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Research Highlight
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LncRNAs and paraspeckles predict cell fate in early mouse embryo†

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How develop into a whole body is a fundamental question in developmental biology, and this process involves a series of cell-fate decisions. In mammals such as mouse and human, after fertilization, the first embryonic fate decision emerges after several rounds of cleave division when two distinct cell populations arise: the pluripotent inner cell mass (ICM) that contributes to the fetus and the trophectoderm (TE) that develops into a placenta. However, it remains unclear when and how asymmetry emerges in mammalian early embryos, as there are two different viewpoints existing on the mechanism underlying the first cell-fate decision. The first view considered that the blastomeres begin to differentiate only after the 8-cell stage, initiated by cell polarity, positional clues, and asymmetric cell division followed by deterministic molecular signals, while the other view argued that cell fate begins to bias before the 8-cell stage when blastomeres are still morphologically indistinguishable, and such cell-fate inclination is initiated by transcriptional and epigenetic heterogeneity [1]. In support of the later model, previous studies have revealed that the heterogeneous H3R26me2 level driven by Carm1 [2] and the CARM1/PRDM14/SOX2-SOX21 signal axis are the earliest molecular heterogeneity markers at the 4-cell embryo stage, which predispose cell fate toward ICM [1]. However, it remains unknown how such molecular heterogeneity in the 4-cell embryo arises, and could there be a molecular factor at the 2-cell stage responsible for this heterogeneity?

In fact, single-blastomere transcriptomic analyses have revealed that the initial symmetry-breaking process already begins as early as the 2-cell stage, driven by both chance separation and defined transcriptional circuits [3]. Also, it has been shown that when the identical-looking blastomeres from a 2-cell embryo are separated, usually only one of them can develop to term, suggesting the existence of intrinsic differences between them [4]. However, there is still lack of experimental evidence showing what molecules at 2-cell blastomeres can drive cell-fate segregation. Recently, two back-to-back works published in *Cell* [5, 6] provided new evidence showing long-noncoding RNAs and their nuclear compartmentalization are the earliest known markers to bias cell fate in mammalian 2-cell embryos (Figure 1).

In one of the studies, Zhou and colleagues firstly found a long noncoding RNA, *LincGET* (a LincRNA that is GLN-, MERVL-, ERVK- associated), transiently localized in the nucleus of 2- to 4-cell mouse embryos [5, 7], where it shows a heterogeneous distribution since the late 2-cell embryo, and its heterogeneity at the 4-cell stage correlates with CARM1 [5]. Overexpression of *LincGET* in one of the 2-cell blastomeres by injecting *LincGET* RNA has a similar effect to that of Carm1 mRNA injection, which can elevate the level of H3R26me2 in blastomeres of the 8-cell stage and can bias the progeny cells toward an ICM fate. Knocking down *LincGET* can either block embryo development (complete depletion) or inhibit blastomeres from undergoing an ICM fate (partial depletion) [5, 7]. These data suggest that a differential expression of *LincGET* in 2-cell embryos can bias cell fate. The authors further found that *LincGET* can physically interact and form a complex with CARM1. By using ATAC-seq and other approaches, they showed that the *LincGET*/CARM1 complex can increase chromatin accessibility and activate ICM-specific genes rather than TE-specific genes; and this process relates to transposon activation in the early embryos. Interestingly, once *Carm1* is depleted in one of the two blastomeres, and the overexpression of *LincGET* can no longer bias its progeny toward ICM, demonstrating that *LincGET*'s effect in biasing cell fates depends on CARM1. On the other hand, the developmental arrest phenotype by *LincGET* depletion cannot be rescued by *Carm1* overexpression, suggesting *LincGET* is upstream of CARM1 and it has other essential functions in addition to interacting with CARM1.

In an accompanying study by Zernicka-Goetz and colleagues, they found CARM1 accumulated in nuclear granules in the 2- to 4-cell stage, and these granules began to show a heterogeneous distribution since the late 2-cell stage [6], and they could be responsible for the heterogeneity of histone H3R26me2 modification in 4-cell embryos. The authors further found that most of CARM1 granules were associated with paraspeckles—a nuclear structure that is

