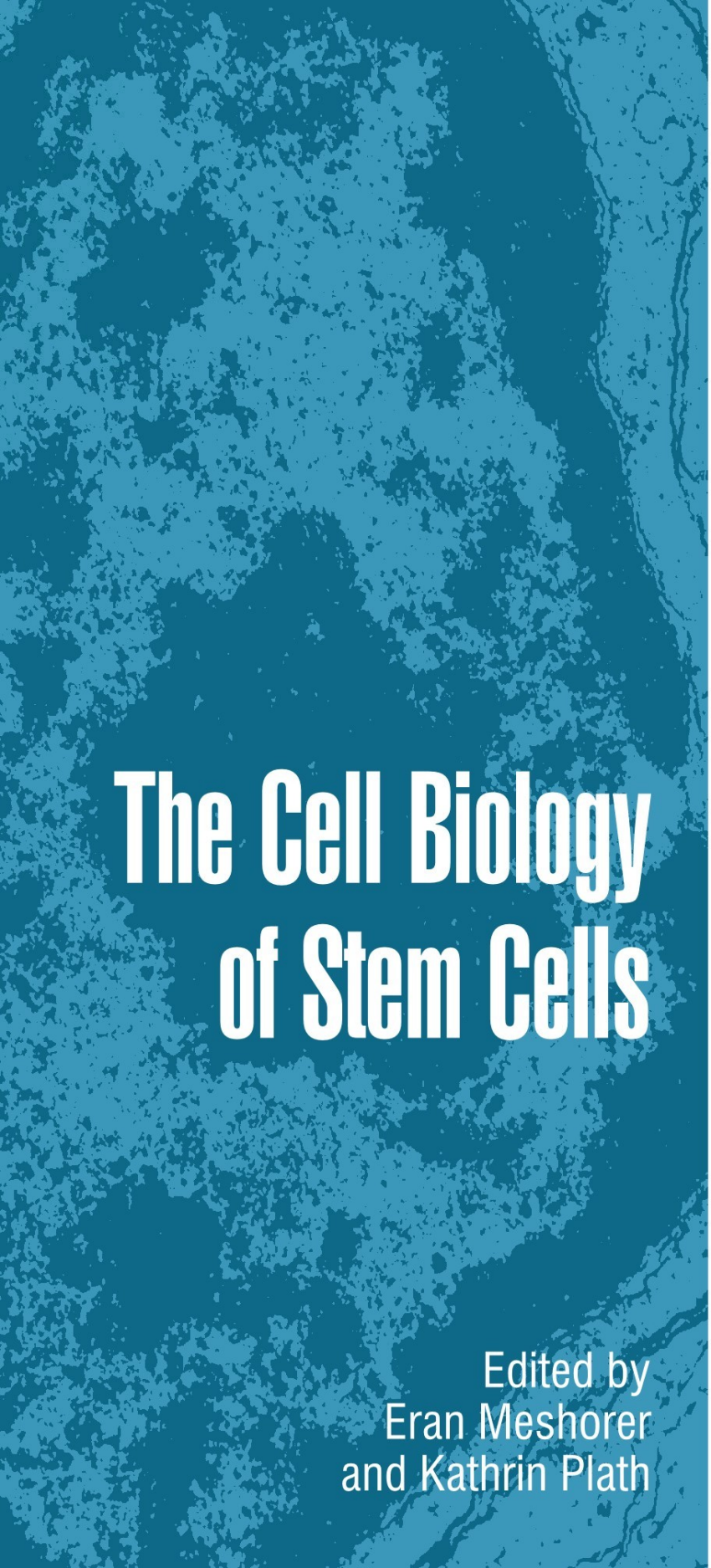

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Volume 695

The Cell Biology of Stem Cells

Edited by
Eran Meshorer
and Kathrin Plath

The Cell Biology of Stem Cells

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Volume 695
THE CELL BIOLOGY OF STEM CELLS
Edited by Eran Meshorer and Kathrin Plath

The Cell Biology of Stem Cells

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PREFACE

Stem cells have been gaining a lot of attention in recent years. Their unique potential to self-renew and differentiate has turned them into an attractive model for the study of basic biological questions such as cell division, replication, transcription, cell fate decisions, and more. With embryonic stem (ES) cells that can generate each cell type in the mammalian body and adult stem cells that are able to give rise to the cells within a given lineage, basic questions at different developmental stages can be addressed. Importantly, both adult and embryonic stem cells provide an excellent tool for cell therapy, making stem cell research ever more pertinent to regenerative medicine.

