Making a firm decision: multifaceted regulation of cell fate in the early mouse embryo

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Abstract | The preimplantation mammalian embryo offers a striking opportunity to address the question of how and why apparently identical cells take on separate fates. Two cell fate decisions are taken before the embryo implants; these decisions set apart a group of pluripotent cells, progenitors for the future body, from the distinct extraembryonic lineages of trophectoderm and primitive endoderm. New molecular, cellular and developmental insights reveal the interplay of transcriptional regulation, epigenetic modifications, cell position and cell polarity in these two fate decisions in the mouse. We discuss how mechanisms proposed in previously distinct models might work in concert to progressively reinforce cell fate decisions through feedback loops.

Blastocyst
A preimplantation embryo that contains a fluid-filled cavity (the blastocoel), a focal cluster of cells from which the embryo will develop (the inner cell mass) and peripheral trophoblast cells, which form the placenta.

Trophectoderm
The outer layer of the blastocyst stage embryo that will give rise to the extraembryonic ectoderm after implantation and will provide the bulk of the embryonic part of the placenta.

Inner cell mass
A small group of undifferentiated cells in the blastocyst, which gives rise to the entire fetus and some of its extraembryonic tissues.

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How do we get from a single cell, the fertilized egg, to a blastocyst comprised of three distinct types of cell with differing biological potential and function? The answer lies in the emergence of transcriptional programmes characteristic of these cell types. The programmes depend on mutually reinforcing or antagonizing interactions between key transcription factors. These transcription factors in turn influence and respond to epigenetic marks in chromatin that reflect cellular ancestry, cell positional history, cell polarity and division orientation. In this Review we discuss how these different factors might act in concert to control cell fate decisions.

As in other organisms, mammalian embryos inherit a pool of maternal transcripts that are progressively degraded and replaced by the products of zygotic transcription. In mammals, zygotic transcription is initiated at a very early stage when cells still exhibit developmental flexibility and can switch their fate. Nevertheless, when differential patterns of transcription first become evident these can be predictive of the first two cell fate decisions: the setting apart of trophectoderm from the inner cell mass (ICM) and the subsequent formation of primitive endoderm and epiblast as the blastocyst prepares for implantation (FIG. 1a,b).

The factors influencing how transcriptional programmes are initiated and maintained have only become evident with the advance of technology. It is only now that we can film and trace each cell in the embryo using fluorescently labelled reporters and so discover cells' origins, behaviour and fate, all of which can be correlated with profiles of gene expression and epigenetic modifications. By changing gene expression in individual cells at specific times we can investigate gene function in a clone of cells whose development can be traced in the normally developing embryo. Similarly, specific events faced by any cell or by the entire embryo during its history, such as the development of cellular or embryonic polarity or the emergence of the embryonic–abembryonic axis, can be correlated with the origins and fates of individual cells. The integration of the resulting information is giving invaluable insight into the cellular and molecular understanding of when and how cells make fate decisions, and is helping to resolve previously controversial issues.

These novel perspectives are beginning to blur the hitherto sharp edges between previously proposed alternative models of cell fate determination in mammalian development. We discuss how these models might be compatible with each other, and suggest that multiple mechanisms could work in concert. The core theme of this Review is how cells first gain and then maintain their identity in the mouse embryo. We start by looking at zygotic genome activation and then examine how the three distinct lineages in the blastocyst progressively develop.

Onset of zygotic transcription

At the earliest stages of development cells are pluripotent. Their transcriptional circuits have no apparent role in differentiation but rather they ensure the switch from reliance on maternally provided transcripts to active

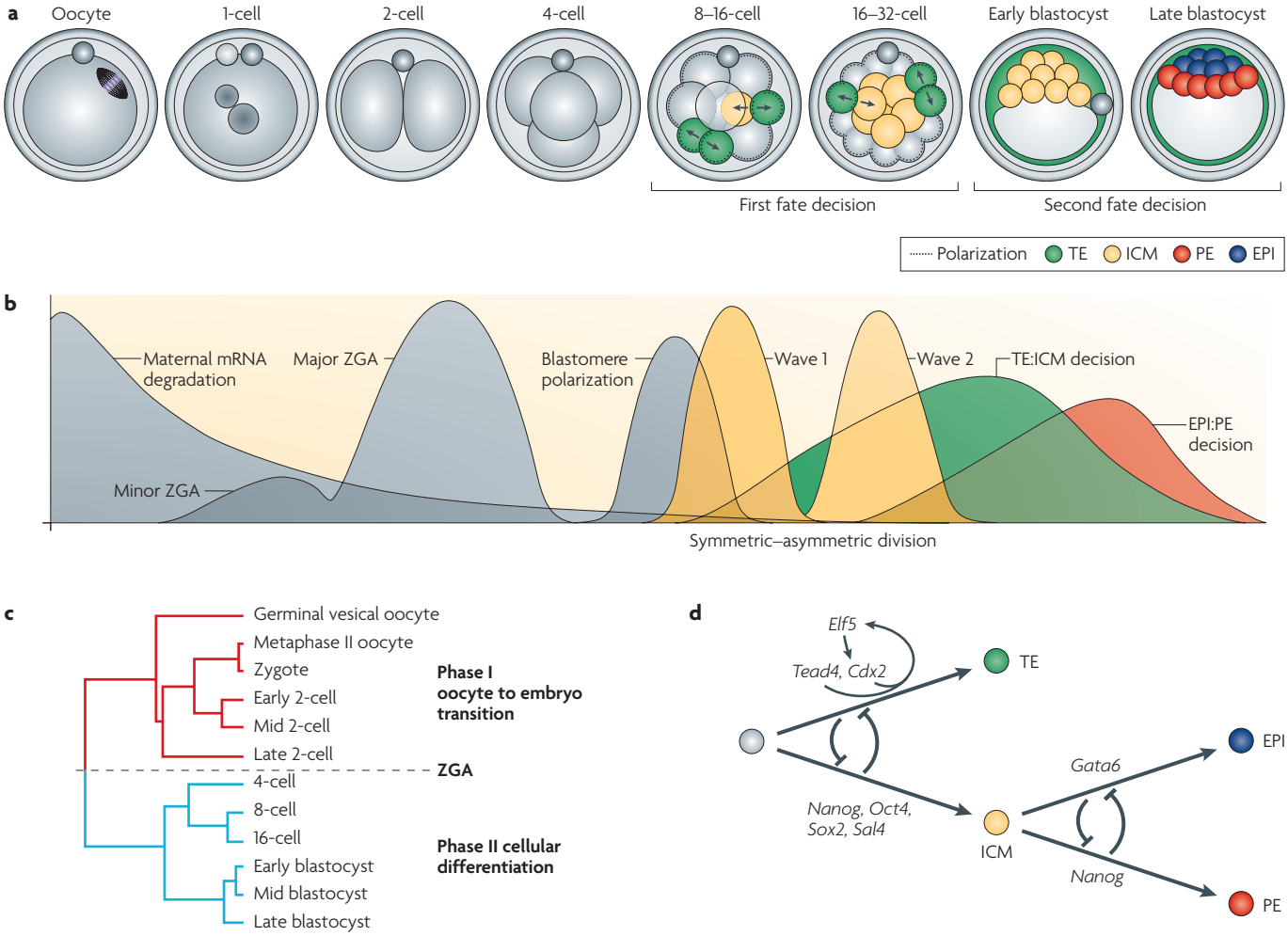


Figure 1 | **Transcriptional regulation and cell fate decisions in preimplantation development. a** | The stages of preimplantation development. Inner cell mass (ICM) progenitor cells are set aside from outer cells in two successive waves of asymmetric cell division commencing at the 8–16-cell stage transition. The outer cells become trophectoderm (TE) in the first cell fate decision. The second cell fate decision involves the formation of primitive endoderm (PE) at the surface of the ICM and the formation of the epiblast (EPI) in the deeper layer. **b** | Representation of major events during preimplantation development that line up with the stages shown in part **a**. Maternal mRNA degradation, the minor and major phases of zygotic genome activation (ZGA), cell polarization and the waves of asymmetric divisions and the temporal onset of gene expression patterns associated with the first and second cell fate decisions. **c** | Hierarchical clustering of global mRNA expression levels throughout preimplantation development reveals two distinct mRNA populations: the 'oocyte-to-embryo' and 'cellular differentiation' populations. These two populations mark the transition between maternal and zygotic transcriptional control in the embryo. **d** | Transcriptional circuitry of cell fate decisions. ICM-specific gene expression (yellow; such as *Nanog*, *Oct4*, *Sox2* and *Sall4*) represses TE-specific genes (green; such as *Tead4*, *Cdx2* and *Elf5*) that in turn could repress ICM genes. The ICM then differentiates into EPI (blue; for example, *Nanog*) and PE (red; such as *Gata6*), where there is similar reciprocal antagonism of gene expression.

zygotic transcription. Even at these early stages, many factors control transcription: specific transcriptional regulators, regulatory RNAs and chromatin remodelling machinery. Clues to the identity of some of these factors can be found in microarray profiling of all transcripts throughout murine preimplantation development[1,2]. This profiling reveals that the early transcriptome is divided into two temporal clusters: the first representing the mature oocyte until the late 2-cell embryo, and the second representing the subsequent stages up to the blastocyst stage (FIG. 1c). These populations of transcripts straddle the period of zygotic genome activation (ZGA) and the destruction of many of the maternally provided mRNAs (FIG. 1b,c). Although this article will not explore ZGA in detail, as comprehensive reviews can be found elsewhere[3,4], we will highlight some recent insights into ZGA.

