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Review

Totipotency or plenipotency: rethinking stem cell bipotentiality☆

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The term 'totipotency' has often been misapplied in stem cell research to describe cells with embryonic and extraembryonic bipotentiality, despite a lack of evidence that they can generate an entire organism from a single cell. Additionally, no specific term currently distinguishes bipotential stem cells from pluripotent cells, which contribute poorly to extraembryonic tissues. This review examines the developmental continuum from totipotency to pluripotency in early embryos and revisits the previously proposed concept of plenipotency in preimplantation development. We evaluate emerging stem cell models that exhibit bipotentiality but have lost the ability to autonomously initiate and sustain the sequential fate decisions necessary to develop into a complete organism. Unlike totipotent embryonic cells, which retain the information required to initiate fate decisions at the correct timing and cell numbers, these stem cells have lost that capacity. This loss of critical developmental information distinguishes totipotency from plenipotency, with bipotential stem cells aligning more closely with the latter. By distinguishing plenipotency from totipotency and pluripotency, we aim to refine terminology, enhance our understanding of early embryonic development, and address ethical considerations in human research.

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Distinct developmental stages in mouse preimplantation embryos

Upon fertilization, the inactive sperm and oocyte genomes unite to create a diploid zygotic genome, which undergoes extensive epigenetic reprogramming, leading to zygotic genome activation and the initiation of embryonic development [1–4]. This single-cell zygote possesses the remarkable ability to develop into a complete organism, a capacity known as *totipotency* [5]. During preimplantation development, the zygote divides asynchronously without altering the cytoplasmic volume, resulting in progressively smaller cells called blastomeres. These blastomeres eventually form a blastocyst capable of implanting in the uterus. The blastocyst consists of an outer epithelial layer of trophectoderm (TE), which serves as the precursor to the placental embryonic component, and a fluid-filled cavity containing the inner cell mass (ICM), marking the first lineage specification (Figure 1). Subsequently, the ICM progresses to a second lineage specification, giving rise to two distinct populations of cells: the primitive endoderm, which differentiates into epithelial cells that serve as yolk sac precursors, and the epiblast (EPI), which gives rise to the entire embryo, including the three germ layers: ectoderm, mesoderm, and endoderm. This ability, termed *pluripotency*, refers to the capacity to generate all cell types of the embryo proper but not the extraembryonic annexes [5] (Figure 1).

At the 2-cell (2C) stage, mouse blastomeres are considered fully totipotent. While recent studies suggest imbalanced lineage contributions from 2C blastomeres in both mice and humans [6–8], the two individual mouse blastomeres at this stage are still thought to retain totipotency and possess equal developmental potential. This is supported by evidence showing that separated 2C-stage blastomeres can generate normal twin pups *in utero* [9–11]. However, totipotency decreases in the 4-cell (4C) stage and is completely lost in the 8-cell (8C) stage, without carrier blastomeres, a single 8C stage mouse blastomere cannot generate any live offspring [5,10,11]. Growing evidence suggests that functional asymmetry and molecular heterogeneity emerge among 4C-stage mouse blastomeres [10–16]. Our recent study [17] provides clear evidence that

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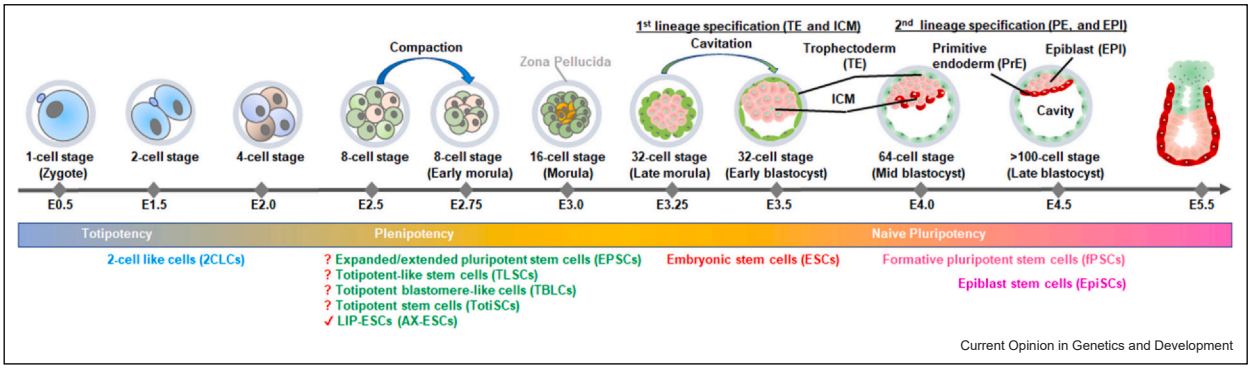
Figure 1



