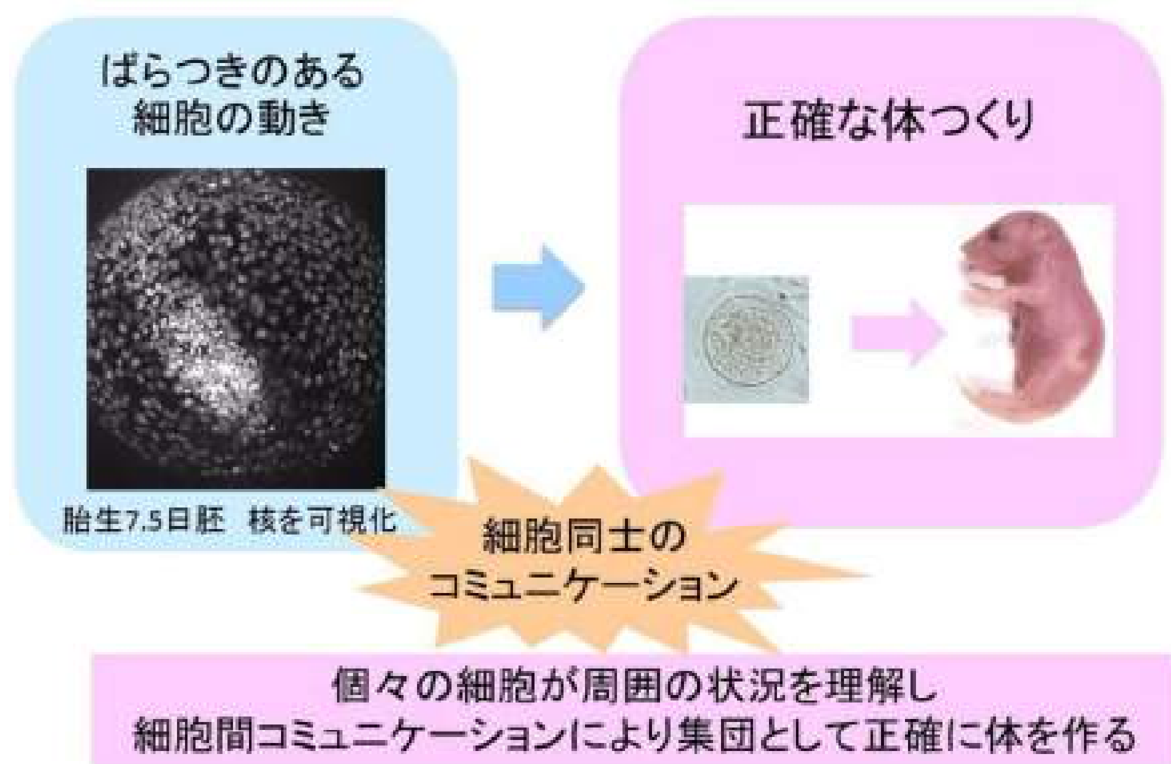
分化制御分野
Department of Cell Fate Control

正確であることは、発生の重要な性質の一つである。当分野では、マウス初期胚を研究対象として、正確な発生を可能にする細胞間のコミュニケーションの役割とそのしくみについて研究する。具体的には、細胞間コミュニケーションに重要な働きをしている Hippo シグナル経路に注目して、Hippo シグナルの制御機構、Hippo シグナルによる着床前胚の細胞分化の制御機構、Hippo シグナルによる細胞競合機構とその胚発生における役割について研究を行うとともに、Hippo シグナルの制御にもかかわる、物理的な力が胚発生の制御に果たす役割、についても明らかにする。正確性は発生だけでなく、組織の恒常性の維持にも重要な働きをしており、本研究の成果を広く個体という細胞集団の制御機構の解明につなげたいと考えている。

Accuracy is an important feature of development. In our laboratory, we focus on the intercellular communication, which support accurate development, and study the roles and the mechanisms underlying it. Particularly, we focus on the Hippo signaling pathway, which plays central roles in intercellular communication, and address the mechanisms of regulation of the Hippo signaling, cell fate control in preimplantation embryos by Hippo signaling, cell competition mediated by Hippo signaling. We also study the roles of physical forces in regulation of embryonic development, because forces regulates the Hippo signaling.



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当分野は、２０１０年９月に教授着任、２０１１年４月に研究室開設、２０１５年３月に転出。

研究概略　Projects

正確であることは、発生の重要な性質の一つである。発生中の胚内の個々の細胞はダイナミックに変化しており、その挙動や状態にはばらつきがある。一方で、最終的に作られる、体や臓器は常に正確であり、正確性の破綻は奇形や発生停止につながる。このように発生が正確であるためには、個々の細胞が、隣接細胞とコミュニケーションすることで、細胞が自身が置かれた環境や隣接細胞の状態を認識し、自身や隣接細胞の挙動を制御することで、全体として調和のとれたものにすることが必要である。我々のこれまでの研究から、その様な細胞間のコミュニケーションに Hippo シグナル経路が関わっていることが明らかになってきた。そこで、と研究室では、Hippo シグナル経路に注目して、その制御機構や細胞間コミュニケーション機構、マウス初期胚発生における役割の研究を中心に研究することで、発生の正確性の分子基盤を明らかにすることを目指す。細胞間のコミュニケーションによる正確性保証機構は胚発生だけでなく、組織の恒常性の維持にも重要な働きをしており、本研究の成果を広く個体という細胞集団の制御機構の解明につなげることを視野に研究を進めている。

1. Hippo シグナルによる着床前胚の細胞分化の制御

着床前のマウス胚は胚盤胞初期までに胎盤を作る栄養外胚葉と胚の体を作る内部細胞塊の 2 つの細胞を作る。その 2 つの細胞の分化は、細胞系譜によらず、胚の中の細胞の位置によって決まる。我々はこれまでに、この細胞分化は、細胞の位置依存的な Hippo シグナルの活性化によって制御されていることを明らかにした

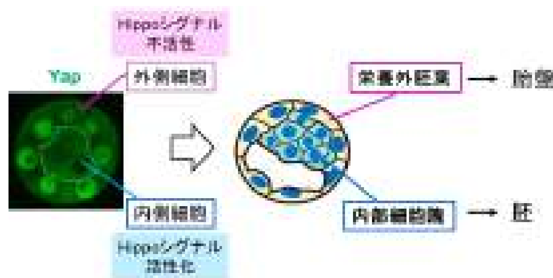

図1. 細胞の位置依存的なHippoシグナル活性化が細胞分化を制御する

(Nishioka et al. *Dev Cell* 2009) (図 1)。すなわち、外側の細胞は Hippo シグナルが働かないため、転写共役因子 Yap が核移行して転写因子 Tead4 が活性化して栄養外胚葉特異的遺伝子を発現させる。逆に内側の細胞は Hippo シグナルが働くことで Yap が核に入らず Tead4 が活性を持たないために内部細胞塊へと分化する。

我々は、細胞位置依存的な Hippo シグナルの制御機構として、以下のことを見出した。Hippo シグナルの活性化は接着結合における細胞間接着によること。一方で、外側の細胞は Par-aPKC 系による頂端—基底の上皮様の細胞極性を持っており、極性化していることで細胞間接着による Hippo シグナルの活性化が抑制されている。即ち、胚内の細胞は、接着と極性化の情報の組合せにより、細胞の位置情報を Hippo シグナルの制御に用いていることが分った (Hirate et al. *Curr Biol* 2013) (図 2) 。

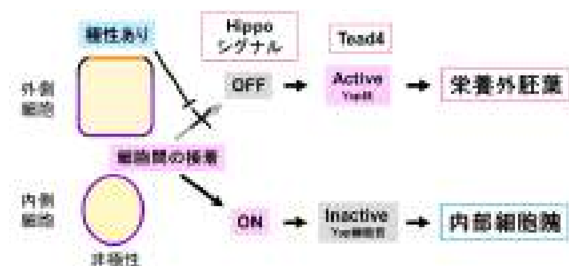

図2. 細胞間接着と細胞極性による細胞位置依存的なHippoシグナル制御

上記の研究は 32 細胞期胚を用いて行ったが、最初に内側の細胞がつくられる 16 細胞期胚においては、若干異なる制御が行われていることもわかってきた。16 細胞期胚では Par-aPKC 系を破壊しても Hippo シグナルの弱い制御が見られ、Hippo シグナルの制御には Par-aPKC 依存的な物と非依存的なものとが協調的に働いていることが明らかになった。また、8 細胞期から 16 細胞期への分裂の多くが極性化細胞と非極性化細胞とを作る非対称分裂であり、それが Hippo シグナルの制御に大きな役割をしていることも明らかになった (Hirate et al *Develop Growth Differ* 2015)。