The degradation of many maternal RNAs relies on members of the RNA-induced silencing complex in both mouse and zebrafish embryos[5,6]. The first, so-called minor phase of ZGA requires specific transcriptional regulators, such as transcription intermediate factor 1α

Primitive endoderm
An early differentiated cell type that lines the inner surface of the blastocyst cavity. It gives rise to the visceral and parietal extraembryonic endoderm after implantation.

(TIF1α) and nucleosome remodelling complex subunit SNF2H (also known as ISWI or SMARCA5)[7]. BAF155 and BRG1 (also known as SMARCC1 and SMARCA4, respectively), which are subunits of the Swi–Snf-type nucleosome remodelling complex[8], are enriched in the transcriptionally more active male pronucleus[9]. The male pronucleus is depleted, unlike the female pronucleus, of the transcriptionally repressive epigenetic marks of histone H3 lysine 9 dimethylation (H3K9me2) and trimethylation (H3K9me3)[10,11]. In addition, embryos derived from *Brg1*$^{-/-}$ oocytes exhibit a characteristic ZGA phenotype of 2-cell embryo arrest and reduced transcription[12]. Thus, growing evidence for the involvement of nucleosome remodelling complexes in the early stages of development points to the importance of epigenetic regulation of chromatin and a need for an understanding of the role of such complexes in decisions to retain pluripotency or differentiate.

The first cell fate decision

The first fate decision in the mouse embryo is taken as two populations of cells are set apart. Cells positioned inside (the ICM) retain pluripotency and cells on the outside develop into extraembryonic trophectoderm. This first set of extraembryonic cells will support the development of the embryo in the uterus and provide signalling sources to pattern the embryo before gastrulation[13–17]. The generation of inside cells requires outer cells to divide in an orientation such that one daughter cell is directed inwards during the 8–16-cell and 16–32-cell stages[18–20] (FIG. 1a). These divisions, named 'differentiative', are in contrast to 'conservative' divisions in which both daughter cells remain on the outside[21]. Because inside and outside cells will follow different fates, differentiative divisions could be considered asymmetric — and indeed recently it has been revealed that they are likely to distribute cell fate-determining factors asymmetrically between the daughters[22].

Once these populations of cells are set apart, inner cells develop a stable regulatory circuit in which the OCT4 (also known as POU5F1)[23,24], SOX2 (REF. 25) and NANOG[26,27] transcription factors promote pluripotency and resist differentiation. By contrast, in outside, trophectoderm-destined cells, transcription factors such as CDX2 and EOMES become upregulated[28–30]. Reciprocal repression of trophectoderm targets by OCT4, SOX2 and NANOG in the pluripotent lineage[31,32], together with the autoregulatory properties of OCT4 and CDX2 (REFS 33,34), ensure that lineage segregation is maintained (FIG. 1d). SALL4, which establishes and maintains ICM integrity by promoting *Oct4* and *Nanog* expression[35,36], and TEAD4, which acts upstream of CDX2 in trophectoderm development[37,38], are new and important additions to the circuits of the first cell fate decision.

In order to understand the initiation of lineage segregation, we need to understand how inside and outside cells become different, and how their formation is regulated. Specifically, what makes some cells in an embryo divide symmetrically and others asymmetrically: is this simply by stochastic (random) events, or are there differences between cells that tip the balance?

The first hypothesis that was proposed to explain how inside and outside cells become different stressed the importance of cell position. By changing the position of cells, it was found that inside cells tended to develop into the ICM and outside ones into trophectoderm[39]. The resulting 'inside–outside' hypothesis proposed that cell-specific environment somehow induces cell fate. But what governs cell position, and does cell fate depend on position alone? It was subsequently realized that blastomeres become polarized along their apical–basal axis before the inner cell-generating divisions begin[21,40]. Thus, asymmetric divisions would generate inside and outside daughters that differ in their polarization properties. The resulting 'polarization' hypothesis therefore implied a crucial role for cell polarity in establishing the developmental properties of cells. As we will discuss later, recent experiments using new technologies to follow development by time-lapse microscopy combined with methods to monitor and perturb gene expression in individual cells indicate that these two hypotheses, originally thought of as alternatives, might work in concert to specify cell fate.

Interplay between cell polarity, position and regulation of cell fate genes. More recently, it emerged that the conserved portioning defective (Par) gene family provides the molecular basis for cell polarity in the mouse embryo[41–43]. Members of the Par complex, including JAM1 (REF. 42), aPKC and PAR3 (REF. 41), become localized apically at the 8-cell stage, whereas PAR1 is localized in basolateral regions[43]. When cells divide asymmetrically the outer daughters retain this polarity, but inside ones inherit mainly the basal pole of the cell[44]. Tight junctions that progressively develop between the cells will separate the apical from basal regions by the blastocyst stage, and this will result in formation of the polarized epithelium of the trophectoderm and the apolar ICM[45]. As asymmetric divisions position cells differentially and result in differential inheritance of their polarization properties, it becomes difficult to distinguish the effect of cell position from polarity. Moreover, not only will changing cell position affect cell polarity and fate, but the converse is also true. Thus, when inside cells are transplanted to outside positions they become polarized and develop as trophectoderm[46–48]. Reciprocally, when polarity molecules such as PAR3 or aPKC are downregulated, progeny cells adopt an inside position by either preferentially dividing asymmetrically or by being 'out-competed' for outside position by more polarized neighbouring cells — consequently, they develop as part of the ICM[41]. Finally, directly affecting the levels of pluripotency genes also affects cell position, presumably through an effect on cell polarity[49]. When the expression of pluripotency genes is enhanced in a cell, its progeny are sorted to the inside to join other cells that express pluripotency genes at high levels. Thus, these recent studies indicate that cell polarity and cell position have a powerful interrelationship with transcriptional networks.

The origins of inside–outside asymmetry. By the mature blastocyst stage the ICM and trophectoderm have established mutually exclusive transcriptional

Epiblast
The epithelial tissue that develops from the inner cell mass of the blastocyst and that gives rise to all three definitive germ layers of the embryo during gastrulation: the ectoderm, mesoderm and endoderm.

Embryonic–abembryonic axis
The side of blastocyst on which the inner cell mass (containing progenitor cells for the body proper) is localized is defined as the embryonic pole, with the opposing pole (containing the cavity) defined as abembryonic. Accordingly, these poles define the embryonic–abembryonic axis.

Chromatin remodelling
Changes in the structural properties of chromatin (either covalent post-translational modifications or architectural properties) that ultimately affect its accessibility to protein factors, such as transcription factors or RNA polymerase, that can result in underlying gene expression changes.

RNA-induced silencing complex
A complex made up of an Argonaute protein and small RNA that inhibits translation of target RNAs through degradative and non-degradative mechanisms.

Blastomere
An early embryonic cell that is derived from the cleavage divisions of a fertilized egg.

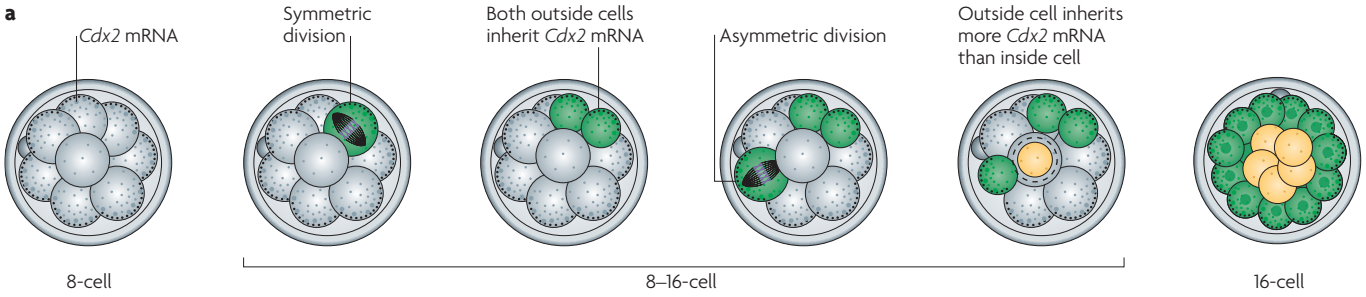
Polarization
Generation of morphological and molecular differences along the apical–basal axis of cells such as blastomeres.

circuits[25–28,50] and yet, before blastocyst formation, the pluripotency factors OCT4, SOX2 and NANOG are present in both inside and outside cells. Expression of pluripotency genes is downregulated by CDX2 (REF. 50) and, in its absence, the expression of these genes continues in outside cells[51]. CDX2 therefore seems to have a crucial role in breaking inside–outside symmetry, which raised the important question of how expression of CDX2 is first regulated. Does its expression become asymmetric between inside and outside cells as a result of cell position, cell polarity or both? Two recent papers reveal the possible mechanisms behind inside–outside asymmetry in CDX2, and provide insights into these questions[22,52].

Several explanations for establishment of the inside–outside asymmetry in CDX2 are possible: *Cdx2* transcription might be upregulated in outside cells after asymmetric cell division; *Cdx2* mRNA could be translated more efficiently in the outside cells; or *Cdx2* might be already expressed before cell division, but its mRNA or protein might be asymmetrically distributed in asymmetric divisions. Two of these possibilities have recently found experimental support. *Cdx2* mRNA has been shown to become enriched at the apical poles of polarized late 8-cell blastomeres[22], raising the possibility of differential inheritance of CDX2 transcripts between daughters of asymmetric divisions (FIG. 2a). This would point to a true asymmetry of this division. Not only does cell polarity affect the spatial distribution of *Cdx2* transcripts but, reciprocally, *Cdx2* expression strengthens cell polarity: upregulation of CDX2 in a cell increases the amount of apically localized aPKC[22]. Taken together, these results suggest a model of a positive feedback loop between cell polarity and CDX2 to reinforce the first cell fate decision and to ensure that a functional trophectoderm is established by the blastocyst stage (FIG. 2b). Thus, interaction between CDX2 and cell polarity could provide a molecular platform for the polarization hypothesis.

