression of normal protein.

**10:4**

**Akt activation in dystrophin-deficient muscle dramatically improves pathology and prevents muscle degeneration**

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Dystrophic muscle undergoes repeated cycles of degeneration/regeneration, characterized by the presence of hypertrophic fibers with central nucleation. We have previously shown that Akt is activated at very early, prenecrotic stages of disease pathogenesis and maximal activation is achieved during peak stages of muscle hypertrophy in dystrophin-deficient *mdx* mice. Duchenne muscular dystrophy patients exhibit a similar pattern of Akt activation. In order to test whether Akt plays a direct role in skeletal muscle hypertrophy and disease pathogenesis, we introduced constitutively active (caAkt) and dominant-negative (dnAkt) Akt into dystrophin-deficient mice. We find that increasing caAkt levels causes dramatic improvements in *mdx* pathology, including reductions in central nucleation and membrane fragility. caAkt treatment normalized myofiber cross-sectional fiber diameter in *mdx* mice providing further evidence that Akt signaling is a key regulator of fiber size in dystrophic muscle. Conversely, expression of dnAkt exacerbated the *mdx* phenotype, resulting in early onset of myofiber necrosis. Our data demonstrate that Akt signaling plays an important role in preventing degeneration in dystrophin-deficient skeletal muscle. Importantly, we show that hyper-activation of Akt signaling in *mdx* muscle improves dystrophic pathology.

**10:5**

***TPM3* is a recurrent cause of congenital fibre type disproportion**

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The key diagnostic features of congenital fibre type disproportion (CFTD) are uniform type 1 fibre hypotrophy (without other diagnostic histological abnormalities), together with clinical features of a congenital myopathy. To date, CFTD has been associated with mutations in the *ACTA1*, *SEPN1* and *TPM3* genes but the molecular cause in most patients remains uncertain. We sequenced all coding exons of *TPM3* gene in 11 unrelated patients with CFTD. In four CFTD families we identified novel missense mutations in *TPM3*. The p.100L > M mutation (previously reported) followed clear autosomal dominant inheritance. The remaining mutations were likely de novo, affecting single children in each family (168R > G, 169K > E, 245R > G). The changes affect highly conserved amino acids and were not found in 200 control chromosomes. There was a common pattern of muscle weakness, with variable severity, that included a waddling gait with foot drop, accentuated lumbar lordosis, weakness of neck movements, and mild facial weakness and ptosis. Three out of 8 patients require nocturnal ventilatory support though all remain ambulant. Type 1 fibres were 50–70% smaller than type 2 fibres. Mutations in *TPM3* accounted for a quarter of patients in our CFTD cohort, suggesting this gene may a relatively frequent cause. Phenotypic clues may be useful in directing mutation analysis toward *TPM3*.

## 10:6

### Defining diagnostic boundaries for congenital fibre type disproportion

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Uniform type 1 fibre hypotrophy, relative to type 2 fibre size is the principal histological feature of congenital fibre type disproportion (CFTD) but is relatively non-specific. Therefore care is needed when diagnosing CFTD. At present there is no consensus about diagnostic boundaries. To consider the most appropriate diagnostic guidelines for CFTD we ascertained a cohort of 93 patients with relative type 1 fibre hypotrophy, confirmed fibre measurements from ATPase slides and collected clinical data. We selected 37 patients with idiopathic muscle weakness for closer study. The likely or possible genetic basis was identified in 11 families amongst six genes or loci, most of which have been implicated in other congenital myopathies. We compared the 11 families with the full cohort to identify the clinical and histology parameters that may best distinguish CFTD from other neuromuscular disorders. The best histological and clinical features in this regard were mean type 1 fibre diameter at least 40% smaller than mean type 2 fibre diameter, type 1 fibres uniformly smaller than normal, CK less that 3 times upper limit of normal, at least 50% of fibres are type 1, and presentation under 1 year. Alternative pathologies must be carefully excluded before CFTD is diagnosed. We propose clinical guidelines to aid the clinician and pathologist in this process.