As the title *The Cell Biology of Stem Cells* suggests, our book deals with multiple aspects of stem cell biology, ranging from their basic molecular characteristics to the in vivo stem cell trafficking of adult stem cells and the adult stem-cell niche, and ends with a visit to regeneration and cell fate reprogramming. In the first chapter, "Early embryonic cell fate decisions in the mouse", Amy Ralson and Yojiro Yamanaka describe the mechanisms that support early developmental decisions in the mouse pre-implantation embryo and the current understanding of the source of the most immature stem cell types, which includes ES cells, trophoblast stem (TS) cells and extraembryonic endoderm stem (XEN) cells. From the derivation of these stem cell types, we turn to examining the nuclear architecture and genome organization of pluripotent ES cells in the second chapter "Nuclear architecture in stem cells" by Kelly Morris, Mita Chotalia and Ana Pombo. The chapter addresses the structure and function of the three-dimensional space of the nucleus in ES cells, emphasising the unique properties of chromatin, nuclear bodies and gene positioning in these cells. ES cell epigenetics is analyzed in more depth in the third chapter "Epigenetic regulation of pluripotency" by Eleni Tomazou and Alexander Meissner. The authors describe the epigenetic profiles of key chromatin modifications, including DNA methylation and histone modifications, and discuss functional aspects of these epigenetic marks. Remaining at the DNA level, the fourth chapter, "Autosomal lyonization of replication domains during early mammalian development", by Ichiro Hiratani and David Gilbert, illustrates the dynamics and regulation of DNA replication in ES cells by taking us through 50 years of research history of this exciting field, reviving the old concept of 'autosomal lyonization' to explain the process of heterochromatinization.

Genomic DNA, the fundamental unit of life, is constantly being damaged and repaired. Peter Stambrook and Elisia Tichy discuss mutation rates, signaling pathways and the mechanisms of DNA damage and repair in ES cells in their chapter, "Preservation of genomic integrity in mouse embryonic stem cells". Having talked about DNA packaging, replication and damage, the book now turns to focus on RNA with the sixth chapter, "Transcriptional regulation in embryonic stem cells", by Jian-Chien Dominic Heng and Huck-Hui Ng. This chapter discusses the transcriptional networks that are at the heart of the pluripotent state and describes the recent technological advances that allow a systemic look at transcriptional regulation in ES cells and during their differentiation. From transcriptional control, we continue to RNA splicing. David Nelles and Gene Yeo authored the seventh chapter entitled "Alternative splicing in stem cell self-renewal and differentiation", in which they review the recent literature on splicing, highlighting several key examples of alternatively spliced genes in ES cells, and address novel genome-wide approaches to analyze splicing and alternative splicing patterns at a global scale. Chapter eight, "MicroRNA regulation of embryonic stem cell self-renewal and differentiation" by Collin Melton and Robert Blelloch, elucidates microRNA regulation in ES cells, emphasizing several prominent examples of microRNAs, including Let-7, Lin-28, miR-134, miR-296 and others, that regulate self-renewal and/or pluripotency of ES cells. Chapter 9, "Telomeres and telomerase in adult stem cells & pluripotent embryonic stem cells" by Rosa Marión and Maria Blasco gives an overview of telomere biology and telomerase regulation in multipotent and pluripotent cells, discussing the potential mechanisms enabling the remodeling of telomeric chromatin during nuclear reprogramming from somatic cells to pluripotency. In the mouse, nuclear reprogramming to pluripotency also entails the reactivation of the somatically silenced X chromosome in female cells. The next chapter, "X chromosome inactivation and embryonic stem cells" by Tahsin Stefan Barakat and Joost Gribnau discusses the regulation of X chromosome inactivation (XCI) as female ES cells are induced to differentiate and explains the cis-and trans-acting mechanisms that act in concert to precisely orchestrate this transcriptional silencing of an entire chromosome, while presenting hypotheses for why this intriguing process occurs in female cells only.

Having covered the molecular biology in the nucleus of pluripotent ES cells, the next three chapters deal with somatic or adult stem cells. While pluripotent cells only exist during a brief phase in early embryonic development, adult stem cell populations are maintained throughout the entire lifespan of the organism until they are required for tissue homeostasis and/or repair. The signals that keep adult stem cells in check and regulate their differentiation versus self-renewal are thought to be controlled by interactions with the cells and extracellular matrix that constitute the stem cell niche. In Chapter 11, "Adult stem cells and their niches", Francesca Ferraro, Cristina Celso and David Scadden explain the niche concept, discuss the signaling pathways that operate at different mammalian niches, and link the current understanding of niche biology to carcinogenesis and aging. In Chapter 12 "Adult stem cell differentiation and trafficking and their implications in disease", Ying Zhuge, Zhao-Jun Liu and Omaida Velazquez present trafficking of hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and endothelial progenitor cells (EPCs) and discuss the mechanisms that control their regulated movement in mammals. Zhuge et al. also explain how understanding these fundamental processes may translate into therapeutic applications.

