New insights into the epitranscriptomic control of pluripotent stem cell fate

Young Hyun Che1,5, Hojae Lee2,5 and Yong Jun Kim1,3,4✉

© The Author(s) 2022

Each cell in the human body has a distinguishable fate. Pluripotent stem cells are challenged with a myriad of lineage differentiation options. Defects are more likely to be fatal to stem cells than to somatic cells due to the broad impact of the former on early development. Hence, a detailed understanding of the mechanisms that determine the fate of stem cells is needed. The mechanisms by which human pluripotent stem cells, although not fully equipped with complex chromatin structures or epigenetic regulatory mechanisms, accurately control gene expression and are important to the stem cell field. In this review, we examine the events driving pluripotent stem cell fate and the underlying changes in gene expression during early development. In addition, we highlight the role played by the epitranscriptome in the regulation of gene expression that is necessary for each fate-related event.

INTRODUCTION

Stem cells exhibit the potential to differentiate into various cell types1. Therefore, stem cells have been useful in biological and medical research2. Stem cells have the advantage of inducibility to obtain and utilize the cell types suitable for a particular research purpose. In particular, stem cell types affect different developmental stages and differentiation lineages3,4. The usefulness of stem cells is based primarily on their ability to undergo various cell development stages, allowing researchers to obtain specific cell types with phenotypes at the appropriate developmental time based on the stage of acquisition5. Using lineage differentiated stem cells has enabled research model establishment for the direct investigation of molecular mechanisms, phenotypes, and therapeutic strategies in phenotype-relevant human cells6–8. Stem cells in the early stages of development exhibit a wide range of differentiation properties2. For example, zygote cells proliferate to form a morula, a group of stem cells with the same multicellular differentiation potential, including embryonic cells that form an organism and supporting cells needed to maintain proper embryonic development6. This broad differentiation potential and totipotency of stem cells gradually decrease as stem cells proliferate and acquire the necessary phenotypes during development, eventually progressing to a state called pluripotency9. Embryonic stem cells (ESCs) extracted from the inner cell mass (ICM) of a blastocyst are stem cells in the pluripotent state10. Although these cells have lost the ability to differentiate into cells that form extraembryonic tissues, such as the placenta and yolk sac, they retain the ability to differentiate into cells that can form fetal tissues and organs11. Thus, human ESCs (hESCs) in a pluripotent state are actively employed in developmental and pathological research that integrates aspects of human body pathophysiology13,14. However, both the totipotent and pluripotent states of cells are dynamic, and the range of differentiation potential changes gradually with the progressive maturation of stem cells15. Furthermore, whereas totipotent stem cells are characterized by their homogenous proliferation, human pluripotent stem cells (hPSCs) gradually show cellular diversity in the ICM of a blastocyst16,17. The pluripotency of stem cells is thus limited, leading to the generation of three heterogeneous germ populations through pattern and structural formation events and gastrulation18. Thus, understanding the biological events and molecular mechanisms that control the properties of hPSCs is critical. However, only a subset of the molecular pathways governing stem cell pluripotency is understood.

The state of stem cells is determined by the influence of spatiotemporal location and ambient environmental conditions that change on the basis of developmental processes19. In a proliferating mass of stem cells, the topological information of each cell leads to diverse cell polarity and plasticity20,21. Both the matrix surrounding stem cells and various environmentally induced signaling pathways trigger alterations in stem cell activity and behavior22. Through the control of gene expression, these factors eventually lead to biological changes, functioning as intrinsic fate regulators operated by the stem cells themselves23,24. Gene expression patterns regulate the biological properties of cells and define cell types25. Similar to those of somatic cells, the functions of stem cells are controlled by the molecular actions of proteins encoded by cell-type-specific genes26. Gene expression control, therefore, is a key factor in determining the state and function of stem cells, regardless of intrinsic or extrinsic triggers24,27. To ensure the timely and accurate regulation of required gene expression, stem cells engage well-established transcription machineries and epigenetic regulatory systems28. For instance, recent studies have suggested that an epitranscriptomic...
gene regulatory system changes the protein translation efficiency and persistence of messenger RNA (mRNA) through chemical modifications of transcribed mRNAs. Compared with somatic cells, hPSCs have immature genetic and epigenetic regulatory mechanisms, which can affect precise gene expression control, resulting in alterations in protein expression patterns that can be fatal. Nonetheless, PSCs in the early stages of development preserve an accurate fate decision process based on tight gene expression control. In addition to conventional gene regulation, other molecular mechanisms may play a role in the control of gene expression in PSCs.

In this review, we summarize changes in the state of PSCs during the early stages of development and the regulatory mechanisms underlying these changes. Additionally, we discuss recent research on gene regulation at the transcriptome level in relation to the regulation of PSC fate. Although a number of studies have reported distinct properties of hPSCs compared with mouse PSCs due to interspecies differences, the use of mouse embryos can provide sufficient knowledge for understanding human embryonic development. Here, we present studies on both mice and human cells and point out whether the results have been derived from human or animal models.

**STATE OF PLURIPOTENT STEM CELLS (PSCS) DURING EARLY DEVELOPMENT**

Stem-cell development generates diverse groups of cells with different properties via the generation of identical multiple-twin cells from a starting single cell (Fig. 1a). Immediately after fertilization, zygote cells continue to divide, forming a group of 16-homogenous cells that comprise the early morula. As this cell group continues to divide, differences in environmental conditions, including topographical forces applied to each cell in the mass, are created. These differences result in the generation of the first heterogeneous population: the ICM and trophectoderm (TE) (Fig. 1b). The morphogenetic events include the polarization of the epiblast, which forms the central lumen that develops into the amniotic cavity; creation of the amniotic epithelium, which forms the amniotic sac membrane; and differentiation of primordial germ cells, which are precursors of eggs or sperm. Moreover, extraembryonic mesenchyme cells derived from the hypoblast surround the generated structure to isolate it from the outer cell membrane (OCM) formed by the trophoblast. The ectoderm, mesoderm, and endoderm are organized surrounding structures such as the chorion, which supports embryogenesis, whereas the ICM is critical for the formation of the embryo. After implantation of a blastocyst in the maternal endometrial epithelium, the ICM undergoes subsequent morphogenetic changes. The ICM of a postimplantation blastocyst contains epiblasts and hypoblasts (Fig. 1a). The morphogenetic events include the polarization of the epiblast, which forms the central lumen that develops into the amniotic cavity; creation of the amniotic epithelium, which forms the amniotic sac membrane; and differentiation of primordial germ cells, which are precursors of eggs or sperm. Moreover, extraembryonic mesenchyme cells derived from the hypoblast surround the generated structure to isolate it from the outer cell membrane (OCM) formed by the trophoblast. Thereafter, epiblasts in the ICM form a primitive streak, gastrulate and differentiate into three germ layers: The ectoderm, mesoderm, and endoderm.

