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論文内容の要旨
Summary of thesis contents

Physical forces are considerable regulatory factors for a broad range of cellular processes. Studies using cultured cells have shown that cells can sense various mechanical stresses, and that these mechanical stimuli affect cell proliferation, differentiation, polarity, and migration. In addition to molecules, such physical forces have recently been found to have important functions in tissue morphogenesis and animal development. Animal development involves successive cell movements and tissue rearrangements that transform a mass of embryonic cells into complex organ structures. Thus, given that these dynamic events occur under the spatial constraint of the embryo size, shape changes and movements of particular cells and tissues are thought to generate physical forces that deform neighboring cells and tissues. However, important questions regarding to the force-involved processes have not been fully answered, such as 1) which tissue generates the force, 2) how much force is generated during tissue movements, and 3) which tissue and biological events were affected by the generated force. For better understanding of how physical forces are involved in the regulation of early embryogenesis, an integrated analysis of tissue movements and characterization of the force in morphogenetic events is necessary.

In this study, I investigated the involvement of the physical force in the gastrulation process. Gastrulation is a dynamic process of tissue remodeling during early development in various kinds of animals. Gastrulation involves complex and dramatic cell shape changes and tissue rearrangements to form organs at the right time and location and to establish the proper body plan. Thus, it has been thought that those tissue remodeling involved in gastrulation could generate physical forces and that play important roles for later morphogenesis, in addition to chemical signals. The cell movements and signaling pathways involved in gastrulation have been extensively studied using *Xenopus laevis*. In addition, relatively large *Xenopus* embryos are easily handled, which is advantageous for measuring and applying physical forces making the *Xenopus* gastrula as an excellent model for addressing the above questions. Here, I focused on an actively migrating mesodermal tissue, the leading-edge mesoderm (LEM) which shows directional migration during *Xenopus* gastrulation.

The LEM consists of mesendodermal cells derived from the peripheral region of the blastocoel floor and is attached to the blastocoel roof (BCR) of the early gastrula embryo. Firstly, the most dorsal LEM involutes and contacts to the BCR. Next, LEM migrates collectively toward the animal pole as a cell stream on the substrate that coats the inner surface of the BCR, and then subsequent dorsal tissues invaginate. It has previously been shown that collective cell migration can generate and exert forces on the trailing cells. Therefore, I presumed that the actively migrating LEM could generate physical forces during *Xenopus* gastrulation, and attempted to investigate about the magnitude and possible functions of the force which had not been

characterized to date but important to understand the possible role of the force in gastrulation.

First, I constructed an assay system that measures the physical force generated by the anterior migration of LEM. It was previously reported that the substrate coats on the basal side of BCR could be transferred on culture dishes, and thus I used this method with minor modifications. On the copied BCR substrate (BCR-coating), LEM explant isolated from early-gastrula migrated unidirectionally, which reproduced the movement in vivo, suggesting that the assay system I established is suitable for the force measurement of LEM migration.

Next, I directly measured the actual force generated by LEM using a micro-needle assay. To determine the amplitude of the force generated by the migrating LEM, I prepared thin and flexible micro-glass needle whose spring constant was pre-determined prior to the experiments. The needle was placed in front of the migrating LEM, and the needle was bent by the migrating LEM. As the LEM migrated anteriorly on the BCR-coated dish, the deflection of the needle increased. Based on the maximum deflection of the glass needle and its known spring constant, the maximum force generated by the LEM was estimated. Through this experiment, I estimated that the physical force generated by LEM explant (500 x 500 μm; almost equal to the size of the LEM which locates at dorsal region) was approximately 40 nN on average. Interestingly, I also found that the tissue-generated force increased in a tissue-size dependent manner. These results indicated that LEM indeed act as a considerable force generator during gastrulation.

The finding that LEM has an ability to generate the force by its migration also suggested the possibility that LEM influences the force distribution of the neighbor tissues during gastrulation. I next focused on the axial mesoderm (AM) which is the prospective notochord located and connected at the posterior side of the LEM. It has been known that the AM undergoes convergent extension (CE) movement from mid-gastrula stage, that is a key process of body axis elongation during gastrulation. However, in early to mid gastrulation stage, the AM neither show directional migration toward the animal pole nor cause active elongation while the LEM actively migrates anteriorly. Therefore, there was the possibility that the LEM pulls the AM along anterior-posterior (A-P) direction by their directional migration in early-mid gastrula. In order to investigate that, I performed laser ablation experiments and observed the recoils generated on the tissues after ablation because the magnitude of such recoils become large when the tissue receives tensions. In this experiment, I prepared two types of explants: one containing migratory LEM, and one lacking it. I found a high deformation field in the AM with LEM, but less recoil in the cutting edges of the AM that lacked LEM. These results demonstrated that LEM indeed exerts pulling forces on AM and affect the force distribution of the AM along A-P direction. I also measured the force generated in the explants in which the connections between the LEM, AM, and ectoderm were maintained (LAE explants). The magnitude of the LEM-generated force in the LAE explants was smaller than in the LEM-only explants,

suggesting that the AM consumed the force while LEM migrated. Thus, I proposed that LEM has an ability to pull the AM along the A-P direction, like train with an engine pulls passenger cars.