In the next chapter, "Vertebrates that regenerate as models for guiding stem cells", Christopher Antos and Elly Tanaka focus on the mechanisms of regeneration in several vertebrate animal models such as frog, fish and salamander. They describe the extensive cellular plasticity involved in the regeneration of several structures: the eye, heart, nervous system and appendages, and they summarize some of the molecules that underlie transdifferentiation and dedifferentiation in select tissues. The final chapter of the book "Reprogramming of somatic cells to pluripotency" by Masato Nakagawa and Shinya Yamanaka comes to the most recent exciting development in stem cell biology: cellular reprogramming to pluripotency. The authors give a brief history of somatic cell nuclear transfer experiments conducted in frog oocytes in the '50s and '60s, discuss cell fusion experiments leading to reprogrammed cells, albeit tetraploid, and describe their lab's own seminal contribution to the reprogramming field—the generation of pluripotent cells from somatic cells upon expression of a specific set of transcription factors—leading to the new thriving field of induced pluripotent stem (iPS) cells.

Human adult and embryonic stem cells, and now induced pluripotent stem cells, could be used for the generation of cells and tissues for cell-based therapies. With iPS cells, one is now able to generate patient-specific pluripotent cells with tremendous potential for disease studies and drug screenings. To be able to take full advantage of the huge capacity of stem cells, our knowledge of the underlying biology still needs to grow. In its 14 chapters, *The Cell Biology of Stem Cells* provides much of the current understanding of the cell biology of stem cells and discusses many of the open questions that remain to be answered.

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CONTENTS

CHAPTER 1

EARLY EMBRYONIC CELL FATE DECISIONS IN THE MOUSE

Yojiro Yamanaka* and Amy Ralston*

Abstract: During development, initially totipotent cells of the embryo specialize to form discrete tissue lineages. The first lineages to form in the mouse are the extraembryonic tissues. Meanwhile, cells that do not become extraembryonic retain a pluripotent fate since they can give rise to all the germ layers of the fetus. Pluripotent stem cell lines have been derived from the fetal lineage at several stages of development. Interestingly, multipotent stem cell lines have been derived from the extraembryonic lineages around the same time. Examining the regulation of early embryonic cell fate decisions is therefore a rare opportunity to examine establishment of stem cell progenitors. Classical studies have provided considerable insight into specification of the first three lineages and use of modern molecular and imaging techniques has advanced this field further. Here we describe current understanding of the diverse molecular mechanisms that lead to establishment and maintenance of the first three lineages during mouse development.

INTRODUCTION

During the earliest days of mouse development, initially totipotent cells become restricted in their developmental potential to give rise to the first lineages of the mouse. While in nonmammalian species the first lineage decisions might involve specification of the major body axes, mammals have an altogether different first priority: implantation. Thus discrimination between fetal and extraembryonic tissue lineages comprises the first two lineage decisions (Fig. 1) and precedes establishment of the germ layers (ectoderm, mesoderm, endoderm) and the germline by several days. This uniquely mammalian developmental strategy involves unique cell types that can be isolated and expanded

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in culture as stable stem cell lines. Understanding the origins of the extraembryonic tissues therefore illuminates our understanding of establishment and differentiation of stem cells. Classical studies provided considerable insight into specification of the first three lineages and use of modern molecular and imaging techniques has advanced this field further.

Three days after fertilization, the mouse embryo, or blastocyst contains three tissue lineages: epiblast (EPI), trophectoderm (TE) and primitive endoderm (PE). Isolation and study of stem cell lines from these lineages has reinforced and extended our understanding of early embryonic cell fate decisions. Three types of stem cell lines have been derived from the blastocyst: embryonic, trophoblast and extraembryonic endoderm stem cells (ES, TS and XEN cells). Each of these exhibits stem cell properties, such as the ability to either self-renew or to differentiate into multiple mature cell types. Yet each stem cell line exhibits features of the lineage from which it derives, including tissue-specific developmental potential, morphology, transcription factor expression and growth factor requirements.[1] These stem cell lines not only provide an expandable source of pure cell populations for studies requiring large amounts of starting material, but they provide an opportunity to understand where stem cells come from.