In addition to polarization of *Cdx2* transcripts, it has been recently shown that, following asymmetric divisions, cells in the embryo can 'sense' whether they reside in the inside or outside compartments through the Hippo signalling cascade[52]. This mechanism enables *Cdx2* expression to be enhanced when a functional transcriptional complex containing TEAD4 is present in outside cells but not in inside cells. Although the exact mechanism underlying this phenomenon remains unknown, one possibility involves sensing the degree of cell–cell contacts. Thus, the Hippo signalling pathway could provide a molecular platform for the inside–outside hypothesis. It will be of future interest to dissect the functional significance or synergy of differential *Cdx2* transcript localization and enhancement of *Cdx2* transcription. One might speculate, however, that earlier events might also influence *Cdx2* expression as its transcripts are already localized at the 8-cell stage[22], before any inner population of cells is generated and thus before Hippo signalling is likely to operate.

Are cell fate decisions random or are they biased? Zygotic expression of *Cdx2* begins at the 8-cell stage but, interestingly, several groups have found that initiation of CDX2 expression is not uniform[22,37,51,53]. Typically, both *Cdx2* mRNA and protein first appear in only a few, often just two, 8-cell stage blastomeres. Progressively, the number of cells expressing CDX2 increases. How can such asymmetry be explained if all blastomeres at this stage have the same relative position in the embryo? Are all cells equal or are some cells more equal than others? (After G. Orwell's *Animal Farm*.)



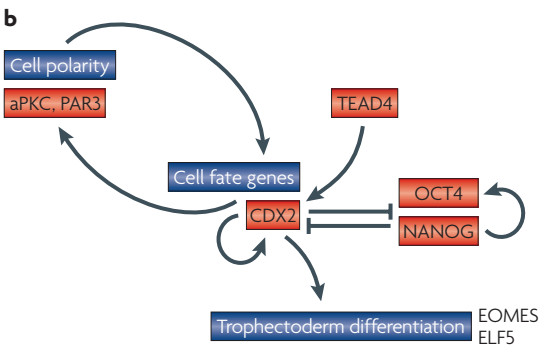


Figure 2 | **Transcriptional circuits in the first cell fate decision. a** | Cell polarization helps create a symmetry-breaking event. mRNA for the *Cdx2* transcription factor (small grey dots) becomes asymmetrically localized at the cortex of polarized blastomeres[22]. Thus, when these cells divide symmetrically this mRNA is equally partitioned between the daughter cells, but when they divide asymmetrically outer daughters inherit more *Cdx2* mRNA than inner daughters. When, after asymmetric divisions, cells reach their inside (yellow) or outside (green) position, molecular mechanisms that sense cell position can further influence transcription from the *Cdx2* locus. **b** | Cell polarity and trophectoderm fate are mutually reinforcing in symmetrically dividing cells. Increased *Cdx2* expression increases cell polarity and cell polarity leads to asymmetric localization of *Cdx2* mRNA. Decreased *Cdx2* transcripts in inner cells, as a result of the mechanisms outlined in **a**, relieves CDX2-mediated repression of the mutually reinforcing *Nanog* and *Oct4* genes that establish or retain pluripotency.

Animal pole
The position on the oocyte, and later on the embryo, in which the two asymmetric meiotic divisions take place. The first of these meiotic divisions takes place during oocyte maturation and the second after fertilization, both lead to extrusion of small cells called polar bodies. The second polar body remains attached and marks the animal–vegetal axis.

Vegetal pole
The position on the oocyte, and later on the embryo, opposite where the two asymmetric meiotic divisions take place.

Cavitation
The process by which the fluid-filled vesicular cavity (the blastocoel) is generated in approximately 32-cell stage embryos, forming a morphologically recognizable blastocyst.

The debate of recent years (BOX 1), as to whether cells are truly identical before the inside and outside populations are set apart, has to some extent been resolved with an increasing number of groups finding that there is differential gene expression in blastomeres before they come to occupy different positions[22,51,53]. This moves the debate away from the question of whether there are differences between early blastomeres and towards what the origins and significance of these differences might be. Thus, to consider just CDX2 as an example, is the development of differences in CDX2 expression stochastic (random) or probabilistic (biased)? In the latter case, do particular blastomeres have a greater chance of initiating *Cdx2* expression owing to their past history — that is, their specific division orientation, division order and relative position?

Precise tracking of the origins, division patterns, cell cycle lengths, movements and relative positions of all cells from the time they are born to the time their fate is fixed, combined with quantitative gene expression profiling in individual cells, has revealed some of the origins of the differences between the cells[22,54]. These studies showed that blastomeres with significantly higher levels of CDX2 are daughters of cells that show the lowest levels of specific chromatin modifications — asymmetric dimethylation of arginine residues 17 and 26 of histone H3 (H3R26me2a and H3R17me2a)[22,49]. Moreover, the differences in these epigenetic modifications at the 4-cell stage are a result of cell history. Namely, the blastomeres that inherit both animal and vegetal components of the zygote have higher levels of H3R17me2a and H3R26me2a, whereas the blastomeres that inherit solely vegetal material have lower levels of these modifications[49] (BOX 2). These vegetal blastomeres are also significantly less pluripotent than other cells[55]. Together, the conclusions that higher levels of CDX2 predispose cells to differentiation and restrain pluripotency in embryos mirror the findings of the effect of *Cdx2* expression in embryonic stem cells[50]. The difference is that the spatial organization of the embryo provides a cell with a unique history that influences CDX2 levels differentially between blastomeres. It is important to note that such differences do not have to be initially particularly strong to have a profound effect on cell properties, and thus on cell fate and cell potential, because they can be amplified by positive feedback loops.

Consequences of differences in CDX2 expression levels. Cells inheriting the vegetal part of the zygote at the second cleavage have maximal levels of CDX2 at the 8-cell stage and preferentially divide symmetrically, contributing more to trophectoderm than other cells do[22,54]. To understand the reason behind this we need to understand whether the CDX2 level can affect division orientation and, if so, by what mechanism. Two complementary approaches have been used to address this question. In the first, CDX2 expression was enhanced in half of the embryo, through the injection of *Cdx2* mRNA, and the development of this clone of cells was compared with neighbouring cells. This revealed that higher levels of CDX2 lead cells to divide symmetrically significantly more often, thereby retaining cells with higher CDX2 levels in an outside position[22]. This would reinforce transcriptional programming along the differentiation pathway to trophectoderm. In the second approach, *Cdx2* was downregulated in half of the embryo. This led to the opposite effect: lowering the levels of CDX2 resulted in more asymmetric divisions that directed cells to the ICM. This effect of CDX2 on division orientation might work through cell polarity, because increasing CDX2 levels enhances the apical localization of cell polarity markers such as aPKC[22]. Thus, the extent of cell polarization might be affected by the expression level of factors, such as CDX2, that promote differentiation into epithelium (trophectoderm). Interestingly, *Cdx2* downregulation at the 2-cell stage leads to a stronger effect on cell polarity and cell allocation (inside versus outside) than zygotic knockout of *Cdx2* (REFS 22,51). One possibility to explain this is that there is a pool of maternal *Cdx2* mRNA that would be affected by RNAi at these early stages but would not be affected by zygotic knockout. It would be interesting to examine in future if such a pool of maternal *Cdx2* exists in the mouse egg.

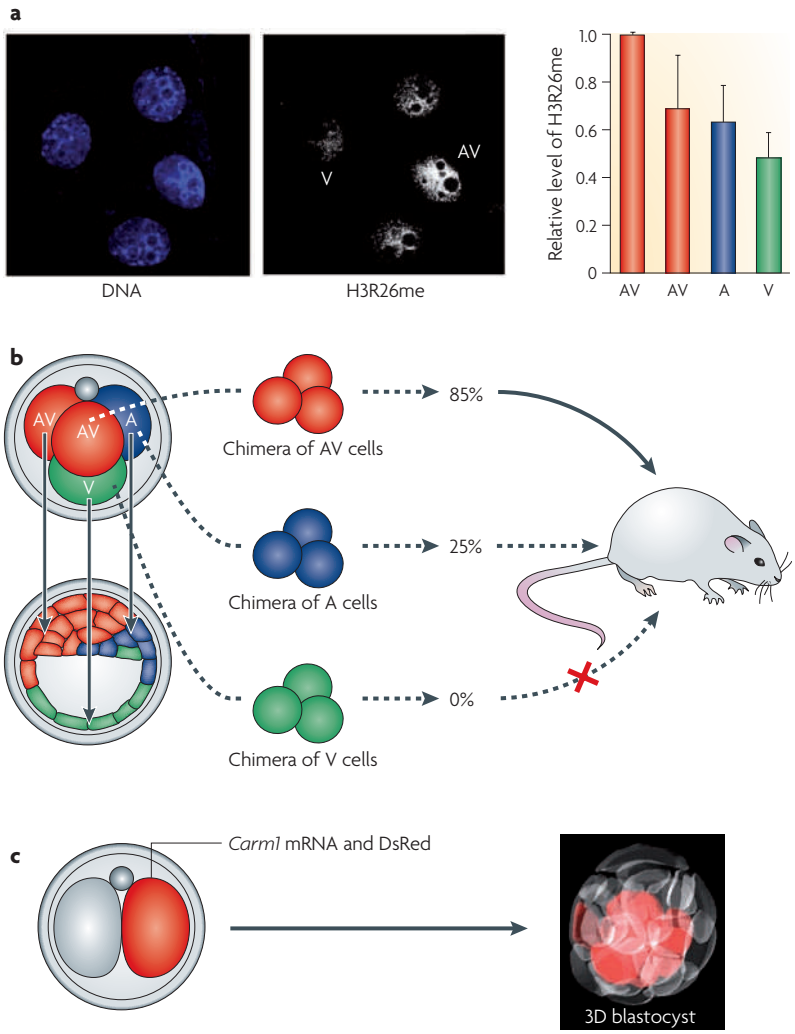
In summary, correlating the division orientation of every blastomere in normal development with its gene expression profile reveals that division orientation can

Box 1 | All blastomeres are born equal but are some more equal than others?