2. Hippo シグナルの制御機構

着床前胚の研究より、Hippo シグナルの活性

化には接着結合が必要であることが分ったが、その分子基盤として、細胞接着因子 E-cadherin の細胞内ドメインに Nf2, Angiomotin (Amot)が結合することが必須であることを見出した。特に Amot (及びその関連因子 Amotl2)のリン酸化が鍵となっており、Amot はアクチン結合能を持つが、E-cadherin 複合体に結合すると Lats によって Ser176 がリン酸化されてアクチン結合能を失い、同時に Lats との結合が安定化して Hippo シグナル経路を活性化する。さらに、着床前胚の外側の細胞で接着による Hippo シグナルの活性化が起こらないのは、極性化すると Amot が側方基底の接着結合から排除されてしまうためであることも見出した(Hirate et al. *Curr Biol* 2013) (図 3)。また、Amot が頂端面に存在することが Amotl2 による Hippo 経路の活性化の抑制に関わっていることも見出した (Hirate & Sasaki *Tissue Barriers* 2014)。細胞極性化による Amot の局在制御機構の解明がこれからの課題である。

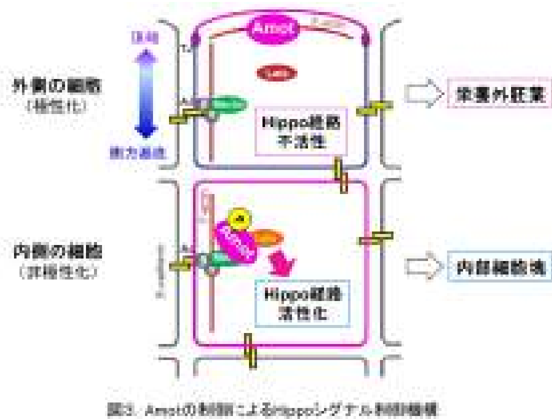

図3. Amotの制御によるHippoシグナル制御機構

3. Hippo シグナルによる細胞競合

古くから、培養細胞の増殖は細胞の密度に依存していることが、細胞増殖の接触阻止として知られていたが、我々はこれまでのマウス胚由来の線維芽細胞株 NIH3T3 を用いた研究において、細胞増殖の接触阻止の分子基盤は、細胞密度による Hippo シグナルの制御、さらにその結果としておこる Tead の転写活性の制御であることを示していた (Ota & Sasaki *Development* 2008)。その研究では Tead 活性の強弱が細胞増殖速度の増減とコンフルエント時の細胞数の増減につながることを示したが、このような Tead 活性を操作した細胞と正常細胞とを混ぜて共培養すると、細胞間コミュニケーションが起こり、単独培養時と比べて増殖が大きく変化することを見出した(Mamada et al *J Cell Sci* 2015)。Tead 活性を弱くした細胞は、正常細胞と共培養すると細胞の増殖が強力に抑制されてコンフルエントになると死にはじめ、逆に Tead 活性を強くした細胞は、正常細胞と共培養すると細胞は増殖を続け、コンフルエントを過ぎると共培養している正常細胞が死んで数が減少した。すなわち相対的に Tead 活性の高い細胞が勝者になるような細胞競合が起こった。さらに Tead は Myc の発現を制御しており、Tead 活性の異なる細胞が接する所において勝者細胞において Myc の高発現が見られた。Myc の過剰発現細胞は細胞競合の勝者になった。Myc の発現は Tead の制御を受けているが、細胞増殖の促進と細胞競合活性には Tead 活性と Myc の発現の両方が必要であり、両者が協調的に働いて細胞競合を制御していると考えられた(Mamada et al *J Cell Sci* 2015) (図 4)。この性質は上皮組織であるショウジョウバエの成虫原基で見られる細胞競合機構と類似したものであり、生物種・細胞種を問わない普遍的な細胞競合制御機構であると考えらえた。今後は、細胞競合のしくみを明らかにすること、さらに、このような細胞競合が、実際に生体内で起こっているのか、もし起こっているとすれば、マウス胚の発生どのような役割をはたしているのかを明らかにすることが重要な課題である。

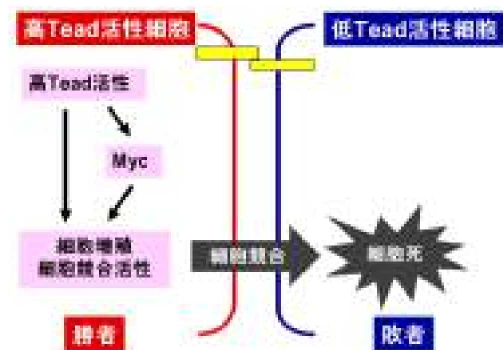

図4. NIH3T3細胞におけるTead活性とMycによる細胞競合機構

4. 物理的な力による胚の形態形成の制御

発生中の胚内の細胞の動態を観察するために、ノード、脊索、内胚葉で発現する *Foxa2* 遺伝子のコーディング配列の下流に 2A 配列を用いて核に局在する蛍光タンパク質 H2B-EGFP の

cDNA を導入したノックインマウス系統を樹立した (Imuta et al. *Genesis* 2013)。このマウスを用いて胎生 7.5 日胚を顕微鏡下で培養しながら脊索形成の様子をライブイメージングしたところ、脊索が収斂伸長を起こす際に、同時に各細胞の向きが左右方向から前後方向へと次第に変化した。この様子から、脊索脊索が前後方向の張力を受けることで収斂伸長が起こっていると考え、その張力の発生源として、発生に伴う羊膜腔の膨張力を考えた。実際、脊索細胞は前後軸方向へ偏った張力を受けていることが示され、羊膜腔の膨張を抑えて培養すると収斂伸長と細胞向きの変化が抑制された。力学モデルを作成すると羊膜腔は均等に膨張するが脊索が前後方向に長い形をしていることで、前後方向への張力を受けることが示された。また、張力が収斂伸長に関わる平面細胞極性を制御していることもわかった。これらのことから、羊膜腔の膨張力が脊索を前後に引っ張る力となり、マウス初期胚の形態形成に重要な役割をはたしていることがあきらかになった (Imuta et al. *Mech Dev* 2014)。(図 5)

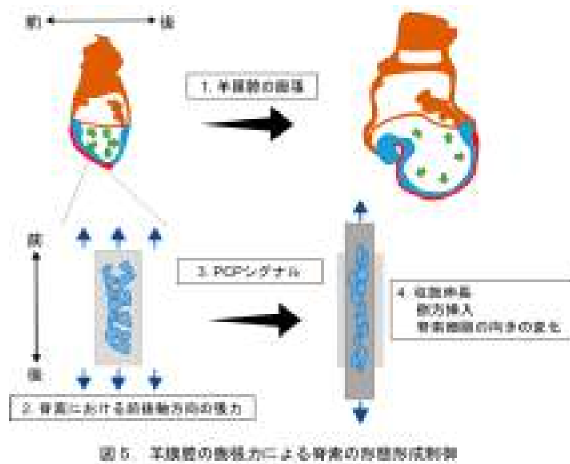

図5 羊膜腔の膨張力による脊索の形態形成制御

Accuracy is an important feature of embryonic development. In developing embryos, cells dynamically changes their behaviors, and variations are present in behaviors and conditions (e.g. gene expression, shape etc.) among cells. In spite of such variations, the final organs and bodies formed through development are always very accurate, and disruption of developmental accuracy should cause malformation, dysfunction and/or developmental arrest. To achieve such an accurate development, intercellular communications are important. With intercellular communications, cells recognize their environment and/or the status of their neighboring cells, and control their behaviors to coordinate cellular behaviors as a whole. We previously showed that the Hippo signaling plays key roles in such communications. We aim to reveal the molecular mechanisms that support accurate development. For this purpose, we focus on the Hippo signaling pathway and study its roles in development and the mechanisms controlling it.

1. Regulation of differentiation in preimplantation mouse embryos.

During preimplantation development, mouse embryos forms blastocyst which consists of two types of cells: the trophectoderm (TE), which later forms a placenta, and the inner cell mass (ICM), which form an embryo proper. Differentiation of these two cell types is controlled by cell positions within the embryo. Previously, we revealed that this cell position-dependent differentiation is controlled by cell position-dependent activation of the Hippo signaling pathway.

We studied the mechanisms underlying regulation of the Hippo signaling by cell position, and found the following mechanisms. Cell-cell adhesions via adherens jucntions activate the Hippo pathway, while polarization of the outer cells via Par-aPKC system inhibits activation of the pathway by adherens junctions (AJs). Thus, the cells use information of cell-cell adhesion and polarization to convert the information of cell position to Hippo signaling.