Studies performed in ES cells have enabled deeper molecular analysis of the role of genes in cell fate selection. Manipulation of levels of certain lineage-regulating genes causes corresponding changes in stem cell fate. For example, the trophoblast transcription factor Cdx2 is sufficient to convert ES cells to TS-like cells.[2] These kinds of observations demonstrate the remarkable plasticity of ES cells, as well as the central role of genes such as *Cdx2* as lineage-determining factors. ES cells also provide an opportunity to examine molecular interactions between lineage-determining genes and thus serve as a model for understanding cell fate selection in the embryo. However, examination of the role of lineage-determining genes in the embryo has revealed that lineage-determining genes play a relatively late role in lineage specification, raising the question as to how the first three lineages are initially specified.

A variety of mechanisms are probably involved in specifying the first lineages, including cell position, shape, polarization, signaling and division plane. A new paradigm is emerging, in which an early pre-stem cell program specifies the tissue lineages as the blastocyst forms. Later, around the time of implantation and thereafter, cell fates are maintained by a program that is active in stem cell lines (Fig. 2).

LINEAGE ESTABLISHMENT AND THE PRE-STEM CELL PROGRAM: FORMATION OF THE BLASTOCYST

Here we will consider the first phase of lineage specification: establishment of the TE and inner cell mass (ICM) as the blastocyst forms. The TE will give rise to placenta, while the ICM contains a mixture of fetal and primitive endoderm progenitors. In the blastocyst, the TE surrounds the ICM and hollow blastocoel and lineage-tracing experiments have shown that TE and ICM populations begin as the outside and inside cell populations of the embryo.[3] That is, as cell cleavage partitions the zygote into two, four, eight and sixteen cells, a small number of cells become enclosed by outside cells. Continued cleavages increase numbers of inside and outside cells, the TE epithelializes and the blastocoel expands, forming the blastocyst structure. The mechanism by which topology becomes linked to cell fate has been elusive. Several models have been put

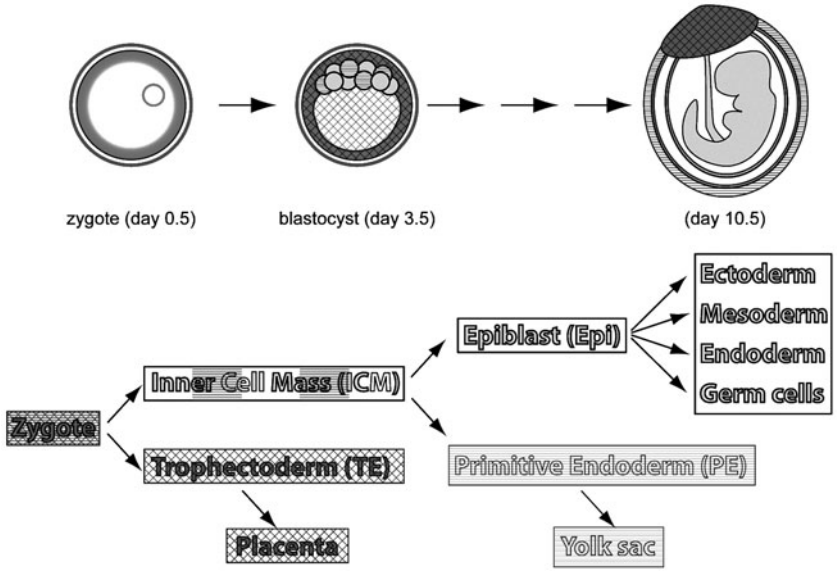

Figure 1. Overview of the first two lineages decisions during mouse development. The initially totipotent zygote develops to the blastocyst, which contains three lineages: EPI (blue), TE (red, crosshatched) and PE (yellow, lined). These lineages will give rise to the fetus, the placenta and a portion of the yolk sac at later stages of development. A color version of this figure is available at www.landesbioscience.com/curie.