Whether blastomeres are truly equivalent has been controversial partly because it is not an easy problem to address. This is because the mouse embryo is regulative and can recover from removal or addition of cells. Regulative development does not preclude the possibility of early bias, but does makes it extraordinarily difficult to detect. The paucity of endogenous markers with which to orient the mouse embryo has also confounded attempts to study the origins of heterogeneity. Although some groups have been able to identify the animal–vegetal axis (that is, the axis defined by the animal pole and vegetal pole) because its marker, the second polar body, remains firmly attached throughout preimplantation development[84,85], others reported they were unable to do so as the second polar body became detached in their cultures[86]. It has also been reported that when embryos become extensively elongated (experimentally or naturally) embryo shape influences the site of cavitation and thus the orientation of the embryonic–abembryonic axis[85,87,88]. In such elongated embryos, blastomeres were reported to 'dance' to adjust their position to the shape of the zona[88]. However, the role of extrinsic factors, such as the zona, in blastocyst axis specification remains a matter of controversy[88,89]. Counter evidence suggests that the blastocyst cavity will form in the vicinity of symmetrically dividing cells, thus determining the orientation of the embryonic–abembryonic axis[41,54]. To help resolve this longstanding impasse, it would be interesting to examine whether cells in elongated embryos are more similar to each other or whether they also develop differences that bias their fates.

The separation of animal and vegetal cytoplasm provides a natural bias in a large proportion of embryos and in some way affects the methylation of arginine residues in histone H3 to influence potency (BOX 2). However, it is likely that, when the position of cells is changed or when the animal and vegetal parts are not separated, development will follow a more stochastic path. It is now a widely debated issue that cell-to-cell variation in transcription, so-called transcriptional noise, can generate variability that can be of selective advantage to individual members of a cell population[90]. It is thus possible that such variable (that is, stochastic) expression would need very little to send it in a direction in which it could be reinforced by natural feedback loops. Therefore, this biological decision could, like many others, be a continuum from stochastic to biased, thus accounting for the great difficulty in pinning down the exact mechanism.

Box 2 | **Histone methylation and developmental potential**



The differential methylation of arginine 26 and arginine 17 of histone H3 (H3R26me and H3R17me) (see the figure, part **a**) was only discovered through a subtle difference in the developmental history of blastomeres, namely a dependence on whether the vegetal (V) and animal (A) parts of the zygote have become separated by an equatorial division by the 4-cell stage. Methylation of H3R26 and H3R17 is elevated in 4-cell blastomeres containing animal and vegetal components as a result of meridional (that is, parallel to the animal–vegetal axis) divisions of the zygote[55]. The level of H3R26me and H3R17me is significantly decreased in blastomeres that divide last to enter the 4-cell stage and that inherit vegetal components after the equatorial division. Blastomeres with animal and vegetal components (AV) contribute more cells to the ICM as a result of dividing asymmetrically significantly more often than blastomeres that inherit vegetal components.

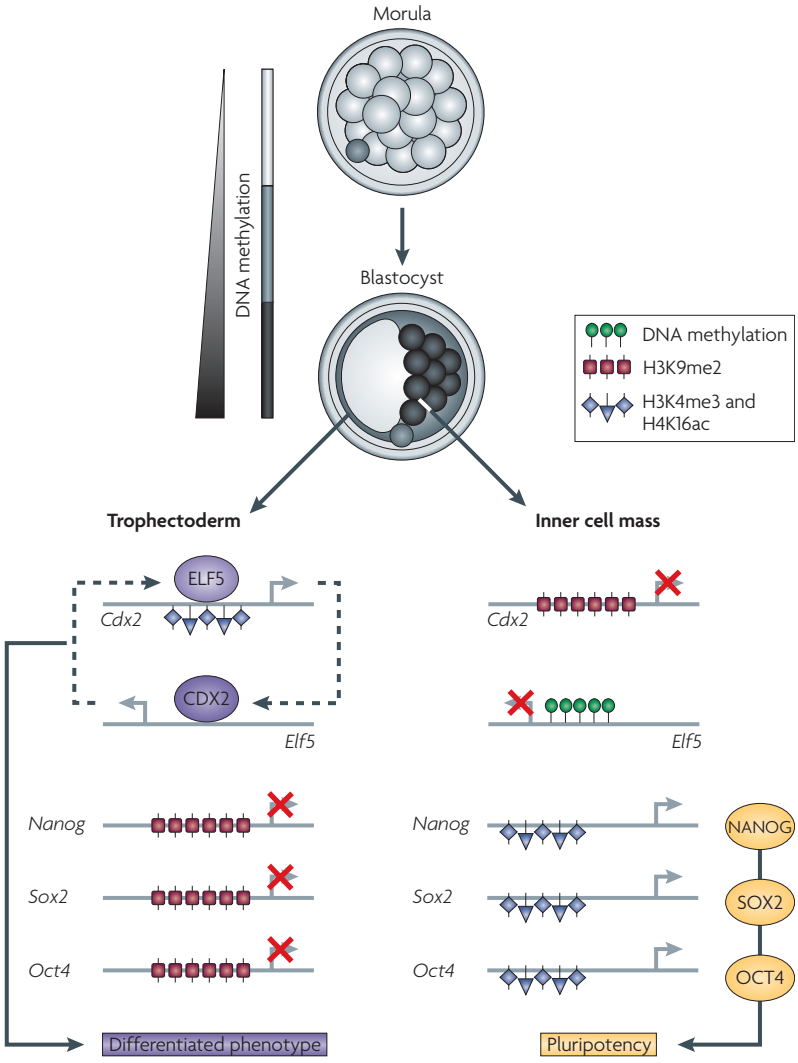
Chimeras of AV blastomeres (see the figure, part **b**) that have elevated histone H3R26me show greater developmental potential than chimeras of just animal or just vegetal blastomeres, which have lower levels of histone H3R26me[55]. Chimeras consisting of three AV blastomeres develop to birth with high efficiency, chimeras consisting of three animal blastomeres develop less efficiently, and chimeras consisting of three vegetal blastomeres are significantly smaller and arrest their development shortly after implantation[55]. Direct evidence that this specific epigenetic modification affects pluripotency came from the finding that overexpression of CARM1, the methyltransferase responsible for methylating H3R17 and H3R26 (REF. 91), leads to elevated *Nanog* and *Sox2* expression and causes cells to make a greater contribution to the ICM (revealed in red by the DsRed lineage marker) in the image of reconstructed blastocysts (see the figure, part **c**)[55]. It now seems that CARM1 also modulates the pluripotency of embryonic cells[92].

be affected by cell origin and expression levels of factors such as CDX2. This provides an insight into the long-standing question of why some blastomeres divide symmetrically at the 8-cell stage and others divide asymmetrically. Furthermore, as the CDX2 expression level can strengthen cell polarization, and cell polarity can affect CDX2 distribution, CDX2 and cell polarity might form a positive feedback loop. After differentiative cell divisions have accomplished their task and inside and outside cell populations emerge, molecular mechanisms sensing cell position can influence transcription from the *Cdx2* locus. From all that we know so far, it seems that both cell polarity and position affect cell fate, but the exact underlying mechanisms remain to be determined.

Epigenetic events and transcriptional programmes. When the two blastocyst lineages become separated they exhibit epigenetic asymmetries. For example, histone marks such as H3K27me3 are enriched in the ICM compared with the trophectoderm[56]. New methods that allow chromatin immunoprecipitation to be performed on small numbers of cells have now enabled the examination of loci-specific histone modifications in these two lineages[57]. The results imply that epigenetic regulation of chromatin is important for lineage segregation (BOX 3). The *Cdx2* gene is associated with repressive H3K9me2 marks in the ICM but not in the trophectoderm, where it is enriched for the transcriptionally activating marks of trimethylation of H3 lysine 4 (H3K4me3) and H4K16 acetylation. Conversely, the pluripotency-related genes *Nanog* and *Oct4* show the reciprocal relationship[57]. H3K9me2 has also been implicated in earlier developmental functions; the zygotic pronuclei show an asymmetry in the level of H3K9me2 with the maternal pronucleus having a higher level, which is coincident with the erasure of DNA methylation from the paternal genome[10,11,58]. The more extensive re-establishment of DNA methylation in the ICM compared with the trophectoderm suggests the potential importance of DNA methylation for regulating gene expression at these later stages[58]. Differential DNA methylation of the *Elf5* transcription factor gene might function to maintain trophectodermal fate decisions in the trophectoderm through a feedback loop to *Cdx2* and *Eomes*, but restrict expression of these genes in the pluripotent ICM[59].

