# Department of Cell Physiology

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## General Summary

The aim of research in our laboratory is to understand the regulatory mechanism of the cardiovascular system. In particular, we are interested in the development of the cardiovascular system, the mechanics of sarcomere contraction, $Ca^{2+}$ homeostasis in the cardiac sarcoplasmic reticulum, and the pathophysiology of cardiac fibrosis. We established an experimental system to investigate small fetal arteries, such as the rat fetal ductus arteriosus (DA) and pulmonary vein. In addition, we developed an *in vivo* nanoimaging system to observe sarcomere contraction in the ventricles of small animals, such as rat and mouse.

## Research Activities

*Molecular mechanism of closure of the DA*
The DA is an essential artery that connects the main pulmonary artery and the descending aorta in fetus. The DA closes immediately after birth in accordance with its smooth muscle contraction and vascular remodeling. We are investigating molecular mechanisms of DA closure after birth. The incidence of patent ductus arteriosus (PDA) is known to be higher in premature neonates with infection than in those without infection. However, the detailed mechanism has not been investigated. We found that lipopolysaccharide delays closure of the rat ductus arteriosus by induction of inducible nitric oxide synthase but not prostaglandin $E_2$.

*Causal factors of aortic coarctation*
Aortic coarctation is a congenital heart disease whereby the descending aorta is narrow, usually in the area where the DA connects. In some case re-narrowing of the aorta develops after definitive operation. We found that DA smooth muscle cells were straying into aortic smooth muscle cells of narrow area and further extent. We are also investigating the long-term use of prostaglandin $E_2$ on the human DA. We are collaborating with Hyogo Prefectural Kobe Children's Hospital in this study.

*Regulation of sarcoplasmic reticulum ATPase activity*
We are interested in regulation of the sarcoplasmic reticulum $Ca^{2+}$-ATPase and $Ca^{2+}$ homeostasis in the sarcoplasmic reticulum. We found that sarcolipin-knockout mice improved the impairment of muscle function in mdx mice that are an animal model of muscular dystrophy. We are collaborating with National Center of Neurology and Psychiatry in this study.

*Regulation of cardiac metabolism*

Cardiac metabolism plays an essential role in maintaining cardiac function. Vitamin B1 (VitB1, thiamine) deficiency causes Beriberi, which is characterized by peripheral sensory and motor neuropathy, and congestive heart failure. Dr. Kenehiro Takaki who founded Jikei University, eliminated Beriberi from the Imperial Japanese Navy by improving dietary habit (thiamine supplementation). We found that pretreatment with VitB1 preserved cardiac function in ischemic-reperfusion injury.

*Pathophysiological mechanisms of organ fibrosis*

Organ fibrosis is a maladaptive response to pathophysiological conditions, such as in impaired organ perfusion and ischemic diseases. However, the effects of pressure-overloaded interstitial fibrosis in the heart and liber in myocardium remain unclear. We prepared pulmonary artery banding (PAB) rats as a model of cardiac hypertrophy. We found that several factors including fibroblast growth factor 23 (FGF23), which is known to play a role in the regulation of osteogenesis, was up-regulated in the interstitial fibrosis group. We also found that low cardiac output was an important determinant that promoted liver fibrosis.

*Mechanism of sarcomere contraction in cardiac muscle*

1. Simultaneous imaging of local $Ca^{2+}$ and single sarcomere length in rat neonatal cardiomyocytes using yellow Cameleon-Nano140

We developed a novel experimental system for simultaneous nano-imaging of the dynamics of the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and single sarcomeres in the subcellular partition, via expression of a FRET-based yellow Cameleon-Nano140 (YC-Nano140) fused into α-actinin for the localization at Z-disks in primary-cultured rat neonatal cardiomyocytes. The system enabled quantitative analyses of local $Ca^{2+}$ transient (CaT) and the ensuing sarcomere dynamics at low and high temperatures during spontaneous beating and at electric stimulation (5 Hz) at 37°C. Local $Ca^{2+}$ waves were observed between CaT, and induced local sarcomeric contractions. There was a positive correlation between an increase in local $[Ca^{2+}]_i$ and the magnitude of sarcomere shortening. This experimental method will be widely applied for elucidating the molecular mechanisms of cardiac excitation-contraction coupling under physiological and pathophysiological conditions (*J. Gen Physiol.*, 2016-2).