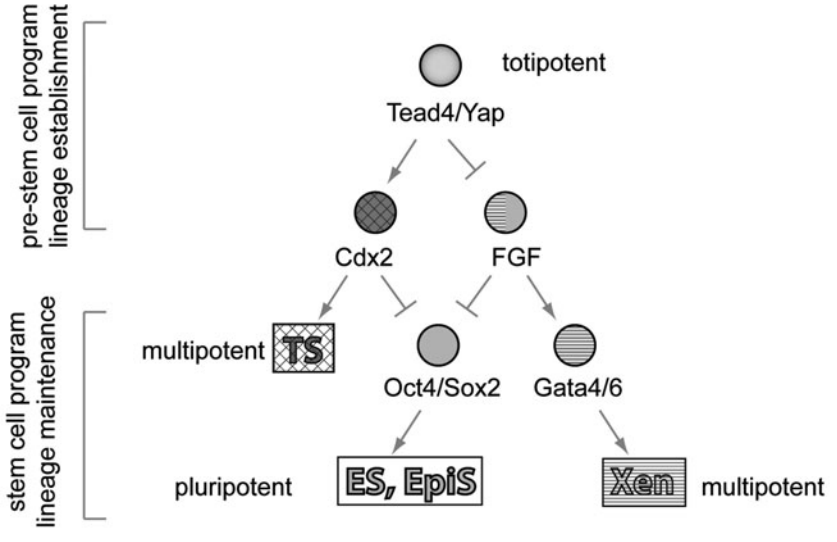

Figure 2. Overview of molecular interactions leading to cell fate specification and maintenance during early mouse development. The Tead4/Yap complex selects TE fates (red, crosshatched) from initially totipotent cells (grey). Cells that do not become TE, then adopt a mixture of EPI (blue) and PE (yellow, lined) fates. Signaling within this lineage facilitates the sorting out of EPI and PE fates. Lineage-specific transcription factors participate in maturation of each lineage. A color version of this figure is available at www.landesbioscience.com/curie.

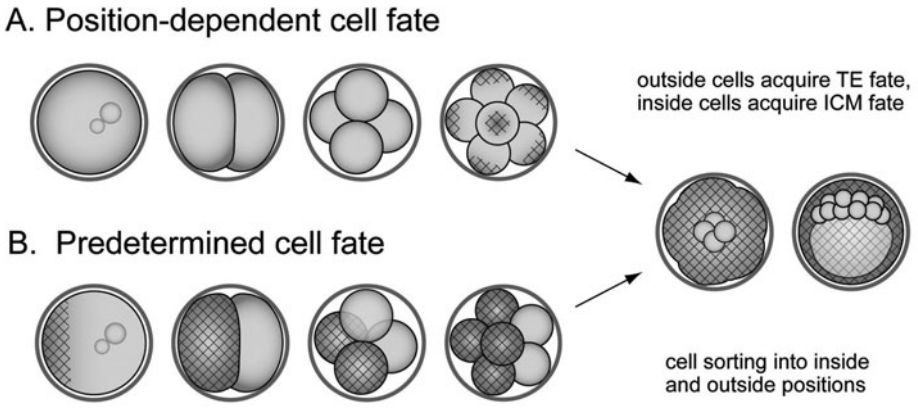

Figure 3. Two possible models of TE specification. A) Cell position dictates cell fate, as outer cells, or outer portions of cells, adopt TE cell fate (red, crosshatched). B) TE fate is predetermined and a specific subset of cells inherits TE fate-determining molecules. A color version of this figure is available at www.landesbioscience.com/curie.

forward. For example, cell fate could be a consequence of cell position (Fig. 3A). Alternatively, predetermined cell fates could drive cells into appropriate topological positions (Fig. 3B). This latter mechanism predicts that pre-inside and pre-outside cells would be detectable prior to formation of overt inside and outside cell populations. In spite of extensive effort in the field, however, there is currently no support for this predetermination mechanism.

Two main strategies have been used to look for evidence of predetermination among cells prior to the blastocyst stage: lineage tracing and molecular analysis. In terms of lineage tracing, reports of biased developmental potential among cells at the two-cell stage[4-13] are not relevant to the TE/ICM lineage decision since these studies demonstrate contribution of both cells to the TE and ICM. Likewise, all cells of four and eight-cell embryos can also contribute to both TE and ICM lineages.[14,15] Although one group reported restricted lineage potential from the four-cell stage,[7] extraembryonic lineages were incompletely scored. Thus there is no evidence from lineage tracing experiments to suggest that cells are predetermined to make TE or ICM prior to formation of inside and outside groups. In terms of molecular analyses, no protein has been detected within a subset of cells prior to the 16-cell stage that instructs the TE/ICM lineage decision. The level of one type of histone methylation is reported to exhibit uneven distribution among blastomeres at the 4-cell stage and correlates with reduced potential to contribute to viable mice in chimeras.[16] The functional importance of these observations in TE/ICM lineage specification needs to be clarified. Therefore, no molecular evidence supports the existence of pre-TE or pre-ICM cells prior to formation of inside and outside cell populations. Rather, inside and outside cells could acquire fates once they have acquired their positions within the embryo.