Thus, epigenetic asymmetries may serve to reinforce the molecular identity of blastocyst lineages. However, recent demonstrations of epigenetic asymmetry in H3R17me2a and H3R26me2a at the 4-cell stage and its association with blastomere pluripotency[49] open the attractive possibility that at least some epigenetic modification could also precede transcriptional circuits in the first fate decision and thus steer lineage separation. It seems unlikely that the H3R17me2a or H3R26me2a modifications act alone in early development, rather that they are just a single cog in the epigenetic machinery controlling the balance between differentiation and pluripotency.

Box 3 | **Epigenetic events and transcriptional programmes**



During the transition from morula to blastocyst, *de novo* DNA methylation is preferentially established in the inner cell mass (ICM) rather than the trophectoderm (TE) lineage[58]. In these two lineages, the *Elf5* gene promoter becomes differentially methylated — in the ICM it is substantially methylated (see the figure, green symbols), and in the TE it remains free of DNA methylation. This lack of DNA methylation in the TE allows the *Elf5* gene to be transcriptionally activated (in part by the TE-specific factor CDX2). This in turn allows the establishment of a mutually reinforcing positive feedback transcriptional circuit, in which ELF5 transcriptionally activates the *Cdx2* gene, which then activates the *Elf5* gene. This transcriptional relationship ultimately maintains a TE-specific transcriptional programme. In the ICM the establishment of this TE-specific circuit is inhibited by the presence of *Elf5* gene promoter DNA methylation, precluding transcriptional activation[59]. Furthermore, the gene locus for the TE-specific factor *Cdx2* is also enriched for the transcriptionally repressive post-translational histone modification H3K9me2, and is depleted for the transcriptionally activating post-translational histone modifications H3K4me3 and H4K16 acetylation (H4K16ac)[57]. This contributes to the transcriptional silencing of the *Cdx2* gene and a failure to establish the same feedback loop observed in the TE. This, coupled with the fact that the pluripotency-related gene loci *Nanog*, *Sox2* and *Oct4* are enriched for the activating marks and depleted for the repressive ones[57], ensures that transcriptional programmes that promote pluripotency are favoured in the ICM. Consistent with this, the pluripotency-related gene loci are marked by transcriptionally repressive chromatin marks[57] in the TE, further contributing to TE identity.

Integrating the earlier models of development. How can we assimilate recent discoveries into the larger picture? Historically there have been three black and white viewpoints on the first cell fate decision. The 'early asymmetry' hypothesis proposed that asymmetry in the egg would generate differences between the cells that influence their fate[60] (modified in REF. 61). The early extreme and unrealistic interpretation of this hypothesis, that such differences would be deterministic, proved unfounded following the finding that repositioning a cell changes its fate. The next proposal, the inside–outside hypothesis[39], was also often over-interpreted to mean that cells are identical before they reach and respond to differential positions in the embryo. This interpretation also became vulnerable when cells were shown to be polarized before the inside and outside populations were delineated[21]. In this way, the polarization hypothesis (discussed previously) was born.

New technology, when applied to studying living mouse embryos and their individual cells, suggests that in reality the situation is not so black and white. Each of these hypotheses holds some truth, thereby offering opportunity for their integration (FIG. 3). It now seems that the mouse egg is not perfectly symmetric, most probably reflecting the highly asymmetric divisions during meiosis. However, the developmental stage at which this asymmetry is revealed depends on subsequent division orientations in the embryo, leading to differences between cells in specific epigenetic modifications at the 4-cell stage and in the expression levels of transcription factors such as CDX2. Such differences could in turn lead to differing levels of cell polarity that affect whether cells divide symmetrically or asymmetrically. This would affect cell position in the embryo, and cell position reinforces cell fate. Overall, this argues for a model in which the development of polarity and the segregation of lineages in the mouse embryo occurs progressively rather than in quantum leaps at one particular stage. Importantly, the existence of feedback loops reinforcing cell fate decisions means that even a small initial bias is sufficient to break the symmetry. This model sits well with, and in fact contributes to, an explanation of 'regulative' development of the mouse embryo, because the progressive acquisition of differences between cells through multiple mechanisms exemplifies the plasticity of cells and of the embryo as a whole.

Is bias helpful in guiding cell fate? Bias in successive developmental steps provides an ideal way of directing cells along particular pathways in a window of restricted developmental time. Such bias can arise either through the vagaries of stochastic events or it can be influenced by the organization of the early embryo. In either case, a developmental inclination induced by bias can be amplified through positive feedback loops. Cultured embryonic stem cells or other stem cells differ from the developing organism as they do not face the same spatial and temporal constraints, and thus they are more likely to follow stochastically biased patterns of gene expression. The asymmetry of the egg, which is a memory of meiosis, and the subsequent geometric constraints on

the developing embryo, bias its organization and are thus likely to have some impact on development. However, exposing such bias has not been trivial because the regulative properties of the embryo can mask its existence. Thus, for example, when clearly polarized outside cells are repositioned alongside non-polarized inside neighbours, they adopt a different fate[62]. The transcriptional and epigenetic changes that occur during such cell repositioning, and the mechanisms that ensure its plasticity, will be interesting topics of future study. Could they involve similar mechanisms to those suggested for embryonic stem cells, whereby pluripotent transcriptional programmes are propagated between generations of cells and yet the cells retain the ability to efficiently respond to differentiative cues? In such cells, functionally opposed methylation of specific histone H3 lysine residues (so-called bivalent chromatin domains[63–65]) have been proposed to maintain key differentiation genes in transcriptionally poised states. It is possible that similar mechanisms could contribute to the observed plasticity of blastomeres in the embryo.

The second cell fate decision

In the second fate decision, cells of the ICM that are in contact with the blastocyst cavity are set aside to form the second extraembryonic tissue, the primitive endoderm (PE). Deeper ICM cells escape differentiation, express pluripotency genes and become progenitors for all cells of the future body (FIG. 1a). PE differentiation necessitates the activation of the *Gata4* and *Gata6* transcription factor genes[66,67], and perhaps of genes encoding other factors yet to be discovered. These transcription factors are proposed to antagonize the expression of pluripotency transcription factors, such as *Nanog*[68] (FIG. 1d). Following *Gata4* and *Gata6* expression, proteins required for PE integrity become upregulated[68–71].

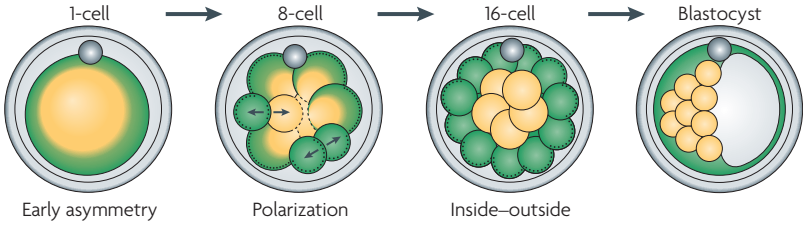


Figure 3 | **Integration of three hypotheses for the emergence of inside and outside cell differences.** The combined model we propose here suggests that the core elements of each of the three models — early asymmetry, polarization and inside–outside — that have been proposed for the first cell fate decision identify concepts that are not exclusive. The mouse egg has some asymmetry, possibly reflecting previous asymmetric meiotic divisions at the animal part of the egg, which leads to heterogeneity between the cells. The extent of this heterogeneity would depend on when cleavage divisions separate animal and vegetal parts of the embryo. Heterogeneity is revealed through asymmetry in epigenetic modifications at the 4-cell stage and through the expression levels of transcription factors such as CDX2 at the 8-cell stage. Such heterogeneity could generate differences in the timing or extent of blastomere polarization along the apical–basal axis that, in turn, would affect whether a cell divides symmetrically or asymmetrically. Asymmetric divisions generate inherently different inside and outside cells that will occupy different positions in the embryo. Cell position further reinforces cell fate, possibly owing to the different environment of inside (yellow) and outside (green) cells. This combined model proposes that the development of polarity to affect cell fate occurs progressively. Feedback loops reinforcing cell fate decisions ensure that even a small initial bias is sufficient to break the symmetry.

Old and new outlooks. Views of how the second lineage segregation occurs are also now shifting (FIG. 4a). The 'positional induction' model provided an early view that was conceptually similar to the inside–outside hypothesis for the first lineage segregation. According to this model, cells on the surface ICM adjacent to the cavity (outside) respond to and are triggered to differentiate by a hypothetical inductive signal that cannot be transmitted to the deeper (inside) cells. This model supposes ICM cells to be both homogeneous and bipotent, that is, able to form either epiblast or PE. Support for this model came from the finding that the outside cells of isolated ICM would differentiate into PE[72] — although whether this is the case has been controversial[73]. The more recent 'cell sorting' model arose from the finding of differences among the cells of the early ICM in expression levels of the genes encoding the PE-specific transcription factor *Gata6*, and the epiblast-specific transcription factor *Nanog*[68,74]. Thus, a key feature of the cell sorting model is that the early ICM is not composed of cells that can give rise to both lineages but of a mixed population of epiblast and PE progenitors that later segregate into their composite layers[68,75]. However, how this heterogeneity is established and whether progenitors for both epiblast and PE do indeed sort have remained unknown.