2. Regulation of Hippo signaling pathway

Studies in preimplantation embryos revealed importance of AJs for activation of the Hippo pathway. We also found that binding of angiomotin (Amot) and Nf2 proteins to the intracellular domain of E-cadherin is required for Hippo pathway activation by AJs. Phosphorylation of Amot is the key step for pathway activation. Amot has an actin-binding activity, but, upon binding to E-cadherin complex, Ser176 of Amot is phosphorylated by Lats protein kinase. This phosphorylation inhibits actin-binding activity, stabilizes interaction with Lats, and activates the Hippo pathway. In the outer cells, cell polarization sequesters Amot from basolateral AJs to free apical domains, thereby inhibit Hippo pathway activation by AJs. The mechanisms by which cell polarization controls Amot remains to be elucidated.

3. Cell competition via Hippo signaling.

We previously showed that the molecular basis of contact inhibition of cell proliferation is the regulation of transcriptional activity of the Tead family transcription factors by controlling Hippo signaling. We used NIH3T3 mouse embryonic fibroblast cell line for this study, and showed that increase or decrease of activities of Tead transcription factors increased or decreased proliferation of cells, respectively. We found that co-culture of Tead activity-manipulated cells with normal cells dramatically altered proliferation of both cells. In the co-culture of low Tead-activity cells and normal cells, proliferation of the low Tead-activity cells was strongly suppressed, and the cells started to die when the total cell number reached to the confluence. In contrast, in the co-culture of the high Tead-activity cells and normal cells, the high Tead-activity cells continued proliferation without stopping, and the co-cultured normal cells start to die once the total cell number exceeded the confluence. These results indicate that co-culture of the cells with different Tead activities causes cell competition, in which the cells with relatively higher Tead activity become the winners. Tead regulates expression of Myc, and higher Myc expression is induced in the winner cells at the interface of different Tead activity cells. The competitive activity and proliferation of the cell is regulated by both Tead activity and Myc expression. Such mechanism is similar to that of cell competition in *Drosophila* imaginal discs. The important questions to be addressed are whether Tead activity triggered cell competition also takes place in developing embryos, and if this is the case, what is the role of it.

4. Mechanical control of morphogenesis.

To study the dynamic cell behaviors in mouse embryos, we established the transgenic mouse line, in which a fluorescent protein H2B-EGFP is expressed in the node, notochord and endoderm from *Foxa2* locus. Live imaging of postimplantation embryos of this mouse line revealed that the convergent-extension morphogenetic movement of the notochord is accompanied by alteration of cell orientation from the left-right axis to the antero-posterior (A-P) axis. We hypothesized that such cellular behaviors are caused by application of an anisotropic stretching force along A-P axis to the notochord. Indeed, we found that expansion of amniotic cavity provide the extension force to the notochord. In addition, mathematical simulation and physical modeling revealed that the rectangular morphology of the notochord caused application of the anisotropic force along long axis (A-P axis) from isotropic expansion of the amniotic cavity. This mechanical force is also an upstream regulator of planar cell polarity signaling, which is required for convergent-extension morphogenesis of the notorhocd.

論文目録　Publications

1. Hirate Y, Hirahara S, Inoue K-i, Kiyonari H, Niwa H, Sasaki H. Par-aPKC-Dependent and -Independent Mechanisms Cooperatively Control Cell Polarity, Hippo Signaling, and Cell Positioning in 16-Cell Stage Mouse Embryos. ***Dev Growth Differ*** 57: 544-556, 2015.

2. Mamada H, Sato T, Ota M, Sasaki H. Cell competition in mouse NIH3T3 embryonic fibroblasts is controlled by the activity of Tead family proteins and Myc. ***J Cell Sci*** 128: 790-803, 2015.

3. Wicklow E, Blij S, Frum R, Hirate Y, Lang RA, Sasaki H, Ralston A. HIPPO pathway members restrict SOX2 to the inner cell mass where it promotes ICM fates in the mouse blastocyst. ***PLoS Genet*** 10: e1004618, 2014.

4. Rayon T, Menchero S, Nieto A, Xenopoulos P, Crespo M, Cockburn K, Cañon S, Sasaki H, Hadjantonakis AK, de la Pompa JL, Rossant J, Manzanares M. Notch and hippo converge on cdx2 to specify the trophectoderm lineage in the mouse blastocyst.Mechanical control of notochord morphogenesis by extra-embryonic tissues in mouse embryos. ***Dev Cell*** 30: 410-422, 2014.

5. Imuta Y, Koyama H, Shi D, Eiraku M, Fujimori T, Sasaki H Mechanical control of notochord morphogenesis by extra-embryonic tissues in mouse embryos. ***Mech Dev*** 132: 44-58, 2014.

6. Taguchi A, Kaku Y, Ohmori T, Sharmin S, Ogawa M, Sasaki H, Nishinakamura R. Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells. ***Cell Stem Cell*** 14: 53-67, 2014.

7. Hirate Y, Hirahara S, Inoue K-i, Suzuki A, Alarcon VB, Akimoto K, Hirai T, Hara T, Adachi M, Chida K, Ohno S, Marikawa Y, Nakao K, Shimono A, Sasaki H. Polarity-dependent distribution of angiomotin localizes Hippo signaling in preimplantation embryos. ***Curr Biol*** 23: 1181-1194, 2013.

8. Imuta Y, Kiyonari H, Jang CW, Behringer RR, Sasaki H. Generation of knock-in mice that express nuclear EGFP and tamoxifen-inducible Cre recombinase in the notochord from Foxa2 and T loci. ***Genesis*** 51: 210-218, 2013.

9. Hirate Y, Cockburn K, Rossant J, Sasaki H Tead4 is constitutively nuclear, while nuclear vs. cytoplasmic Yap distribution is regulated in preimplantation embryos. ***Proc Natl Acad Sci USA*** 109:E3389-90, 2012

10. Yoshiba S, Shiratori H, Kuo IY, Kawasumi A, Shinohara K, Nonaka S, Asai Y, Sasaki G, Belo JA, Sasaki H, Nakai J, Dworniczak B, Ehrlich BE, Pennekamp P, Hamada H. Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. ***Science*** 338: 226-231, 2012

11. Tao H, Inoue KI, Kiyonari H, Bassuk AG, Axelros JD, Sasaki H, Aizawa S, Ueno N Nuclear localization of Prickle2 is required to establish cell polarity during early mouse embryogenesis. ***Dev Biol*** 364: 138-148, 2012.

著書・総説目録　Publications

1. Sasaki H. Position- and polarity-dependent Hippo signaling regulates cell fates in preimplantation mouse embryos. ***Semin Cell Dev Biol***: 47-48:80-7, 2015.

2. 佐藤卓史、佐々木洋. Hippo シグナルによる細胞競合の制御. **実験医学** 33: 2920-2925, 2015.

3. Hirate Y, Sasaki H. The role of angiomotin phosphorylation in the Hippo pathway during preimplantation mouse development. ***Tissue Barriers***:2, e28127, 2014.

4. Kondoh H, Kuroiwa A (eds.) New Principles in Developmental Processes, Springer. Sasaki H "Position-dependent Hippo signaling controls cell fates in preimplantation mouse embryos" pp 249-264, 2014.

5. Oren M., Aylon Y. (eds.) The Hippo Signalin Papthway and Cancer, Springer. Sasaki H. "Role of Hippo signaling in early mouse development" pp 41-54, 2013.

学会・研究集会 発表目録　Meeting Presentations

1. Sasaki, H. Tead and Myc cooperatively regulates cell competition in mouse NIH3T3 embryonic fibroblasts. 1st International Symposium on Cell Competition,"Cell Competition in Development and Cancer", Shiran Kaikan, Kyoto Univ, Kyoto, Japan, Sep 10th, 2015.

2. 佐々木洋　マウス胚由来線維芽細胞 NIH3T3 における細胞競合. 第 67 回日本細胞生物学会　シンポジウム　細胞社会を支える適者生存システム―細胞競合の分子基盤とその役割― タワーホール船堀、東京, 2015 年 7 月 1 日.

3. Sasaki, H. Roles of Tead family proteins in cell competition in mouse NIH3T3 embryonic fibroblasts. Keystone Symposium, "The Hippo Pathway: Signaling, Development and Disease", Sagebrush Inn & Suites, Taos, New Mexico USA, May 17 - 21, 2015.

4. 佐々木洋　細胞極性依存的な angiomotin の局在制御が着床前胚の Hippo シグナルを制御する. 第 87 回日本生化学会大会、国立京都国際会館, 2014 年 10 月 15-18 日.

5. Sasaki H. Mechanisms of cell fate specification in preimplantation mouse embryos. KEY Forum: From Stem Cells to Organs, Kumamoto City Medical Association Hall, Kumamoto, Japan, Sep 4-5, 2014.