If cell position acts upstream of cell fate, mechanisms must exist for cells to sense their position within the embryo. Longstanding evidence that cells polarize around the 8-cell stage[17] supports the claim that there are differences along the inside/outside axis at the cellular level. Polarization by conserved polarity proteins such as atypical PKC (aPKC),

Par3 and Par6 is required for maintaining cell position[8] and cell contact has been shown to be required for cell polarization.[17] However the link between position, polarization and cell fate has not been examined at the molecular level. This area is challenging to study using conventional knockout techniques. Many of the proteins involved in cell position and cell contact, such as aPKC, are members of large gene families, suggesting that genetic redundancy may mask their requirements in single gene knockout studies. In addition, this early developmental stage may be regulated in part by maternally supplied protein, requiring germline gene deletion to detect a phenotype. Finally, many of these proteins are involved in basic cellular processes, such as cell division, making it difficult to study their effects during development. On the other hand, overexpression of dominant-negative or siRNA constructs leads to only short-term or partial loss of function, which can also impede phenotype resolution.

Ultimately, to convert inside/outside differences into changes in gene expression, a differentially localized transcription factor is needed. Several strategies have led to the identification of transcription factors involved in early lineage development. Candidates have been identified by microarray analysis of transcripts expressed in pre-implantation development, followed by in situ hybridization to screen for those with restricted expression in the blastocyst.[18] Alternatively, candidates have been identified by microarray comparison of blastocyst-derived stem cell lines.[19] Advances have also come from fortuitous discovery of an unexpectedly early lethal phenotype in knockouts,[20-22] which led to identification of *Cdx2* and *Tead4.*

While required for TE development, *Cdx2* probably does not play an instructive role in TE formation.[23,24] Nevertheless, *Cdx2* mRNA,[25] but not protein,[24,26] has been reported to localize to the outside surface of cells at the 8-cell stage. Since *Cdx2* is not required for specification of TE at either morphological[23,24] or molecular levels, evidenced by the continued expression of the TE marker Gata3 in *Cdx2* null embryos,[19] it is difficult to imagine that localized *Cdx2* mRNA plays an instructive role in lineage establishment. Recently, a new pathway, involving Tead4 and cofactors, has been shown to play an instructive role in the first lineage decision. The transcriptional coactivator Yap and related protein Taz, exhibit cell position-sensitive changes in activation of Cdx2 expression.[27] Prior to the blastocyst stage, Yap/Taz localize to nuclei of outside cells and cytoplasms of inside cells. This localization is regulated by phosphorylation by the Hippo signaling pathway members Lats1/2. In addition, manipulation of cell position led to corresponding changes in Yap localization: outside cells embedded inside an aggregate of cells lost nuclear Yap, while inside cells stripped of surrounding outer cells acquired nuclear Yap. Yap/Taz interact directly with Tead4 a DNA binding protein required for expression of Cdx2[21,22] and other trophectoderm markers.[19] The identity or nature of Yap/Taz-regulating signals that can sense cell position are unknown, but probably involve the Hippo signaling pathway and possibly proteins involved in cell contact such as cadherins. This will undoubtedly be an exciting area of research to follow in the future.

Besides what is working upstream of Yap/Tead4, it is not entirely clear what is working downstream. *Tead4* is required for Cdx2 expression, but *Tead4* null embryos die prior to blastocyst formation, while *Cdx2* null embryos die after blastocyst formation. *Tead4* is not required in the ICM,[21,22] so additional genes must operate in parallel to *Cdx2* in the TE. Some of these, such as *Gata3* are beginning to be identified.[19] It will be important to identify Tead4 targets that participate in promoting outside cell proliferation and construction of the blastocyst.

LINEAGE MAINTENANCE AND THE STEM CELL PROGRAM: BEYOND THE BLASTOCYST

In the blastocyst, interactions between lineage-determining transcription factors reinforce TE and ICM fates established at earlier stages. Central players at this stage are *Oct4* (*Pou5f1*) and *Cdx2*. *Oct4* is required for maturation of the ICM,[28] while *Cdx2* is required for maturation of the TE.[23] Mutual antagonism between these two factors was initially speculated to cause the first lineage decision. *Cdx2* is required for repression of *Oct4* and other ICM genes in the TE of the blastocyst.[23] But the TE still forms in *Cdx2* null embryos and other TE markers are still expressed.[19] Similarly, Oct4 represses Cdx2 in the ICM, but not until implantation, a full day after blastocyst formation.[19] Thus lineage specification is initially normal in the absence of either *Oct4* or *Cdx2*, but embryos fail to maintain correct expression of lineage genes. Nevertheless, in spite of adoption of ICM gene expression, *Cdx2* null TE does not fully adopt ICM fate. The TE marker Gata3 is still expressed in the TE of *Cdx2* null embryos[19] and *Cdx2* null embryos exhibit higher levels of apoptosis in the TE than do wild type embryos.[23] Cdx2 must therefore enable survival and/or proliferation of cells that are already committed to being TE. This is consistent with its continued expression in the proliferative region of the trophoblast at later stages.[29] The reason for the lethality of *Oct4* null embryos is currently unclear.