To understand PE and epiblast specification requires a complete knowledge of a number of factors: where the cell type of both tissues comes from; how the cells move between deep and surface ICM; whether they change their expression patterns; and whether the two cell layers can be refined by the apoptosis of cells that have an expression pattern inappropriate to their position. Studies of embryos expressing a PE reporter, *Pdgfra*, showed not only that some cells transit from the deep layer to the surface layer but also that deep cells can stop expressing *Pdgfra*[76]. The former finding is consistent with the cell sorting model and the latter could be explained through an effect of cell position on cell fate. However, as this study could only track the subpopulation of ICM cells that become PE, the origins of the epiblast and its ability to sort remained unknown. A more recent time-lapse study following the dynamics of all ICM cells from the early to late blastocyst stage revealed that some progenitors for both lineages move between the surface and deep layers[77]. Moreover, some ICM cells are bipotent, contributing to both lineages, indicating a degree of flexibility of early ICM fate. Although both of these time-lapse studies detect cell death within the ICM, computer modelling suggests that apoptosis is not the major mechanism that refines the final pattern[77]. This recent study also shows that, although GATA6 is required for cells to remain on the surface, GATA6 alone is insufficient to direct cell sorting to the surface, suggesting that additional signals are involved in this process. Taken together, this indicates that the cell sorting model on its own might not be enough to account for specification of PE. Rather, elements of both the cell sorting and positional induction

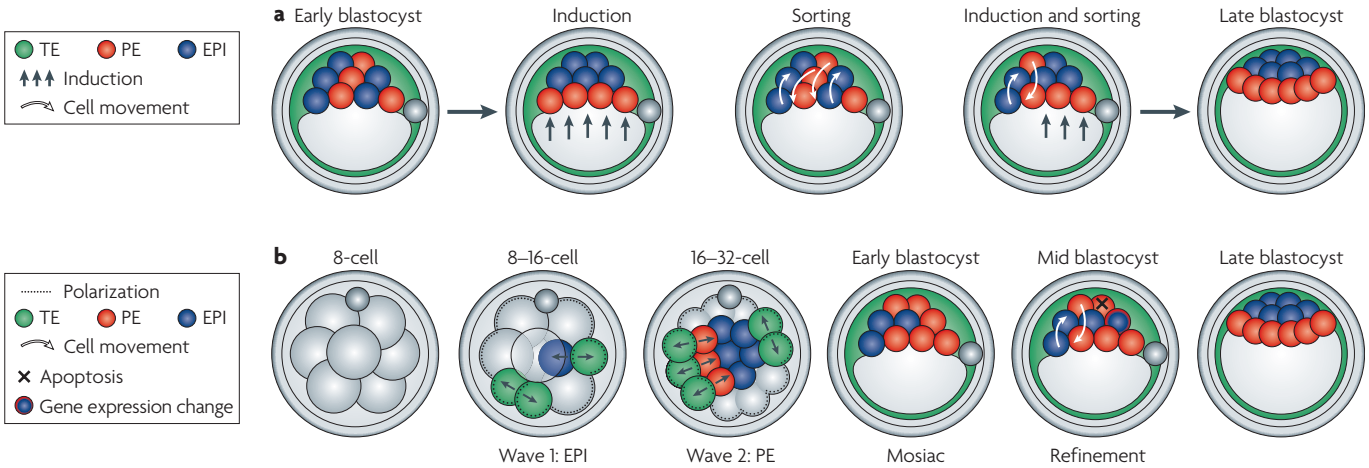


Figure 4 | **Second cell fate decision — primitive endoderm formation.** Following the first cell fate decision between the trophectoderm (TE) and the inner cell mass (ICM), the second cell fate decision designates cells that form the primitive endoderm (PE) and those that form epiblast (EPI). **a** | Three different models for PE formation are illustrated. The induction model proposes that cell position establishes whether cells differentiate into PE owing to an inductive signal — for example, from the cavity. The sorting model proposes that cells are pre-specified for the EPI or PE lineage in random positions and are then sorted according to their gene expression patterns. The combination model suggests that cells are affected by cell position from the beginning of cavity formation, but that cells do change their position and switch their fate. **b** | The social mobility model of EPI and PE formation. This model proposes that waves of asymmetric cell divisions have a large effect on whether a cell will develop as epiblast or primitive endoderm. The first wave of asymmetric divisions generates most of the EPI lineage, and the second wave of asymmetric divisions generates most of the PE lineage. Most of the cells are appropriately positioned from the beginning, but most of the cells that are not appropriately positioned move in an actin-dependent way between the surface and deeper layers and others contribute progeny to both compartments. Cells that do not change their position and remain inappropriately located either change their expression profile or die. Thus, patterning of the ICM into distinct PE and EPI layers is refined through a combination of cell movement, apoptosis and changes in gene expression.

models might act together in lineage segregation in the blastocyst. Interestingly, this would be analogous to the first fate decision that also involves more than one mechanism for lineage segregation.

Impact of early waves of divisions on the second cell fate. One of the important questions not answered by the studies just described is how the heterogeneous 'salt and pepper' distribution of epiblast and PE progenitors becomes established. The predominant view in recent years has been that the heterogeneity of the blastocyst ICM arises stochastically. Another view is that properties of the two different cell types might reflect different properties of their ancestors. Differential distribution of cytokeratin filaments could suggest inner cells generated at the 8–16-cell and 16–32-cell stages might differ[75,78]. However, the experimental evidence that the waves of asymmetric divisions could contribute cells of different properties in respect to second fate choice has been lacking.

The attraction of a model in which epiblast progenitors are generated in one set of asymmetric divisions and PE in the other set is that the inside cells would have identity as they arise. Thus, it would only be inappropriately positioned cells, when the cavity forms, that sort. These could be either epiblast or PE progenitors, which could account for the finding that both surface and deep cells can sort into the other layer[77]. The preliminary results of our recent studies to trace the origins, dynamics and final fate of every inside cell from the 8-cell stage (M.Z.-G. and S.A.M., unpublished observations) support this model. We therefore propose that the final position, and thus the fate, of the inside cells is determined both by the order in which they were generated, in successive waves of asymmetric divisions, and their subsequent mobility. We also propose that this decision-making process is multifactorial and involves position-dependent induction. This was first apparent from our earlier lineage studies following the pedigrees of individually labelled surface ICM cells[79,80] that showed that, although the majority of surface early ICM cells are destined to the PE lineage, some can give rise to epiblast and some are bipotent. Fibroblast growth factor (FGF) signalling could be implicated in the induction process as elimination of FGF4 affects PE development[81–83]. However, the role of FGF4 or other signalling cascades in lineage segregation remains to be determined.

The 'social mobility' model we propose here posits that the two successive waves of divisions position most of the inner cells according to which of the two classes they belong. Those cells that are not positioned appropriately move to join their fellows of similar class, or switch classes, or die. It would mean that, whereas the fate of most cells would be 'assigned' following internalization, cells retain the flexibility of being able to respond to some form of positional induction. Thus, in this proposed model, a combination of the positional history of cells,

specific cell division orientation, cell movement, cell death and induction contribute to the segregation of PE from epiblast lineages (FIG. 4b).

Conclusions

Careful consideration of old and new discoveries leads us to suggest that the first and second fate decisions may be inextricably linked. The onset of cell polarization is the first step on the road to becoming extraembryonic trophectoderm, and cells can only escape this fate by going inside. Those inside cells arising from the first wave of asymmetric divisions will give rise predominantly to epiblast, possibly because at this stage they still have a higher state of pluripotency. Those arising from the second wave will contribute mainly to extraembryonic PE. These decisions are influenced by biased expression of key transcription factors that guides rather than determines cell fate. Thus, a higher level of *Cdx2* predisposes a cell to form trophectoderm, a higher level of NANOG guides a cell to form epiblast, and a higher level of GATA6 tips it towards forming PE. But we still have more questions than answers. How might higher levels of CDX2 affect cell polarity, and thus division orientation, so that cells tend to divide more symmetrically than asymmetrically to contribute to trophectoderm? How exactly is this reinforced by positional signalling? In the first fate decision, cell polarity influences cell position and both influence gene expression, and vice versa. What, if any, are the influences of cell polarity on the decision to make PE rather than epiblast? In other words, how might the outside cells in the fourth cleavage cycle become different from those in the fifth cycle to endow the two waves of asymmetric division with different properties?

We now have a stronger conceptual framework on which to assemble a molecular and cellular picture of segregation of three distinct lineages by the blastocyst stage. We have learnt that cellular history can differentially influence epigenetic modifications and, in turn, the expression of key transcription factors to favour specific differentiation pathways. At first these shifts in gene expression are slight, but the interrelationships between transcription factors ensure their amplification. Thus, the emerging theme is that, once generated, differences are self-reinforcing. But until a certain point development is flexible and can be redirected to reflect changing circumstances. Another challenge for the future will be to uncover how all of the contributing factors to the cell fate decision process become re-established when an embryo regulates its own recovery from perturbation. Will the first changes include epigenetic reprogramming? Will repositioning a cell affect cell polarity to guide new patterns of gene expression, or can cell position guide gene expression independently of cell polarity? Understanding further the mechanisms behind natural bias in developmental cell fate decisions may guide us in the search for answers.

