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Review

The XXXIII European Muscle Conference: ELBA Sept. 04

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The thirty-third European Muscle Conference was held on the island of Elba from September 19th to the 23rd. It was organized by Vincenzo Lombardi, Gabriella Piazzesi, Corrado Poggesi, Chiara Tesi and Marco Linari of the University of Firenze. The site of the conference was the Hotel Hermitage, located adjacent to a beach on a small bay on the North coast of the Island. The location was magnificent. The weather was excellent, and participants were able to swim in the warm, clear waters of the beach between sessions. Lunches, featuring many samples of local seafood and other delicacies, were served each day in a picturesque open air setting beside one of the swimming pools on the conference grounds. On Wednesday the conferees had their choice of three excursions: to visit Napoleon's former dwelling, a tour of Elba or a boat trip to the adjacent island of Pianosa, a former penal colony with uncontaminated environments. On Wednesday night there was a concert followed by a gala banquet and more live music. The setting was among the best in the history of these conferences. In spite of the distractions, the sessions were well attended.

The meeting began on Sunday night with a lecture by Clara Franzini-Armstrong (Philadelphia) on EC coupling followed by a welcome party and dinner at the Hotel Hermitage. Clara gave both a historical perspective and an up to the date summary of our knowledge of the structure of the machinery responsible for translating a depolarization of the membrane into the release of calcium from the sarcoplasmic reticulum. Increased resolution and more sophisticated analysis of electron micrographs have elucidated the structures, and the changes that occur in them, of the two large membrane proteins, DHPR and RyR, that couple T-tubule depolarization to the sarcoplasmic reticulum. Although high resolution structures of these proteins are not yet available, recent results provide strong proof that they communicate via a direct mechanical link.

Session I (Structural Dynamics) was chaired by Ken Holmes (Heidelberg). The first three sessions were concerned with the structure and mechanics of the actomyosin interaction, mainly in skeletal muscle. Ken, the first speaker in session I, summarized our current knowledge of the conformational changes that occur in myosin during the contractile cycle. He integrated the latest structures, which show an opening of the nucleotide pocket of myosin upon binding to actin, with previous results to suggest how the nucleotide communicates with the lever arm to produce a power stroke. Roger Cooke (San Francisco) described spectroscopic studies that also monitored conformational changes at the nucleotide site. David Thomas (Minneapolis) reported measurements of the motion of the 50 kDa cleft of myosin and some initial spectroscopic measurements refining the structure of the actomyosin complex. Malcolm Irving (London) described further studies of the fine structure of the X-ray diffraction pattern, which measures the position of the myosin heads in a muscle fiber with high accuracy. The position of the myosin heads changes during steady fiber shortening, providing a measure of the working stroke, which increases up to a maximum of 4–5 nm with the reduction of the load from the isometric force to ½ the isometric force. However at lower loads the position of the heads recovers towards the isometric value, as detached myosin heads reattach near the beginning of the working stroke in weakly binding states. Hugh Huxley (Waltham) described similar results with whole muscles, showing that the position and dispersion of the myosin heads change little between isometric and slow isotonic active fibers. This result is puzzling because in the isometric case the myosin heads are thought to be clustered near the beginning of the power stroke, while at even slow velocities, the known rate of release of myosin from actin (determined from the isometric ATPase activity) suggests that myosin heads are distributed throughout the power stroke. It is clear that these high-resolution X-ray diffraction patterns contain a wealth of information on the configuration of myosin heads in active fibers, but optimal extraction of this information requires sophisticated models, possibly incorporating information obtained from other techniques. Together the results of high-resolution crystal structures, X-ray diffraction, improved reconstructions of EM images of the actomyosin complex and spectroscopy (both EPR and fluorescence, see Session III) have produced a reasonably complete picture of the mechanism of force production by actomyosin. The major missing pieces

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to this picture are the structures of the actin–myosin complexes.