## 11:1

### Period 3 of tropomyosin (Tm) is essential to enhance isometric tension in the thin-filament reconstituted cardiac muscle fibers

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Previous investigations on reconstituted cardiac muscle fibers demonstrated that Tm applies the positive allosteric effect on actin to enhance force generated by each cross-bridge (Fujita et al. 2002). We reconstituted fibers with *E. coli*-expressed mutant Tm in which periods 2 (Δ2Tm: res 47–88) or 3 (Δ3Tm: res 89–123) of Tm's 7 quasiequivalent repeats was deleted. We studied isometric tension, 6 kinetic constants, and pCa-tension relationship in reconstituted fibers. Compared to the control, isometric tension was unchanged for Δ2Tm, but ~50% in Δ3Tm. There were changes in the kinetic constants ( < 3X), but the occupancy of strongly attached cross-bridges was 60% for the fibers with control Tm and Δ2Tm, and 75% for Δ3Tm. The Hill factor was similar in Δ2Tm (1.79 ± 0.19) and the control (1.73 ± 0.21), but reduced in Δ3Tm (1.35 ± 0.02). The $Ca^{2+}$ sensitivity ($pCa_{50}$) decreased slightly in Δ2Tm (5.11 ± 0.07) from the control (5.28 ± 0.04), and increased significantly in Δ3Tm (5.57 ± 0.09). These results imply that Tm's period 3 is essential for the positive allosteric effect that enhances the actin and myosin interaction and increases the isometric force each cross-bridge generates, whereas region 2 is not important for this effect. The results agree with the effects of the deletion mutants in vitro (Hitchcock-DeGregori et al. 2002).

## 11:2

### Over-expression of uncoupling protein-3: where does the energy go?

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Mice over-expressing uncoupling protein-3 (UCP-3) are hyperphagic but lean. We investigated this phenotype by comparing muscle fibres from wild type (WT) and over-expressor mice (OE). Male OE mice weighed less than WT, whereas females OE and WT body weights were not different. The rate oxygen consumption by resting soleus fibres from male OE mice (12.4 ± SEM1.7 nmol g wet weight$^{-1}$ s$^{-1}$, $n = 7$) was significantly higher than that of female OE (7.0 ± 1.5, $n = 6$) and of WT (male: 4.9 ± 1.9, $n = 5$; female:

4.1 ± 0.9, $n = 5$). The resting heat production was also greater in OE than WT muscle. The oxygen cost of isometric contraction was similar for OE and WT muscle. We conclude that the phenotypic difference between male OE mice and the other groups (female OE and males and female WT) is due to the higher resting metabolic rate, and is not due to a higher cost of contraction.

**11:3**

**Crossbridge properties during force enhancement by stretch in activated frog muscle fibres**

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It is well known that slow stretching of activated skeletal muscles enhances the force well above the isometric value. It is not clear however if force enhancement is due to an increase of crossbridge strain or crossbridge number or both. This point was investigated here by measuring the force needed to forcibly detach the cross-bridges ensemble (critical tension, $P_c$) by very fast stretches. If the rupture force of the individual bridge is constant, $P_c$ is expected to be directly proportional to crossbridge number while sarcomere length at $P_c$ (critical length, $L_c$) should vary with the individual bridge tension. Fast test stretches were applied to single intact fibres at 4 °C at various tension levels during the tetanus rise and $P_c$ and $L_c$ values were compared with those obtained when the stretch was applied during slow fibre lengthening. The results showed that during slow stretching which enhanced force by 100%, crossbridge number increased by about 15% while crossbridge mean extension increases by 85%. Force enhancement is therefore mainly due to an increases of crossbridge strain with a small contribution from crossbridge number increase.

**11:4**

**Intensity changes of equatorial 1,1 1,0 and meridional 14.3 nm X-ray reflections during slow stretching of activated frog muscle fibre bundles**

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14.3 nm meridional ($I_{M3}$) and 1.1 1.0 equatorial X-ray reflection intensities were measured on tetanized frog fibre bundles of toe muscle at SAXS beam line at Elettra Synchrotron, with a two dimensions CCD detector located 2.3 m from the preparation. Bundles were subjected to ramp stretches, which enhanced the isometric force by about 100% and exposed to X-ray beam (dimension 3 × 0.4 mm) during the isometric plateau and during lengthening for periods of 30–300 ms at 4°C. Data were averaged for 10–20 contractions from the same bundle. Preliminary analysis showed that, compared to isometric, stretching induced a drop of all the reflection intensities. The 1.1/1.0 intensity ratio, a putative index of attached crossbridge number, decreased during stretching. The drop of $I_{M3}$ and of the 1.1/1.0 ratio suggest that force enhancement during stretch is not accompanied by a significant crossbridge number increase. 1.1 1.0 spacing varied according to bundle constant volume.