1. Wang, Q. T. *et al.* A genome-wide study of gene activity reveals developmental signaling pathways in the preimplantation mouse embryo. *Dev. Cell* **6**, 133–144 (2004).
2. Hamatani, T., Carter, M. G., Sharov, A. A. & Ko, M. S. Dynamics of global gene expression changes during mouse preimplantation development. *Dev. Cell* **6**, 117–131 (2004).
3. Schultz, R. M. The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. *Hum. Reprod. Update* **8**, 323–331 (2002).
4. Schier, A. F. The maternal–zygotic transition: death and birth of RNAs. *Science* **316**, 406–407 (2007).
5. Giraldez, A. J. *et al.* Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* **312**, 75–79 (2006).
6. Lykke-Andersen, K. *et al.* Maternal Argonaute 2 is essential for early mouse development at the maternal–zygotic transition. *Mol. Biol. Cell* **19**, 4383–4392 (2008).
7. Torres-Padilla, M. E. & Zernicka-Goetz, M. Role of TIF1α as a modulator of embryonic transcription in the mouse zygote. *J. Cell Biol.* **174**, 329–338 (2006).
8. Sun, F., Tang, F., Yan, A. Y., Fang, H. Y. & Sheng, H. Z. Expression of SRG3, a chromatin-remodelling factor, in the mouse oocyte and early preimplantation embryos. *Zygote* **15**, 129–138 (2007).
9. Aoki, F., Worrad, D. M. & Schultz, R. M. Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. *Dev. Biol.* **181**, 296–307 (1997).
10. Yeo, S., Lee, K. K., Han, Y. M. & Kang, Y. K. Methylation changes of lysine 9 of histone H3 during preimplantation mouse development. *Mol. Cell* **20**, 423–428 (2005).
11. Santos, F., Peters, A. H., Otte, A. P., Reik, W. & Dean, W. Dynamic chromatin modifications characterise the first cell cycle in mouse embryos. *Dev. Biol.* **280**, 225–236 (2005).
12. Bultman, S. J. *et al.* Maternal BRG1 regulates zygotic genome activation in the mouse. *Genes Dev.* **20**, 1744–1754 (2006).
13. Yamamoto, M. *et al.* Antagonism between Smad1 and Smad2 signaling determines the site of distal visceral endoderm formation in the mouse embryo. *J. Cell Biol.* **184**, 323–334 (2009).
14. Soares, M. L., Torres-Padilla, M. E. & Zernicka-Goetz, M. Bone morphogenetic protein 4 signaling regulates development of the anterior visceral endoderm in the mouse embryo. *Dev. Growth Differ.* **50**, 615–621 (2008).
15. Rodriguez, T. A., Srinivas, S., Clements, M. P., Smith, J. C. & Beddington, R. S. Induction and migration of the anterior visceral endoderm is regulated by the extra-embryonic ectoderm. *Development* **132**, 2513–2520 (2005).
16. Richardson, L., Torres-Padilla, M. E. & Zernicka-Goetz, M. Regionalised signalling within the extraembryonic ectoderm regulates anterior visceral endoderm positioning in the mouse embryo. *Mech. Dev.* **123**, 288–296 (2006).
17. Lawson, K. A. *et al. Bmp4* is required for the generation of primordial germ cells in the mouse embryo. *Genes Dev.* **13**, 424–436 (1999).
18. Graham, C. F. & Deussen, Z. A. Features of cell lineage in preimplantation mouse development. *J. Embryol. Exp. Morphol.* **48**, 53–72 (1978).
19. Graham, C. F. & Lehtonen, E. Formation and consequences of cell patterns in preimplantation mouse development. *J. Embryol. Exp. Morphol.* **49**, 277–294 (1979).
20. Pedersen, R. A., Wu, K. & Balakier, H. Origin of the inner cell mass in mouse embryos: cell lineage analysis by microinjection. *Dev. Biol.* **117**, 581–595 (1986).
21. Johnson, M. H. & Ziomek, C. A. The foundation of two distinct cell lineages within the mouse morula. *Cell* **24**, 71–80 (1981).
22. Jedrusik, A. *et al.* Role of Cdx2 and cell polarity in cell allocation and specification of trophectoderm and inner cell mass in the mouse embryo. *Genes Dev.* **22**, 2692–2706 (2008).
The first paper to indicate positive feedback between *Cdx2* expression and cell polarization in the mouse embryo and its importance for trophectoderm specification. Also shows that 8-cell stage heterogeneity between cells in the levels of factors such as CDX2 depends on the cell origins and affects cell fate.
23. Scholer, H. R., Ruppert, S., Suzuki, N., Chowdhury, K. & Gruss, P. New type of POU domain in germ line-specific protein Oct-4. *Nature* **344**, 435–439 (1990).
24. Nichols, J. *et al.* Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* **95**, 379–391 (1998).
25. Avilion, A. A. *et al.* Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* **17**, 126–140 (2003).
26. Chambers, I. *et al.* Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* **113**, 643–655 (2003).
27. Mitsui, K. *et al.* The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell* **113**, 631–642 (2003).
28. Strumpf, D. *et al.* Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst. *Development* **132**, 2093–2102 (2005).
The first study to show that CDX2 is required for trophectoderm differentiation and the maintenance of the cavity in the mouse embryo. It also shows that the elimination of *Cdx2* prevents downregulation of pluripotency factors in outside cells.
29. Beck. *et al.* A study of regional gut endoderm potency by analysis of *Cdx2* null mutant chimeric mice. *Dev. Biol.* **255**, 399–406 (20003).
30. Russ, A. P. *et al.* Eomesodermin is required for mouse trophoblast development and mesoderm formation. *Nature* **404**, 95–99 (2000).
31. Loh, Y. H. *et al.* The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nature Genet.* **38**, 431–440 (2006).
32. Boyer, L. A. *et al.* Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* **122**, 947–956 (2005).
33. Chew, J. L. *et al.* Reciprocal transcriptional regulation of *Pou5f1* and *Sox2* via the Oct4/Sox2 complex in embryonic stem cells. *Mol. Cell Biol.* **25**, 6031–6046 (2005).
34. Beland, M. *et al. Cdx1* autoregulation is governed by a novel Cdx1-LEF1 transcription complex. *Mol. Cell Biol.* **24**, 5028–5038 (2004).
35. Elling, U., Klasen, C., Eisenberger, T., Anlag, K. & Treier, M. Murine inner cell mass-derived lineages depend on Sall4 function. *Proc. Natl Acad. Sci. USA* **103**, 16319–16324 (2006).

