STEM CELLS

Bridging naïve and primed pluripotency

Pluripotent cells generate all types of cells in the body and have largely been classified dichotomously into two types: naïve and primed. Arguing against a binary classification system, a study now discovers a unique transition state between naïve and primed pluripotency and describes the signals that control this transition.

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Pluripotent cells, by virtue of their unique ability to generate all cell types in the adult body, are the foundation of mammalian development and are widely used for regenerative therapies[1]. However, not all pluripotent cells are the same. Until recently, two main types of pluripotency have been recognized in mouse: "naïve" cells that exist in an earlier developmental stage (the peri-implantation blastocyst, at embryonic day 4.5 (E4.5)) and "primed" cells present in the later, post-implantation embryo (from E5.5 onwards)[1–4] (Fig. 1a). The distinction between naïve and primed pluripotency was a conceptual landmark for the field. Although naïve and primed pluripotent cells both seemingly harbor the ability to generate all cell types, they are distinguished by differences in gene expression signatures, chromatin states, reliance on extracellular signals and other properties[1–4]. The transition from naïve to primed pluripotency is a pivotal step in mammalian development that sets the stage for the generation of germ layers and organs, and has thus attracted great attention from researchers[5]. The nature of the transition between naïve and primed pluripotency and possible intermediate states has been the subject of intensive study. In this issue of *Nature Cell Biology*, Neagu, van Genderen, Escudero et al. provide evidence for the existence of an intermediate state between naïve and primed pluripotency[6]. This state, which they designate "rosette pluripotency"[6], raises exciting questions regarding pluripotency itself and about how we classify discrete cell states within the developmental continuum of cellular differentiation.

Previous studies have posited intermediate stages between naïve and primed pluripotency, largely on the basis of in vitro experiments[7–9]. However, the developmental authenticity of these in vitro cell states (namely, whether they have a clear in vivo counterpart) remained unresolved. Past studies have described "naïve-primed intermediate"[7], "poised"[8] or "formative" pluripotent cells[9] arising in vitro that seemed to be distinct from either naïve or primed cells. By contrast, Neagu, van Genderen, Escudero et al. took an in vivo approach to search for an intermediate pluripotent state. Given that naïve pluripotent cells appear in the E4.5 peri-implantation blastocyst and primed pluripotent cells are present in the E5.5 post-implantation blastocyst in the mouse[1,2], they decided to analyze recently implanted E5.0 embryos during the timespan between when naïve and primed cells are expected to exist.

The authors relied on the observation that naïve pluripotent cells take the form of a disorganized ball within the implanting E4.5 blastocyst[10]. Shortly after implantation, however, pluripotent cells rearrange themselves into a spatially polarized "rosette" structure with apical–basal polarity[10].

