© 2015. Published by The Company of Biologists Ltd | Development (2015) 142, 9-12 doi:10.1242/dev.115451

SPOTLIGHT

Mouse and human blastocyst-derived stem cells: vive les differences

Janet Rossant[1,2,*]

ABSTRACT

Lessons learned from conserved vertebrate developmental pathways have catalyzed rapid advances in pluripotent stem cell differentiation towards therapeutically relevant cell types. The most highly conserved phases of development are associated with the early patterning of the body plan – the so-called phylotypic stage. Both prior to and after this stage there is much more divergence across species. Developmental differences between human and mouse at the blastocyst and early post-implantation stages might help explain the differences among the different stem cell lines derived from these embryos. A better understanding of these early stages of human development will aid our ability to generate and manipulate human stem cells and their derivatives.

Introduction

Differentiation protocols designed to generate specialized cell types or even mini-organs from human pluripotent stem cells are becoming increasingly sophisticated. These approaches offer hope for future cell-based therapies for many degenerative diseases and devastating injuries. They also provide new tools for drug discovery and toxicology, and open up opportunities to study stages of human development not accessible by any other means. Developmental biologists have been able to show that the mechanisms of germ layer formation at gastrulation, anterior-posterior body axis patterning, somite formation, limb development and formation of organ anlage are fundamentally conserved across vertebrate evolution. Knowledge of such pathways has been translated into stepwise differentiation protocols to take embryonic stem cells (ESCs) from pluripotency to formation of a variety of fetal-type specialized cells, without direct knowledge of the relevant stages of human development *in vivo*.

However, when it comes to the next key phases of maturation of these fetal progenitors into fully functional adult cells, there are still too few cases in which this has been successfully achieved. This is still a real challenge for the field [see, for example, the discussion on generating lung cells elsewhere in this issue (Snoeck et al., 2015)]. In part, this reflects the fact that these stages tend to show more variation among species. Thus, studying maturation of cell types in the mouse or in mouse ESCs might not adequately inform human stem cell differentiation. More direct analysis of human fetal and postnatal physiology may provide some more clues to help in the quest for the perfect human stem cell differentiation protocol.

It should come as no surprise to developmental biologists that the early phases of tissue differentiation from ESCs seem to be relatively conserved, whereas final maturation pathways vary across species. This typifies the concept of the phylotypic phase of development (Richardson, 1995), in which action of conserved developmental genes, including the *Hox* genes, leads to the fundamental patterning of the body axis and organ rudiments. Prior to and after this stage of development, much more variation in morphology and transcriptional profiles are seen across vertebrate evolution – the so-called 'hourglass model' of development (Duboule, 1994; Raff, 1996). This model also predicts that there will be potential differences among ESCs from different mammalian species, as ESCs are derived from the blastocyst stage, before the phylotypic stage. Indeed, there is considerable variation even in the capacity to derive ESCs across different mammalian species and, in the two species most well characterized, the mouse and the human, there are clear differences between the properties of ES or induced pluripotent stem (iPS) cell lines derived under standard conditions.

In this Spotlight article, I argue that these differences might reflect the fact that ESCs arise from a stage of development that is mostly concerned with making extra-embryonic cell types that play no permanent role in the formation of the fetus itself, but are undergoing highly divergent selection pressures related to variation in implantation and placentation across mammalian species.

Blastocyst: conservation and divergence of lineage functions

Formation of the blastocyst represents the first lineage segregation event during embryonic development of every eutherian mammal. Morphologically, all blastocysts contain three distinct cell types: (1) an outer monolayer of polarized cells, the trophectoderm (TE), enclosing the inner cell mass (ICM), which consists of (2) a layer of primitive endoderm (PE) covering (3) a compact group of cells, the epiblast (EPI) (Fig. 1). Extensive studies in the mouse have shown that these lineages are specified to their future fates by the late blastocyst stage [reviewed by Rossant and Tam (2009)]. The EPI cells are the pluripotent cells of the blastocyst producing all germ layers of the fetus itself, whereas the TE gives rise to the trophoblast layers of the placenta and the PE largely gives rise to the endoderm of the yolk sacs. Although experimental lineage analysis is not possible in humans, it seems likely that these general lineage relationships also hold true in the human blastocyst.