Session II (Mechanochemistry and Kinetics) was chaired by Mike Geeves (Kent). Mike described the growing knowledge of the kinetics of the various isoforms of the myosin superfamily. He categorized the myosins into three classes, based on the kinetics of their hydrolysis of ATP, with emphasis on the rate of product release. One class is specialized for rapid movement, one for maintaining force and one for sensing tension. Bernhard Brenner (Hannover) reported the isolation and characterization of a new complex of actin-myosin–ADP–Pi in a stable state in which the actomyosin bond is stronger than that of previously characterized forms of this complex. This complex would be a good candidate for structural studies to characterize the transitory and elusive structures that occur at the beginning of the power stroke. Structural changes in actin upon binding of myosin, a 5° rotation, were reported by Olga Pronina (St. Peterburg), reminding us that myosin is not the only protein involved in force production. Two presentations, one by K. W. Ranatunga (Bristol) and one by Valérie Decostre (Firenze) explored the effect of increasing temperatures on the endothermic actomyosin interaction. Valérie found the actomyosin interaction produces considerably greater work in the power stroke, 40%, on raising the temperature of frog muscle from 2 to 17 °C. Tim West (London) used a new method of rapidly measuring ADP release using a fluorescently labeled form of nucleotide diphosphate kinase. The amount of ADP released decreased with temperature indicating a shift from more weakly binding actin-myosin–ADP–Pi states towards more strongly binding actin–myosin–ADP states. The results of these last three presentations suggest that the contractile proteins should be studied at the temperatures where they have evolved to be maximally efficient and not, as is often the case, at the temperatures where the studies are more easily done.

Session III (Molecular Motors) was chaired by Yale Goldman (Philadelphia). Yale continued the discussion of the nature of the weakly binding actomyosin states at the beginning of the power stroke. He first summarized evidence that there are two forms of the actin–myosin–ADP–Pi complex, one that generates force and one that does not, a topic also discussed in session I. He described an improved single molecule force measurement, that shows that a force generating actin–myosin–ADP–Pi complex can be mechanically dissociated near the beginning of the power stroke, demonstrating the dynamic nature of the actomyosin interaction. This was followed by an ingenious proposal by Mike Reedy (Durham) that myosin walks processively backward using both heads during stretch of muscle fibers; a very interesting proposal in search of some experimental proof. The final QED that myosin moves by using the light chain as a lever arm was provided by Jim Sellers (NIH, Bethesda). High resolution measurements of the position of fluorescently labeled light chains, made in collaboration with Paul Selvin (Urbana), provided a quantitative measure of the lever arm motion. The temperature dependence of the actomyosin interaction was revisited by Monica Canepari (Pavia), who showed that the *in vitro* assays used to measure filament velocities are more sensitive to temperature than are the fibers from which the proteins came. Tatiana Nenasheva (Ekaterinburg) followed the movements of a GFP-myosin X construct, demonstrating that real time data can be obtained in living cells. The brave new world of nanotechnology, and the promise of the motor proteins in this realm, was entered by Alf Månsson (Kalmar), who manufactured nano tracks of myosin, along which actin filaments could be driven unidirectionaly.

Session IV (Membrane and EC Coupling) was chaired by Clara Franzini-Armstrong (Philadelphia). Bernhard Flucher (Innsbruck) discussed the first evidence for a functional role of the $\alpha_2\delta$-1 subunits of L-type Ca^{2+} channels in muscle by silencing the $\alpha_2\delta-1$ gene using specific siRNA in cultured myotubes. Quantitative RT-PCR and Western blot analysis confirmed the overall reduction of $\alpha_2\delta-1$ message and protein, respectively. Absence of $\alpha_2\delta$-1 did not affect expression of the endogenous α_{1S} subunit and its targeting and clustering at triads. Parallel recordings of L-type Ca^{2+} currents and of transient changes in cytoplasmic free Ca^{2+} concentrations showed that depletion of $\alpha_2\delta-1$ did not affect the ability of the DHPR to conduct Ca^{2+} and to activate SR Ca^{2+} release. Interestingly, however, $\alpha_2\delta$-1 depleted DHPRs have altered kinetics: they are rapidly activating and show a significant inactivation within the period of the 200 ms test pulses. Thus, the function of the $\alpha_2\delta$-1 subunit in α_{1S} channel complexes (CaV1.1) is to stabilize the slow mode of current activation. Kurt Beam (Fort Collins) presented ingenious experiments using FRET to explore the proximity between the specific sites of the DHPR and RyRs of skeletal muscle that allow the two molecules to reciprocally influence each other. Using cyan- and yellow-fluorescent proteins (CY tandem) and expression of various labeled constructs in normal, dysgenic (lacking DHPR) and dyspedic (RyR1 null) myotubes, he defined the relative positioning of DHPRs within tetrads. More importantly, Kurt provided initial evidence for a close proximity between RyR1 and both the N-terminal of β1a and the C-terminal of α1s. A further confirmation of the close proximity between the C-terminal of α1s and RyR1 was obtained by fusing a biotin acceptor domain to the same sites used for the CY tandem. Comparison between dysgenic and dyspedic myotubes indicated that the C terminal of α1s was occluded in the presence of RyR1. Peter Szentesi (Bern) discussed Ca^{2+} induced inactivation of the L-type Ca^{2+} channel (DHPR) that limits Ca^{2+} influx during cardiac activity. Caged Ca^{2+} was used to study kinetics and voltage dependence of Ca^{2+} inactivation in cardiac myocytes, showing that