6. Sasaki H. Mechanical control of notochord morphogenesis by extra-embryonic tissues in mouse embryos. International Symposium on Mechanobiology 2014, Okayama University, Japan, May 20-23, 2014.

7. 佐々木洋　Hippo pathway regulation by cell morphology and stress fibers. 第 34 回日本分子生物学会年会、福岡国際会議場・マリンメッセ福岡、2012 年 12 月 11-14 日.

8. 佐々木洋　Tead regulated contact-mediated cell competition by controlling Hippo signaling and Myc expression. 第 85 回日本生化学会大会、福岡国際会議場、2012 年 12 月 14-16 日.

9. 佐藤卓史、儘田博志、大田光徳、佐々木洋 Molecular mechanisms of contact-mediated cell competition regulated by activity of a transcription factor Tead. 第 34 回日本分子生物学会年会、福岡国際会議場・マリンメッセ福岡、2012 年 12 月 11-14 日.

10. Sasaki H. Mechanisms of trophectoderm fate specification in preimplantation embryos. Hong Kong Society for Developmental Biology Symposium "From Embryology to Disease Mechanisms" In honor of Patrick Tam "60 years & still gastrulating." Hong Kong Academy of Medicine, Hong Kong, China, November 26-27, 2012.

11. Sasaki H. Mechanisms of position-dependent specification of cell fates in preimplantation embryos. Swiss-Japanese Developmental Biology Meeting, Kyoto, Japan, November 5-9, 2012.

12. Sasaki H. Mechanisms of position-dependent specification of cell fates in preimplantation embryos. EMBO/EMBL Symposium: Germline Immortality through Totipotency. Heidelberg, Germany, October 13-16, 2012.

13. Sasaki H. Mechanisms of position-dependent specification of cell fates in preimplantation embryos. Mouse Molecular Genetics Meeting. Asilomar Conference Center in Pacific Grove, CA, USA, October 2-6, 2012.

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Original Article

Par-aPKC-dependent and -independent mechanisms cooperatively control cell polarity, Hippo signaling, and cell positioning in 16-cell stage mouse embryos

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In preimplantation mouse embryos, the Hippo signaling pathway plays a central role in regulating the fates of the trophectoderm (TE) and the inner cell mass (ICM). In early blastocysts with more than 32 cells, the Par-aPKC system controls polarization of the outer cells along the apicobasal axis, and cell polarity suppresses Hippo signaling. Inactivation of Hippo signaling promotes nuclear accumulation of a coactivator protein, Yap, leading to induction of TE-specific genes. However, whether similar mechanisms operate at earlier stages is not known. Here, we show that slightly different mechanisms operate in 16-cell stage embryos. Similar to 32-cell stage embryos, disruption of the Par-aPKC system activated Hippo signaling and suppressed nuclear Yap and Cdx2 expression in the outer cells. However, unlike 32-cell stage embryos, 16-cell stage embryos with a disrupted Par-aPKC system maintained apical localization of phosphorylated Ezrin/Radixin/Moesin (p-ERM), and the effects on Yap and Cdx2 were weak. Furthermore, normal 16-cell stage embryos often contained apolar cells in the outer position. In these cells, the Hippo pathway was strongly activated and Yap was excluded from the nuclei, thus resembling inner cells. Dissociated blastomeres of 8-cell stage embryos form polar–apolar couplets, which exhibit different levels of nuclear Yap, and the polar cell engulfed the apolar cell. These results suggest that cell polarization at the 16-cell stage is regulated by both Par-aPKC-dependent and -independent mechanisms. Asymmetric cell division is involved in cell polarity control, and cell polarity regulates cell positioning and most likely controls Hippo signaling.

Key words: asymmetric cell division, cell polarity, Hippo signaling, Par-aPKC, preimplantation embryo.

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Introduction

Before implantation in the uterus, mouse embryos undergo several rounds of cell division and form a cyst-like structure called the blastocyst (Yamanaka *et al.* 2006; Sasaki 2010, 2015). The early blastocyst contains two types of cells, the trophectoderm (TE) and the inner cell mass (ICM). The TE is an outer epithelial structure required for implantation that later gives rise to placental tissues, whereas the ICM is a mass of pluripotent cells surrounded by the TE that later forms the embryo proper and some extraembryonic tissues.

Formation of the TE and ICM is the first cell fate specification in mouse development, and it has been

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RESEARCH ARTICLE

Cell competition in mouse NIH3T3 embryonic fibroblasts is controlled by the activity of Tead family proteins and Myc

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ABSTRACT

Cell competition is a short-range communication originally observed in *Drosophila*. Relatively little is known about cell competition in mammals or in non-epithelial cells. Hippo signaling and its downstream transcription factors of the Tead family, control cell proliferation and apoptosis. Here, we established an *in vitro* model system that shows cell competition in mouse NIH3T3 embryo fibroblast cells. Co-culture of Tead-activity-manipulated cells with normal (wild-type) cells caused cell competition. Cells with reduced Tead activity became losers, whereas cells with increased Tead activity became super-competitors. Tead directly regulated *Myc* RNA expression, and cells with increased Myc expression also became super-competitors. At low cell density, cell proliferation required both Tead activity and Myc. At high cell density, however, reduction of either Tead activity or Myc was compensated for by an increase in the other, and this increase was sufficient to confer 'winner' activity. Collectively, NIH3T3 cells have cell competition mechanisms similar to those regulated by Yki and Myc in *Drosophila*. Establishment of this *in vitro* model system should be useful for analyses of the mechanisms of cell competition in mammals and in fibroblasts.

KEY WORDS: Cell competition, Tead, Myc, NIH3T3 cells

INTRODUCTION

Communication among cells plays important roles in the regulation of embryonic development and maintenance of tissue homeostasis. Among the various types of communication, cell competition is a unique type of short-range communication originally identified in *Drosophila*. Cells compare their fitness level with those of neighboring cells, and cells with relatively higher fitness become winners, and cells with relatively lower fitness are eliminated as losers (for reviews, see Baker, 2011; Levayer and Moreno, 2013; Vincent et al., 2013; Amoyel and Bach, 2014).

In *Drosophila* imaginal discs, several genes affecting cell proliferation cause cell competition. *Minute* mutants, which have mutations in ribosomal genes, were originally reported to show cell competition (Morata and Ripoll, 1975; Simpson, 1979). Subsequently, *Myc* involvement was identified (de la Cova et al., 2004; Moreno and Basler, 2004). Cells with additional copies of *Myc* become 'super-competitors' and can eliminate neighboring wild-type cells. More recently, another growth regulator, Hippo, was found to be involved in cell competition (Neto-Silva et al., 2010; Ziosi et al., 2010). Hippo signaling suppresses cell proliferation by repressing nuclear localization of the coactivator protein Yorkie (Yki) (for reviews, see Pan, 2010; Halder and Johnson, 2011; Yu and Guan, 2013). Yki-overexpressing cells become super-competitors and *Yki* mutant cells become losers. Together, Yki and the transcription factor Scalloped (Sd) induce Myc, and Yki–Sd and Myc cooperate in growth regulation (Neto-Silva et al., 2010).

Regulators of apico-basal cell polarity also play important roles in cell competition-like selection of cells. Cells mutant for the tumor suppressor genes *scribble (scrib)*, *lethal giant larvae* (*lgl*), and *discs large* (*dlg*) lose cell polarity, and these cells are eliminated by surrounding normal epithelial cells (Brumby and Richardson, 2003; Grzeschik et al., 2007; Ohsawa et al., 2011). This differs slightly from cell competition as described above, and is sometimes referred to as intrinsic tumor suppression (Igaki, 2009). Defects in cell polarity regulate the Hippo pathway (Chen et al., 2010; Grzeschik et al., 2010; Ling et al., 2010; Robinson et al., 2010), which is a major inducer of selection. An Lgl-interacting protein, Mahjong (Mahj; also known as VprBP in mammals), is also a downstream effector of selection (Tamori et al., 2010).

Although Myc-regulated cell competition has been most studied, other mechanisms exist. Steep differences in Wingless (Wg) signaling trigger cell competition independently of Myc (Vincent et al., 2011). Differences in the activity of the transcription factor STAT also induce cell competition independently of Myc, Yki and Wg (Rodrigues et al., 2012).