36. Zhang, J. *et al.* Sall4 modulates embryonic stem cell pluripotency and early embryonic development by the transcriptional regulation of *Pou5f1*. *Nature Cell Biol.* **8**, 1114–1123 (2006).
37. Yagi, R. *et al.* Transcription factor TEAD4 specifies the trophectoderm lineage at the beginning of mammalian development. *Development* **134**, 3827–3836 (2007).
38. Nishioka, N. *et al. Tead4* is required for specification of trophectoderm in preimplantation mouse embryos. *Mech. Dev.* **125**, 270–283 (2008).
39. Tarkowski, A. K. & Wroblewska, J. Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. *J. Embryol. Exp. Morphol.* **18**, 155–180 (1967).
40. Fleming, T. P. & Johnson, M. H. From egg to epithelium. *Annu. Rev. Cell Biol.* **4**, 459–485 (1988).
41. Plusa, B. *et al.* Downregulation of Par3 and aPKC function directs cells towards the ICM in the preimplantation mouse embryo. *J. Cell Sci.* **118**, 505–515 (2005).
The first paper to show that the downregulation of cell polarity molecules, such as PAR3 and aPKC, leads cells to change their position from outside to inside, where they develop as ICM.
42. Thomas, F. C. *et al.* Contribution of JAM-1 to epithelial differentiation and tight-junction biogenesis in the mouse preimplantation embryo. *J. Cell Sci.* **117**, 5599–5608 (2004).
43. Vinot, S. *et al.* Asymmetric distribution of PAR proteins in the mouse embryo begins at the 8-cell stage during compaction. *Dev. Biol.* **282**, 307–319 (2005).
44. Johnson, M. H. & Ziomek, C. A. Cell interactions influence the fate of mouse blastomeres undergoing the transition from the 16- to the 32-cell stage. *Dev. Biol.* **95**, 211–218 (1983).
45. Fleming, T. P., Sheth, B. & Fesenko, I. Cell adhesion in the preimplantation mammalian embryo and its role in trophectoderm differentiation and blastocyst morphogenesis. *Front. Biosci.* **6**, D1000–D1007 (2001).
46. Handyside, A. H. Time of commitment of inside cells isolated from preimplantation mouse embryos. *J. Embryol. Exp. Morphol.* **45**, 37–53 (1978).
47. Spindle, A. I. Trophoblast regeneration by inner cell masses isolated from cultured mouse embryos. *J. Exp. Zool.* **203**, 483–489 (1978).
48. Rossant, J. & Lis, W. T. Potential of isolated mouse inner cell masses to form trophectoderm derivatives *in vivo*. *Dev. Biol.* **70**, 255–261 (1979).
49. Torres-Padilla, M. E., Parfitt, D. E., Kouzarides, T. & Zernicka-Goetz, M. Histone arginine methylation regulates pluripotency in the early mouse embryo. *Nature* **445**, 214–218 (2007).
50. Niwa, H. *et al.* Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. *Cell* **123**, 917–929 (2005).
51. Ralston, A. & Rossant, J. Cdx2 acts downstream of cell polarization to cell-autonomously promote trophectoderm fate in the early mouse embryo. *Dev. Biol.* **313**, 614–629 (2008).
52. Nishioka, N. *et al.* The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass. *Dev. Cell* **16**, 398–410 (2009).
Identifies the signalling pathway that is likely to sense cell position from the 16-cell stage, which is important to maintain the required high levels of *Cdx2* expression in the outside, but not inside, cells.
53. Dietrich, J. E. & Hiiragi, T. Stochastic patterning in the mouse pre-implantation embryo. *Development* **134**, 4219–4231 (2007). .
Reports co-expression of pluripotency genes with genes required for cell differentiation at the morula stage. Proposes that cell fate decisions in the mouse embryo occur randomly.
54. Bischoff, M., Parfitt, D. E. & Zernicka-Goetz, M. Formation of the embryonic–abembryonic axis of the mouse blastocyst: relationships between orientation of early cleavage divisions and pattern of symmetric/asymmetric divisions. *Development* **135**, 953–962 (2008).
The first complete analysis of lineage tracing from the 2-cell to blastocyst stages. It monitors cell division orientations, cell cycle length and the origins and fates of all cells. It reveals that cell origins affect division orientation and thus can bias allocation of cells to different lineages at the blastocyst stage.
55. Piotrowska-Nitsche, K., Perea-Gomez, A., Haraguchi, S. & Zernicka-Goetz, M. Four-cell stage mouse blastomeres have different developmental properties. *Development* **132**, 479–490 (2005).
56. Erhardt, S. *et al.* Consequences of the depletion of zygotic and embryonic enhancer of zeste 2 during preimplantation mouse development. *Development* **130**, 4235–4248 (2003).
57. O'Neill, L. P., VerMilyea, M. D. & Turner, B. M. Epigenetic characterization of the early embryo with a chromatin immunoprecipitation protocol applicable to small cell populations. *Nature Genet.* **38**, 835–841 (2006).
58. Santos, F., Hendrich, B., Reik, W. & Dean, W. Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev. Biol.* **241**, 172–182 (2002).
59. Ng, R. K. *et al.* Epigenetic restriction of embryonic cell lineage fate by methylation of *Elf5*. *Nature Cell Biol.* **10**, 1280–1290 (2008).
60. Dalcq, A. M. [Cytochemistry of the 1st Stages of Development in Various Mammals.] *Annee Biol.* **59**, 129–155 (1965) (in French).
61. Gardner, R. L. Can developmentally significant spatial patterning of the egg be discounted in mammals? *Hum. Reprod. Update* **2**, 3–27 (1996).
A critical review of the literature that resurrected a previously dismissed possibility that there might be some differences between early blastomeres in the mouse embryo that relate to the animal–vegetal polarity of the egg.
62. Suwinska, A., Czolowska, R., Ozdzenski, W. & Tarkowski, A. K. Blastomeres of the mouse embryo lose totipotency after the fifth cleavage division: expression of *Cdx2* and *Oct4* and developmental potential of inner and outer blastomeres of 16- and 32-cell embryos. *Dev. Biol.* **322**, 133–144 (2008).
63. Bernstein, B. E. *et al.* A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315–326 (2006).
64. Mikkelsen, T. S. *et al.* Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* **448**, 553–560 (2007).
65. Ku, M. *et al.* Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. *PLoS Genet.* **4**, e1000242 (2008).
66. Morrisey, E. E. *et al.* GATA6 regulates HNF4 and is required for differentiation of visceral endoderm in the mouse embryo. *Genes Dev.* **12**, 3579–3590 (1998).
67. Koutsourakis, M., Langeveld, A., Patient, R., Beddington, R. & Grosveld, F. The transcription factor GATA6 is essential for early extraembryonic development. *Development* **126**, 723–732 (1999).
68. Chazaud, C., Yamanaka, Y., Pawson, T. & Rossant, J. Early lineage segregation between epiblast and primitive endoderm in mouse blastocysts through the Grb2-MAPK pathway. *Dev. Cell* **10**, 615–624 (2006).
This study originally identified the 'salt and pepper' pattern of expression of *Nanog* and *Gata6* in the early ICM. This led to the first suggestion that cell sorting might be involved in PE specification.
69. Yang, D. H. *et al.* Disabled-2 is essential for endodermal cell positioning and structure formation during mouse embryogenesis. *Dev. Biol.* **251**, 27–44 (2002).
70. Miner, J. H., Li, C., Mudd, J. L., Go, G. & Sutherland, A. E. Compositional and structural requirements for laminin and basement membranes during mouse embryo implantation and gastrulation. *Development* **131**, 2247–2256 (2004).
71. Gerbe, F., Cox, B., Rossant, J. & Chazaud, C. Dynamic expression of Lrp2 pathway members reveals progressive epithelial differentiation of primitive endoderm in mouse blastocyst. *Dev. Biol.* **313**, 594–602 (2008).
72. Dziadek, M. Cell differentiation in isolated inner cell masses of mouse blastocysts *in vitro*: onset of specific gene expression. *J. Embryol. Exp. Morphol.* **53**, 367–379 (1979).
73. Gardner, R. L., Lyon, M. F., Evans, E. P. & Burtenshaw, M. D. Clonal analysis of X-chromosome inactivation and the origin of the germ line in the mouse embryo. *J. Embryol. Exp. Morphol.* **88**, 349–363 (1985).
74. Kurimoto, K. *et al.* An improved single-cell cDNA amplification method for efficient high-density oligonucleotide microarray analysis. *Nucleic Acids Res.* **34**, e42 (2006).
75. Rossant, J., Chazaud, C. & Yamanaka, Y. Lineage allocation and asymmetries in the early mouse embryo. *Philos. Trans. R. Soc. Lond. B* **358**, 1341–1348 (2003); discussion 1349.
76. Plusa, B., Piliszek, A., Frankenberg, S., Artus, J. & Hadjantonakis, A. K. Distinct sequential cell behaviours direct primitive endoderm formation in the mouse blastocyst. *Development* **135**, 3081–3091 (2008).
This study presents time-lapse dynamics of the expression patterns of a PE marker and is the first to show that progenitor cells for PE sort from deep to surface compartments. However, it also shows that some cells expressing this PE marker change their expression pattern or undertake apoptosis if they stay deep.
77. Meilhac, S. *et al.* Active cell movements coupled to positional induction are involved in lineage segregation in the mouse blastocyst. *Dev. Biol.* 5 May 2009 (doi:10.1016/j.ydbio.2009.04.036).
This paper shows that progenitors for both PE and epiblast sort and that this is an active, actin-dependent process. However, it also shows that a fraction of early ICM cells contribute to both lineages. It leads to the proposal that both cell movement and induction participate in lineage segregation.
78. Chisholm, J. C. & Houliston, E. Cytokeratin filament assembly in the preimplantation mouse embryo. *Development* **101**, 565–582 (1987).
79. Weber, R. J., Pedersen, R. A., Wianny, F., Evans, M. J. & Zernicka-Goetz, M. Polarity of the mouse embryo is anticipated before implantation. *Development* **126**, 5591–5598 (1999).
80. Perea-Gomez, A. *et al.* Regionalization of the mouse visceral endoderm as the blastocyst transforms into the egg cylinder. *BMC Dev. Biol.* **7**, 96 (2007).
81. Feldman, B., Poueymirou, W., Papaioannou, V. E., DeChiara, T. M. & Goldfarb, M. Requirement of FGF-4 for postimplantation mouse development. *Science* **267**, 246–249 (1995).
82. Chai, N. *et al.* FGF is an essential regulator of the fifth cell division in preimplantation mouse embryos. *Dev. Biol.* **198**, 105–115 (1998).
83. Arman, E., Haffner-Krausz, R., Chen, Y., Heath, J. K. & Lonai, P. Targeted disruption of fibroblast growth factor (FGF) receptor 2 suggests a role for FGF signaling in pregastrulation mammalian development. *Proc. Natl Acad. Sci. USA* **95**, 5082–5087 (1998).
84. Gardner, R. L. The early blastocyst is bilaterally symmetrical and its axis of symmetry is aligned with the animal–vegetal axis of the zygote in the mouse. *Development* **124**, 289–301 (1997).
This is the first paper to show that the second polar body remains firmly attached to the embryo in undisturbed development, and therefore provides an enduring marker of the animal–vegetal axis. It is also the first paper to show the correlation of polarity between the zygote and the blastocyst.
85. Plusa, B. *et al.* The first cleavage of the mouse zygote predicts the blastocyst axis. *Nature* **434**, 391–395 (2005).
86. Hiiragi, T. & Solter, D. First cleavage plane of the mouse egg is not predetermined but defined by the topology of the two apposing pronuclei. *Nature* **430**, 360–364 (2004).
87. Zernicka-Goetz, M. The first cell-fate decisions in the mouse embryo: destiny is a matter of both chance and choice. *Curr. Opin. Genet. Dev.* **16**, 406–412 (2006).
88. Kurotaki, Y., Hatta, K., Nakao, K., Nabeshima, Y. & Fujimori, T. Blastocyst axis is specified independently of early cell lineage but aligns with the ZP shape. *Science* **316**, 719–723 (2007).
89. Gardner, R. L. The axis of polarity of the mouse blastocyst is specified before blastulation and independently of the zona pellucida. *Hum. Reprod.* **22**, 798–806 (2007).
90. Raj, A. & van Oudenaarden, A. Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell* **135**, 216–226 (2008).
91. Chen, D. *et al.* Regulation of transcription by a protein methyltransferase. *Science* **284**, 2174–2177 (1999).
92. Wu, Q. *et al.* CARM1 is required in ES cells to maintain pluripotency and resist differentiation. *Stem Cells* (in the press).

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DATABASES
UniProtKB: http://www.uniprot.org
CDX2 | NANOG | OCT4 | SOX2

FURTHER INFORMATION
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