Mechanistically, local FGF/ERK signaling levels are crucial for the development of all three lineages and their derived stem cells in mouse (Lanner and Rossant, 2010). FGF4 is produced by a subset of cells in the early ICM, under the control of the pluripotency factor partners, OCT4 and SOX2 (Yuan et al., 1995), and signals to adjacent cells within the ICM, directing them towards the PE pathway. Blocking FGF/ERK signaling or enhancing FGF action during ICM development can transform all ICM cells to EPI or PE, respectively (Nichols et al., 2009; Yamanaka et al., 2010). The same FGF signal from ICM cells also signals to overlying TE cells and promotes their proliferation and self-renewal (Goldin and

---

[1] Program in Developmental and Stem Cell Biology, Hospital for Sick Children, Peter Gilgan Centre for Research and Learning, 686 Bay Street, Toronto, Ontario, Canada M5G OA4. [2] Department of Molecular Genetics, University of Toronto, Peter Gilgan Centre for Research and Learning, 686 Bay Street, Toronto, Ontario, Canada M5G OA4.

*Author for correspondence (janet.rossant@sickkids.ca)

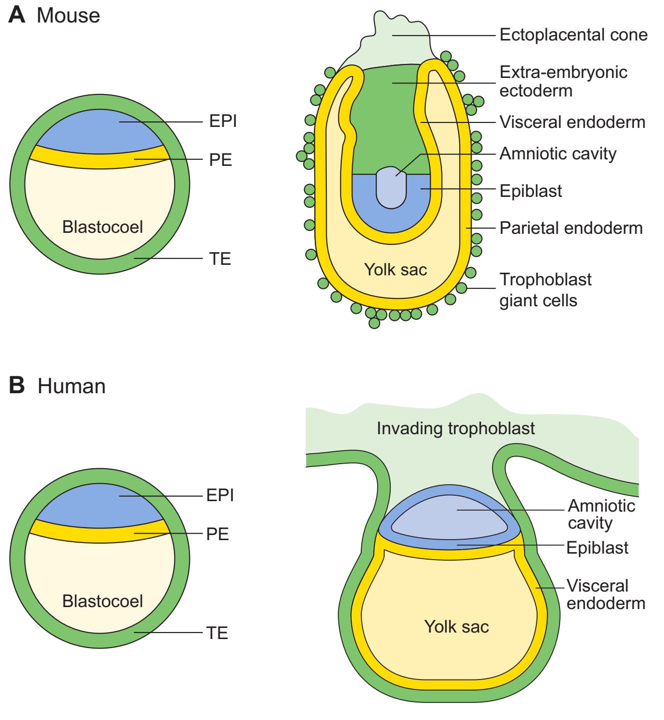

Fig. 1. Comparison of blastocyst and early post-implantation development between mouse and human. Whereas the blastocysts of the two species look very similar, later stages show significant differences, particularly in the extra-embryonic tissues. (A) The trophectoderm of the mouse blastocyst after implantation undergoes a proliferative phase stimulated by FGF4 signals from the epiblast, to form the extra-embryonic ectoderm (green) and ectoplacental cone (light green). There is limited invasion of the maternal uterus by trophoblast cells until much later in placental development. (B) The trophectoderm of the human blastocyst does not stay in close contact with the epiblast (EPI, blue) after implantation, but invades into the endometrium, where it will later form the chorionic villi. PE, primitive endoderm; TE, trophectoderm.

Papaioannou, 2003). This heavy reliance on the FGF pathway is a very parsimonious use of a single signal from a single cell type to promote lineage specification and proliferation at the blastocyst stage in the mouse.