Distinct developmental stages of mouse preimplantation embryos. A putative developmental coordinate of plenipotency together with well-established embryonic stages of totipotency and naive/formative/primed pluripotency.

the maternal genome in 4C-stage mouse blastomeres maintains distinct epigenetic states. Specifically, two blastomeres inherit the repressive epigenetic memories of the oocyte genome, encoded by the histone variant H3.3 (referred to as oH3.3, denoting H3.3-containing nucleosomes within the oocyte genome). These two oH3.3-retaining blastomeres do not contribute to embryo formation but instead participate in trophoblast development or are eliminated during embryonic development. In contrast, the remaining blastomeres, which acquire nascent histones, undergo full reprogramming and maternal genome activation [17]. Our results suggest that, by the 4C stage, two blastomeres have already undergone distinct fate determination. In contrast, individual mouse 4C and 8C-stage blastomeres that do not inherit oH3.3 exhibit partial totipotency, enabling them to contribute to both embryonic and extraembryonic lineages (i.e., they are bipotential) in a chimeric embryo, although they cannot develop into a complete organism independently. While 4C/8C-stage blastomeres are bipotential, their developmental potency is distinct from that of zygote/2C-stage blastomeres. Accordingly, the term '*plenipotency*' was previously introduced to describe the bipotential capacity of mouse 4C/8C blastomeres [18], distinguishing it from 'totipotency', which refers to the capacity of zygote/2C blastomeres to self-organize and generate a complete organism from a single cell [10,11], and from 'pluripotency', which characterizes epiblast cells with embryonic trilineage differentiation potential and a restricted ability to differentiate into extraembryonic tissues. Notably, blastomeres from the 4C stage to cells of the early blastocyst ICM up to the 64-cell stage have been reported to maintain embryonic/extraembryonic bipotentiality [19]. Accordingly, plenipotency can be applied to a broader range of developmental stages than initially proposed [18] (Figure 1).

Pluripotent cells in mice exist from embryonic day (E) 3.5 to 7.5, and during this period, pluripotency gradually diminishes (naïve→formative→primed) as cells differentiate into specialized lineages. The pluripotent population undergoes changes characterized by modifications in gene expression, epigenomic profiles, and functional properties [20]. Although 'naive', 'formative', and 'primed' pluripotent states have been defined for pre- and post-implantation epiblasts, the early stages of development before pluripotency (i.e., totipotency and plenipotency) remain largely undefined because of the rapid progression of *in vivo* development and limited material availability. The precise characterization of these states requires high-resolution gene expression and epigenomic profile of these embryonic stages, and it is imperative to have *in vitro* stable cell lines that can accurately model these states for further research in this area. Establishing distinct transcriptomic and epigenomic signatures is crucial to clearly distinguish plenipotency from both totipotency and pluripotency.

Bipotential stem cells: totipotency or plenipotency?

The question of whether stem cells can fully recapitulate totipotency remains a critical and unresolved issue in developmental and stem cell biology. Pluripotent stem cells (PSCs) can differentiate into cells of all three germ layers, mimicking the pluripotency of their *in vivo* counterparts. However, stem cells that fully recapitulate the totipotency of zygotes or 2C blastomeres have not yet been established. In 2012, Macfarlan discovered that a small percentage (~1%) of mouse ESCs (mESCs) expressing zinc finger and SCAN domain–containing 4 (ZSCAN4) and murine endogenous retrovirus-like genes display a transcriptional profile and bipotential-like early-stage (2C) blastomeres. These two-cell-like ESCs (2CLCs) contributed to both the TE and ICM in a blastocyst when injected into morula-stage embryos. However, the 2CLC population is transient and cannot be stably maintained in ESC cultures [21]. Nevertheless, recent efforts to capture cells with greater developmental potency have resulted in a flurry of

Table 1

Mouse PSCs established with or without bipotentiality.