The antagonistic relationship between Oct4 and Cdx2 is borne out by stem cells from the blastocyst. ES cells cannot be derived from *Oct4* null embryos and TS cells cannot be derived from *Cdx2* null embryos.[23,28] Loss of *Oct4* from existing ES cell lines leads to upregulation of *Cdx2* and formation of TS-like cells in the presence of TS cell culture medium.[30] Similarly, overexpression of *Cdx2* in ES cells leads to repression of *Oct4* and formation of TS-like cells.[2] Other trophoblast factors, such as Eomes and Gata3 can also induce trophoblast gene expression in ES cells[2,19] and these also play relatively late roles in trophoblast maturation rather than allocation.[23,31,32] Maintenance of the TE/ICM lineage restriction in stem cells therefore appears to use genetic programs that become active once the blastocyst has formed. This makes sense given that stem cell derivation requires culture beyond the blastocyst stage. Understanding the further development of the ICM, however, requires a look at the second lineage decision in development, discussed next.

THE SECOND LINEAGE DECISION: SUBDIVIDING THE ICM

Three days after fertilization, the ICM of the blastocyst contains two cell types: the epiblast (EPI) and the primitive endoderm (PE). Only the EPI gives rise to the fetus, whereas the PE is an extraembryonic lineage, which contributes to the yolk sac (Fig. 1).[33-36] The PE lineage plays two important roles just after implantation. The first is that it provides nutrients to the embryo and the second is that it serves as a signaling center that helps confer anterior-posterior polarity upon the gastrulating embryo.[37] As for the TE lineage, a special stem cell line can be derived from the PE lineage. Multipotent stem cell lines, called XEN cells, have been derived from the PE lineage (Fig. 2).[38] In addition, PE-like cells can be induced from ES cells by overexpression of PE transcription factors, such as *Gata4* and *Gata6.*[39] Yet *Gata4/6* act relatively late in PE development,[40,41] suggesting that, as for the TE lineage, the PE is specified by a mechanism acting upstream of the stem cell genes. Insight into specification of the PE lineage has revealed a unique cell signaling-based strategy.

Heterogeneity and Progenitor Sorting

Four days after fertilization, the blastocyst implants. At this stage, the PE appears as a distinct monolayer on the blastocoel surface of the ICM. For this reason, the PE was originally assumed to arise from ICM cells directly facing the blastocoel around the time of implantation. Microenvironmental differences between blastocoel-facing and deeper cells were postulated to participate in lineage specification at this stage. However, recent studies have shown that EPI and PE progenitors can be detected in the blastocyst one full day before implantation.[36,42,43] At this stage, the ICM appears as a mixed population of EPI and PE progenitors, expressing lineage-specific transcription factors. Prior to this stage, Nanog and Gata6 are coexpressed in all cells of the ICM and expression gradually becomes mutually exclusive to specify the two progenitors in a position-independent manner during blastocyst expansion.[36,44] Notably, there is no stereotyped pattern of distribution of the two progenitors within the ICM. Rather, they are sprinkled randomly throughout the ICM like salt and pepper.

These results suggest that the two randomly distributed lineage progenitors sort out to form two morphologically distinct layers by implantation. Indeed, support for this model has been provided by live imaging of blastocyst expansion in transgenic mice expressing fluorescent lineage markers. In the *Pdgfra*$^{H2B\text{-}GFP}$ mouse line histone H2B-GFP is expressed in the PE and revealed that separation of the two lineages involves both apoptosis and cell migration.[36] Cells within the growing ICM appear to rearrange constantly,[36,45] but once PE progenitors come to the ICM surface they stay there. Consistent with this, the maturation of the PE takes place progressively and this is correlated with position within the ICM.[46] One outstanding question is whether PE cells sort out by directional cell movement or a combination of random movement and position recognition.

Several mutants exhibit a defect in formation of a cohesive PE layer.[47-51] In these mutants, Gata4-expressing, presumptive PE cells, are found clustered within the middle of the ICM, suggesting that PE progenitors are specified but fail to form a morphologically distinct surface layer. This contrasts with the TE, in which lineage allocation (position) precedes lineage specification. For the PE, lineage specification precedes allocation. Understanding how PE fates are selected from within the ICM is therefore key to understanding PE/EPI lineage choice.