Cell competition is also present in mammals. Cells from the mouse *Minute* mutant *Belly spot and tail* (*Bst*), which contains a mutation in the ribosomal protein gene *Rpl24* (Oliver et al., 2004), became losers when chimeric mice were created with wild-type embryonic stem cells. In MDCK epithelial cells, cells knocked down for the polarity regulators Mahj (Tamori et al., 2010) or Scribble (Norman et al., 2012), or expressing active Ras (Hogan et al., 2009) or Src (Kajita et al., 2010) are selectively eliminated through intrinsic tumor suppression. In mouse hematopoietic stem and progenitor cells, p53-mediated cell competition selects the least damaged cells (Bondar and Medzhitov, 2010). More recently, an involvement of Myc in cell competition has been identified. In the epiblast of early post-implantation stage mouse embryos and in embryonic stem cells, Myc-driven cell competition eliminates unfit cells at the onset of differentiation (Clavería et al., 2013; Sancho et al., 2013). In spite of recent progress, data on cell competition in mammals remains limited. Additionally, because cell competition has primarily been studied in epithelial cells, it is unknown whether cell competition also takes place in fibroblasts.

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HIPPO Pathway Members Restrict SOX2 to the Inner Cell Mass Where It Promotes ICM Fates in the Mouse Blastocyst

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Abstract

Pluripotent epiblast (EPI) cells, present in the inner cell mass (ICM) of the mouse blastocyst, are progenitors of both embryonic stem (ES) cells and the fetus. Discovering how pluripotency genes regulate cell fate decisions in the blastocyst provides a valuable way to understand how pluripotency is normally established. EPI cells are specified by two consecutive cell fate decisions. The first decision segregates ICM from trophectoderm (TE), an extraembryonic cell type. The second decision subdivides ICM into EPI and primitive endoderm (PE), another extraembryonic cell type. Here, we investigate the roles and regulation of the pluripotency gene *Sox2* during blastocyst formation. First, we investigate the regulation of *Sox2* patterning and show that SOX2 is restricted to ICM progenitors prior to blastocyst formation by members of the HIPPO pathway, independent of CDX2, the TE transcription factor that restricts *Oct4* and *Nanog* to the ICM. Second, we investigate the requirement for *Sox2* in cell fate specification during blastocyst formation. We show that neither maternal (M) nor zygotic (Z) *Sox2* is required for blastocyst formation, nor for initial expression of the pluripotency genes *Oct4* or *Nanog* in the ICM. Rather, Z *Sox2* initially promotes development of the primitive endoderm (PE) non cell-autonomously via FGF4, and then later maintains expression of pluripotency genes in the ICM. The significance of these observations is that 1) ICM and TE genes are spatially patterned in parallel prior to blastocyst formation and 2) both the roles and regulation of *Sox2* in the blastocyst are unique compared to other pluripotency factors such as *Oct4* or *Nanog*.

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Introduction

To create and use pluripotent stem cells, it is essential to understand the origins of pluripotency during normal development. During mouse blastocyst formation, pluripotent epiblast (EPI) cells are established by two cell fate decisions that segregate pluripotent progenitors from extraembryonic tissues [1,2]. During the first cell fate decision, trophectoderm (TE) is segregated from inner cell mass (ICM) prior to blastocyst formation. During the second cell fate decision, the ICM is subdivided into EPI and primitive endoderm (PE) lineages after blastocyst formation. Recent studies have examined the roles and regulation of pluripotency genes, such as *Oct4*, *Nanog*, and *Sox2*, during establishment of EPI cells in the blastocyst [3–12], but aspects of the roles and regulation of *Sox2* in the blastocyst are unresolved. For example, several studies reported that *Sox2* is restricted to the ICM by the blastocyst stage [3,13–15], but the molecular mechanisms regulating *Sox2* expression in the blastocyst are unknown.

In addition to the unresolved mechanism by which *Sox2* expression is patterned, the functional roles of *Sox2* in the blastocyst are not yet clear. ES cells cannot be derived from embryos lacking zygotic (Z) *Sox2* [5], indicating that *Sox2* is essential for pluripotency. In ES cells, *Sox2* is required for the expression of pluripotency genes, such as *Oct4* and *Nanog*, and for the repression of TE genes [16–20]. Therefore, *Sox2* might be required for initial expression of pluripotency genes and repression of TE genes in the ICM. However, the expression of pluripotency and TE genes in *Sox2* Z null blastocysts has not yet been examined at the level of individual cells. Moreover, maternal (M) *Sox2* is also thought to participate in blastocyst formation, which could partially compensate for loss of Z *Sox2*. RNAi knockdown of M and Z *Sox2* in the zygote was reported to disrupt blastocyst formation [6]. However, RNAi knockdown embryos do not always phenocopy MZ null embryos [3,21]. Because understanding the regulation and roles of SOX2 in the blastocyst is key to understanding the molecular regulation of preimplantation

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Notch and Hippo Converge on *Cdx2* to Specify the Trophectoderm Lineage in the Mouse Blastocyst

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SUMMARY

The first lineage choice in mammalian embryogenesis is that between the trophectoderm, which gives rise to the trophoblast of the placenta, and the inner cell mass, from which is derived the embryo proper and the yolk sac. The establishment of these lineages is preceded by the inside-versus-outside positioning of cells in the early embryo and stochastic expression of key transcription factors, which is then resolved into lineage-restricted expression. The regulatory inputs that drive this restriction and how they relate to cell position are largely unknown. Here, we show an unsuspected role of Notch signaling in regulating trophectoderm-specific expression of *Cdx2* in cooperation with TEAD4. Notch activity is restricted to outer cells and is able to influence positional allocation of blastomeres, mediating preferential localization to the trophectoderm. Our results show that multiple signaling inputs at preimplantation stages specify the first embryonic lineages.

INTRODUCTION

Upon fertilization, the unicellular mammalian zygote undergoes a series of equal cell divisions that in 4 days produces a 60- to 100-cell blastocyst, in which the first embryonic lineages—the trophectoderm (TE) and the inner cell mass (ICM)—have been established (Stephenson et al., 2012). How this initial lineage choice occurs and what genetic components constitute the system that controls this process have been actively studied for the past 20 years. Key transcriptional regulators of these early lineages identified in the mammalian preimplantation embryo include OCT4, NANOG, and SOX2 for the ICM and CDX2 for the TE (Cockburn and Rossant, 2010). These factors determine blastocyst lineages, but the onset of their expression is stochastic (Dietrich and Hiiragi, 2007), and later restriction to a particular cell type occurs as a downstream effect of earlier events related to the position of blastomeres in the embryo (Rossant and Tam, 2009).

This process is believed to involve differences in polarity and adhesion between inner and outer cells that are related to differential activation of the Hippo signaling pathway (Hirate et al., 2013; Nishioka et al., 2009), resulting in sustained and restricted expression of genes, such as *Cdx2* in the outer cells of the future trophectoderm (Cockburn and Rossant, 2010). Hippo signaling is switched off in outer cells, leading to nuclear localization of the transcriptional coactivator YAP, which can then activate downstream target genes through interaction with the transcription factor TEAD4. Embryos lacking *Tead4* fail to develop the TE, and *Cdx2* expression is not maintained (Nishioka et al., 2008; Yagi et al., 2007). Correspondingly, overexpression of LATS2 kinase (an activator of Hippo) reduces expression of CDX2 in outer cells, whereas embryos lacking *Lats1* and *Lats2* express CDX2 in inner cells (Lorthongpanich et al., 2013; Nishioka et al., 2009). Similar results are obtained when other components of the pathway, such as *Nf2* and *Amot*, are disrupted (Cockburn et al., 2013; Hirate et al., 2013). How these components are integrated to fully define lineage restriction in the blastocyst is largely unknown, and additional inputs remain to be identified (Wennekamp et al., 2013). Reconstruction of this process will require detailed understanding of the transcriptional control of the key lineage regulators acting in the preimplantation embryo.

With this aim in mind, we have searched for *cis*-regulatory elements responsible for trophectoderm-restricted expression of *Cdx2*, and through the analysis of one such enhancer, we have uncovered a role of the Notch signaling pathway. We find

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Mechanical control of notochord morphogenesis by extra-embryonic tissues in mouse embryos

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ABSTRACT

Mammalian embryos develop in coordination with extraembryonic tissues, which support embryonic development by implanting embryos into the uterus, supplying nutrition, providing a confined niche, and also providing patterning signals to embryos. Here, we show that in mouse embryos, the expansion of the amniotic cavity (AC), which is formed between embryonic and extraembryonic tissues, provides the mechanical forces required for a type of morphogenetic movement of the notochord known as convergent extension (CE) in which the cells converge to the midline and the tissue elongates along the antero-posterior (AP) axis. The notochord is stretched along the AP axis, and the expansion of the AC is required for CE. Both mathematical modeling and physical simulation showed that a rectangular morphology of the early notochord caused the application of anisotropic force along the AP axis to the notochord through the isotropic expansion of the AC. AC expansion acts upstream of planar cell polarity (PCP) signaling, which regulates CE movement. Our results highlight the importance of extraembryonic tissues as a source of the forces that control the morphogenesis of embryos.
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1. Introduction

Mammalian embryos develop inside of the liquid-filled cavities that are formed by extraembryonic tissues (Fig. 1A).