There is still incomplete information on the molecular and cellular events of human blastocyst formation, compared with the mouse, but what is known suggests that there are differences in timing and potentially in mechanisms of lineage formation and function [reviewed by De Paepe et al. (2014)]. The blastocyst stage of development looks superficially similar between mouse and human (Fig. 1), but the human blastocyst undergoes at least one more round of cell divisions before implanting in the uterus. Concomitant with this extended free-living phase prior to implantation, it seems that lineage commitment is delayed when compared with the mouse. Expression of the lineage-specific transcription factors known to be associated with cell fate specification in the mouse blastocyst begins later in human than in mouse. For example, the expression of CDX2, a key transcription factor required for mouse TE specification, only begins after blastocyst formation in humans, and overlaps in expression with the pluripotency factor OCT4, which is not restricted to the ICM until just prior to implantation (Niakan and Eggan, 2013). Recent studies have shown that both inside and outside cells from fully expanded human blastocysts can separately reconstitute a blastocyst, and that totipotency is only lost just prior to implantation (De Paepe et al., 2013). Thus, the morphological events of formation of a polarized outer epithelium, a blastocoelic cavity and an ICM occur prior to actual lineage specification. In the mouse, lineage restriction is complete by the mid-blastocyst stage (Rossant and Tam, 2009), and blastocyst formation can begin in the absence of lineage commitment in embryos mutant for genes such as *Cdx2* (Strumpf et al., 2005) and components of the HIPPO signaling pathway (Cockburn et al., 2013; Hirate et al., 2013; Nishioka et al., 2009). However, the time between first formation of the blastocyst and implantation is shorter in the mouse, perhaps explaining earlier segregation of gene expression and cell fate.

Interestingly, the mechanisms of lineage specification in the human embryo seem to differ significantly from the mouse: the ICM is not sensitive to inhibition of FGF signaling in the same manner as in the mouse. When human pre-implantation embryos were grown in the presence of ERK inhibitors to the blastocyst stage, there was no apparent effect on the formation of the PE (Kuijk et al., 2012; Roode et al., 2012; Van der Jeught et al., 2013), as would have been predicted from the mouse, suggesting that the formation of EPI versus PE is not under the tight control of FGF levels in the human. In addition, levels of FGF receptors are low in the human TE, which does not proliferate in response to FGF (Kunath et al., 2014). Does this reflect a general downplaying of the importance of this pathway in the human? More understanding of the relative availability and roles of different signaling pathways in the two embryos is still needed.

Implantation and placental variation between mouse and human

The timing and invasiveness of implantation of the mammalian blastocyst varies remarkably across species, from the relatively early, deep implantation seen in mouse and human to the very late, superficial implantation seen in pigs, cows and horses. But even between mouse and human, there are differences. In the mouse, the implantation site is surrounded by a rapid expansion of the uterine stroma, the decidua, but the TE cells themselves do not invade into the stroma. Rather, the TE overlying the ICM proliferates in response to FGF to form the solid structure of the extra-embryonic ectoderm – the stem cells for the later placenta (Fig. 1A). In humans, the first TE of the blastocyst is highly invasive, and only later are there proliferative cores of trophoblast cells in the chorionic villi that might act as stem cells for the developing placenta (Fig. 1B). This might explain why the human TE is unresponsive to FGF-promoted cell proliferation.

The relative importance of the yolk sac in mouse and human might also play a role in apparent differences in the regulation of primitive endoderm versus epiblast formation. In the mouse, the visceral yolk sac plays a crucial role as the major interchange between the fetus and mother before the placenta is established. In humans, the few data available suggest that the yolk sac is rather vestigial and that the early invasive trophoblast plays a stronger nutritive role. These early functional differences could drive different pathways of TE and PE differentiation between mice and human that could also lead to differences in the properties of the remaining pluripotent cells by default.

Finally, another important difference between mouse and human is that only the mouse blastocyst can enter diapause – a state of ‘suspended animation’ that occurs naturally during lactational delay or artificially by removal of the ovaries. This process is dependent on the LIF signaling pathway (Nichols et al., 2001), which also enhances ESC self-renewal. Diapause embryos contain all three cell types of the blastocyst and are held in this state until activated by a hormonal signal from the mother.

Deriving stem cell lines from the early embryo

In the mouse, it has proven possible to isolate permanent self-renewing stem cell lines from all three lineages of the blastocyst. These cell lines retain the lineage restriction shown in the embryo itself, as assessed by chimera formation. Mouse ESCs derive from the EPI cells of the ICM (Boroviak et al., 2014), whereas XEN cells derive from the PE (Kunath et al., 2005) and trophoblast stem (TS) cells derive from the TE (Tanaka et al., 1998) (Fig. 2).