either the binding site for the block is located inside the pore, or that a voltage dependent step is involved. In a presentation by Amani Cheick, from Cognard's laboratory (Poitiers), the M1 fraction of the scorpion venom was found to affect the nicotinic acetyl choline receptor, inducing a cytoplasmic calcium release. The effect of a second scorpion venom component (maurocalcine, MCa) was presented by Laszlo Csernoch (Debrecen). Laszlo showed that a positively charged area of the molecule plays a critical role in inducing long lasting RyR openings. Emiliana Giacomello (Siena) presented her exploration of the developmental stages in the interaction between the SR ank1.5 and the peripheral myofibrillar component obscurin, indicating that association of SR to the myofibrils is a secondary developmental event. Simona Boncompagni (Chieti) showed that aging muscles present a significant decrease and a noticeable rearrangement of the SR/T tubule associations or calcium release units, suggesting a role in the age related deterioration of muscle performance. Aurelie Vandebrouk (Poitiers) presented evidence for a relationship between dystrophin expression and the control of store operated calcium entry and showed a moderating effect of minidystrophin expression on calcium accumulation by mitochondria.

Session V (Regulation) was chaired by Earl Homsher (Los Angeles). The first theme emerging from this session was the examination of the effects of regulatory proteins on the mechanical behavior of cardiac muscle. In the first presentation, John Solaro (Chicago) showed that modification of the subunit structure of troponin (TnC, TnI, or TnT) could modulate the isometric force, the calcium sensitivity, and the maximal and minimal ATPase rate, the relaxation rate, and the unloaded shortening velocity. These alterations of contractile behavior have been well established in both *in vitro* assay systems (skinned fibers, motility assays, microneedle assays) and in cardiac tissue from transgenic animals. The data imply that these effects are modulated at the level of the sarcomere by altering cross bridge kinetics (as opposed to modulation in calcium release or sequestration). John reported that phosphorylation of serine or threonine residues at TnI 23, 24, at TnI 44, and 45, and at the carboxy-terminus of TnT (180–220) significantly alter calcium sensitivity, force, and shortening velocity in cardiac muscle. Another issue John noted was the need to measure the force–velocity curve of contractile systems. This followed from his work demonstrating that although replacement of cardiac TnI with slow skeletal TnI (a TnI lacking the N-terminal 30 amino acids of cardiac troponin) produced little change of either the maximal isometric force or the unloaded sliding speed, while pressure–volume loops from heart containing the slow skeletal TnI exhibited alterations. These alterations may stem from changes in the curvature of the force–velocity curve. In a second paper examining the mechanical sequelae of alterations of the regulatory protein isoforms, Alexandra Belus (Firenze) described experiments in which cardiac troponin containing a truncated cardiac troponin I ($cTnI_{1-192}$) isoform (an isoform expressed in 'stunned' and in certain failing hearts) was exchanged into single human cardiac myofibrils. Control experiments showed that human cardiac myofibrils exhibited reproducible and representative mechanical behavior when troponin was exchanged in the fiber lattice, as is the case in myofibrils isolated from skeletal muscle. Further, when a cardiac Tn containing $cTnI_{1-192}$ replaced the endogenous cTnI, the maximal isometric force and rate of isometric force production fell by 25% and the rate of muscle relaxation was prolonged. These results suggest that $cTnI_{1-192}$ may contribute to the mechanical deficits seen in stunning and heart failure.

