# 大学院先導機構　佐藤研究室
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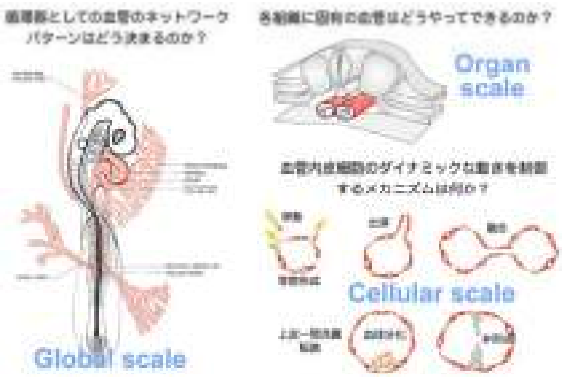
血管は、酸素や栄養を運搬するという重要な機能を果たしながら、胚のかたち作りにあわせて絶えずリモデリングを繰り返し、最終的な成体型の血管ネットワークへと近づいていくことが知られている。しかし、それらの過程がどのような内皮細胞のダイナミックなふるまいによって支えられているのかは謎である。血管網が形成されるしくみを個々の細胞の挙動の観点から直接的に理解する為、血管内皮細胞を特異的に蛍光タンパク質で標識するトランスジェニックウズラ胚を用いて、イメージング解析を行っている。血管形成を支える個々の細胞の動きを明らかにするとともに、それらの動きを制御する分子メカニズムを解明する。

Blood vessels play an important role to deliver oxygen and nutrition in the body. Their network pattern is formed by the organization of the endothelial cells provided by mesodermal tissues during embryonic development. Although a number of molecules required for blood vessel formation have been identified mainly by gene inactivation studies in mice, a detailed understanding of normal blood vessel formation and the direct effects of the genetic abnormalities have not been well performed. To take advantage of the easy accessibility to the developing embryo, Japanese quail is exploited as a model system to study blood vessel formation of higher vertebrates by a dynamic imaging approach. A transgenic quail carrying nuclear-localizing fluorescent protein with endothelial-specific promoter enables vital imaging of the endothelial cells *in vivo*. Using this model animal, we are studying molecular mechanisms of dynamic cell behaviors during blood vessel formation.

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## アウトリーチ活動　Outreach activity

1. 佐藤有紀． GE ヘルスケア はじめてのライフサイエンス基礎講座, 5 月 13 日 2015 福岡.

2. 佐藤有紀． GE ヘルスケア はじめてのライフサイエンス基礎講座, 6 月 20 日 2014 福岡.

3. 佐藤有紀． 熊本県立八代中学校 熊本大学発生医学研究所訪問 6 月 8 日 2012 熊本.

## マスメディアによる研究成果の報道・発信

1. 九大など、細胞の外側に柱状の構造体を発見しその形成機構を解明． 日本経済新聞電子版. 1 月 17 日 2017.

Original Article

Sexual Development

Sex Dev
DOI: 10.1159/000477452

Accepted: March 28, 2017
by M. Schmid
Published online: July 27, 2017

# Androgen Regulates Dimorphic F-Actin Assemblies in the Genital Organogenesis

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**Keywords**
Cell migration · Extracellular matrix · F-actin · Masculinization programming window · Urethral mesenchymal cells

**Abstract**
Impaired androgen activity induces defective sexual differentiation of the male reproductive tract, including hypospadias, an abnormal formation of the penile urethra. Androgen signaling in the urethral mesenchyme cells (UMCs) plays essential roles in driving dimorphic urethral development. However, cellular events for sexual differentiation remain virtually unknown. In this study, histological analyses, fluorescent staining, and transmission electron microscopy (TEM) were performed to reveal the cellular dimorphisms of UMCs. F-actin dynamics and migratory behaviors of UMCs were further analyzed by time-lapse imaging. We observed a prominent accumulation of F-actin with poorly assembled extracellular matrix (ECM) in female UMCs. In contrast, thin fibrils of F-actin co-aligning with the ECM through membrane receptors were identified in male UMCs. Processes for dimorphic F-actin assemblies were temporally identified during an androgen-regulated masculinization programming window and spatially distributed in several embryonic reproductive tissues. Stage-dependent modulation of the F-actin sexual patterns by androgen in UMCs was also demonstrated by time-lapse analysis. Moreover, androgen regulates coordinated migration of UMCs. These results suggest that androgen signaling regulates the assembly of F-actin from cytoplasmic accumulation to membranous fibrils. Such alteration appears to promote the ECM assembly and the mobility of UMCs, contributing to male type genital organogenesis.