HESC cultures have been established by the in vitro cultivation of cells isolated from a morula, an entire blastocyst, or an ICM. HESC- and blastocyst-derived hESCs are thought to remain in a naive state, showing unbiased differentiation potential, including the generation of trophoblasts, whereas blastocyst- or ICM-derived cells are considered to exhibit a primed-like state, retaining comparably late-stage stem cell characteristics (Fig. 1c). Despite differences in cell properties, both types of hESCs exhibit the developmental spectrum that enables the generation of all somatic and germline cells. In contrast, murine naive and primed ESCs have shown to exhibit clear differences in the molecular and cellular properties. Previously, murine ESCs were believed to show better differentiation plasticity than human ESCs, but the limited pluripotent state epiblast stem cells (EpiSCs) was revealed. In contrast to mouse ESCs, known to be in the naive state, mouse EpiSCs in the primed state clearly show not only differences in response to activation by external signals and a decrease in self-renewal capacity but also a massive reduction in whole-animal generation efficiency because of a tetraploid composition. Although the definition of the naive and primed state of hPSCs remains ambiguous, evidence of a naive state of mammalian stem cells, including that of primates, has been reported, suggesting the need to understand the conversion of hPSCs to the pluripotent state.
MECHANISMS THAT CONTROL PSC FATE

The state of PSCs, including their development and differentiation, is controlled by both intrinsic and extrinsic conditions. Deciphering the complicated signaling pathways governing the pluripotent state of stem cells is challenging, as multiple biological events, such as maturation, fate determination, and differentiation, concurrently affect stem cell development. In this context, the biological events regulating the pluripotent state of stem cells are categorized into two processes: “acquisition of heterogeneity from a homogeneous population” and “conversion from a naïve to a primed state.” Each process is accompanied by an explanation of the mandating molecular pathways.

Acquisition of heterogeneity in a homogeneous population

Among the major questions in stem cell biology, when and how is the control of clonal cells within a homogeneous population changed to produce cells with distinct and orderly fates? To form a highly organized body from an embryo comprised of symmetrical cells, the embryonic symmetry must be disrupted59. The importance of acquiring asymmetric fate during gastrulation to enable balanced development of the human body has been investigated in previous studies60,61. Complex signaling at the cellular level, as well as geometrical and topological transformation at the embryonic level, are necessary for asymmetry62,63. Specifically, the process that ultimately disrupts embryonic radial symmetry and triggers anteroposterior axis specification, has been found to be driven by a morphogen signaling gradient64. Notably, the first disruption to the symmetric organization of human cells occurs much earlier than previously thought: during the division of stem cells in the totipotent state. The emergence of the TE in blastocyst-stage embryos is the first morphological variation during embryogenesis. TE and primitive endoderm lineages generate the placenta and yolk sac extraembryonic cells, whereas cells inside the blastocyst form the ICM, the cells of which eventually divide to form the primitive endoderm and pluripotent epiblast cells. According to the most recent understanding, each cell in the early embryonic state retains identical developmental potential. Therefore, in the absence of stimulants such as morphogens, cell fate has been assumed to be determined randomly, making the existence of initiating factors driving cellular heterogeneity enigmatic. Based on single-cell transcriptome analyses, recent studies with PSCs and mouse embryos have suggested another possibility65-67. Differences were detected in the totipotency between cells constituting murine or human blastomeres, with each cell in the 16-cell stage exhibiting a transcriptome that differs from that in the 2-cell stage immediately after the first cleavage66. The gradual increase in the differential tendency of genes to contribute to lineage specification suggests that the symmetry is disrupted earlier than traditionally thought; that is, it distinguishes trophoblasts from embryoblasts after the morula stage. This finding was consistent with the results observed in several studies suggesting that only one of the cells separated in the 2-cell stage can develop into a mouse44,46-71.

The molecular mechanism causing this initial heterogeneity has not yet been clearly elucidated. A recent study reported that individual cells in the 4-cell-stage blastomere exhibit differential expression of epigenetic modifiers, such as that of the histone methyltransferase CARM1 (Fig. 1a)72,73. Differences in the expression of CARM1 regulate the DNA-binding ability of the pluripotency transcription factors SOX2 and OCT4, with activity that is affected by histone H3 arginine 26 dimethylation (H3R26me2)74,75. A differential increase in the expression of genes downstream of SOX2, such as Sox21, at the 4-cell stage has been reported to suppress the expression level of Cdx276, a lineage specifier for TE differentiation in the 8-cell stage77,78. Subsequently, the reduced expression of Cdx2 results in the decreased expression of polarity markers that define the apical domain, in turn leading to TE differentiation. Interestingly, Cdx2 mRNA has been demonstrated to be asymmetrically localized to the apical pole in the 8-cell stage, when the embryo is undergoing apical-basal polarization79,80. Due to the apical localization of Cdx2 transcripts, differential inheritance during subsequent division to the 16-cell stage might result in cells on the outside of the apical domain possessing more Cdx2 transcripts than inner cells. Therefore, the determination of timing of these differences in gene expression between cells is important. The mechanisms underlying the determination of early mammalian stem cell fate remain unclear, largely because whether the first bifurcation in the cell fate sequence is a random event in mammalian embryo during the morula stage or is based on molecular features of differentiation that emerge prior to morphological changes remains unclear75. Depending on the true pathway, either the role played by an intrinsic gene regulatory mechanism or the cellular environment condition will be predominant. An analysis of the transcriptome in multiple blastomeres revealed that after the first cleavage each cell exhibited different gene expression patterns from the pattern evident in the 2-cell stage66. Although evidence supports an autonomous molecular cascade for the regulation of cell fate that directs differences between cells in blastomeres early in embryonic development, the mechanisms that control precise gene expression in the open chromatin structure of early stem cells remain unclear.