The second major theme of this session concerned the development of new or improved methods or assays for studying the mechanism of regulation. Larry Tobacman (Iowa City) described a method he and his colleagues devised to create short fragments of thin filaments each containing 12–14 actin monomers, two Tropomyosin and two Troponin molecules. Studies of calcium binding to the Tn on the filaments and the acto-S1 ATPase activity of these single regulatory unit particles showed that Ca^{2+} bound non-cooperatively to the particle's Tn. This suggests normal thin filament Ca^{2+} binding cooperativity reflects interactions along the long filament axis, absent in the particles. Despite non-cooperative Ca^{2+} binding to the particles in the absence of myosin, Ca^{2+} cooperatively activated the myosin S1-particle ATPase rate. This suggests troponin-troponin interactions can also occur across the thin filament, between its two strands, when myosin is present. Earl Homsher described experiments with Yasuharu Takagi and Henry Shuman (Philadelphia) in which a single thin filament (with or without Tn and Tm) was attached to two latex beads whose position was controlled by separate optical traps. Using a position sensitive feedback system designed to prevent movement of the thin filament, the thin filament was allowed to interact with a single skeletal myosin molecule while the maximal force and duration of cross bridge attachments were measured. The frequency of interactions was calcium sensitive, but at pCa 5 both the duration of attachment and ensemble averages of the force produced during the attachments were independent of the presence or absence of Tm/Tn. These results suggest that Tn/Tm induced alteration of maximal isometric force are exerted by control of the number of interacting cross bridges, not the unitary force. Finally Madoka Suzuki (Tokyo) described a method that permits the experimenter to measure the force exerted on a single thin filament as it passes into the thick filament lattice in a single myofibril fragment. In this method a single actin filament is tethered to a bead held in an optical trap and the thin filament and bead are brought into position to interact with the thick filament lattice. The initial data is encouraging, showing

that it may be possible using this method to critically examine the nature of the length–tension relation.

Session VI (Smooth Muscle) was chaired by Gabriele Pfitzer (Köln). The session covered several aspects of both vertebrate and invertebrate smooth muscle contraction. Vertebrate smooth muscle contraction is primarily switched on by the phosphorylation of the regulatory light chains (RLC) of myosin II at Ser19, the regulation of which was topic of the first two talks. Avril Somlyo (Charlottesville) showed that surprisingly there is still a Ca^{2+}-dependent contraction and phosphorylation of RLC at Ser19 in blood vessels from embryonic mice in which the MLCK gene expression was disrupted. Whether this exciting new finding is due to a novel Ca^{2+}-dependent kinase phosphorylating RLC or a Ca^{2+}-independent kinase which is activated by an upstream Ca^{2+}-dependent mechanism remains to be seen. Telokin deficient mice on the other hand display a leftward shift of the force-calcium relation and a reduced response to cGMP confirming the suggested function of telokin as a regulator of the activity of MLCP. Regulation of MLCP in resistance arteries by monomeric GTPases of the Rho family was the topic of the presentation by Steffen-Sebastian Bolz (München).

In vertebrate and invertebrate smooth muscle, crossbridges may be 'locked' in a force generating state (so called latch and catch respectively) occuring at low [Ca^{2+}] which gives rise to tension maintainance with very little energy expenditure. Marion Siegman (Philadelphia) presented evidence that catch in invertebrate smooth muscle, such as in ABRM of *Mytulis edulis*, may be due to force generating myosin crossbridges which appear to be in a 'locked' ADP bound state whereby unphosphorylated twitchin prevents exit from this state. Upon phosphorylation of twitching by a cAMP-dependent protein kinase, crossbridges leave this state both by completion of the cycle and by reversal to low-force states thereby allowing relaxation from catch. The mechanisms regulating the latch state in vertebrate smooth muscle differ. The intriguing hypothesis that non-muscle myosin II may participate in the molecular mechanism of the latch state put forward by Ingo Morano (Berlin) a few years ago was corroborated by the observation of Frank Brozovich (Cleveland) that a small molecule inhibitor of non-muscle myosin II, blebbistatin, inhibited tonic contraction in smooth muscle. Other players suggested to regulate the attachment of dephosphorylated crossbridges include caldesmon. The stimulation dependent temporal pattern of caldesmon and telokin phosphorylation was monitored by Alexander Vorotnikov (Moscow). Phosphorylation of caldesmon would be compatible with a role of caldesmon in the regulation of the tonic phase of arterial contraction while phosphorylation of telokin was associated with relaxation. The role of the myosin binding subunit of caldesmon was investigated by Veronika Hasse (Düsseldorf) in a mouse mutant deficient in the strong myosin binding subunit. The half time of relaxation of triton skinned smooth muscle was increased in these mice but unexpectedly passive tension in cardiac myofibrils at a given sarcomere length was decreased giving rise to the interesting hypothesis that caldesmon which is located in the I-band region may participate in regulating passive force length relation in the heart.