The development of embryos is coordinated with extraembryonic tissue formation, which support embryonic development by implanting embryos into the uterus (Cha et al., 2012), supplying nutrition (Bielinska et al., 1999), providing a confined

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Redefining the In Vivo Origin of Metanephric Nephron Progenitors Enables Generation of Complex Kidney Structures from Pluripotent Stem Cells

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SUMMARY

Recapitulating three-dimensional (3D) structures of complex organs, such as the kidney, from pluripotent stem cells (PSCs) is a major challenge. Here, we define the developmental origins of the metanephric mesenchyme (MM), which generates most kidney components. Unexpectedly, we find that posteriorly located T^+ MM precursors are developmentally distinct from $Osr1^+$ ureteric bud progenitors during the postgastrulation stage, and we identify phasic Wnt stimulation and stage-specific growth factor addition as molecular cues that promote their development into the MM. We then use this information to derive MM from PSCs. These progenitors reconstitute the 3D structures of the kidney in vitro, including glomeruli with podocytes and renal tubules with proximal and distal regions and clear lumina. Furthermore, the glomeruli are efficiently vascularized upon transplantation. Thus, by reevaluating the developmental origins of metanephric progenitors, we have provided key insights into kidney specification in vivo and taken important steps toward kidney organogenesis in vitro.

INTRODUCTION

Despite the successful achievement of various types of tissues from pluripotent stem cells (PSCs), kidney generation in vitro has remained a challenge for developmental biology and regenerative medicine (Murry and Keller, 2008; Williams et al., 2012). This is partly because the specification of the kidney lineage in vivo has not been well clarified, owing to its complex processes involving the formation of three consecutive primordia (pronephros, mesonephros, and metanephros) during embryogenesis (Saxen, 1987). The pronephros and mesonephros eventually degenerate in females, whereas a portion of the mesonephros in males contributes to a drainage system for the future testis, including the epididymis. The kidney derives from the embryonic metanephros, which develops at the most posterior part of the body trunk. The metanephros is formed by reciprocally inductive interactions between two precursor tissues, namely the metanephric mesenchyme (MM) and the ureteric bud. Cell fate analyses have shown that both the MM and ureteric bud derive from the intermediate mesoderm, which appears around embryonic day 8.5 (E8.5) and expresses the transcription factor *Osr1* (Mugford et al., 2008b). However, the mechanisms underlying how the nascent mesoderm becomes committed to the intermediate mesoderm and how the MM and ureteric bud lineage segregate from one another have not been clarified, despite many reports showing the importance of various growth factors during kidney development (Costantini and Kopan, 2010; Fleming et al., 2013; Kim and Dressler, 2005; Moriya et al., 1993; Poladia et al., 2006). Moreover, it remains to be elucidated how the anteroposterior axis is formed along the intermediate mesoderm and gives rise to the posteriorly located metanephros. In the present study, we addressed these questions by in vivo lineage-tracing experiments together with in-vitro-directed differentiation systems utilizing sorted embryonic kidney precursors at each developmental stage. Importantly, by establishing conditions for the later stages first, and then moving backward to the earlier stages, we were able to optimize the multistep culture conditions from embryonic precursors toward nephron progenitors. Finally, the protocol established by these strategies was successfully applied to the induction of metanephric nephron progenitors from mouse embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs), thereby advocating a model for kidney lineage specification.

RESULTS

The $Osr1^+$/$Integrina8^+$/$Pdgfra^-$ Population Represents Colony-Forming Nephron Progenitors

The MM gives rise to the epithelia of glomeruli (i.e., podocytes) and renal tubules, which constitute the major parts of the nephrons, as shown by cell fate analyses involving labeling of

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Article

Polarity-Dependent Distribution of Angiomotin Localizes Hippo Signaling in Preimplantation Embryos

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Summary

Background: In preimplantation mouse embryos, the first cell fate specification to the trophectoderm or inner cell mass occurs by the early blastocyst stage. The cell fate is controlled by cell position-dependent Hippo signaling, although the mechanisms underlying position-dependent Hippo signaling are unknown.
Results: We show that a combination of cell polarity and cell-cell adhesion establishes position-dependent Hippo signaling, where the outer and inner cells are polar and nonpolar, respectively. The junction-associated proteins angiomotin (Amot) and angiomotin-like 2 (Amotl2) are essential for Hippo pathway activation and appropriate cell fate specification. In the nonpolar inner cells, Amot localizes to adherens junctions (AJs), and cell-cell adhesion activates the Hippo pathway. In the outer cells, the cell polarity sequesters Amot from basolateral AJs to apical domains, thereby suppressing Hippo signaling. The N-terminal domain of Amot is required for actin binding, Nf2/Merlin-mediated association with the E-cadherin complex, and interaction with Lats protein kinase. In AJs, S176 in the N-terminal domain of Amot is phosphorylated by Lats, which inhibits the actin-binding activity, thereby stabilizing the Amot-Lats interaction to activate the Hippo pathway.
Conclusions: We propose that the phosphorylation of S176 in Amot is a critical step for activation of the Hippo pathway in AJs and that cell polarity disconnects the Hippo pathway from cell-cell adhesion by sequestering Amot from AJs. This mechanism converts positional information into differential Hippo signaling, thereby leading to differential cell fates.

Introduction

During preimplantation development, mouse embryos form blastocysts that comprise two cell types: the outer epithelial trophectoderm (TE) layer and the inner cell mass (ICM). TE is required for implantation and later contributes to the placenta. ICM further differentiates into the pluripotent epiblast, which later forms the embryo proper and the primitive endoderm.

Historically, two models have been proposed for the first cell fate specification process: the inside-outside (or positional) model [1], in which the cell position within the embryo specifies the cell fate, and the polarity model [2], in which the acquisition of cell polarity at the eight-cell stage is a critical step in the establishment of differential cell fates. The polarity model was further developed to include the promotion of TE fate based on the presence of the apical domain [3, 4]. We recently found that Hippo signaling pathway components, i.e., the TEAD family transcription factor Tead4 [5–7], its coactivator proteins Yap (encoded by *Yap1*) and Taz (encoded by *Wwtr1*), and the protein kinases Lats1/2, play critical roles in this cell fate specification process [6, 8]. In the inner cells, cell-cell adhesions activate Hippo signaling, which inactivates Tead4 by suppressing the nuclear accumulation of Yap. In the outer cells, weak Hippo signaling facilitates the nuclear accumulation of Yap. The resulting active Tead4-Yap complex induces the TE-specific transcription factors Cdx2 and Gata3, which promote differentiation into TE [6, 9]. Therefore, establishment of position-dependent Hippo signaling is a critical step during differential cell fate specification, which supports the inside-outside model [6, 8]. We previously proposed that a possible mechanism for differential Hippo signaling may be differences in the degrees of cell-cell contacts between the inner and outer cells [6]. However, the exact mechanisms underlying position-dependent Hippo signaling remain largely unknown.

In support of the polarity model, several recent studies have suggested the importance of cell polarity during TE development. The Par-aPKC system plays central roles in the regulation of the apicobasal polarity of cells (see reviews in [10–12]). Knockdown of *Pard6b* resulted in the reduced expression of Cdx2 and the failure of functional TE formation [13]. The complete absence of E-cadherin disrupted cell polarization, while the membrane localization of PKCζ correlated with the nuclear accumulation of Yap and the expression of Cdx2 [14]. These observations suggest that cell polarity is probably important for cell fate specification and the regulation of Hippo signaling in preimplantation embryos. Studies in *Drosophila* also suggest that the cell polarity regulators Crumbs and aPKC control Hippo signaling in epithelial cells

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TECHNOLOGY REPORT

Generation of Knock-in Mice That Express Nuclear Enhanced Green Fluorescent Protein and Tamoxifen-inducible Cre Recombinase in the Notochord from *Foxa2* and *T* loci

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Summary: The node and the notochord are important embryonic signaling centers that control embryonic pattern formation. Notochord progenitor cells present in the node and later in the posterior end of the notochord move anteriorly to generate the notochord. To understand the dynamics of cell movement during notochord development and the molecular mechanisms controlling this event, analyses of cell movements using time-lapse imaging and conditional manipulation of gene activities are required. To achieve this goal, we generated two knock-in mouse lines that simultaneously express nuclear enhanced green fluorescent protein (EGFP) and tamoxifen-inducible Cre, CreER^{T2}, from two notochord gene loci, *Foxa2* and *T* (*Brachury*). In *Foxa2*$^{nEGFP\text{-}CreERT2/+}$ and *T*$^{nEGFP\text{-}CreERT2/+}$ embryos, nuclei of the *Foxa2* or *T*-expressing cells, which include the node, notochord, and endoderm (*Foxa2*) or wide range of posterior mesoderm (*T*), were labeled with EGFP at intensities that can be used for live imaging. Cre activity was also induced in cells expressing *Foxa2* and *T* 1 day after tamoxifen administration. These mice are expected to be useful tools for analyzing the mechanisms of notochord development. genesis 51:210–218, 2013. © 2013 Wiley Periodicals, Inc.