Cell conversion from a naïve to a primed state

The differentiation capacity of stem cells refers to the ability to generate a range of differentiated cells, which is the basis of classifying cells as totipotent, pluripotent, and multipotent10. Although pluripotency is a transient property of stem cells in vivo, regulation of signaling pathway activity involved in fate maintenance enables stem cells to remain in a pluripotent state for an extended period in vitro81. The pluripotent stage involves multiple stem cells in dynamically changing states, and the differentiation potential of each cell is gradually limited; therefore, the stem cell potential is determined on the basis of the developmental stage of the blastocyst from which the ESCs are derived. Due to the limited accessibility to human embryos82, studies using murine cells first elucidated the correlation between the origin, status, and differentiation capacity of PSCs. Two types of PSCs have been established from mouse blastocysts (Fig. 1c); ESCs83,84 and EpiSCs85. ESCs derived from the ICM of preimplanted mouse blastocysts were the naïve state and were capable of generating all three germ layers and primordial germ cells86-88; in contrast, EpiSCs isolated from postimplanted epiblasts were in the primed state and capable only of generating the three germ layers53. Although both ESCs and EpiSCs show the ability to form the three germ layers, these two types of stem cells are clearly distinguished by their developmental potential, as only ESCs, not EpiSCs, can efficiently generate chimeric progeny from injected blastocysts53,54,90. Differences in the ability of PSCs to generate chimeric animals are representative of stem cell potential in the in vivo counterparts of pre- or postimplantation blastocysts89. Various efforts have been made to elucidate the mechanism underlying the differences in pluripotency attributed to differences in PSC origin. In contrast to ESCs, which express a variety of pluripotency genes, including Oct4, Nanog, Sox2, ESRRB, Rex1, Klf2, and Klf4, EpiSCs exhibit elevated expression levels of differentiation-inducing genes, such as Otx2 and Zic2, and low levels of Nanog, Rex1, Klf2, or Klf484,89. Studies designed to optimize in vitro culture conditions to maintain naïve or primed PSCs have suggested that the endogenous activity of genes is regulated by signals transduced from outside a cell, such as fibroblast growth factor (FGF)90, bone morphogenic protein (BMP)91, transforming growth factor β (TGFβ)92, and WNT93. Therefore, early methods for culturing naïve murine ESCs from mitotically inactive mouse
In general, the regulation of gene expression required by cells is described by the central dogma. Specifically, transcription, translation, posttranslational modifications or subsequent proteolytic processes precisely control the levels of molecular expression. In contrast to other processes that require the systematic organization and regulation of subsequent processes to control the level of a final molecular product, transcriptional regulation is an efficient process for regulating PSC fate because the molecular control mechanisms are immature. Although the epigenome shapes stem cell hierarchies, differential transcriptomes only partially explain protein abundance. As hPSCs are characterized by completely open chromatin conditions and immature epigenetic systems, posttranscriptional control is thought to play a key role in the functional output of genetic programs. A zygote survives and functions through the support of molecular components passed down through gametes, which carried these traits before fertilization. In particular, PSCs in the early stages of development undergo zygotic gene activation (ZGA), which allows precise regulation of selective expression of genes necessary for survival. ZGA of hPSCs begins in the 2-cell stage and proceeds to the morula stage, which is still characterized by immature chromatin reorganization, even in the ICM of a preimplanted blastocyst. Although epigenetic control is thought to occur during this stage, epigenetic modifications are deposited during the heterogeneity acquisition process of the cell transition from a primed state to three germ layer development. This timeline has been supported by studies showing de novo heterochromatin formation and X-chromosome inactivation in hPSCs after ICM formation. In addition, as described above, WNT, FGF, or activin signaling regulates the transcription of molecular factors that affect global methylation through the activity of OCT4 and NANOG in the naive state. This regulatory program requires additional mechanisms to compensate for coarse transcriptional level changes caused by immature transcriptional control prior to the completion of epigenome-driven transcriptional regulatory mechanisms. Increasing data have suggested a role for posttranscriptional modifications in the early stages of stem cell development that enables the selective regulation of required functional outputs (i.e., proteome content) based on the global transcriptome.

The epitranscriptome in development and stem cells

In addition to reported posttranscriptional modifications during oocyte development, more than 100 chemical modifications in addition to typical posttranscriptional modifications, such as 5'-capping, polyadenylation, and splicing, have been identified. Methylation is the most common enzyme-catalyzed modification, with N6-methyladenosine (m6A) being reported. However, the role played by RNA m6A in posttranscriptional regulation during stem cell development is unknown.

**N6-methyladenosine (m6A)**

m6A is the most prevalent modification of the 3' untranslated regions (UTRs), long internal exons, intergenic regions, and 5' UTRs in human mRNAs. The functional m6A network is accurately orchestrated through a series of protein effector molecules, including "writers" for methylation, "erasers" for demethylation, and "readers" for interpretation of methylated mRNAs. A microRNA-guided protein complex consisting of Wilms tumor 1-associating protein (WTAP), methyltransferase-like protein 3 (METTL3), and methyltransferase-like protein 14 (METTL14) targets specific regions on mRNA, depositing a methyl group at the N6-position of adenosine. Subsequent demethylation by eraser proteins, such as fat mass and obesity-associated protein (FTO) or human AlkB homolog H5 (ALKD5H), results in m6A being highly
enriched in specific transcriptome sites\textsuperscript{146,147}. The presence of eraser proteins that correct a methylation pattern suggests that m\textsuperscript{6}A-associated posttranscriptional control might be a dynamic process that can quickly reflect environmental changes such as developmental progression and cellular stress. An m\textsuperscript{6}A-loaded mRNA is recognized by RNA-binding protein YTH domain-containing families 1 (YTHDF1), 2 (YTHDF2), or 3 (YTHDF3) after being delivered to the cytoplasm through 5\textsuperscript{′} capping and polyadenylation\textsuperscript{30,148,149}. Emerging evidence has suggested that YTHDF1, YTHDF2, and YTHDF3 target distinct mRNAs for cap-dependent translation, acceleration of mRNA decay, or promotion of translation and decay, respectively. In contrast to the prevailing model, recent studies have reported that all 3 YTHDFs act redundantly to mediate biological functions through specific m\textsuperscript{6}A sites and shared target mRNAs\textsuperscript{150}.