Session VII (Cytoskeletal Proteins) was chaired by Miklos Kellermayer (Pècs). The first presentation was given by Carol Gregorio (Tucson), who talked about length regulation of thin filaments in the sarcomere. She described functional experiments in cultured cardiomyocytes, where interference with the pointed end actin capping protein tropomodulin or with nebulin by either antibody injection or RNAi led to seemingly continuous actin filaments, indicating that both proteins are important for thin filament length regulation. Pauline Bennett (London) talked about the localization of the alpha2 spectrin isoform in cardiomyocytes *in situ*. This isoform is present at the level of the Z-disk and also at the intercalated disk, the specialized type of cell–cell contact between cardiomyocytes, where it localizes as a doublet, distinct from vinculin. Pauline proposed that alpha2 spectrin might be important for the organization of the actin filaments that anchor the myofibrils in the intercalated disk. Attila Nagy (Pècs) and Wolfgang Linke (Müster) described their recent experiments on the elastic filament protein titin. Attila investigated the PEVK segment from soleus titin, which is the longest one known and probed the elasticity as well as the actin binding ability of different subsegments of this PEVK domain. He found slightly different elastic properties and could show that the actin binding strength depends on the content of polyE stretches in a given segment. Wolfgang studied the contribution of titin versus actomyosin on passive shortening velocity in human myofibrils. By degrading titin with a brief trypsin digest, he could show that 20% of the viscous drag is actually due to actomyosin and that titin is mainly responsible for the fast recoil. Fly muscle was the topic of the talks by Jim Vigoreaux (Burlington) and Kevin Leonard (Heidelberg). Jim described his analysis of the fly muscle proteome, where he was able to pull out many proteins that are well known from vertebrate muscle. Analysis of the protein expression pattern of flies either in actin or myosin expression revealed that these mutations also affect the expression of actin and myosin interacting proteins, respectively. Kevin presented his analysis of the open reading frame of the SLS locus in flies, that encodes kettin. Several other transcripts can be generated from this locus, which also contains a PEVK region. Interestingly, these transcripts localize differently in the sarcomeres of flight versus non-flight muscles.

Workshop on stem cells. This year's workshop was dedicated to stem cells and was chaired by Vincenzo Sorrentino (Siena). This area of research is attracting much interest because of many unanswered questions

in basic research and because of the interest in using stem cell based technologies to treat human diseases, including muscle dystrophy and heart diseases, for which current treatment is not satisfactory. Vincenzo provided a general introduction to the topic by relating current research on stem cells to studies on regeneration and tissue repair, in humans and other species. He then highlighted recent developments and success on purification, characterization and initial clinical trials with stem cells, but then purposely underlined how little we still know on the biology of bone marrow cells that are being used in patients to help heal cardiac infarcts. Following this introduction, Antonio Musarò (Roma) reported on the role of soluble mediators of inflammation, various growth factors and cytokines, in the process of homing, which is expected to guide transplanted or endogenous stem cells to the site of injury. Within this complex world of peptides that are sending messages to cells, IGF-1 (insulin like growth factor-1) appears to play a significant role in recruiting circulating stem cells to sites of damage. Maurilio Sampaolesi (Milano) provided a critical review of past experience in the treatment of muscular dystrophy with stem cell therapy. He then introduced mesangioblasts, which, among the stem cells, are currently the top gun in the war on muscular dystrophy. He discussed results obtained after intra-arterial delivery of wild type mesoangioblasts in morphologically and functionally correcting the dystrophic phenotype in a muscle model for limb-girdle muscular dystrophy. He also presented recent results obtained after transduction of mesangioblasts of dystrophic mice with a lentiviral vector expressing alpha sarcoglycan. In this case, the ability of mesangioblast stem cells to help dystrophic muscles appears to depend on their ability to exit the vascular system and enter the muscular tissue. This presentation was followed by one by Giuseppe D'Antona (Pavia) who moved to isolation of human stem cells by purification of AC133+ cells and their use in skeletal muscles of *scid/mdx* mice. In addition to verifying the morphological and functional outcome of this treatment they went on to measure the contractile properties of single fibers of treated and untreated muscle. They found that treatment with human AC133+ cells improved the force generation of skeletal muscle of treated mice. The last presentation was by Christiana Velloso (London) on the *in vitro* response of cultured muscle cells to factors that enhance hypertrophy, like IGF-1. Cultured C2C12 myoblasts upregulate the expression of members of the IGF-1 family, while treatment with IGF-1 abolishes expression of all the IGF-1 isoforms suggesting the existence of a negative feedback in regulating this pathway.

