with lipid binding short tail in both porcine aorta smooth muscle and A10 cell. An appreciable proportion of the myosin I existed in the cytosol of the A10. (Hasegawa, Y. et al; 1996, 1998) We have attempted to find out the role of the cytosolic myosin I with respect to the actin filament organization in vascular smooth muscle cell. During the study of binding between the aorta myosin I and F-actin, an appreciable proportions of F-actin has been sedimented with small amounts of myosin I under physiological ionic strength. This phenomena was attributed to the occurrence of myosin I induced F-actin bundling. During the studies on myosin I, we have also found IQGAP1 in aorta smooth muscle. IQGAP1 has been known as a scaffolding protein of F-actin along with small GTPase, calmodulin. Both myosin I and IQGAP1 in aorta smooth muscle can be key players for actin reorganization during contraction and relaxation cycles of vascular smooth muscle. We have made a fluorescently labeled calmodulin for further characterization of F-actin bundling with myosin I or F-actin gel forming with IQGAP1.

1PS007 ホッキ牽引筋 NAM Mg-ATPase 活性に対する 2 種類の TM (TM1, TM2) の影響

The effects of two kinds TM(TM1, TM2) to Mg-ATPase activity of hokki retractor muscle NAM

Yoichi Yazawa

We have studied Hokki clam, *spisula sachalinensis*, protein components of muscle natural actomyosin (NAM) and Mg-ATPase activities (= Ca-sensitivities).

In autumn(october and november), Ca sensitivity of hokki clam retractor muscle NAM ATPase activities showed low values (15~50%). Them of NAM prepared in other three seasons were higher than autumn(88~97%).

SDS-PAGE patterns of two kinds of NAM.

We was different from TM(TM1 and TM2). The difference between TM1 and TM2 is the first report about two types of TM function to Mg-ATPase activity.

1PS008 クライオ電子顕微鏡法を用いたアクチン-ミオシン硬直複合体の高分解能構造への試み

Approach to higher-resolution structure of actin-myosin rigor complex by electron cryo-microscopy

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Actin-myosin interaction is involved in muscle contraction, cell motion, and intercellular transport, and the interaction mechanism in detail is not figured out yet. We convince it necessary to obtain high-resolution structure of the rigor complexes to elucidate the mechanism to produce strong binding between actin and myosin. The previous studies have reported their 13 Å resolution density maps but their resolution isn't good enough to discuss the rigor structure at the atomic level. We also have been challenging to obtain high resolution maps beyond 4 Å by cryo-electron microscopy and helical particle analysis. The obtained filaments formed arrowhead every 36 nm. It is due to the behavior of actin-myosin rigor complex: actin filaments take double-helical architecture with a half pitch of 36 nm, and actin molecule bind to myosin molecule in pairs. Therefore, those filaments were obtained as full-decoration. This time, we could get to about 30 filaments (~2000 units) in addition to existing 5500 units from earlier study (Tsukada, 2010), but need 10000 units or more number of units in order to get high-resolution structure beyond 4 Å. We calculated that it is important to increase the number of better quality complex filament.

1PS009 In vivo マウス心臓におけるサルコメア長のリアルタイム計測 Real-time measurement of sarcomere length in the mouse heart in vivo by using α -actinin-GFP

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The heart is a unique organ in which cardiomyocytes repeat contraction and relaxation in response to electrical stimulation, thereby ejecting blood. Despite numerous studies conducted thus far under various experimental settings, the molecular mechanisms of contraction and relaxation of cardiomyocytes till remain elusive in vivo. In the present study, we expressed GFP at sarcomeric Z-discs (α -actinin) by using the adenovirus vector system in adult mice, and conducted real-time imaging of the movement of single sarcomeres in

cardiomyocytes in the left ventricle under fluorescence microscopy at 10 nm precision (at 100 fps). First, we found that sarcomere length (SL) was ~2.0 μ m in the isolated heart when perfused with 30 mM BDM (hence during diastole). This value is close to what was obtained previously by other investigators in rats using various experimental techniques. When perfused with Tyrode's solution containing 1 mM Ca²⁺, the heart started to exhibit beating with the diastolic and systolic SLs ~2.2 and ~1.7 μ m in the left ventricle, respectively. Finally, we attempted to visualize single sarcomeres in vivo in open chest mice under anesthesia. We found that SL was ~2.0 and ~1.7 μ m during diastole and systole, respectively, but varied by ~300 nm even in the same left ventricular cell. We also found that sarcomere contraction occurred at the T-wave end point on the electrocardiogram, followed by an increase in the left ventricular pressure. At the meeting, we discuss these findings at the molecular level.

1PS010 チャネルロドプシンを発現した骨格筋細胞における筋収縮と成熟の光操作

Optogenetic manipulation of myogenic contraction and maturation in channelrhodopsin-expressing skeletal muscle myotubes

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Traditionally, neuron and muscle have been electrically stimulated to be induced the artificial activity and examined the specific functions. Although, this technique is simple and convenient, it is often nonuniform, nonspecific and invasive with the electrical field stimulation using metal electrodes placed in the extracellular space. In this study, to improve the spatiotemporal resolution and to reduce the invasiveness, mouse muscle cell lines, C2C12 myoblasts were genetically introduced a variant of light-gated ion channel, channelrhodopsin-green receiver (ChRGR), and subsequently induced into multi-nucleated myotubes according to the standard differentiation conditions. The LED light pulses depolarized the membrane potential of a ChRGR-expressing myotube and eventually evoked action potentials dependent on the intensity and duration of the illumination. The light-activated contraction of the photosensitive myotube was obvious in synchronous to the light pulses of the given frequency and the pattern (1-10 Hz, 20-100 ms). We also found that the optical stimulation using pulsed flash light promoted the sarcomere assembly and the contractile ability of the ChRGR-expressing myotubes. It is suggested that the myogenic contraction and the maturation of skeletal muscle cells can be manipulated by light using optogenetics. This technique could facilitate the studies such as the fundamental biological processes during myogenic development, the stem cell therapy for muscular disorder, and the bioengineering using the muscle-powered actuator.

1PS011 軟体動物横紋筋のコネクチン様タンパク質 Connectin-like protein in molluscan striated muscle

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Connectin is an elastic muscle protein in vertebrate striated muscle. Invertebrate striated muscle is also known to possess several connectin-like proteins. Because the results of our SDS PAGE and immunoblot analyses have suggested the presence of a connectin-like protein in molluscan muscle, we examined its primary structure and compared it with those of some known connectin and connectin-like proteins.

Using the genome information of *Lottia gigantea*, we searched for a connectin-like protein and determined its sequence by RT-PCR. As a result, the connectin-like protein of the limpet was estimated to be 54 kbp and have a molecular weight of 2,020,000. The domain structure of this protein was similar to that of vertebrate connectin in that it possessed tandem Ig domains, a PEVK region at the N-terminus and a kinase domain at the C-terminus. However, the protein had characteristics substantially different from those of the already known connectin and connectin-like proteins. In particular, it was found to possess a tandem Fn region consisting of 45 repeated Fn domains at the C-terminus and to lack a super-repeat region.

Immunoblot analysis using a polyclonal antibody for the recombinant protein showed that the antibody against the PEVK region reacted with a 3000-kDa band of the limpet heart muscle. Furthermore, the observation with fluorescence microscopy revealed that the PEVK region of limpet connectin-like protein is localized in the I-band of the striated muscle sarcomere.