Recent findings have also demonstrated the role played by the m\textsuperscript{6}A modification in various molecular processes, such as translation efficiency, stability, localization, and splicing, which are involved in stem cell development and fate control (Fig. 2)\textsuperscript{151–153}. Deficient METTL3 and METTL14 have been reported to inhibit the expression of pluripotent genes such as SOX2, NANOG, and DPPA3 but to promote the expression of developmental regulators such as FGF5, CDX2, and SOX17\textsuperscript{151}. Furthermore, deletion of Mettl3 and Mettl14 increased mRNA stability in a HuR- and miRNA-dependent manner\textsuperscript{152}, a finding was further supported by a subsequent study showing that ZC3H13 forms biochemical complexes with WTAP, VIRMA, and CBLL1\textsuperscript{154}. This complex formation led to a decrease in global levels of the m\textsuperscript{6}A modification and self-renewal ability, triggering the differentiation of murine ESCs. These results differed from those of a study reporting that Mettl3-knockout mice presented with embryonic lethality between embryonic days 3.5 and 6.5 when PSCs lost lineage differentiation potential\textsuperscript{153}. In Mettl3-knockout murine ESCs, stabilized FGF5 mRNA activated pErk and downregulated Nanog expression, FGF5-mediated concurrently coactivated pAkt to reestablish the expression of Nanog. The induction of differentiation and a concurrent delay in pluripotency loss might explain the discrepancy in these studies\textsuperscript{155}. Ultimately, m\textsuperscript{6}A loss

---

**Fig. 2** Various features of pluripotent stem cells are regulated by epitranscriptomic modifications. Molecular regulatory functions of epitranscriptomic modification m\textsuperscript{6}A (red), m\textsuperscript{1}A (yellow), pseudouridine (green), and m\textsuperscript{5}C (blue) are associated with each biological phenotype in pluripotent stem cells.
causes confusion in determining whether cells maintain and advance in their pluripotent state and undergo differentiation. Another study investigating the interacome of SMAD2/3 revealed that SMAD2/3 promoted m^2^A deposition on nuclear RNA by interacting with the METTL3-METTL14-WTAP complex in response to the activation of activin A/TGF^β^ signaling in primed hESC. Accumulation of the mRNA of NANO2, NODAL, and LEFTY1, which are activin A signaling targets, likely affects processes such as the transition from homogeneity to heterogeneity or from the naive to the primed state by dynamically reflecting extracellular signaling in hPSCs.

**Pseudouridine (ψ)**

Pseudouridine is the most abundant modification on transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs); it has also been found on mRNAs, long noncoding RNAs (lncRNAs), and small nuclear RNAs (snRNAs). RNA is modified by pseudouridine synthases (PUSs) in a guide-RNA-independent manner affecting RNA structures by promoting base stacking interactions via a hydrogen bond donor to increase RNA backbone rigidity, thereby regulating the interaction of other biomolecules with RNAs.

Ultimately, pseudouridylation plays a role in increasing tRNAs stability and simultaneously alters translation termination, thereby regulating protein synthesis. Interestingly, guide-RNA-dependent pseudouridylation is regulated by dyskerin (DKC1), which promotes the elongation of telomeres and increases the expression of OCT4 and SOX2. However, mutations in DKC1 result in failed iPSC reprogramming. In line with this outcome, DKC1 mutations have been found to be involved in the pathogenesis of human diseases characterized by short telomeres. Moreover, deletion of Dkc1 led to early embryonic lethality in a mouse model. Although its association with the state of PSCs has not yet been clarified, pseudouridine might be related to stem cell development, maturation, and aging.

**N1-methyladenosine (m^1^A)**

In human cells, m^1^A is widely distributed on tRNAs. The m^1^A modification is mediated by TRMT10C, TRMT61B, and TRMT6/61A methyltransferases and is associated with the maintenance of the structure, stability, and function of tRNAs in mitochondria or cytoplasm. Less than 0.1% of mRNAs contain an m^1^A modification, which is generally located near the 5′ UTR close to a translation initiation site. Although the role played by TRMT6/61A in depositing m^1^A modifications in cytoplasmic mRNAs carrying the GUUUCRA tRNA-like motif is known, its contribution is minor. Interestingly, similar to the m^2^A modification, the m^1^A modification involves a dynamic and reversible process. m^1^A demethylation is mediated by ALKBH1 and ALKBH3, and m^2^A and m^1^A share a reader protein. YTHDF2 and YTHDF3 are known to contribute to the interpretation of m^1^A, causing the rapid decay of modified mRNAs. Recent studies have suggested that YTHDF3 promotes the degradation of IGFR1 mRNA, leading to the inhibition of trophoblast invasion in trophoblast-associated pregnancy disorders. Although neither the biological role nor the molecular mechanism of m^1^A has been established to date, it is thought to be involved in the pathophysiology of stem cells because of the similarity in the dynamics and shared molecular machineries of the m^2^A and m^1^A modifications.

**CS-methylcytosine (m^5^C)**

Similar to m^2^A, the m^5^C modification is reversible and controlled by methyltransferases (writers: NSUNs, TRDMT1, and DNMT2) and demethylases (erasers: TETs and ALKBH1). The molecular function of m^5^C is mediated by reader proteins (ALYREF and YBX1). Although the m^5^C modification is highly enriched on tRNAs and rRNAs, it reportedly plays a role in mRNA export and stability maintenance, and translation. In zebrafish, deletion of maternal Ybx1 enhanced translation and triggered the unfolded protein response, resulting in oogenesis and embryogenesis defects. In addition, Ybx1 has been shown to play a role in maintaining maternal m^2^C-containing mRNAs during the maternal-to-zygotic transition (MZT) during early embryogenesis. These mRNAs have been reported to facilitate the MZT in early zebrafish development. A pathological association between m^5^C and the activation of JAK-STAT signaling and dysregulation of tRNAs has been reported in various cancers and neurodegenerative disorders in humans. Although the role played by the m^5^C modification in the regulation of early human stem cell properties has not been elucidated, further research into the function of m^5^C in health and disease is warranted because of its effect on early zebrafish embryogenesis and importance of TET proteins in early stem cell development.