Stem cell types	Mouse PSCs			References
	Genome stability (> P20)	Bipotentiality	4n-competency	
SL-ESCs	√	X	√ (low)	[35,36]
2i- or 2iL-ESCs	X	X	X	[21,35,36]
2iLA- or LIP-ESCs	√	√	√ (high)	[38,40]
fPSCs	ND	ND	X	Suppl. Ref. [32,53,54]
EpiLSCs	ND	ND	ND	Suppl. Ref. [55]
EpiLCs (transient)	ND	ND	ND	Suppl. Ref. [56]
EpiSCs	ND	X	ND	Suppl. Ref. [57,58]
2CLCs (transient)	ND	√	ND	[21]
Hex+ 2iL-ESCs (transient)	ND	√	ND	Suppl. Ref. [59]
EPSCs	ND	√	ND	Suppl. Ref. [45,46]
MLCs	ND	√	ND	Suppl. Ref. [22]
TBLCs	ND	√	ND	Suppl. Ref. [60]
TLSCs	ND	√	ND	Suppl. Ref. [61]
TotiSCs	ND	√	ND	Suppl. Ref. [62]
iPSCs (partially reprogrammed)	ND	√	X	Suppl. Ref. [44]
H2A.X KO ESCs	ND	√	ND	Suppl. Ref. [44]
H3.3 dKO ESCs	X	√	X	Suppl. Ref. [42,43]

ND: not determined, P20: passage 20, 4n: tetraploid complementation.

literature reporting the derivation and stabilization of expanded/extended pluripotent stem cells (EPSCs), totipotent stem cells (TotiSCs), totipotent blastomere-like cells (TBLCs), totipotent-like stem cells (TLSCs), and morula-like cells (MLCs) [22] from 2C/4C/8C/morula early embryos or adaptation of existing ESCs in specialized culture conditions with a common functional feature of bipotentiality (reviewed by Ref. [23] and summarized in Table 1). Although many of these stem cells have been described as totipotent or totipotent-like due to their transcriptional similarity to 2C blastomeres and bipotentiality, they do not exhibit strict totipotency, as there is currently no evidence demonstrating their ability to generate a complete organism from a single stem cell. In this context, these stem cells are functionally more like 4C/8C blastomeres, indicating that they may represent a state of plenipotency rather than totipotency, compared to *in vivo* embryos. It remains possible that these stem cells could be reverted to functional totipotency through blastoid [24,25] or embryoid [26] generation, thus enabling the creation of live animals using only stem cells without the need for eggs, a topic currently under active pursuit in the field.

Interestingly, a recent report from the Du group demonstrated that mouse totipotent blastomere-like cells (mTBLCs) can model early embryonic development [25], offering a valuable model in assessing stem cell potential for totipotency. This system recapitulates key events such as cell fate determination, compaction, and polarization of stem cell aggregates, even when starting from a single cell, before forming blastoids. These blastoids can implant in the uterus and induce decidualization to develop a placenta-like structure. However, no viable embryos have been observed beyond the gastrulation stage, a milestone that has only been achieved using assembled blastoids with naïve mESCs [27,28]. It would be informative to test the mTBLCs using tetraploid complementation to determine whether fully stem cell–derived mice can be generated, a stringent assay for evaluating true pluripotency.

Symmetry breaking is a pivotal event in both embryo development and stem cell differentiation, marking the transition from a homogeneous cell population to distinct lineages [29–31]. Embryonic totipotency is intrinsic and preprogrammed, requiring no external stimuli beyond fertilization. In contrast, stem cells acquire self-renewal capacity, producing identical daughter cells through mitosis but losing the ability to break symmetry autonomously. While totipotent embryonic cells are inherently programmed to undergo symmetry breaking, as seen in the epigenetic differences emerging among 4C stage blastomeres [15,17], stem cells maintain transcriptomic and epigenomic uniformity through continuous self-renewal. This loss of intrinsic heterogeneity necessitates artificial re-establishment of symmetry-breaking mechanisms to unlock totipotency in stem cells. Additionally, totipotent embryonic cells, which retain the information required to initiate fate decisions at the correct timing and cell numbers, contrast with stem cells that have lost that capacity due to their self-renewal property. This raises a fundamental question: Are totipotency and stemness inherently compatible? Inducing a single stem cell to differentiate into mutually exclusive lineages is a formidable challenge, as it requires a series of sequential, distinct stimuli to drive differentiation into embryonic and extraembryonic