The reproductive organs possess the sexually specific structures to perform the reproductive function. External genitalia are one of the representative organs showing the sexual differences, with the penile urethra being responsible for copulation and urination in the male. The genital tubercle (GT) is the common anlage for the external genitalia of both the male and the female. Androgen regulates masculinization processes of the male reproductive organs contributing to the dimorphic development of the GT. 5α-dihydrotestosterone (DHT) is the

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© 2017. Published by The Company of Biologists Ltd | Development (2017) 144, 281-291 doi:10.1242/dev.141259

The Company of Biologists

RESEARCH ARTICLE

# Basal filopodia and vascular mechanical stress organize fibronectin into pillars bridging the mesoderm-endoderm gap

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**ABSTRACT**

Cells may exchange information with other cells and tissues by exerting forces on the extracellular matrix (ECM). Fibronectin (FN) is an important ECM component that forms fibrils through cell contacts and creates directionally biased geometry. Here, we demonstrate that FN is deposited as pillars between widely separated germ layers, namely the somitic mesoderm and the endoderm, in quail embryos. Alongside the FN pillars, long filopodia protrude from the basal surfaces of somite epithelial cells. Loss-of-function of Ena/VASP, α5β1-integrins or talin in the somitic cells abolished the FN pillars, indicating that FN pillar formation is dependent on the basal filopodia through these molecules. The basal filopodia and FN pillars are also necessary for proper somite morphogenesis. We identified a new mechanism contributing to FN pillar formation by focusing on cyclic expansion of adjacent dorsal aorta. Maintenance of the directional alignment of the FN pillars depends on pulsatile blood flow through the dorsal aortae. These results suggest that the FN pillars are specifically established through filopodia-mediated and pulsating force-related mechanisms.

**KEY WORDS: Fibronectin, Filopodia, Somite, Endoderm, Dorsal aorta**

## INTRODUCTION

Three-dimensional (3D) organization of the extracellular matrix (ECM) is crucial for configuring tissue and organ shape and function (Rozario and DeSimone, 2010). Understanding how the ECM is patterned and how cells sense and react to ECM patterning are key to understanding the cellular and molecular mechanisms driving tissue morphogenesis. Several studies have shown that physical properties of the ECM, including geometry, rigidity and tension, may influence the forces exerted on cells, which in turn affect cell shape and ultimately lead to the modification of fundamental cellular processes such as migration and differentiation *in vitro* (DuFort et al., 2011). However, with regard to dynamic morphogenesis during embryo development, the interplay between cell cytoskeletal architecture and ECM properties and related tensional forces is poorly understood.

Fibronectin (FN) is an ECM component that is essential for the morphogenesis of various tissues and organs (Rozario and DeSimone, 2010). The generation of FN fibrils is connected to dynamic cell rearrangement events during tissue morphogenesis (Jülich et al., 2015; Dzamba et al., 2009; Martins et al., 2009). For example, binding of FN to α5β1-integrins and their associated cytoplasmic adaptor proteins (talin, vinculin) promotes FN polymerization into fibrils. In addition, intrinsic and/or extrinsic mechanical forces can promote FN fibrillogenesis and fibril alignment to the direction of force (Baneyx et al., 2001, 2002; Little et al., 2008; Hytönen and Wehrle-Haller, 2016). Given that FN fibers can stretch 5- to 6-fold *in vitro* (Little et al., 2008; Smith et al., 2007), it is enticing to speculate that they could be involved in matrix elasticity between separated tissues.

In early amniote embryos, the somitic mesoderm and endoderm are separated by a 40-50 µm gap. The paired dorsal aortae arise and migrate through this space (Sato, 2013). Here, we report that the gap between somitic mesoderm and endoderm is spanned by pillar-like arrangements of FN fibrils, together with long filopodia emanating from the basal surfaces of somitic epithelial cells. We discovered these FN pillars by studying FN distribution in a transgenic quail embryo model system, using tissue-specific DNA electroporation and time-lapse imaging (Bower et al., 2011). The PGK:H2B-mCherry transgenic quail embryo enables us to track every cell and ECM dynamics over time in living embryos, and to study cell-autonomous roles of electroporated genes (Huss et al., 2015). This approach allowed us to visualize the previously unknown pattern of FN in the somite-endoderm gap. In striking contrast to previously reported patterns of FN distribution near the basal surfaces of cells, we found that the FN pillars extend far away from cell bodies into the gap space. The directional arrangement of the FN pillars and their accompanying somite cell filopodia suggested that they might be patterned by a site-specific tensional force. Subsequent experiments suggest that cyclic tensional forces associated with pulsation of the dorsal aortae are involved in formation of the FN pillars.