**CONCLUSIONS AND PERSPECTIVES**

Although somatic cells control chromatin accessibility to transcription machineries through their well-organized heterochromatin structure and epigenetic control, hPSCs accommodate the uncontrolled accessibility of transcriptional machineries to euchromatin throughout the nucleoplasm. However, PSCs strictly control the levels of expression of certain proteins, which are the functional end products of strictly controlled gene expression. More specifically, the molecules that determine the differentiation potential and developmental stage of PSCs are controlled at the epitranscriptomic level. Epitranscriptomic regulation, which controls the stability and translational efficiency of transcribed mRNAs, overcomes unfavorable conditions faced by PSCs. In particular, m^2^A is the most prevalent epitranscriptomic modification of mRNAs and is involved in the regulation of major signaling pathways in hPSCs. However, a number of studies have shown that mRNA modifications in addition to m^2^A were correlated with early developmental changes in animal models or modulated by molecular mechanisms during early developmental changes in PSCs. Therefore, future studies should be directed to investigating the complex epitranscriptomic gene regulatory system, which involves the mutual interaction of multiple types of RNA modifications, as well as the molecular mechanism and biological implications of certain types of RNA modifications.

**REFERENCES**

1. Fuchs, E. & Segre, J. A. Stem cells: a new lease on life. Cell 100, 143–155 (2000).
2. Winickoff, D. E., Saha, K. & Graff, G. D. Opening stem cell research and development: a policy proposal for the management of data, intellectual property, and ethics. Yale J. Health Policy Law Ethics 9, 52–127 (2009).
3. Stent, G. S. The role of cell lineage in development. Philos. Trans. R. Soc. Lond. B Biol. Sci. 312, 3–19 (1985).
4. Wobus, A. M. & Boehler, K. R. Embryonic stem cells: prospects for developmental biology and cell therapy. Physiol. Rev. 85, 615–678 (2005).
5. Yun, W., Kim, Y. J. & Lee, G. Direct conversion to achieve gial cell fates: oligodendrocytes and Schwann cells. Int. J. Stem Cells 15, 44–25 (2022).
6. Lee, G. et al. Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. Nature 461, 402–406 (2009).
7. Lee, G. et al. Large-scale screening using familial dysautonomia induced pluripotent stem cells identifies compounds that rescue KBKAP expression. Nat. Biotechnol. 30, 1244–1248 (2012).
8. Kim, Y. J. et al. Generation of multipotent induced neural crest by direct reprogramming of human postnatal fibroblasts with a single transcription factor. Cell Stem Cell 15, 497–506 (2014).
9. Larsen, W. J., Sherman, L. S., Potter, S. S. & Scott, W. J. Human Embryology. 3rd edn, 20 (Churchill Livingstone, 2001).
10. Mitalipov, S. & Wolf, D. Totipotency, pluripotency and nuclear reprogramming. Adv. Biochem. Eng. Biotechnol. 114, 185–199 (2009).
11. Thomson, J. A. et al. Embryonic stem cell lines derived from human blastocysts. Science 282, 1145–1147 (1998).
12. Popovic, M., Azpiazu, F. & Chavez de Sousa Lopes, S. M. Engineered models of the human embryo. Nat. Biotechnol. 39, 918–920 (2021).
13. Choi, I. Y., Lim, H. T., Che, Y. H., Lee, G. & Kim, Y. J. Inhibition of the combinatorial signaling of transforming growth factor-beta and NOTCH promotes myotube formation of human pluripotent stem cell-derived skeletal muscle progenitor cells. *Cell* 10, 1649 (2021).

14. Lyoo, K. S. et al. Direct neuronal infection of SARS-CoV-2 reveals cellular and molecular pathology of chemosensory impairment of COVID-19 patients. *Emerg. Microbes Infect.* 11, 405–411 (2022).

15. Weinberger, L., Ayash, M., Novershtern, N. & Hanna, J. H. Dynamic stem cell states: naive to primed pluripotency in rodents and humans. *Nat. Rev. Mol. Cell Biol.* 17, 155–169 (2016).

16. Morris, S. A. et al. Origin and formation of the extraembryonic tissues in the mice. *Int. Rev. Exp. Pathol.* 24, 63–133 (1983).

17. Takahashi, K. et al. Induction of pluripotent stem cells from adult human fibroblasts. *Science* 318, 596–600 (2007).

18. Lunyak, V. V. & Rosenfeld, M. G. Epigenetic regulation of stem cell fate. *Nat. Rev. Mol. Cell Biol.* 7, 701–711 (2006).

19. Avila-Gonzalez, D. et al. Unraveling the spatiotemporal human pluripotency in individual isolated early mouse inner cell masses in culture. *Stem Cell Rep.* 8, 1019–1044 (2020).

20. Bhattacharya, B., Puri, S. & Puri, R. K. A review of gene expression profiling in human pre-gastrulation development. *Stem Cell Res.* 145, 133 (1983).

21. Wamaitha, S. E. & Niakan, K. K. Human pre-gastrulation development. *Hum. Reprod.* 26, 577–603 (2011).

22. Lander, A. D. et al. What does the concept of the stem cell niche really mean today? *BMC Biol.* 19, 19 (2012).

23. Desrosiers, R., Friderici, K. & Rottman, F. Identification of hoPSG lines for sustained endogenous gene activation. *Stem Cell Rep.* 8, 1019–1044 (2020).

24. Bhattacharya, B., Puris, S. & Puri, R. K. A review of gene expression profiling of human embryonic stem cell lines and their differentiated progeny. *Curr. Stem Cell Res. Ther.* 4, 98–106 (2009).

25. Messmer, T. et al. Transcriptional heterogeneity in naive and primed pluripotent stem cells at single-cell resolution. *Cell Rep.* 26, 815–824 e814 (2019).

26. Takahashi, K. et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861–872 (2007).

27. Luykx, V. V. & Rosenfeld, M. G. Epigenetic regulation of stem cell fate. *Hum. Mol. Genet.* 17, R28–R36 (2008).

28. Desrosiers, R., Friderici, K. & Rottman, F. Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. *Proc. Natl. Acad. Sci. USA* 71, 3971–3975 (1974).