CELL SIGNALING REGULATES PE/EPI SPECIFICATION

Early heterogeneity in the ICM suggests that position-independent mechanisms regulate specification of PE and EPI lineages. FGF signaling has been shown to be necessary for PE formation in vivo and in vitro.[52-54] How extracellular signaling pathways, such as the FGF signaling pathway, could participate in the generation of a salt and pepper distribution of PE and EPI within the ICM is not clear. For example, certain pre-PE cells within the embryo could be predisposed to respond to signals, or cells could randomly receive signals and thereby become PE progenitors.

These possibilities are summarized in two models: the origin-dependent model and the signaling-dependent model (Fig. 4A,B).[17,55] The origin-dependent model relies on understanding the process of inner cell generation during the cleavage stages.[56] Inner cells of the morula, which will become the ICM of the blastocyst, are generated from two rounds of asymmetric divisions at 8-16 and 16-32 cell stages.[20] According to the

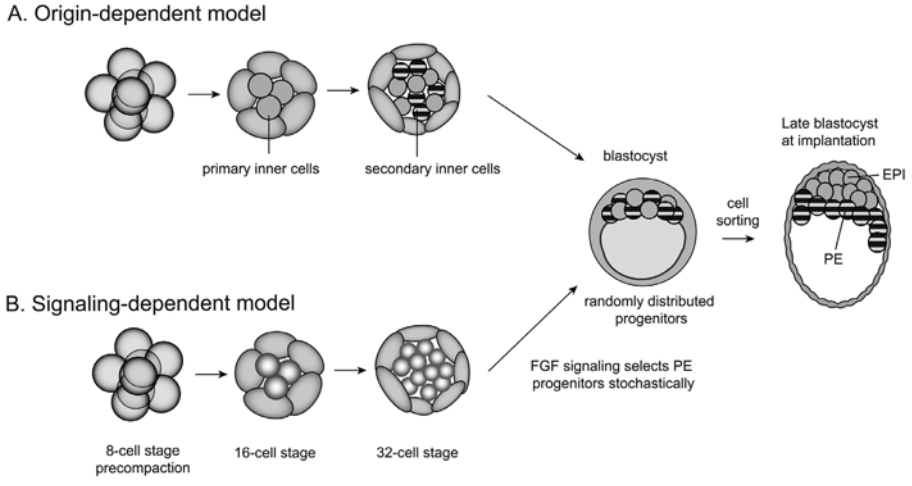

Figure 4. Two models of PE/EPI formation in the mouse embryo. A) Origin-dependent model in which the developmental origin of ICM cells regulates EPI/PE specification. ICM cells are generated from two rounds of asymmetric divisions after the 8-cell stage. Primary inner cells (blue) give rise to the EPI lineage and secondary inner cells (yellow, lined) to the PE lineage. B) Signaling-dependent model in which no difference in lineage potential exists between primary and secondary inner cells. Each inner cell is stochastically capable of responding to FGF signaling. Responding cells become the PE lineage and nonresponding cells become the EPI lineage. After the PE/EPI lineage decision, EPI and PE progenitors express lineage-specific transcription factors, Nanog or Gata6 and are distributed randomly in the ICM of the blastocyst. These two progenitors then sort out to form the two distinct layers of EPI and PE by day 4.5 at implantation. A color version of this figure is available at www.landesbioscience.com/curie.

origin-dependent model, the developmental origins of individual ICM cells determine their fate. That is, inner cells generated in the first round of divisions (primary inner cells) would preferentially adopt the EPI fate, whereas cells generated in the second round (secondary inner cells) would preferentially become PE (Fig. 4A).[42,57] Secondary inner cells would be predisposed to become extraembryonic due to their prolonged external position since TE cells are also external.[17] To test the origin-dependent model, generation of inner cells was first directly observed in live embryos and then the contribution of their progeny to EPI and PE lineages was analyzed at later stages.[44] No difference in lineage potential was detected between primary and secondary inner cells since both primary and secondary inner cell progeny contributed to EPI and PE lineages without an obvious bias. These observations therefore suggest that the origin-dependent model is unlikely.

The second model is a signaling-dependent model, in which individual ICM cells stochastically respond to certain levels of FGF signaling to choose EPI or PE fates (Fig. 4B). As described above, FGF signaling is necessary for PE formation in the embryo.[52-54] When FGF signaling is blocked, using either chemical inhibitors or by gene knockouts, all ICM cells adopt EPI fates.[42,58] Interestingly, high doses of exogenous FGF4 can induce the converse phenotype: all ICM cells adopt PE fates.[44] This suggests that all early ICM cells have the potential to respond to FGF signaling and become PE. During normal development, however, limited amounts of endogenous FGFs would restrict the proportion of FGF-responding ICM cells (Fig. 5). Whether or not individual ICM cells