Session VIII (Plasticity) was chaired by Carlo Reggiani (Padova). In his introductory address Carlo underlined how much our knowledge of muscle adaptive responses has gained in the last few years from the development of new technology of high throughput mRNA expression analysis. Microarrays have allowed the study of thousands of transcripts in the same experiment, leading to a global description of the changes in gene expression which form the basis of muscle plasticity. Martin Fluck (Bern) gave a rich updating of the studies on muscle metabolic adaptation. Starting from the early observations on mitochondrial adaptability to endurance-type training, Martin described the recent applications of high-throughput gene expression profiling which yield a highly resolved picture of the skeletal muscle and mitochondrial plasticity and the discovery of sensors of the physiological stimuli given by exercise and of signaling pathways which control gene transcription at nuclear and mitochondrial level in a highly co-ordinated way.

The neural pattern of excitation is known to be one of the most effective factors that induce muscle fiber adaptation. The effects of innervation during development were discussed by Henriette de Jonge (Utrecht), whereas the changes in muscle fibers following denervation were the object of two presentations given by Paolo Laveder (Padova) and Feliciano Protasi (Chieti). Paolo described early changes in gene transcription taking advantage of microarray technology, while Feliciano described muscle ultrastructural changes after several years of denervation and the effects of repetitive electrical stimulation. Richard Jaspers (Amsterdam) reported results obtained in a unusual model of muscle plasticity: single fibers kept in culture for 2–4 weeks and exposed to factors such insulin or IGF which are able to induce an increase of fiber size. The mechanisms responsible for fiber size regulation and the relevance of the nuclear number to this process was discussed by Jo Bruusagaard (Oslo), reporting data on mice carrying the *MSVski* proto-oncogene and showing muscle hypertrophy without changes in nuclear number.

Session IX (Intracellular Transduction Signals) was chaired by Stefano Schiaffino (Padova). The session was focussed on signaling pathways that mediate the effect of muscle activity on skeletal muscle. Grahame Hardie (Dundee) introduced the AMP-activated protein kinase (AMPK) cascade, which acts as a sensor of the muscle energy status. AMPK is activated when muscle contraction leads to a fall of the ATP:ADP ratio, which is amplified by adenylate kinase into an increase in the AMP:ATP ratio. AMP activates AMPK both by direct allosteric activation and by promoting the phosphorylation of the upstream kinase, the tumor suppressor LKB1. AMPK is partly, but not entirely, responsible for the acute metabolic responses of muscle to exercise, including the switch from the anaerobic metabolism of glycogen to oxidative metabolism of blood glucose and fatty acids. It is also responsible for the long-term metabolic adaptations to endurance training, particularly the up-regulation of mitochondrial content and oxidative metabolism and the increased expression of the insulin-dependent glucose transporter GLUT4. The notion that AMPK may be a

potential target to attenuate insulin resistance in type II diabetes, is supported by the finding that AMPK is activated by metformin, one of the most commonly used drugs for the treatment of type II diabetes.