29. Wang, X. et al. N6-methyladenosine-dependent regulation of messenger RNA stability. *Nature* 505, 117–120 (2014).

30. Shen, G. Epiblast morphogenesis before gastrulation. *Dev. Biol.* 401, 17–24 (2015).

31. Sasaki, K. et al. The germ cell fate of cynomolgus monkeys is specified in the nascent amnion. *Dev. Cell* 39, 169–185 (2016).

32. Chen, D. et al. Human primordial germ cells are specified from lineage-primed progenitors. *Cell Rep.* 29, 4566–4582 e4565 (2019).

33. Kobayashi, T. & Surani, M. A. On the origin of the human germ line. *Development* 145, dev150433 (2018).

34. Yung, S. et al. Large-scale transcriptional profiling and functional assays reveal important roles for Rho-GTPase signalling and SCL during haematopoietic differentiation of human embryonic stem cells. *Hum. Mol. Genet.* 20, 4932–4946 (2011).

35. Petropoulos, S. et al. Single-cell RNA-Seq reveals lineage and X chromosome dynamics in human preimplantation embryos. *Cell* 165, 1012–1026 (2016).

36. Shahbazi, M. N. et al. Self-organization of the human embryo in the absence of maternal tissues. *Nat. Cell Biol.* 18, 700–708 (2016).

37. Xiang, L. et al. A developmental landscape of 3D-cultural human pre-gastrulation embryos. *Nature* 577, 537–542 (2020).

38. Wamaitha, S. E. & Niakan, K. K. Human pre-gastrulation development. *Curr. Top. Dev. Biol.* 128, 295–338 (2018).

39. Stirparo, G. G. et al. Correction: Integrated analysis of single-cell embryo data yields a unified transcriptome signature for the human pre-implantation epiblast. *Development* 145, dev150672, https://doi.org/10.1242/dev.150672 (2018).

40. Mole, M. A., Weberling, A. & Zernicka-Goetz, M. Comparative analysis of human and mouse development: from zygote to pre-gastrulation. *Curr. Top. Dev. Biol.* 136, 113–138 (2020).

41. Tarrade, A. et al. Characterization of human villous and extravillous trophoblasts isolated from first trimester placenta. *Lab. Investig.* 81, 1199–1211 (2001).

42. Trounson, A. O. & de Sousa, M. G. Human Embryonic Stem Cells. *EMBO J.* 240 (1984).

43. Yung, S. et al. Large-scale transcriptional profiling and functional assays reveal important roles for Rho-GTPase signalling and SCL during haematopoietic differentiation of human embryonic stem cells. *Hum. Mol. Genet.* 20, 4932–4946 (2011).

44. Petropoulos, S. et al. Single-cell RNA-Seq reveals lineage and X chromosome dynamics in human preimplantation embryos. *Cell* 165, 1012–1026 (2016).

45. Shahbazi, M. N. et al. Self-organization of the human embryo in the absence of maternal tissues. *Nat. Cell Biol.* 18, 700–708 (2016).

46. Xiang, L. et al. A developmental landscape of 3D-cultural human pre-gastrulation embryos. *Nature* 577, 537–542 (2020).

47. Wamaitha, S. E. & Niakan, K. K. Human pre-gastrulation development. *Curr. Top. Dev. Biol.* 128, 295–338 (2018).

48. Stirparo, G. G. et al. Correction: Integrated analysis of single-cell embryo data yields a unified transcriptome signature for the human pre-implantation epiblast. *Development* 145, dev150672, https://doi.org/10.1242/dev.150672 (2018).

49. Mole, M. A., Weberling, A. & Zernicka-Goetz, M. Comparative analysis of human and mouse development: from zygote to pre-gastrulation. *Curr. Top. Dev. Biol.* 136, 113–138 (2020).