The culture conditions required for derivation and self-renewal of these cell lines can be related back to the signaling pathways involved in lineage specification and maintenance in the embryo itself (Fig. 2). Consistent with the EPI-promoting effect of FGF/ERK inhibition in the embryo, derivation of stable naïve ESCs from mouse embryos is promoted by inhibition of FGF/ERK signaling, along with inhibition of GSK3 and activation of the LIF/Jak/Stat pathway [2iLIF conditions, see Ying et al. (2008)] (Fig. 2). In the presence of LIF alone, mouse ESCs show dynamic heterogeneity of cell states (Chambers et al., 2007; Hayashi et al., 2008; Toyooka et al., 2008), because endogenous FGF produced by undifferentiated cells is constantly driving other cells away from the naïve state. Consistent with this, ESCs that are heterozygous for *Oct4*, the pluripotency factor that regulates FGF4 production, are actually more stable than wild-type cells (Karwacki-Neisius et al., 2013), and FGF4-mutant ESCs are resistant to differentiation *in vitro* (Kunath et al., 2007). Whereas mouse ESCs thrive under conditions of FGF/ERK inhibition, derivation of XEN cells and maintenance of TS cells actually requires active FGF/ERK signaling, as predicted from the embryo itself (Fig. 2).

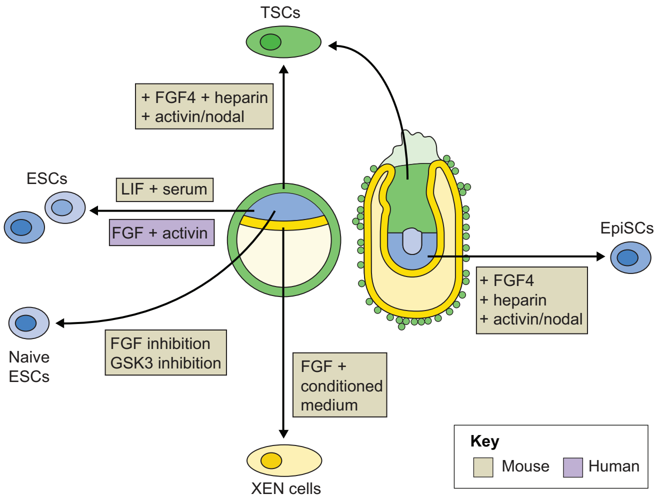

Fig. 2. Growth factor conditions promote different stem cell lines from early mouse and human embryos. In the mouse (beige labels), varying responses to FGF promote derivation and maintenance of different stem cell states from the inner cell mass (ICM, blue), the trophectoderm (green) or the later epiblast (blue). In the presence of leukemia inhibitory factor (LIF) and serum, embryonic stem cells (ESCs) are in a dynamic equilibrium between the naïve state and states that are primed for differentiation. Blocking endogenous FGF signaling (plus blocking GSK3) stabilizes the naïve state derived from epiblast progenitors in the ICM. XEN cells derive from the primitive endoderm (yellow) and are favored by activating FGF signaling. Epi stem cells (EpiSCs) derive from the early post-implantation epiblast, and require FGF and activin signaling for their maintenance. Trophoblast stem cells (TSCs) derive from the trophectoderm of the blastocyst (green) or the early post-implantation extra-embryonic ectoderm (green) and require FGF/ERK signaling for their maintenance, as is also the case in the embryo itself. In contrast to mouse ESCs, human ESCs (purple labels) are more efficiently derived from very late blastocysts, and require FGF and activin for self-renewal in culture. Permanent human TSCs and XEN lines have not yet been reported.

In humans, permanent TS and XEN lines have not yet been reported. Consistent with the absence of early proliferation of the TE in the human blastocyst, TS cells cannot be derived directly from human embryos under the conditions used in the mouse (Kunath et al., 2014) (Fig. 2B). XEN-like cells have been reported to be produced following overexpression of SOX7 in human ESCs (hESCs) (Séguin et al., 2008), but no-one has reported direct derivation from the blastocyst. hESCs were derived first in 1998 (Thomson et al., 1998) and were shown to require FGF and activin for self-renewal in culture, conditions that are antithetical to the maintenance of mouse ESCs (Ying et al., 2008) (Fig. 2). Does this relate to differences in blastocyst development and the function of the early lineages in the two species? Many studies have suggested that hESCs more closely resemble mouse EpiSCs, which are pluripotent stem cell lines derived from early post-implantation epiblast under FGF/activin conditions (Brons et al., 2007; Tesar et al., 2007) (Fig. 2). hESCs are most successfully derived from the ICM of very late human blastocysts (Chen et al., 2009). At this point, the EPI cells of the ICM might be more similar to post-implantation epiblast, given that TE development is delayed and implantation is different from the mouse.

