# 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). In addition, we developed an *in vivo* nanoimaging system to observe sarcomere contraction in the ventricles of small animals, such as the rat and mouse.

## Research Activities

*Development and pathogenesis of the great arteries*

1. Molecular mechanism of closure of the DA

The DA is a mysterious artery that is interesting to study. The DA is an essential vascular shunt between the aortic arch and the pulmonary trunk during fetal development. The DA closes immediately after birth in accordance with its smooth muscle contraction and vascular remodeling. When the DA fails to close after birth, the condition is known as patent DA, which is a common problem in premature infants. Although cyclooxygenase inhibitors are often used to treat patent DA, their efficacy is often limited. Both vascular contraction and remodeling, i.e., intimal thickening, are required for complete anatomical closure of the DA. Decreased elastogenesis is a hallmark of DA remodeling and is thought to contribute to intimal thickening of the DA. However, the molecular mechanisms of decreased elastogenesis are not fully understood. We found that prostaglandin $E_2$ ($PGE_2$) receptor EP4 signaling promotes degradation of the mature lysyl oxidase protein, a cross-linking enzyme for elastic fibers, only in the DA, leading to decreased elastogenesis. We also found that phospholipase C, but not phosphokinase A is involved in EP4-mediated degradation of the mature lysyl oxidase protein. In addition, we found that thromboxane A2 plays a role in both vasoconstriction and the promotion of vascular remodeling in the rat DA. Furthermore, using DNA microarray analyses, we examined the transcriptional profiles of the DA in the Brown-Norway rat, which often exhibits patent DA. We newly identified more than 70 DA-dominant genes that may play an important role in DA-specific functional and morphologic characteristics.

2. Molecular mechanism of elastic fiber formation in the great arteries

Abdominal aortic aneurysm (AAA) is a common but life-threatening disease among the elderly. In collaboration with Yokohama City University, we developed smooth muscle cell-derived 3-dimensional multilayers as a new experimental model for vascular elastic fiber formation studies.

*Regulation of cardiac sarcoplasmic reticulum ATPase activity*

Impaired $Ca^{2+}$ reuptake into the sarcoplasmic reticulum is thought to be a primary pathogenic mechanism of heart failure. We are interested in regulation of the sarcoplasmic reticulum $Ca^{2+}$-ATPase and $Ca^{2+}$ homeostasis in the sarcoplasmic reticulum. Using 2 types of transgenic mice that exhibited either sarcoplasmic reticulum $Ca^{2+}$ overload or $Ca^{2+}$ deficiency, the selective modulation of the sarcoplasmic reticulum $Ca^{2+}$-ATPase activity did not change $Ca^{2+}$ leak from the sarcoplasmic reticulum. Our data indicate that $Ca^{2+}$ leak is independent of sarcoplasmic reticulum $Ca^{2+}$ uptake.

*Regulation of cardiac metabolism*

Cardiac metabolism plays an essential role in maintaining cardiac function. The energy of cardiac muscle largely depends on fatty acid oxidation. It has been known that the main cardiac metabolism switches from fatty acid oxidation to glycolysis when the heart has stress. Using capillary electrophoresis and mass spectrometry, we are investigating the key metabolite(s) or enzyme for this switch when the heart begins to fail.

*Pathophysiological mechanisms of cardiac remodeling and fibrosis*

Cardiac fibrosis is a maladaptive response to pathophysiological conditions, such as in cardiac hypertrophy and ischemic heart diseases. However, the effects of interstitial fibrosis on $Ca^{2+}$ handling and contraction in myocardium remain unclear. We prepared pulmonary artery banding (PAB) rats as a model of cardiac hypertrophy. Four weeks after the operation, the right ventricular papillary muscles of the PAB rats were dissected and their tension was measured with intracellular $Ca^{2+}$ transients by means of the photoprotein aequorin. On the basis of histological analysis, papillary muscles after PAB were clearly divided into 2 groups: the interstitial fibrosis group and the nonfibrosis with hypertrophy group. Using DNA microarray analyses, we found that fibroblast growth factor 23, which is known to play a role in the regulation of osteogenesis, was up-regulated in the interstitial fibrosis group. We are now investigating the role of fibroblast growth factor 23 in the development of cardiac fibrosis.