50. Tarrade, A. et al. Characterization of human villous and extravillous trophoblasts isolated from first trimester placenta. *Lab. Investig.* 81, 1199–1211 (2001).
73. Burton, A. et al. Single-cell profiling of epigenetic modifiers identifies PRDM14 as an inducer of cell fate in the mammalian embryo. Cell Rep. 5, 687–701 (2013).
74. White, M. D. et al. Long-lived binding of Sox2 to DNA predicts cell fate for four-cell mouse embryo. Cell 165, 75–87 (2016).
75. Torres-Padilla, M. E., Parfitt, D. E., Kouzairides, T. & Zernicka-Goetz, M. Histonearginine methylation regulates pluripotency in the early mouse embryo. Nature 454, 214–218 (2008).
76. Kuzmichev, A. N. et al. Sox2 acts through Sox21 to regulate transcription in pluripotent and differentiated cells. Curr. Biol. 22, 1705–1710 (2012).
77. 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).
78. Porfai, P. et al. Position- and Hippo signaling-dependent plasticity during line-age segregation in the early mouse embryo. Elife 6, e22905 (2017).
79. 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).
80. Skamagki, M., Wichler, K. B., Jedrusik, A., Ganguly, S. & Zernicka-Goetz, M. Asymmetric localization of CdX2 mRNA during the first cell-fate decision in early mouse development. Cell Rep. 3, 442–457 (2013).
81. Dietrich, J. E. & Hiragi, T. Stochastic patterning in the mouse pre-implantation embryo. Development 134, 4219–4231 (2007).
82. Wennekamp, S. & Hiragi, T. Stochastic processes in the development of pluripotency in vivo. Biotechnol. J. 7, 737–744 (2012).
83. Tabansky, I. et al. Developmental bias in cleavage-stage mouse blastomeres. Curr. Biol. 23, 21–31 (2013).
84. Plachta, N., Bollenbach, T., Pease, S., Fraser, S. E. & Pantazis, P. Oct4 kinetics predict cell lineage patterning in the early mammalian embryo. Nat. Cell Biol. 13, 117–123 (2011).
85. Nichols, J. & Smith, A. Pluripotency in the embryo and in culture. Cold Spring Harb. Perspect. Biol. 4, a008128 (2012).
86. Lovell-Badge, R. The regulation of human embryo and stem-cell research in the United Kingdom. Nat. Rev. Mol. Cell Biol. 9, 998–1003 (2008).
87. Evans, M. J. & Kaufman, M. H. Establishment in culture of pluripotential cells from mouse embryos. Nature 292, 154–156 (1981).
88. Martin, G. R. & Evans, M. J. Differentiation of clonal lines of teratocarcinoma cells: formation of embryoid bodies in vitro. Proc. Natl Acad. Sci. USA 78, 7634–7638 (1981).
89. Hayashi, K., Ohta, H., Kurimoto, K., Aramaki, S. & Saitou, M. Reconstitution of the germ cell specification pathway in culture by pluripotent stem cells. Cell 146, 519–532 (2011).
90. Bradley, A., Evans, M., Kaufman, M. H. & Robertson, E. Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. Nature 309, 255–256 (1984).
91. Brook, F. A. & Gardner, R. L. The origin and efficient derivation of embryonic stem cells in the mouse. Proc. Natl Acad. Sci. USA 94, 5709–5712 (1997).
92. Hackett, J. A. & Surani, M. A. Regulatory principles of pluripotency: from the ground state up. Cell Stem Cell 15, 416–430 (2014).
93. Chen, Y., Blair, K. & Smith, A. Robust self-renewal of rat embryonic stem cells requires fine-tuning of glycogen synthase kinase-3 inhibition. Stem Cell Rep. 1, 209–217 (2013).
94. Smith, Z. D. et al. DNA methylation dynamics of the human preimplantation embryo. Nature 511, 611–615 (2014).
95. Melkourouh, S. et al. Erosion of dosage compensation impacts human iPSC disease modeling. Cell Stem Cell 10, 595–609 (2012).
96. Chia, N. Y. et al. A genome-wide RNAi screen reveals determinants of human embryonic stem cell identity. Nature 468, 316–320 (2010).
97. Hackett, J. A. et al. Synergistic mechanisms of DNA demethylation during transition to ground-state pluripotency. Stem Cell Rep. 1, 518–531 (2013).
98. Shipony, Z. et al. Dynamic and static maintenance of epigenetic memory in pluripotent and somatic cells. Nature 513, 115–119 (2014).
99. Park, I. H. et al. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451, 141–146 (2008).
100. Bayerl, J. et al. Principles of signaling pathway modulation for enhancing human naive pluripotency induction. Cell Stem Cell 28, 1549–1565 e1512 (2021).
101. Taelman, J. et al. WNT inhibition and increased FGF signaling promotes derivation of less heterogeneous primed human embryonic stem cells, compatible with differentiation. Stem Cells Dev. 28, 579–592 (2019).
102. Linneberg-Agerholm, M. et al. Naive human pluripotent stem cells respond to Wnt, Nodal and LIF signalling to produce expandable naive extra-embryonic endoderm. Development 146, dev180620 (2019).
103. Kurek, D. et al. Endogenous WNT signals mediate BMP-induced and spontaneous differentiation of epiblast stem cells and human embryonic stem cells. Stem Cell Rep. 4, 114–128 (2015).
104. Wu, J. et al. An alternative pluripotent state confers interspecies chimeraic competence. Nature 521, 316–321 (2015).
105. Nirenberg, M. Historical review: deciphering the genetic code—a personal account. Trends Biochem. Sci. 29, 46–54 (2004).
106. Player, A. et al. Comparisons between transcriptional regulation and RNA expression in human embryonic stem cell lines. Stem Cells Dev. 15, 313–326 (2006).
107. Fathi, A. et al. Comparative proteome and transcriptome analyses of embryonic stem cells during embryoid body-based differentiation. Proteomics 9, 4859–4870 (2009).
108. Efroni, S. et al. Global transcription in pluripotent embryonic stem cells. Cell Stem Cell 4, 437–447 (2009).
109. Horis, N., Pina, C. & Arias, A. M. Transition states and cell fate decisions in epigenetic landscapes. Nat. Rev. Genet. 17, 693–703 (2016).
110. Chen, Q. & Hu, G. Post-transcriptional regulation of the pluripotent state. Curr. Opin. Genet. Dev. 46, 15–23 (2017).
111. van der Berg PR, Budnik B, Slavov N, Semrau S. Dynamic post-transcriptional regulation during embryonic stem cell differentiation. Preprint at bioRxiv https://doi.org/10.1101/234697 (2017).
112. Zhang, C., Wang, M., Li, Y. & Zhang, Y. Profiling and functional characterization of maternal mRNA translation during mouse maternal-to-zygotic transition. Sci. Adv. 8, eabj3967 (2022).
113. Lukam, D., Shariati, S. A. M. & Skothem, J. M. Zygotic genome activation in vertebrates. Dev. Cell 42, 316–332 (2017).
114. Sha, Q. Q. et al. Dynamics and clinical relevance of maternal mRNA clearance during the oocyte-to-embryo transition in humans. Nat. Commun. 11, 4917 (2020).