2. *In vivo* visualization of sarcomeric motions in the beating mouse heart

The Frank-Starling law predicts that a change in the length of myocardial sarcomeres by only 100 nm dramatically changes the heart's pump functions, indicating the importance of highly accurate measurements of cardiac sarcomere length displacement *in vivo*. In this study, we developed a high speed (100-frames per second), high resolution (20-nm) imaging system for myocardial sarcomeres in living mice. Using this system, we conducted three-dimensional analysis of sarcomere dynamics in left ventricular myocytes during the cardiac cycle, simultaneously with electrocardiogram and left ventricular pressure measurements. We found that (1) the working range of SL was on the shorter end of the resting distribution, and (2) the left ventricular-developed pressure was positively correlated with the SL change between diastole and systole. The present findings provide the

first direct evidence for the tight coupling of sarcomere dynamics and ventricular pump functions in the physiology of the heart (*J. Gen Physiol.*, 2016-1).

3. *In vivo* cardiac excitation-contraction coupling

We developed a novel analysis on the kinetics of $Ca^{2+}$ waves in the mouse heart with the assumption that $Ca^{2+}$ waves expand in a concentric fashion. We found that the velocity of the $Ca^{2+}$ expansion was ～120 μm/s on the confocal *X-Y* plane in a cardiomyocyte, and afterwards, the $Ca^{2+}$ wave propagated at a faster velocity in the longitudinal direction in the myocyte (～170 μm/s). These values were similar to those previously reported in isolated cardiomyocytes. Therefore, our experimental system is usefulness for the analysis of $Ca^{2+}$ waves, and even $Ca^{2+}$ sparks in future studies (*Prog. Biophys. Mol. Biol.* 2017).

## Publications

***Onda A*[1]*, Kono H*[1]*, Jiao Q*[2]*, Akimoto T*[3]*, Miyamoto T*[4]*, Sawada Y*[5]*, Suzuki K*[2]*, Kusakari Y, Minamisawa S, Fukubayashi T*[6] *(*[1]*Teikyo Univ,* [2]*Hangzhou Normal Univ,* [3]*Tokyo Univ,* [4]*Tsukuba Univ,* [5]*National Rehabilitation Center,* [6]*Waseda Univ).*** A new mouse model of skeletal muscle atrophy using spiral wire immobilization. *Muscle Nerve.* 2016; **54:** 788-91.

***Tsukamoto S, Fujii T, Oyama K, Shintani SA*[1]*, Shimozawa T*[2]*, Kobirumaki-Shimozawa F, Ishiwata S*[2]*, Fukuda N (*[1]*Tokyo Univ,* [2]*Waseda Univ).*** Simultaneous imaging of local calcium and single sarcomere length in rat neonatal cardiomyocytes using yellow Cameleon-Nano140. *J Gen Physiol*. 2016; **148:** 341-55.

***Ito K, Hongo K, Date T, Ikegami M, Hano H, Owada M, Morimoto S, Kashiwagi Y, Katoh D, Yoshino T, Yoshii A, Kimura H, Nagoshi T, Kajimura I, Kusakari Y, Akaike T, Minamisawa S, Ogawa K, Minai K, Ogawa T, Kawai M, Yajima J*[1]*, Matsuo S, Yamane T, Taniguchi I, Morimoto S*[2]*, Yoshimura M (*[1]*The Cardiovascular Institute,* [2]*Kyushu Univ).*** Tissue thrombin is associated with the pathogenesis of dilated cardiomyopathy. *Int J Cardiol*. 2016; **228:** 821-7.

***Kusakari Y, Urashima T, Shimura D, Amemiya E, Miyasaka G, Yokota S, Fujimoto Y, Akaike T, Inoue T, Minamisawa S.*** Impairment of excitation-contraction coupling in right ventricular hypertrophied muscle with fibrosis induced by pulmonary artery banding. *PLoS One*. 2017; **12:** e0169564. Epub 2017 Jan 9.

***Shimozawa T*[1]*, Hirokawa E, Kobirumaki-Shimozawa F, Oyama K, Shintani SA*[2]*, Terui T, Kushida Y, Tsukamoto S, Fujii T, Ishiwata S*[1]*, Fukuda N (*[1]*Waseda Univ,* [2]*Tokyo Univ).*** In vivo cardiac nano-imaging: A new technology for high-precision analyses of sarcomere dynamics in the heart. *Prog Biophys Mol Biol*. 2017; **124:** 31-40. Epub 2016 Sep 21.

## Reviews and Books

***Akaike T, Minamisawa S.*** Prostaglandin E-mediated Vascular Remodeling of the Ductus Arteriosus and Ductus-Dependent Congenital Heart Diseases. *J Mol Genet Med*. 2016; **10:** E109.