*Mechanism of sarcomere contraction in cardiac muscle*

1. Sarcomere length nanometry in rat neonatal cardiomyocytes via expression of α-actinin-*Aequorea coerulescens* green fluorescent protein in Z-disks

In cardiac muscle, a change in sarcomere length by a mere 100 nm causes a dramatic change in contractility, indicating the need for the simultaneous measurement of sarcomere length and intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) in cardiomyocytes at high spatial and temporal resolutions. To accurately analyze the motion of individual sarcomeres with nanometer precision during excitation-contraction coupling, we applied nanometry techniques to primary-cultured rat neonatal cardiomyocytes. First, we developed an experimental system for simultaneous nanoscale analysis of single sarcomere dynamics and $[Ca^{2+}]_i$ changes via the expression of *Aequorea coerulescens* green fluorescent protein in Z-discs. We found that the averaging of the lengths of sarcomeres along the myocyte, a method generally now used in myocardial research, caused marked underestimation of sarcomere lengthening speed due to the superposition of different timings for lengthening

between sequentially connected sarcomeres. Then, we found that following treatment with ionomycin, neonatal myocytes exhibited spontaneous sarcomeric oscillations (Cell-SPOC) at partial activation with blockage of sarcoplasmic reticulum functions and that the waveform properties were indistinguishable from those obtained in electric field stimulation. The present experimental system has a broad range of possible applications for unveiling single sarcomere dynamics during excitation-contraction coupling in cardiomyocytes under various settings.

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*. We have developed a high-speed high-resolution *in vivo* cardiac imaging system in mice. This system enables 3-dimensional analysis of sarcomere dynamics during the cardiac cycle, simultaneously with electrocardiography and left ventricular pressure measurements. We demonstrated that the working range of sarcomere length exists on the shorter resting distribution side and that the developed pressure is a linear function of the sarcomere length change between diastole and systole at 100-nm levels.

## Publications

***Yokota T*[1]*, Shiraishi R*[1]*, Aida T*[1]*, Iwai K*[1]*, Liu ML*[1]*, Yokoyama U*[2]*, Minamisawa S (*[1]*Waseda Univ,* [2]*Yokohama City Univ).*** Thromboxane $A_2$ receptor stimulation promotes closure of the rat ductus arteriosus through enhancing neointima formation. *PLoS One*. 2014; **9:** e94895.

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***Aoki R*[1]*, Yokoyama U*[1]*, Ichikawa Y*[1]*, Taguri M*[1]*, Kumagaya S*[2]*, Ishiwata R*[2]*, Yanai C*[1]*, Fujita S*[1]*, Umemura M*[2]*, Fujita T*[2]*, Okumura S*[3]*, Sato M*[4]*, Minamisawa S, Asou T*[5]*, Masuda M*[1]*, Iwasaki S*[1]*, Nishimaki S*[1]*, Seki K*[1]*, Yokota S*[1]*, Ishikawa Y*[1] *(*[1]*Yokohama City Univ,* [2]*Waseda Univ,* [3]*Tsurumi Univ,* [4]*Aichi Univ,* [5]*Kanagawa Child Med Ctr).*** Decreased serum osmolality promotes ductus arteriosus constriction. *Cardiovasc Res*. 2014; **104:** 326-36.

***Jiao Q*[1]*, Sanbe A*[2]*, Zhang X*[1]*, Liu JP*[1]*, Minamisawa S (*[1]*The Affiliated Hosp Hangzhou Normal Univ,* [2]*Iwate Med Univ).*** αB-Crystallin R120G variant causes cardiac arrhythmias and alterations in the expression of $Ca^{2+}$ handling proteins and endoplasmic reticulum stress in mice. *Clin Exp Pharmacol Physiol*. 2014; **41:** 589-99.

## Reviews and Books

***Kobirumaki-Shimozawa F, Inoue T, Shintani SA*[1]*, Oyama K*[1]*, Terui T, Minamisawa S, Ishiwata S*[1]*, Fukuda N (*[1]*Waseda Univ).*** Cardiac thin filament regulation and the Frank-Starling mechanism. *J Physiol Sci*. 2014; **64:** 221-32.