lineages at the correct timing and cell numbers. Even though stem cells can respond to opposing signals, directing them simultaneously toward these different fates is impractical in a single-cell system. Instead, such induction must occur across multiple stem cells, a property more aligned with plenipotency, which lacks the ability to generate a complete organism from a single cell due to the loss of intrinsic information regarding timing and cell counting.

Genetic and epigenetic manipulations, coupled with optimized culture conditions, may facilitate the generation of totipotent-like stem cells. However, fully replicating totipotency within a self-renewing system remains a significant challenge in stem cell biology. One of the key obstacles in using the blastoid approach to model totipotency is the need to apply multiple, contrasting stimuli to a single stem cell aggregate. For example, while one stimulus must promote differentiation into extraembryonic lineages, another must simultaneously sustain pluripotency within the remaining cells — all within the same culture environment. The integration of assembled blastoids with precise genetic manipulations may provide a promising strategy to address this challenge [28].

Stem cell bipotentiality: an intricate property or an artifact?

Various mouse PSC lines [20] have been derived and stably maintained *in vitro*, including embryonic stem cells (ESCs) derived from the ICM of blastocysts at E3.5–E4.5 and epiblast stem cells (EpiSCs) from post-implantation epiblasts (E5.5–E7.5). Moreover, formative pluripotent stem cells (fPSCs) have also been derived from E5.5–E6.5 embryos, characterized by their capacity to immediately respond to inductive cues and execute the lineage specification [32] (Figure 1). fPSCs are pluripotent with superior germline propensity but contribute to blastocyst chimeras less efficiently than naive ESCs [32] and cannot produce all-ESC mice by tetraploid complementation.

Pluripotent mouse ESCs were initially established and cultured in medium supplemented with fetal bovine serum on feeder layers of mouse embryonic fibroblasts [33]. The breakthrough discovery that inhibition of Mek1/2 and Gsk3β (2i) maintains mESCs in a more homogeneous state of naive pluripotency [34] allowed stabilization and expansion of ground-state ESCs *in vitro* in medium supplemented with leukemia inhibitory factor (L). However, prolonged culture of mouse ESCs in 2iL [35,36] or human ESCs in 5iL+Activin medium [37], unfortunately, causes aneuploidy, DNA hypomethylation, and loss of imprinting, impairing developmental potential. We recently discovered that supplementing 2iL medium with lipid-rich albumin (AlbuMAX, AX) significantly improves the genome stability and the developmental potential of mESCs, resulting in a unique pluripotent state of these lipid-induced pluripotent (LIP)-ESCs. LIP-ESCs exhibit exceptional long-term genome stability and robust developmental potency, as evidenced by the generation of both male and female all-ESC mice [38].

Our results reveal that LIP-ESCs are bipotential, capable of contributing to both the ICM and trophoblasts *in vitro* and to the embryo and placenta *in vivo* when injected into 8-cell-stage embryos during chimeric assays. Additionally, LIP-ESCs can be induced to form blastoids using a previously reported protocol [39]. These blastoids can implant and trigger decidualization to develop a placenta-like structure; however, no viable embryos have been observed up to E8.5. Notably, our LIP-ESCs have been demonstrated to generate entirely stem cell–derived mice via tetraploid complementation [38]. This suggests that the current blastoid protocol fails to maintain the pluripotency of the 'epiblast' cells.