The next question was that whether migrating LEM has important functions for the morphogenesis of the *Xenopus* gastrula embryo. To address that, I knocked down fibronectin (FN) in BCR to inhibit the LEM migratory activity with a non-destructive method. I performed BCR-targeted injection of antisense Morpholino oligonucleotide specific to FN (FN-MO). It depleted FN protein exclusively in the BCR region but not in the dorsal mesoderm, suggesting that only the interaction between the BCR and LEM was inhibited. Using the migration assay and laser ablation, I confirmed that BCR-targeted injection of FN-MO could reduce migratory activity of the LEM and removed tension on the AM in both in vivo and in vitro. In this condition, BCR-injected embryos (BCR FN-MO) exhibited a higher frequency of gastrulation defects and wider and shorter notochord. Detailed observations revealed that the notochord malformation in embryos with reduced LEM motility was due to the misorientation and aborted elongation of the AM cells, indicating the CE movement was hampered.

These results suggested that the force generated by the directional migration of LEM is transmitted to AM and supports its elongation in vivo. Taken these results and findings in previous reports together, I finally proposed several interpretations that LEM plays a considerable role in AM elongation through a force-mediated process in addition to the Wnt/PCP pathway.

In summary, this study unveiled the magnitude of force involved in a developmental process and proposed mechano-regulatory mechanisms of *Xenopus* CE movement and notochord formation.

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博士論文の審査結果の要旨

Summary of the results of the doctoral thesis screening

申請者は、アフリカツメガエル原腸胚をモデルとして、生物の発生に含まれる物理的な力の存在、そしてその力が形態形成においてどのような役割を持つかについて研究した。申請者は、ツメガエル原腸胚の組織の中で、背側に位置する中胚葉で、いち早く陥入し動物極側（将来の頭部）へ向かって胞胚腔蓋(BCR)上を積極的に移動する能力を持つ先行中胚葉(leading edge mesoderm, LEM)に着目した。申請者はこの能動的に移動する組織が胚内、とくに後方で移動する脊索中胚葉（axial mesoderm, AM）を牽引する力を生み出している可能性が高いと予想し、LEM の組織移動が生み出す力を計測した。まず、培養皿上に BCR の基質を写しとり、生体外で単離した LEM の移動を再現する系を確立した。次に、マイクロガラスニードルを用いて、移動する LEM が生み出す力の計測を行った。同実験によって LEM が細胞移動によって約 40 nN の力を生み出していることを明らかにした。次に、LEM によって生み出される力が LEM の後方に位置し将来脊索となる AM 組織に影響を及ぼしているかどうかを明らかにするために、レーザーアブレーション実験を行って組織内の力場を推定した。AM 領域における力の分布が前方に接し移動する LEM の有無によって変化するかどうかを調べた結果、LEM 存在下では非存在下に比較して、AM のレーザー焼灼後の反動が大きい傾向にあることがわかった。これにより、LEM の移動が AM の力分布を変化させる能力を持っている事が実験的に示された。この力発生源としての LEM 移動の形態形成における意義を調べるために、LEM の移動に必要なファイブロネクチン(FN)基質を BCR 特異的にノックダウンすることを通して LEM の移動阻害を行った。その結果、LEM の移動能を減じさせた胚では、AM における張力が減少し、全胚レベルでは原腸陥入異常や脊索の伸長異常が見られた。異常胚の AM 領域を細胞レベルで観察すると、正常胚で見られる内外軸方向への細胞の整列や伸長、相互入り込みが起こっていないことが分かった。こうした内外軸に沿って起こる細胞形態変化や運動は正常な前後軸方向への脊索の伸長に必要とされており、これらの結果から LEM の移動やその結果生まれる力が内外軸方向への細胞の整列や相互入り込みの制御をしている可能性が示された。過去の知見より、AM の伸長および脊索形成には細胞増殖因子シグナル Wnt/PCP 経路による細胞骨格やその関連因子の制御が重要であることが知られていることから、同シグナル経路による制御と本研究で見出された LEM による制御の関連を FN のノックダウンと Wnt/PCP シグナル経路の阻害を組み合わせることによって検証した。その結果、それぞれ単独の効果よりも、両者の同時機能阻害によって表現型は重篤になることが分かった。これは、脊索の伸長には AM 内における Wnt/PCP シグナル経路による制御と、LEM から受ける外的な力学制御が必要であることを示唆するものである。

本研究を通して、原腸胚に含まれる細胞移動が力を発生する能力を持っていることが明らかとなり、さらにその生まれる力を計測することに初めて成功した。また、この胚内に生まれた力が後方に接する脊索中胚葉組織の力分布に影響を与え、後の形態形成現象を制御する新しいモデルが示された。以上の結果は、生物の初期胚内において組織の移

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動によって物理的な力が実際に発生し、それによって形態形成が制御されていることを示す重要な結果であり、学位論文審査に十分値する研究であると判断した。