## RESULTS

### FN forms pillars between somites and endoderm

Using confocal laser microscopy of whole-mount immunostained quail embryos at the early somitogenesis stage and subsequent reconstruction of optical sections, we observed many long FN fibrils patterned as pillars (~40-50 µm in length) in the gap between somites and endoderm tissues (lower panels in Fig. 1B,C). FN around the somite epithelium is known to be required for somite boundary formation and cell rearrangement during somite epithelialization in various vertebrate embryos (Girós et al., 2011; Jülich et al., 2015; Koshida et al., 2005; Martins et al., 2009; Rifes

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**Received 17 June 2016; Accepted 29 November 2016**

# Sall1 Maintains Nephron Progenitors and Nascent Nephrons by Acting as Both an Activator and a Repressor

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## ABSTRACT

The balanced self-renewal and differentiation of nephron progenitors are critical for kidney development and controlled, in part, by the transcription factor Six2, which antagonizes canonical Wnt signaling-mediated differentiation. A nuclear factor, Sall1, is expressed in Six2-positive progenitors as well as differentiating nascent nephrons, and it is essential for kidney formation. However, the molecular functions and targets of Sall1, especially the functions and targets in the nephron progenitors, remain unknown. Here, we report that *Sall1* deletion in Six2-positive nephron progenitors results in severe progenitor depletion and apoptosis of the differentiating nephrons in mice. Analysis of mice with an inducible *Sall1* deletion revealed that Sall1 activates genes expressed in progenitors while repressing genes expressed in differentiating nephrons. Sall1 and Six2 co-occupied many progenitor-related gene loci, and Sall1 bound to Six2 biochemically. In contrast, Sall1 did not bind to the *Wnt4* locus suppressed by Six2. Sall1-mediated repression was also independent of its binding to DNA. Thus, Sall1 maintains nephron progenitors and their derivatives by a unique mechanism, which partly overlaps but is distinct from that of Six2: Sall1 activates progenitor-related genes in Six2-positive nephron progenitors and represses gene expression in Six2-negative differentiating nascent nephrons.

*J Am Soc Nephrol* 25: 2584–2595, 2014. doi: 10.1681/ASN.2013080896

The nephron is a basic functional unit of the kidney, which includes the glomerulus, proximal and distal renal tubules, and the loop of Henle. The mammalian kidney, the metanephros, is formed by reciprocally inductive interactions between two precursor tissues, namely the metanephric mesenchyme and the ureteric bud. The mesenchyme contains nephron progenitors that express a transcription factor, Six2. When Six2-positive cells are labeled using *Six2GFPCre*, a mouse strain expressing Cre recombinase fused to green fluorescent protein (GFP) under the control of the *Six2* promoter, they give rise to nephron epithelia *in vivo*.[1] *Six2* opposes the canonical Wnt-mediated differentiation evoked by ureteric bud-derived *Wnt9b*, thereby maintaining the self-renewal of nephron progenitors.[2–4] However, the progenitors gradually lose *Six2* expression

Received August 27, 2013. Accepted February 26, 2014.

S.K., S.T., and T.O. contributed equally to this work.

Published online ahead of print. Publication date available at www.jasn.org.

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Development, Growth & Differentiation — The Japanese Society of Developmental Biologists

*Develop. Growth Differ.* (2013) **55**, 406–421 doi: 10.1111/dgd.12058

Review Article

# Transgenesis and imaging in birds, and available transgenic reporter lines

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Avian embryos are important model organism to study higher vertebrate development. Easy accessibility to developing avian embryos enables a variety of experimental applications to understand specific functions of molecules, tissue–tissue interactions, and cell lineages. The whole-mount *ex ovo* culture technique for avian embryos permits time-lapse imaging analysis for a better understanding of cell behaviors underlying tissue morphogenesis in physiological conditions. To study mechanisms of blood vessel formation and remodeling in developing embryos by using a time-lapse imaging approach, a transgenic quail model, Tg(*tie1*:H2B-eYFP), was generated. From a cell behavior perspective, Tg(*tie1*:H2B-eYFP) quail embryos are a suitable model to shed light on how the structure and pattern of blood vessels are established in higher vertebrates. In this manuscript, we give an overview on the biological and technological background of the transgenic quail model and describe procedures for the *ex ovo* culture of quail embryos and time-lapse imaging analysis.