Key words: node; notochord; *Foxa2*; *T* (*Brachyury*); dual labeling; nuclear EGFP; inducible Cre

During mouse embryogenesis, the organizer and its descendant signaling centers play central roles in the establishment of the correct body plan (Davidson and Tam, 2000; Niehrs, 2004; Tam and Behringer, 1997). The midline mesodermal tissue, the notochord, is the major signaling center that controls trunk and tail development. The notochord expresses both signaling molecules and their antagonists, thereby regulating the differentiation, growth, and patterning of the surrounding tissues (Cleaver and Krieg, 2001). Development of the notochord proceeds from an anterior to posterior direction through continuous addition of the more-posterior cells in coordination with the posterior extension of the body axis. The notochord develops from

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Tead4 is constitutively nuclear, while nuclear vs. cytoplasmic Yap distribution is regulated in preimplantation mouse embryos

Home et al. (1) propose a provocative model of cell fate specification in preimplantation embryos, in which regulated nuclear localization of Tead4 controls trophectoderm vs. inner cell mass (ICM) formation, whereas its coactivator protein, Yap, is present in all nuclei. This model is inconsistent with our model in which position-dependent Hippo/Lats signaling regulates nuclear versus cytoplasmic Yap distribution whereas Tead4 is constitutively nuclear (2). Our model is consistent with the canonical Hippo signaling pathways conserved from flies to mammals (3).

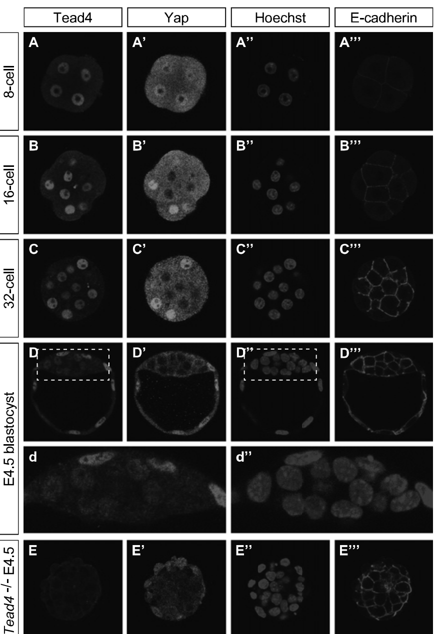

Fig. 1. Distribution of Tead4 proteins in preimplantation mouse embryos. Confocal images of WT embryos stained with anti-Tead4 (ab58310; Abcam; *A–E*), anti-Yap1 (5) (*A′–E′*), Hoechst (*A′′–E′′*), and anti–E-cadherin (*A′′′–E′′′*) antibodies. *d* and *d′′*: Enlarged images of boxed areas of *D* and *D′′*. Confocal images of a single *z*-section are shown for each panel. Similar results were obtained with the following numbers of embryos analyzed for each stage: 8-cell, *n* = 2; 16-cell, *n* = 5; 32-cell, *n* = 6; embryonic day (E) 4.5 Tead4$^{+/+}$, *n* = 5; and embryonic day 4.5 Tead4$^{-/-}$, *n* = 5.

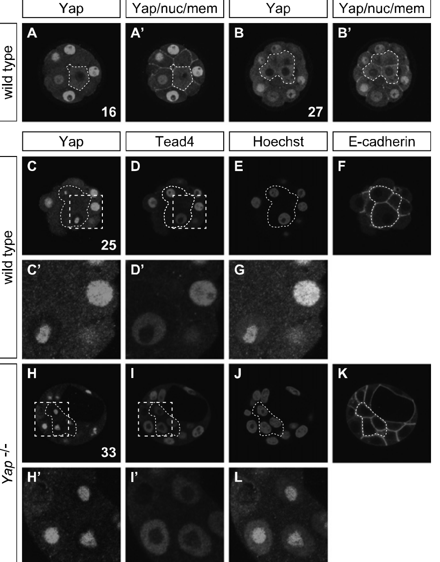

Fig. 2. Comparison of signal distributions with different anti-Yap antibodies. Confocal images of WT (*A–G*) and Yap mutant embryos (*H–L*) stained with anti-Yap1 (H00010413-M01; Abnova; *A*, *A′*, *B*, and *B′*), anti-Yap (no. 4912; Cell Signaling Technology; *C* and *H*), anti-Tead4 (ab58310; Abcam; *D* and *I*), Hoechst (*A′*, *B′*, *E*, and *J*), and anti–E-cadherin (*A′*, *B′*, *F*, and *K*) antibodies. Inner cells are outlined with thin broken lines. *A′* and *B′*: Merged images of anti-Yap staining of *A* and *B* with Hoechst (blue) and anti–E-cadherin (purple) staining. *C′*, *D′*, *H′*, and *I′*: Enlarged images of boxed areas of *C*, *D*, *H*, and *I*, respectively. *G* and *L*: Merged images of *C′* and *D′* and of *H′* and *I′*, respectively. Confocal images of a single *z*-section are shown for each panel. Numbers in *A*, *B*, *C*, and *H* indicate the nuclear numbers in the embryos. Similar results were obtained with the following numbers of embryos for anti-Yap (no. 4912; Cell Signaling Technology): Yap$^{+/+}$, *n* = 3; and Yap$^{-/-}$, *n* = 2.

Because both models are highly dependent on the antibodies used for Tead4 and Yap, we repeated the immunostaining experiments involving preimplantation embryos with the antibodies used by Home et al. (1).

We first examined Tead4 protein distribution in preimplantation embryos using the anti-Tead4 antibody described in the study of Home et al. (1) (ab58310; Abcam). Tead4 signals were detected in the nuclei of all blastomeres, including the inner cells of 16- and 32-cell–stage embryos (Fig. 1 *A–C*). Position-dependent differences in signal intensities gradually became evident from the 32-cell stage onward (Fig. 1 *B* and *C*), leading to clear differences in the ICM and trophectoderm of late blastocysts (Fig. 1*D*). Despite very weak signals in the ICM, the

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Our limited understanding of how genes relate to phenotypes makes it impossible to predict the functional consequences of these changes. However, diseases caused by mutations in genes offer clues as to which organ systems particular genes may affect. Of the 34 genes with clear associations with human diseases that carry fixed substitutions changing the encoded amino acids in present-day humans, four (*HPS5*, *GGCX*, *ERCC5*, and *ZMPSTE24*) affect the skin and six (*RP1L1*, *GGCX*, *FRMD7*, *ABCA4*, *VCAN*, and *CRYBB3*) affect the eye. Thus, particular aspects of the physiology of the skin and the eye may have changed recently in human history. Another fixed difference occurs in *EVC2*, which when mutated causes Ellis–van Creveld syndrome. Among other symptoms, this syndrome includes taurodontism, an enlargement of the dental pulp cavity and fusion of the roots, a trait that is common in teeth of Neandertals and other archaic humans. A Denisovan molar found in the cave has an enlarged pulp cavity but lacks fused roots (*2*). This suggests that the mutation in *EVC2*, perhaps in conjunction with mutations in other genes, has caused a change in dental morphology in modern humans.

We also examined duplicated regions larger than 9 kilobase pairs (kbp) in the Denisovan and present-day human genomes and found the majority of them to be shared (*8*). However, we find 10 regions that are expanded in all present-day humans but not in the Denisovan genome. Notably, one of these overlaps a segmental duplication associated with a pericentric inversion of chromosome 18. In contrast to humans, the Denisovan genome harbors only a partial duplication of this region, which suggests that a deletion occurred in the Denisovan lineage. However, we are unable to resolve if the pericentric inversion is indeed present in Denisovans.

Implications for archaic and modern human history. It is striking that genetic diversity among Denisovans was low although they were present in Siberia as well as presumably in Southeast Asia where they interacted with the ancestors of present-day Melanesians (*6*). Only future research can show how wide their geographic range was at any one time in their history. However, it is likely that they have expanded from a small population size with not enough time elapsing for genetic diversity to correspondingly increase. When technical improvements such as the one presented here will make it possible to sequence a Neandertal genome to a quality comparable to the Denisovan and modern genomes, it will be important to clarify whether the temporal trajectory of Neandertal effective population size matches that of the Denisovans. If that is the case, it is likely that the low Denisovan diversity reflects the expansion out of Africa of a population ancestral to both Denisovans and Neandertals, a possibility that seems compatible with the dates for population divergences and population size changes presented.