The role of the transcription factors NFAT and FoxO was discussed by Stefano Schiaffino. Calcineurin, a calcium–calmodulin regulated protein phosphatase, is selectively activated in skeletal muscle by slow motor neuron firing and leads to the dephosphorylation and nuclear translocation of the transcription factors of the Nuclear Factor of Activated T cells (NFAT) family, that can be visualized *in vivo* using NFAT-GFP fusion proteins. A major role of the calcineurin-NFAT pathway in the activation of slow muscle genes and the repression of the fast gene program is supported by the effect of the peptide inhibitor VIVIT, that blocks the activation of NFAT, and of constitutively active NFAT mutants. Similar approaches, including *in vivo* RNAi, were used to establish the essential role of the transcription factors of the Forkhead box O (FoxO) family in muscle atrophy. Previous studies have shown that the muscle-specific ubiquitin ligase atrogin-1 (also called MAFbx1) is involved in muscle wasting induced by denervation, fasting or cachexia. Atrogin-1 expression is controlled by FoxOs, which are normally repressed by PI3K-PKB/Akt activity. These findings suggest novel approaches for pharmacological interventions to prevent muscle wasting.

The role of MyoD family transcription factors was discussed by Christine Doucet (Montpellier) and Merete Ekmark (Oslo). Christine showed that Myf5 is degraded during mitosis via the ubiquitin-proteasome pathway. Merete presented evidence that myogenin and MyoD can modulate the muscle phenotype in adult animals: a glycolytic-to-oxidative switch is induced by over-expression of myogenin in type IIb fibers, whereas fast myosin is induced in denervated slow fibers over-expressing MyoD. The transcriptional regulation of the human atrial myosin light chain-1 was investigated by Ingo Morano (Berlin) both in the cardiomyocyte H9c2 cell line and in a transgenic rat model, which show significantly improved contractile parameters of the isolated perfused hearts. Haouaria Balghi (Poitiers) characterized Ca^{2+} release events in a dystrophin deficient cell line and found that IP3 plays a substantial role in Ca^{2+} release from SR in dystrophic cells.

Session X (Sarcomeric Diseases and Cardiomyopathies) was chaired by Ger Stienen (Amsterdam). The session was a balanced collection of presentations on cardiac and skeletal dysfunctions. Ger started the session with an overview of studies on contractile dysfunction underlying both systolic and diastolic dysfunctions in human tissue, highlighting the influence of alterations in phosphorylation levels of contractile and regulatory proteins. David Allen (Sydney) continued by presenting novel data on the involvement of stretch-activated channels in muscular dystrophy. Pieter de Tombe (Chicago) presented data obtained by using extraction–reconstitution of troponin in rat skinned myocytes to identify the domain of cardiac troponin I, responsible for the length dependence of myofilament activation. Paul Robinson (Oxford) provided evidence obtained from studies on mutations in fast skeletal regulatory proteins, troponin T and I and alpha-tropomyosin, and suggested that failure to inhibit crossbridge cycling at low Ca^{2+} might be responsible for skeletal muscle deformations in distal arthrogryposis. Theresia Kraft (Hannover) gave an excellent talk on the diverse effects of point mutations in the beta-myosin heavy chain, which cause familial hypertrophic cardiomyopathies, possibly through both an increase and a decrease in Ca^{2+} sensitivity. Elisabeth Ehler (London) studied intercalated disk and M-band proteins and showed that elevated expression of a LIM-domain protein was a marker of dilated cardiomyopathy. Finally Cris dos Remedios (Sydney) illustrated the potential of gene profiling using cardiovascular cDNA arrays in characterising patients with heart failure from differing etiologies. One of the lessons from the presentations and the lively discussions in this session was that the very stimulating interdisciplinary interaction between basic and clinical research generates a huge momentum to all areas of muscle research.

The final session was a summary of the conference chaired by Roger Cooke. Each session was summarized by the chair of the session, or by one of the speakers. Of necessity each presentation was brief. The great breadth and diversity of the conference, which explored the many facets of muscle from high resolution molecular structures to the pathology of human disease, was evident in the close juxtaposition of the descriptions of the various sessions. The session was followed by one last exotic lunch, and a sad departure from this idyllic setting and from the excellent science and camaraderie. This will be one of the truly memorable experiences that makes the life of the academic scientist, with its long hours in the laboratory, worth the effort.

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