**11:5**

**Effects of step exercise on muscle cell damage in young men and women**

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Eccentric exercise often produces severe muscle damage whereas concentric exercise of similar load elicits a minor degree of muscle damage. We analyzed several muscle damage parameters and investigated possible differences between men and women in the response to step exercise.

33 healthy subjects (18 men, 15 women) (18–37 years) performed 30 min of step-exercise involving concentric contractions with one leg and eccentric contractions with the other leg. Muscle $Ca^{2+}$ content, isometric maximum voluntary contraction (MVC) and muscle enzymes in plasma were measured. In a subgroup of the subjects T2 relaxation time was measured by MRI.

We found no significant changes in $Ca^{2+}$ content in biopsies from vastus lateralis, neither in women nor in men. Following step-exercise MVC decreased in both legs of both gender (11–25%). The women had a significantly larger strength decrease in the eccentric leg than the men at day 2 ($P < 0.01$). Plasma creatine kinase increased following step-exercise with a markedly and significantly higher response in women (38-fold) than in men at day 3 ($P < 0.001$). In the women but not in the men, we found a pronounced increase in T2 relaxation time in the eccentric working adductor magnus muscle at day 3 (75%, $P < 0.001$).

In conclusion, step-exercise induces muscle damage preferentially in the eccentric working muscles and considerably more in women than in men.

**11:6**

**Structure and function of skeletal muscle in Zebrafish (*Danio Rerio*) larvae**

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Zebrafish has become an important tool for examining development of organ systems, including muscle. Of great potential is the possibility to manipulate gene expression during early stages, and the availability of mutated strains. However, detailed information of muscle function is lacking. We have therefore examined Zebrafish muscle at an early developmental stage (larvae 5–7 days). Using aluminum clips, preparations (~1.5 mm length, 150 μm diam.) were mounded for force registration. Electrical stimulation elicited very fast and reproducible single twitch contractions. Sarcomeres were oriented mainly in parallel with the preparation long axis and optimal length for active force was 2.17 μm. Small angle X-ray diffraction showed clear equatorial 1.1/1.0 reflections, and showed that myofilaments are predominantly arranged along the preparation long axis. In contrast, reflections from adult fish showed two main filament orientations. Force summation was weak and a smooth tetanus was observed at a stimulation frequency of ~175 Hz. Single twitch/tetanus ratio was ~0.8. A fatigue stimulation protocol with repeated 200 ms/175 Hz tetani revealed that tetanic force decreased to 50% at a train rate of 0.1 $s^{-1}$. In conclusion, Zebrafish larvae muscles can be examined in vitro

using mechanical and X-ray methods and are longitudinally orientated with a significant fast contractile component.

**11:7**

**Blebbistatin inhibits actin–myosin interaction in skinned muscle preparations**

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Blebbistatin, an inhibitor of actin-myosin interaction, has been suggested to have a potency dependent of myosin isoform. Effects of blebbistatin on skinned muscle preparations were examined to determine its specificity and mode of action in the organized contractile system. Studies on striated muscles showed that blebbstatin did not bind in rigor. It inhibited active contractions and bound under relaxed conditions and did not affect phosphate sensitivity of force. The blebbistatin relaxed state was characterized by relaxed X-ray diffraction pattern. The inhibition could be partly reversed by photolytic destruction of blebbistatin. However, recovery was slow and most likely not limited by the rate of force-generating cross-bridge reactions. Blebbistatin inhibited active force to a larger extent in faster muscle: 10 μM blebbistatin inhibited force to 2.8% in mouse EDL, 2.3% in mouse soleus, 29.5% in pig cardiac atrium, 52.8% in pig cardiac ventricle, 26.5% in mouse cardiac muscle, 82.2% in urinary bladder from newborn mice (partly non-muscle myosin) and 96.0% in adult mouse urinary bladder (mainly smooth muscle myosin). In conclusion, blebbistatin traps myosin in a dissociated relaxed state in the organized contractile system. The inhibitory action is dependent on muscle type, possibly reflecting that the apparent binding constant is dependent on myosin isoform and/or on availability of Blebbistatin-binding cross-bridge states.