115. Burton, A. B. & Torres-Padilla, M. E. Chromatin dynamics in the regulation of cell fate allocation during early embryogenesis. Nat. Rev. Mol. Cell Biol. 15, 723–734 (2014).
116. Ayyo, T., Saiho, N., Ichimura, T., Niwa, H. & Nakao, M. Nuclear and chromatin reorganization in the MHC-Oct3/4 locus at developmental phases of embryonic stem cell differentiation. Dev. Biol. 298, 354–367 (2006).
117. Lengner, C. J. et al. Derivation of pre-X inactivation human embryonic stem cells under physiological oxygen concentrations. Cell 141, 872–883 (2010).
118. Kresoja-Rakic, J. & Santoro, R. Nucleolus and RNA gene chromatin in early embryo development. Trends Genet. 35, 868–879 (2019).
134. Berdasco, M. & Esteller, M. DNA methylation in stem cell renewal and multi-
potency. Stem Cell Res. Ther. 2, 42 (2011).
135. Gao, L. et al. Chromatin accessibility landscape in human early embryos and its
association with evolution. Cell 173, 248–259 e215 (2018).
136. Wu, J. et al. Chromatin analysis in human early development reveals epigenetic
transition during ZGA. Nature 557, 256–260 (2018).
137. Ozban, N., Tandler, J. & Srin, J. L. Methylation of nuclear RNA during develop-
ment of the amphibian oocyte. J. Embryol. Exp. Morphol. 129, 373–380 (1964).
138. Bentley, D. L. Coupling mRNA processing with transcription in time and space.
Nat. Rev. Genet. 15, 163–175 (2014).
139. Dominissini, D. et al. The dynamic N(1)-methyladenosine methylome in eukar-
yotic messenger RNA. Nature 530, 441–446 (2016).
140. Carlile, T. M. et al. Pseudouridine profiling reveals regulated mRNA pseudour-
idylation in yeast and human cells. Nature 515, 143–146 (2014).
141. Khodda, V. & Cairns, B. J. Identification of direct targets and modified bases of
RNA cytosine methyltransferases. Nat. Biotechnol. 31, 458–464 (2013).
142. Yue, Y., Liu, J. & He, C. RNA N6-methyladenosine methylation in post-
transcriptional gene expression regulation. Genes Dev. 29, 1343–1355 (2015).
143. Liu, J. et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-
methyladenosine methylation. Nat. Chem. Biol. 10, 93–95 (2014).
144. Ping, X. L. et al. Mammalian WTAP is a regulatory subunit of the RNA N6-
methyladenosine methyltransferase. Cell Res. 24, 177–189 (2014).
145. Chen, T. et al. m6A RNA methylation is regulated by microRNAs and promotes
reprogramming to pluripotency. Cell Stem Cell 16, 289–301 (2015).
146. Ja, G. et al. N6-methyladenosine in nuclear RNA is a major substrate of the
ubiquitylation- and DEAD-box RNA helicase HuR. Mol. Cell 51, 783–794 (2013).
147. Zheng, G. et al. ALKBH5 is a mammalian RNA demethylase that impacts RNA
stability and metabolism and mouse fertility. Mol. Cell 49, 18–29 (2013).
148. Wang, X. et al. N6-methyladenosine modulates messenger RNA translation
efficiency. Cell 161, 1388–1399 (2015).
149. Shi, H. et al. YTHDF3 facilitates translation and decay of N(6)-methyladenosine-
modiﬁed RNA. Cell Res. 27, 315–333 (2017).
150. Zaccara, S. & Jaffrey, S. R. A uniﬁed model for the function of YTHDF proteins
in regulating m6A-modiﬁed mRNA. Cell 181, 1582–1595 e1518 (2020).
151. Batista, P. J. et al. m6A RNA modiﬁcation controls cell fate transition in
mammalian embryonic stem cells. Cell Stem Cell 15, 707–719 (2014).
152. Wang, Y. et al. N6-methyladenosine modiﬁcation destablizes developmental
regulators in embryonic stem cells. Nat. Cell Biol. 16, 191–198 (2014).
153. Geula, S. et al. Stem cells. m6A RNA methylation facilitates resolution of naive
pluripotency toward differentiation. Science 347, 1002–1006 (2015).
154. Weng, H. et al. METTL14 inhibits hematopoietic stem/progenitor differentiation
and promotes leukemogenesis via m6A RNA modification. Cell Stem Cell 22,
191–205 e199 (2018).
155. Jin, K. X. et al. N6-methyladenosine (m6A) depletion regulates pluripotency
exit by activating signaling pathways in embryonic stem cells. Proc. Natl Acad.
Sci. USA 118, e2105192118 (2021).
156. Bertero, A. et al. The SMAD2/3 interactome reveals that TGFbeta controls m6A
methylation in pluripotency. Nature 555, 256–259 (2018).
157. Li, X. et al. Chemical pulldown reveals dynamic pseudouridylation of the
mammalian transcriptome. Nat. Chem. Biol. 11, 592–597 (2015).
158. Nachtergaele, S. & He, C. Chemical modiﬁcations in the life of an mRNA tran-
script. Annu. Rev. Genet. 52, 349–372 (2018).
159. Hamma, T. & Ferre-D’Amare, A. R. Pseudouridine synthases. Chem. Biol. 13,
1125–1135 (2006).
160. Charette, M. & Gray, M. W. Pseudouridine in RNA: what, where, how, and why.
J. Mol. Biol. 49, 341–351 (2000).
161. De Zoya, M. D. & Yu, Y. T. Posttranscriptional RNA pseudouridylation. Enzymes
41, 151–167 (2017).
162. Roundtree, I. A., Evans, M. E., Pan, T. & He, C. Dynamic RNA modiﬁcations in gene
expression regulation. Cell 169, 1187–1200 (2017).
163. Batista, L. F. et al. Telomere shortening and loss of self-Renewal in dyskeratosis
congenita induced pluripotent stem cells. Nature 474, 399–402 (2011).
164. Feng, Y. W., Ho, J. J., Inouye, C. & Tjian, R. The dyskerin ribonucleoprotein
complex as an OCT4/SOX2 coactivator in embryonic stem cells. Elife 3, e03573 (2014).
165. Agarwal, S. et al. Telomere elongation in induced pluripotent stem cells from
dyskeratosis congenita patients. Nature 464, 292–296 (2010).
166. He, J. et al. Targeted disruption of Dkc1, the gene mutated in X-linked dys-
keratosis congenita, causes embryonic lethality in mice. Oncogene 21,
7740–7744 (2002).
167. Li, X. et al. Base-resolution mapping reveals distinct m1A methylome in nuclear-
and mitochondrial-encoded transcripts. Mol. Cell 68, 993–1005 e1009 (2017).
168. Li, X. et al. Transcriptome-wide mapping reveals reversible and dynamic N(1)-
methyladenosine methylome. Nat. Chem. Biol. 12, 311–316 (2016).
169. Macari, F. et al. TRM6/61 connects PKCalpha with translational control through
RNAi(Met) stabilization: impact on tumorigenesis. Oncogene 35, 1785–1796
(2016).
170. Liu, F. et al. ALKBH1-Mediated RNA demethylation regulates translation. Cell
167, 815–828 e816 (2016).
171. Zaccara, S. & Jaffrey, S. R. A uniﬁed model for the function of YTHDF proteins
in regulating m6A-modiﬁed mRNA. Cell 181, 1582–1595 e1518 (2020).
172. Seo, K. W. & Kleiner, R. E. YTHDF2 recognition of N(1)-methyladenosine (m1A)-
modiﬁed RNA is associated with transcript destabilization. ACS Chem. Biol. 15,
132–139 (2020).