It has always been anecdotally agreed that the existence of blastocyst diapause must be related in some way to the relative ease of derivation of ESCs from the mouse compared with any other mammalian species. Recent attempts to derive hESCs with properties more similar to mouse naïve ESCs are still somewhat contradictory and controversial (Chan et al., 2013; Gafni et al., 2013; Takashima et al., 2014; Theunissen et al., 2014; Ware et al., 2014). However, the stability of the naïve pluripotency network in the mouse does not seem to be easily duplicated in human cells. This might relate back to the diapause issue – activation of a regulatory network that can hold cells in a pluripotent state against their natural tendency to differentiate might be more important for species that undergo diapause. In human embryos, pluripotent cells are always rapidly transiting to differentiation and might never normally activate the stable naïve state. Analysis of gene expression data has suggested that human and mouse naïve-type cells resemble their respective blastocysts more closely than each other (Huang et al., 2014). Thus, we start to realize that it might not be possible to identify the exactly same pluripotent stem cell state in mouse and human embryos – so vive les differences!

Implications for replicating developmental pathways from human pluripotent cells

Despite the differences between mouse and human ES and iPS cells, the fundamental conservation of lineage specification and later developmental pathways still ensures that we can learn much from translating mouse findings into the human system. However, we need to be aware that the starting route to pluripotency might differ between the two species, with potential implications for the next phases of differentiation. Further understanding of the different routes towards pluripotency and towards escape from pluripotency in both human and mouse are still needed if we are to learn how to control differentiation pathways relevant to understanding human biology and treating disease.

Competing interests

The author declares no competing financial interests.

References

Boroviak, T., Loos, R., Bertone, P., Smith, A. and Nichols, J. (2014). The ability of inner-cell-mass cells to self-renew as embryonic stem cells is acquired following epiblast specification. *Nat. Cell Biol.* **16**, 516-528.

Brons, I. G. M., Smithers, L. E., Trotter, M. W. B., Rugg-Gunn, P., Sun, B., Chuva de Sousa Lopes, S. M., Howlett, S. K., Clarkson, A., Ahrlund-Richter, L., Pedersen, R. A. et al. (2007). Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature* **448**, 191-195.

Chambers, I., Silva, J., Colby, D., Nichols, J., Nijmeijer, B., Robertson, M., Vrana, J., Jones, K., Grotewold, L. and Smith, A. (2007). Nanog safeguards pluripotency and mediates germline development. *Nature* **450**, 1230-1234.

Chan, Y.-S., Göke, J., Ng, J.-H., Lu, X., Gonzales, K. A. U., Tan, C.-P., Tng, W.-Q., Hong, Z.-Z., Lim, Y.-S. and Ng, H.-H. (2013). Induction of a human pluripotent state with distinct regulatory circuitry that resembles preimplantation epiblast. *Cell Stem Cell* **13**, 663-675.

Chen, A. E., Egli, D., Niakan, K., Deng, J., Akutsu, H., Yamaki, M., Cowan, C., Fitz-Gerald, C., Zhang, K., Melton, D. A. et al. (2009). Optimal timing of inner cell mass isolation increases the efficiency of human embryonic stem cell derivation and allows generation of sibling cell lines. *Cell Stem Cell* **4**, 103-106.

Cockburn, K., Biechele, S., Garner, J. and Rossant, J. (2013). The Hippo pathway member Nf2 is required for inner cell mass specification. *Curr. Biol.* **23**, 1195-1201.

De Paepe, C., Cauffman, G., Verloes, A., Sterckx, J., Devroey, P., Tournaye, H., Liebaers, I. and Van de Velde, H. (2013). Human trophectoderm cells are not yet committed. *Hum. Reprod.* **28**, 740-749.