By providing a comprehensive catalog of features that became fixed in modern humans after their separation from their closest archaic relatives, this work will eventually lead to a better understanding of the biological differences that existed between the groups. This should ultimately aid in determining how it was that modern humans came to expand dramatically in population size as well as cultural complexity while archaic humans eventually dwindled in numbers and became physically extinct.

References and Notes

1. R. E. Green *et al.*, *Science* **328**, 710 (2010).
2. D. Reich *et al.*, *Nature* **468**, 1053 (2010).
3. J. J. Hublin, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 16022 (2009).
4. J. Krause *et al.*, *Nature* **464**, 894 (2010).
5. A. Gibbons, *Science* **333**, 1084 (2011).
6. D. Reich *et al.*, *Am. J. Hum. Genet.* **89**, 516 (2011).
7. H. A. Burbano *et al.*, *Science* **328**, 723 (2010).
8. Materials and methods are available as supplementary materials on *Science* Online.
9. A. W. Briggs *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 14616 (2007).
10. L. Orlando *et al.*, *Genome Res.* **21**, 1705 (2011).
11. M. Kircher, S. Sawyer, M. Meyer, *Nucleic Acids Res.* **40**, e3 (2012).
12. H. Li, R. Durbin, *Bioinformatics* **25**, 1754 (2009).
13. A. McKenna *et al.*, *Genome Res.* **20**, 1297 (2010).
14. R. E. Green *et al.*, *Cell* **134**, 416 (2008).
15. M. Goodman, *Am. J. Hum. Genet.* **64**, 31 (1999).
16. J. Pickrell, J. Pritchard, Inference of population splits and mixtures from genome-wide allele frequency data. *Nature Precedings* (2012); http://precedings.nature.com/documents/6956/version/1.
17. P. Skoglund, M. Jakobsson, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 18301 (2011).
18. M. Currat, L. Excoffier, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 15129 (2011).
19. R. J. Petit, L. Excoffier, *Trends Ecol. Evol.* **24**, 386 (2009).
20. J. A. Coyne, H. A. Orr, in *Speciation and its Consequences*, D. Otte, and J. A. Endler, Eds. (Wiley, New York, 1989), pp. 180–207.
21. J. R. Kidd, F. L. Black, K. M. Weiss, I. Balazs, K. K. Kidd, *Hum. Biol.* **63**, 775 (1991).
22. H. Li, R. Durbin, *Nature* **475**, 493 (2011).
23. D. F. Conrad *et al.*, *Nat. Genet.* **43**, 712 (2011).
24. C. C. Cerqueira *et al.*, *Am. J. Hum. Biol.* **24**, 705 (2012).
25. J. W. IJdo, A. Baldini, D. C. Ward, S. T. Reeders, R. A. Wells, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9051 (1991).
26. R. M. Durbin *et al.*, *Nature* **467**, 1061 (2010).
27. S. C. Vernes *et al.*, *N. Engl. J. Med.* **359**, 2337 (2008).
28. W. Enard *et al.*, *Cell* **137**, 961 (2009).

Acknowledgments: The Denisovan sequence reads are available from the European Nucleotide Archive (ENA) under study accession ERP001519. The present-day human sequence reads are available from the Short Read Archive (SRA) under accession SRA047577. Alignments and genotype calls for each of the sequenced individuals are available at www.eva.mpg.de/denisova/. In addition, the Denisovan sequence reads and alignments are available as a public data set via Amazon Web Services (AWS) at http://aws.amazon.com/datasets/2357/ and as a track in the University of California, Santa Cruz genome browser. We thank D. Falush, P. Johnson, J. Krause, M. Lachmann, S. Sawyer, L. Vigilant and B. Viola for comments, help, and suggestions; A. Aximu, B. Höber, B. Höffner, A. Weihmann, T. Kratzer, and R. Roesch for expert technical assistance; R. Schultz for help with data management; and M. Schreiber for improvement of graphics. The Presidential Innovation Fund of the Max Planck Society made this project possible. D.R. and N.P. are grateful for support from NSF HOMINID grant no. 1032255 and NIH grant GM100233. J.G.S., F.J., and M.S. were supported by NIH grant R01-GM40282 to M.S. P.H.S. is supported by an HHMI International Student Fellowship. F.R. is supported by a German Academic Exchange Service (DAAD) study scholarship. E.E.E. is on the scientific advisory boards for Pacific Biosciences, Inc., SynapDx Corp, and DNAnexus, Inc.

Supplementary Materials
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Materials and Methods
Figs. S1 to S38
Tables S1 to S58
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Cilia at the Node of Mouse Embryos Sense Fluid Flow for Left-Right Determination via Pkd2

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Unidirectional fluid flow plays an essential role in the breaking of left-right (L-R) symmetry in mouse embryos, but it has remained unclear how the flow is sensed by the embryo. We report that the Ca^{2+} channel Polycystin-2 (Pkd2) is required specifically in the perinodal crown cells for sensing the nodal flow. Examination of mutant forms of Pkd2 shows that the ciliary localization of Pkd2 is essential for correct L-R patterning. Whereas *Kif3a* mutant embryos, which lack all cilia, failed to respond to an artificial flow, restoration of primary cilia in crown cells rescued the response to the flow. Our results thus suggest that nodal flow is sensed in a manner dependent on Pkd2 by the cilia of crown cells located at the edge of the node.

Most of the visceral organs in vertebrates exhibit left-right (L-R) asymmetry in their shape and/or position. The breaking of L-R symmetry in the embryos of many vertebrates is mediated by a unidirectional fluid flow in the ventral node (an embryonic cavity

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Nuclear localization of Prickle2 is required to establish cell polarity during early mouse embryogenesis

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A B S T R A C T

The establishment of trophectoderm (TE) manifests as the formation of epithelium, and is dependent on many structural and regulatory components that are commonly found and function in many epithelial tissues. However, the mechanism of TE formation is currently not well understood. *Prickle1* (*Pk1*), a core component of the planar cell polarity (PCP) pathway, is essential for epiblast polarization before gastrulation, yet the roles of *Pk* family members in early mouse embryogenesis are obscure. Here we found that *Pk2*$^{-/-}$ embryos died at E3.0–3.5 without forming the blastocyst cavity and not maintained epithelial integrity of TE. These phenotypes were due to loss of the apical–basal (AB) polarity that underlies the asymmetric redistribution of microtubule networks and proper accumulation of AB polarity components on each membrane during compaction. In addition, we found GTP-bound active form of nuclear RhoA was decreased in *Pk2*$^{-/-}$ embryos during compaction. We further show that the first cell fate decision was disrupted in *Pk2*$^{-/-}$ embryos. Interestingly, Pk2 localized to the nucleus from the 2-cell to around the 16-cell stage despite its cytoplasmic function previously reported. Inhibiting farnesylation blocked Pk2's nuclear localization and disrupted AB cell polarity, suggesting that Pk2 farnesylation is essential for its nuclear localization and function. The cell polarity phenotype was efficiently rescued by nuclear but not cytoplasmic *Pk2*, demonstrating the nuclear localization of Pk2 is critical for its function.

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Introduction

In preimplantation mouse development, the first cell lineages to be established are the trophectoderm (TE) and inner cell mass (ICM) (Marikawa and Alarcón, 2009; Rossant and Tam, 2009; Sasaki, 2010; Zernicka-Goetz et al., 2009). In mouse, these lineages begin to diverge at the 8-cell stage, when blastomeres polarize during compaction. In this process, blastomeres acquire apical–basal (AB) polarity, typified by the apical localization of microvilli and acquisition of cytoplasmic polarity, including the asymmetric distribution of E-cadherin and reorganization of the microtubule (MT) network (Fleming and Johnson, 1988; Johnson et al., 1986; Maro et al., 1990). Next, the blastocoel, a fluid-filled cavity, is formed in the central region of the embryonic cell mass. Blastocoel formation requires two major interrelated features of TE differentiation: intracellular junction biogenesis and a directed transport system, mediated by Na^+/K^+ ATPase (Eckert and Fleming, 2008). These findings suggest that the functional polarity of embryonic cells is essential for proper blastocyst cavity formation. The cell polarity complex (aPKC/PAR) regulates the orientation of cell cleavage planes and cell polarity (Alarcón, 2010; Dard et al., 2009; Plusa et al., 2005). Thus, the aPKC/PAR complex also influences the localization of blastomeres to an outer or inner position in the blastocyst as well as blastocoel formation (Alarcón, 2010; Plusa et al., 2005). However, the connection between these two processes is unclear.

Planar cell polarity (PCP) is manifested as the coordinated, polarized orientation of cells within epithelial sheets, or as the directional cell migration and intercalation during convergent extension (Axelrod, 2009; Goodrich and Strutt, 2011; Simons and Mlodzik,

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