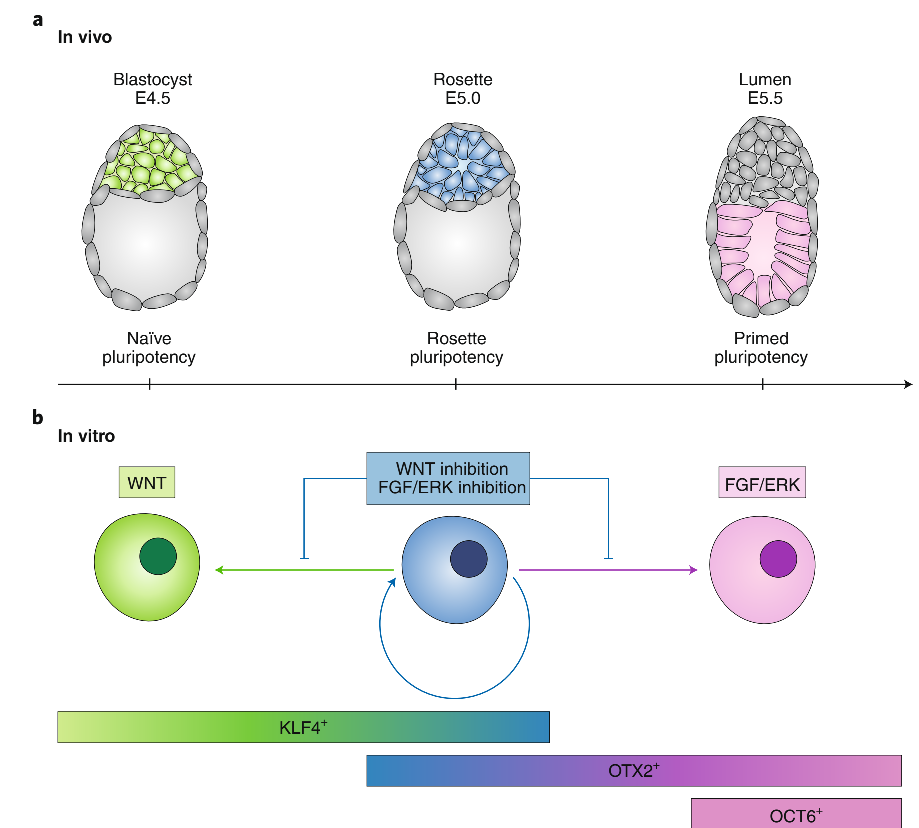

Fig. 1 | A continuum of pluripotent states in the early mouse embryo. a,b, Neagu, van Genderen, Escudero et al.[6] discover an intermediate cell state, designated "rosette pluripotency," that exists both in vivo (**a**) and in vitro (**b**). Previously, two types of pluripotent cells have been characterized in the mouse: naïve pluripotent cells are obtained from the embryonic day 4.5 (E4.5) peri-implantation blastocyst and are maintained by WNT signalling, and primed pluripotent cells exist in the E5.5 post-implantation embryo and are maintained by FGF/ERK signalling. Rosette pluripotent cells are molecularly characterized by overlapping expression of KLF4 and OTX2, and the inhibition of both WNT and FGF/ERK signalling captures the intermediate stage of rosette pluripotency in vitro[6].

The authors hypothesized that this in vivo rosette stage (E5.0) represents a clear intermediate between naïve and primed pluripotent states[6] (Fig. 1a). They defined rosette pluripotent cells on the basis of their coexpression of both naïve (Klf4) and primed (Otx2) pluripotency transcription factors, in addition to their apical–basal polarity (in contrast to the unpolarized nature of naïve pluripotent cells), chromatin state and other distinctive characteristics[6]. Importantly, they found that rosette pluripotency represents a transient state of development, typically lasting just hours and restricted to very recently implanted blastocysts. During this state naïve pluripotency genes continue to be expressed to some degree. Shortly afterwards, such genes are abruptly turned off[11].

The finding that mouse rosette pluripotent cells express naïve pluripotency markers is noteworthy and might inform ongoing efforts to generate human naïve pluripotent cells[1]. Careful evaluation of whether these protocols produce naïve cells or inadvertently generate rosette cells will be critical. Future research on rosette pluripotent cells will benefit from the discovery of specific markers of these transient cells. Currently, they are largely defined by the overlap of naïve and primed markers in addition to the expression of core pluripotency transcription factors (Oct4 and Sox2)[6]. Whether rosette pluripotency exists in vivo in other species will be an important question to explore. Preliminary analyses of human embryos suggest that a similar morphological reorganization of naïve pluripotent cells into a rosette occurs around the time of implantation[12], although further molecular analyses will be required to assess whether these cells fit the criteria for mouse rosette pluripotency. Alternatively, rosette pluripotency may not exist in human embryos, or may exist in a partially conserved form, sharing some but not all the attributes found in the mouse. Assessing the degree of evolutionary conservation will be vital to establishing the generality and significance of the pluripotent rosette across multiple mammalian species.