De Paepe, C., Krivega, M., Cauffman, G., Geens, M. and Van de Velde, H. (2014). Totipotency and lineage segregation in the human embryo. *Mol. Hum. Reprod.* **20**, 599-618.

Duboule, D. (1994). Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. *Dev. Suppl.* 135-142.

Gafni, O., Weinberger, L., Mansour, A. A., Manor, Y. S., Chomsky, E., Ben-Yosef, D., Kalma, Y., Viukov, S., Maza, I., Zviran, A. et al. (2013). Derivation of novel human ground state naive pluripotent stem cells. *Nature* **504**, 282-286.

Goldin, S. N. and Papaioannou, V. E. (2003). Paracrine action of FGF4 during periimplantation development maintains trophectoderm and primitive endoderm. *Genesis* **36**, 40-47.

Hayashi, K., Chuva de Sousa Lopes, S. M., Tang, F. and Surani, M. A. (2008). Dynamic equilibrium and heterogeneity of mouse pluripotent stem cells with distinct functional and epigenetic states. *Cell Stem Cell* **3**, 391-401.

Hirate, Y., Hirahara, S., Inoue, K.-i., Suzuki, A., Alarcon, V. B., Akimoto, K., Hirai, T., Hara, T., Adachi, M., Chida, K. et al. (2013). Polarity-dependent distribution of angiomotin localizes Hippo signaling in preimplantation embryos. *Curr. Biol.* **23**, 1181-1194.

Huang, K., Maruyama, T. and Fan, G. (2014). The naive state of human pluripotent stem cells: a synthesis of stem cell and preimplantation embryo transcriptome analyses. *Cell Stem Cell* **15**, 410-415.

Karwacki-Neisius, V., Göke, J., Osorno, R., Halbritter, F., Ng, J. H., Weisse, A. Y., Wong, F. C. K., Gagliardi, A., Mullin, N. P., Festuccia, N. et al. (2013). Reduced Oct4 expression directs a robust pluripotent state with distinct signaling activity and increased enhancer occupancy by Oct4 and Nanog. *Cell Stem Cell* **12**, 531-545.

Kuijk, E. W., van Tol, L. T. A., Van de Velde, H., Wubbolts, R., Welling, M., Geijsen, N. and Roelen, B. A. J. (2012). The roles of FGF and MAP kinase signaling in the segregation of the epiblast and hypoblast cell lineages in bovine and human embryos. *Development* **139**, 871-882.

Kunath, T., Arnaud, D., Uy, G. D., Okamoto, I., Chureau, C., Yamanaka, Y., Heard, E., Gardner, R. L., Avner, P. and Rossant, J. (2005). Imprinted X-inactivation in extra-embryonic endoderm cell lines from mouse blastocysts. *Development* **132**, 1649-1661.

Kunath, T., Saba-El-Leil, M. K., Almousailleakh, M., Wray, J., Meloche, S. and Smith, A. (2007). FGF stimulation of the Erk1/2 signalling cascade triggers transition of pluripotent embryonic stem cells from self-renewal to lineage commitment. *Development* **134**, 2895-2902.

Kunath, T., Yamanaka, Y., Detmar, J., Macphee, D., Canniggia, I., Rossant, J. and Jurisicova, A. (2014). Developmental differences in the expression of FGF receptors between human and mouse embryos. *Placenta*. (In press)

Lanner, F. and Rossant, J. (2010). The role of FGF/Erk signaling in pluripotent cells. *Development* **137**, 3351-3360.

Niakan, K. K. and Eggan, K. (2013). Analysis of human embryos from zygote to blastocyst reveals distinct gene expression patterns relative to the mouse. *Dev. Biol.* **375**, 54-64.

Nichols, J., Chambers, I., Taga, T. and Smith, A. (2001). Physiological rationale for responsiveness of mouse embryonic stem cells to gp130 cytokines. *Development* **128**, 2333-2339.

Nichols, J., Silva, J., Roode, M. and Smith, A. (2009). Suppression of Erk signalling promotes ground state pluripotency in the mouse embryo. *Development* **136**, 3215-3222.