**Key words:** blood vessels, confocal laser microscopy, quail, time-lapse imaging, transgenic bird.

## Introduction

Time-lapse microscopy is an important approach to study dynamic morphogenetic events during embryogenesis on the cellular level. Confocal laser microscopy enables the capture of high-resolution images of cells in *xyz*-dimensions at regular intervals, permitting us to trace and quantify behaviors of the cells by means of computer image analysis (or a really hard working postdoc can do it by hand). Quantitative analysis of cell behavior helps us to assess how a cell's individual behavior influences tissue shape (i.e. when a gene function is disrupted). Time-lapse imaging and analysis of cultured cells are easy to perform because the cells can be labeled efficiently with fluorescent proteins by DNA transfection. In addition, because they are not multi-layered, the number of *z*-sections is minimized and light scattering is negligible. In contrast, time-lapse imaging of developing amniote embryos is not achieved easily because large body sizes require several *xy* and numerous *z*-sections and the opaque tissue causes light scattering. In addition, drifting or "framing out" of embryos frequently occurs since they are growing in all dimensions. These issues notwithstanding, capturing cell behaviors *in vivo* is vitally important in the field of developmental biology because the tissue shape changes and functions are dynamically governed by physiological circumstances in a body. Time-lapse imaging analysis of developing embryos requires an animal model that enables: (i) fluorescent labeling of desired cells; (ii) undergoes normal development on a microscope stage and is resist to the deleterious effects of excitating light; and (iii) is amenable to experimental manipulations of its molecular and cellular components.

Developing chick and quail embryos are easily observed by creating a small hole in the eggshell; extended observation is possible if the hole is transparently resealed by using a cover slip, saran wrap, Dupont stuff. Researchers have taken advantage of this built-in culture system from hundreds of years ago (see Stern 2004 for a review), using avian embryos as a model for developmental biology. Time-lapse observation of migrating neural crest cells was performed for the first time in chick embryos *in ovo* by using fluorescent dye, for example, Rhodamine dextran-, DiI

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Received 31 December 2012; revised 10 March 2013; accepted 14 March 2013.

Development, Growth & Differentiation
The Japanese Society of Developmental Biologists

*Develop. Growth Differ.* (2013) **55**, 113–129 doi: 10.1111/dgd.12010

Review Article

# Dorsal aorta formation: Separate origins, lateral-to-medial migration, and remodeling

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Blood vessel formation is a highly dynamic tissue-remodeling event that can be observed from early development in vertebrate embryos. Dorsal aortae, the first functional intra-embryonic blood vessels, arise as two separate bilateral vessels in the trunk and undergo lateral-to-medial translocation, eventually fusing into a single large vessel at the midline. After this dramatic remodeling, the dorsal aorta generates hematopoietic stem cells. The dorsal aorta is a good model to use to increase our understanding of the mechanisms controlling the establishment and remodeling of larger blood vessels *in vivo*. Because of the easy accessibility to the developing circulatory system, quail and chick embryos have been widely used for studies on blood vessel formation. In particular, the mapping of endothelial cell origins has been performed using quail-chick chimera analysis, revealing endothelial, vascular smooth muscle, and hematopoietic cell progenitors of the dorsal aorta. The avian embryo model also allows conditional gene activation/inactivation and direct observation of cell behaviors during dorsal aorta formation. This allows a better understanding of the molecular mechanisms underlying specific morphogenetic events during dynamic dorsal aorta formation from a cell behavior perspective.

**Key words:** dorsal aorta, endoderm, notochord, quail-chick chimera, somite.



## Introduction

The developing chick embryo is easily observed by creating a small hole in the eggshell; extended observation is even possible if the hole is resealed by adhesive tape. Researchers have taken advantage of this built-in culture system for more than a century, using chick embryos as a model for developmental biology. The blood vessel network, which can be observed in the chick embryo from day 2 of incubation, is one of the prominent features of vigorous embryogenesis. Moreover, a beating heart and flowing red blood cells are the first visible vital functions of the circulatory system; therefore, many biologists have been drawn to study the blood vessels in the chick embryo (for a review, see Noden 1990). Dorsal aortae are the first intra-embryonic blood vessels to arise in the trunk. Primary dorsal aortae comprise a pair of longitudinal vessels in which the anterior ends are connected to the nascent heart via outflow tracts and the posterior parts are linked to vitelline arteries at the umbilicus level. In addition to its critical function as the largest vessel to circulate blood through the embryo's body, the dorsal aorta is known to be a place of secondary embryonic hematopoiesis (for reviews, see Dieterlen-Lievre & Le Douarin 2004; Jaffredo *et al.* 2005; Adamo & Garcia-Cardena 2012). The dorsal aorta also acts as a signaling center, providing instructive signals to induce pancreas differentiation, migration of neural crest cells, and subsequent specification of cell-types (Reissmann *et al.* 1996; Schneider *et al.* 1999; Lammert *et al.* 2001; Yoshitomi & Zaret 2004; Saito *et al.* 2012). Moreover, the dorsal aorta does not resemble peripheral blood vessels, which are typically seen in small organs and cancer, in terms of its diameter, the number of endothelial cells incorporated within the vessel, and its blood flow volume. Studies of dorsal aorta formation during embryogenesis will provide clues to understanding the mechanisms through which large blood vessel formation is achieved *in vivo*. This review presents the dynamic process of dorsal aorta formation and describes recent discoveries related to regulatory mechanisms that have been revealed by studies using avian (mostly quail and chick) embryo models.

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Received 2 September 2012; revised 19 September 2012; accepted 19 September 2012.