Given that rosette pluripotent cells truly exist in vivo, another important question is whether they can be generated and expanded in vitro. As reported in earlier studies, naïve and primed pluripotent cells are maintained by two different signalling pathways: WNT and FGF/ERK signalling, respectively[1–4]. Starting from naïve pluripotent cells, Neagu, van Genderen, Escudero et al. inhibited both pathways and simultaneously activated LIF signalling, thereby entrapping the intermediates and preventing their progression into either naïve or primed pluripotency[6] (Fig. 1b). Under these conditions, the majority of cells coexpressed KLF4 and OTX2 and could be cultured for dozens of passages[6], although the homogeneity and long-term stability of these rosette pluripotent cells remains to be rigorously assessed. Lending confidence to the premise that rosette cells are still pluripotent, the authors found that rosette pluripotent cells injected into the mouse blastocyst differentiated in vivo to create chimeric mice composed of both the transplanted and recipient cells[6].

Lastly, the authors suggested that rosette pluripotent cells are a uniquely plastic intermediate state with bidirectional developmental potential and can be reverted into naïve cells by WNT activation or further differentiated into primed cells by FGF/ERK activation[6] (Fig. 1b). This plasticity seems to be a unique feature of rosette cells, as the later-stage primed cells cannot be easily reverted into naïve cells without extensive genetic or signalling perturbations[1]. The dormant potential of rosette pluripotent cells to revert back to the naïve state in vitro is fascinating. This characteristic could be rooted in the finding that rosette pluripotent cells still continue to express naïve pluripotency genes[6], perhaps presaging the dormant potential of rosette cells to dedifferentiate into the naïve state. Whether this transition occurs naturally in vivo and what its mechanistic basis may be both warrant further attention, as other mammalian cell types are also able to revert to an earlier state under physiological conditions[13].

More broadly speaking, the authors' definition of a discrete intermediate state between two well-established states[6] indicates a fundamental challenge in biology: cellular differentiation is likely a continuous process that defies categorical labels of distinct steps along the process. Our prevailing view of cell states has thus far been largely shaped by static snapshots of continuous differentiation trajectories, limited, for instance, by cell culture media that stabilize specific stages or mouse embryos obtained from timed pregnancies. In turn, we have subjectively labeled the experimentally accessible steps of cellular differentiation as discrete 'cell types', whereas other stages (such as rosette pluripotency) remain uncharacterized. We anticipate that the advent of single-cell-sequencing and live-imaging technologies will rapidly expose intermediate transition states between well-recognized cell types[14]. This estimation pertains especially to later stages of embryonic development that are typified by exponentially increasing cellular diversity, which must derive from increasing numbers of transition states as progenitors progressively differentiate into more mature cells. However, does each transition state warrant designation as an alternative 'cell type', by analogy to rosette pluripotency?

Consequently, these results raise the question of what we define as a 'cell type'. Following on from the recognition of rosette pluripotency, the classification of pluripotent states is clearly nonbinary. But on a continuum from naïve to rosette to primed pluripotency, why should we stop at three distinct states? Do additional transition states exist and, if so, do they each warrant the moniker of being a unique 'cell type'? There are no simple answers. At one extreme, no two single cells are identical, yet it would be impractical to say that each single cell constitutes its own cell type. How different do two cells need to be before we distinguish them as being of two different kinds? In essence, the gold rush of single-cell RNA-sequencing surveys has relied heavily on transcriptional differences to nominate previously unrecognized cell types. However, beyond their differences in gene expression, Neagu, van Genderen, Escudero et al. show that pluripotent rosette cells differ from naïve and primed cells with regard to their chromatin status, ability to engraft in the mouse blastocyst, apical–basal polarity and requirement for extracellular signals[6]. Although it may seem obvious, cells are more than just their transcriptome. This work provides an instructive example for future studies to confidently classify alternative, unreported 'cell types' based on multiple functional criteria beyond single-cell RNA-sequencing profiling. Many types of cells have historically been viewed through the lenses of binary classification systems. For instance, macrophages have long been categorized based on their M1 or M2 polarization state, a restricted view that does not fully capture their functional diversity[15]. We would anticipate that these binary classification schemes may quickly fall by the wayside in other tissues as well. We must develop an adjusted taxonomy to describe and enumerate cellular diversity that avoids oversimplified, binary distinctions and classifies cell types in a realistic way, based on multiple criteria beyond differences in gene expression. ❐

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Competing interests

The authors declare no competing interests.