Nishioka, N., Inoue, K.-i., Adachi, K., Kiyonari, H., Ota, M., Ralston, A., Yabuta, N., Hirahara, S., Stephenson, R. O. and Ogonuki, N. (2009). The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass. *Dev. Cell* **16**, 398-410.

Raff, R. (1996). *The Shape of Life: Genes, Development and the Evolution of Animal Form*. Chicago: Chicago University Press.

Richardson, M. K. (1995). Heterochrony and the phylotypic period. *Dev. Biol.* **172**, 412-421.

Roode, M., Blair, K., Snell, P., Elder, K., Marchant, S., Smith, A. and Nichols, J. (2012). Human hypoblast formation is not dependent on FGF signalling. *Dev. Biol.* **361**, 358-363.

Rossant, J. and Tam, P. P. L. (2009). Blastocyst lineage formation, early embryonic asymmetries and axis patterning in the mouse. *Development* **136**, 701-713.

Séguin, C. A., Draper, J. S., Nagy, A. and Rossant, J. (2008). Establishment of endoderm progenitors by SOX transcription factor expression in human embryonic stem cells. *Cell Stem Cell* **3**, 182-195.

Snoeck, H.-W. (2015) Modeling human lung development and disease using pluripotent stem cells. *Development* **142**, 13-16.

Strumpf, D., Mao, C.-A., Yamanaka, Y., Ralston, A., Chawengsaksophak, K., Beck, F. and Rossant, J. (2005). Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst. *Development* **132**, 2093-2102.

Takashima, Y., Guo, G., Loos, R., Nichols, J., Ficz, G., Krueger, F., Oxley, D., Santos, F., Clarke, J., Mansfield, W. et al. (2014). Resetting transcription factor control circuitry toward ground-state pluripotency in human. *Cell* **158**, 1254-1269.

Tanaka, S., Kunath, T., Hadjantonakis, A.-K., Nagy, A. and Rossant, J. (1998). Promotion of trophoblast stem cell proliferation by FGF4. *Science* **282**, 2072-2075.

Tesar, P. J., Chenoweth, J. G., Brook, F. A., Davies, T. J., Evans, E. P., Mack, D. L., Gardner, R. L. and McKay, R. D. G. (2007). New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature* **448**, 196-199.

Theunissen, T. W., Powell, B. E., Wang, H., Mitalipova, M., Faddah, D. A., Reddy, J., Fan, Z. P., Maetzel, D., Ganz, K., Shi, L. et al. (2014). Systematic identification of culture conditions for induction and maintenance of naive human pluripotency. *Cell Stem Cell* **15**, 471-487.

Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S. and Jones, J. M. (1998). Embryonic stem cell lines derived from human blastocysts. *Science* **282**, 1145-1147.

Toyooka, Y., Shimosato, D., Murakami, K., Takahashi, K. and Niwa, H. (2008). Identification and characterization of subpopulations in undifferentiated ES cell culture. *Development* **135**, 909-918.

Van der Jeught, M., O'Leary, T., Ghimire, S., Lierman, S., Duggal, G., Versieren, K., Deforce, D., Chuva de Sousa Lopes, S., Heindryckx, B. and De Sutter, P. (2013). The combination of inhibitors of FGF/MEK/Erk and GSK3beta signaling increases the number of OCT3/4- and NANOG-positive cells in the human inner cell mass, but does not improve stem cell derivation. *Stem Cells Dev.* **22**, 296-306.

Ware, C. B., Nelson, A. M., Mecham, B., Hesson, J., Zhou, W., Jonlin, E. C., Jimenez-Caliani, A. J., Deng, X., Cavanaugh, C., Cook, S. et al. (2014). Derivation of naive human embryonic stem cells. *Proc. Natl. Acad. Sci. USA* **111**, 4484-4489.

Yamanaka, Y., Lanner, F. and Rossant, J. (2010). FGF signal-dependent segregation of primitive endoderm and epiblast in the mouse blastocyst. *Development* **137**, 715-724.

Ying, Q.-L., Wray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J., Cohen, P. and Smith, A. (2008). The ground state of embryonic stem cell self-renewal. *Nature* **453**, 519-523.

Yuan, H., Corbi, N., Basilico, C. and Dailey, L. (1995). Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3. *Genes Dev.* **9**, 2635-2645.