While LIP-ESCs are positioned between the naïve and formative pluripotency states, they exhibit a fourfold increase in the 2CLC population compared to naïve 2iL-ESCs, along with significantly elevated expression of *ZSCAN4* [38,40], which may account for their increased bipotential. Additionally, the enhanced responsiveness of LIP-ESCs to differentiation cues [38,40], a hallmark of formative pluripotency [41], could further explain this acquired bipotentiality as assessed in chimeric embryo assays. In mice, the first lineage specification begins at the 32-cell stage (E3.25) and completes by the early blastocyst stage (E4.0). The transition from the 8-cell stage to a blastocyst takes approximately 1.5 days (Figure 1). As LIP-ESCs are immediately responsive to differentiation cues, they can differentiate and contribute to TE when transplanted during this short time window. In contrast, naïve 2iL-ESCs require two days of *in vitro* preparation [38,40], which may explain why LIP-ESCs can contribute to the TE, while naïve 2iL-ESCs cannot, as shown in chimeric embryo assays. Whether 2CLCs or other bipotential stem cells transiently alter their responsiveness to differentiation cues to enable their bipotential contribution in chimeric embryo assays remains an open question.

Furthermore, cells with restricted potential, such as partially reprogrammed iPSCs or mutant ESCs (e.g. H2A.X KO, H3.3A/B double KO), may also exhibit bipotential capacity but lack 4n competency or genome stability (see Table 1 and supplementary Refs. [42–44]). Similarly, re-analysis of previously classified EPSCs [45,46] revealed that these bipotential PSCs are more closely aligned with the E4.5-E5.5 EPI, contrary to earlier assumptions that these cells resemble earlier developmental stages [47]. Thus, these findings suggest

that bipotentiality in PSCs can be induced and is not necessarily an inherent developmental characteristic nor does it correspond to specific developmental stages. Therefore, this raises an important question: does the bipotential contribution of injected stem cells observed in chimeric embryo assays truly reflect specific stem cell potential, or is it an artifact of the assay?

Finally, chimeric assays provide limited insight into stem cell potential. Contribution to a chimeric embryo does not necessarily imply that stem cells can give rise to all cell types; rather, this method often reflects a biased contribution. For example, many developmentally restricted stem cells, such as androgenetic [48,49], parthenogenetic [50,51], and even tetraploid (4n) [52] ESCs, can contribute substantially to chimeric embryos, potentially including germline contribution but are not truly pluripotent. Therefore, to rigorously evaluate the bipotential capacity of stem cells, two key criteria must be met. First, the ability of stem cells to generate all-stem-cell mice should be tested using tetraploid complementation (a method currently applicable only in mice). Second, the capacity of stem cells to produce a functional placenta may be assessed through blastoid formation, where stem cells create a trophoblast vehicle to support the injection of proven 4n-competent ESCs to generate all-ESC live animals. However, this approach remains under intensive investigation. Together, these methods may offer more robust and functional assessments of stem cell bipotentiality.

Concluding remarks

We propose adopting the term 'plenipotency', previously advocated by Dr. Condic [18], to describe those bipotential PSCs, including our LIP-ESCs, and emphasize their full power ('pleni-') of cellular 'potency', which also encompasses long-term genome stability and 4n-competency. This term 'plenipotency' is distinct from 'totipotency', which defines the self-organization capacity of a zygote or a 2C blastomere. Although both terms describe the bipotential capacity, 'totipotency' specifically refers to the ability of a zygote or 2C blastomere to generate a complete organism on its own. In contrast, 'plenipotency' refers to the ability of stem cells to contribute to both embryonic and extraembryonic lineages but without the capacity to generate an entire organism from a single stem cell.

This term of plenipotency in PSCs, characterized by (1) long-term genome stability, (2) robust developmental potency, and (3) bipotentiality, is expected to reduce misuse or confusion of the term 'totipotency' in the field that has been applied to various types of PSCs with bipotentiality but without genome stability and/or 4n-competency. Furthermore, it should alleviate potential public aversion or mistrust toward this line of research in the human system by avoiding the association of totipotency with self-organizing embryo capacity.

Outstanding questions:

1) What are the critical molecular and epigenetic signatures that define totipotency, plenipotency, and pluripotency?
2) What are the transcriptional and epigenetic mechanisms governing totipotency and plenipotency?
3) Can stem cell lines derived from early-stage totipotent embryos (zygote to 2C stage) faithfully recapitulate totipotency?
4) Is it possible to generate live animals solely from stem cells without the involvement of germ cells?

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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