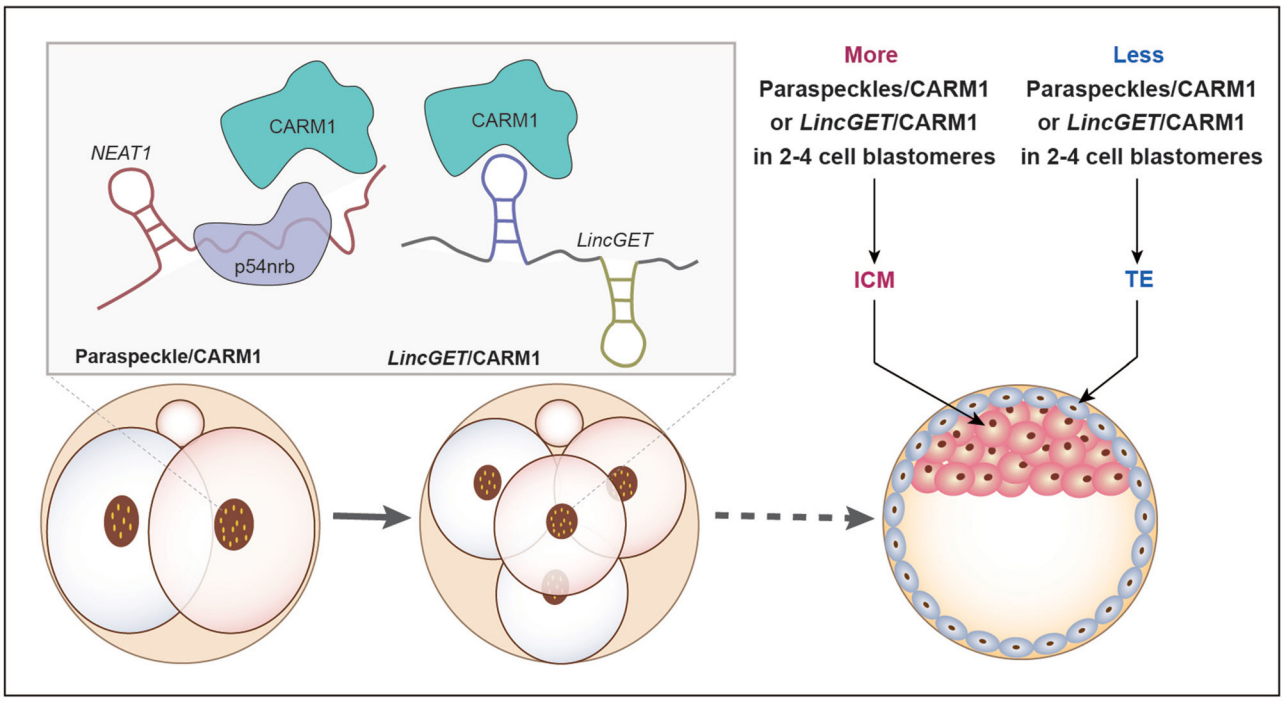

Figure 1. Molecular heterogeneity in 2-cell mouse embryos biases the first cell fate. CARM1 granules and *LincGET* are asymmetrically expressed in the nucleus of 2-cell mouse embryos. CARM1 granules were associated with paraspeckles in 2- to 4-cell embryos and *LincGET* formed a complex with CARM1 in the 4-cell stage. Heterogeneous expression of paraspeckles/CARM1 or *LincGET*/CARM1 biased cell fate contribution to either ICM or TE.

formed by specific LncRNA–protein complex bodies. The essential structural component of paraspeckles, *Neat1* (a LncRNA), and the protein p54nrb are found to regulate CARM1 intensity and its granule formation, as well as the level of H3R26me2 in the 4-cell stage. Also, the levels and activity of CARM1 feed back to paraspeckle formation by regulating the Neat1 transcript and p54nrb location. Although the depletion of p54nrb or Neat1 in the zygote or in one blastomere of 2-cell embryos resulted in embryonic developmental arrest, they also increased Cdx2 mRNA levels in 16-cell embryos, suggesting that these blastomeres are predisposed to a TE fate. Interestingly, the developmental phenotype by knocking down of *Neat1* and p54nrb is more severe than maternal and zygotic *Carm1* knockout embryos, suggesting that paraspeckles may also affect other important developmental events.

As such, both studies have shed new insights into nuclear CARM1 and the associated LncRNAs. They also advanced our understanding of the earliest known lineage regulators to bias cell fate in mammalians toward the 2-cell stage (Figure 1). This is a feat to previous bioinformatic and mathematical analyses [3]. These studies also raised many interesting questions. For example, what is the molecular regulators or signal pathways that are responsible for the CARM1 nuclear entry? Could this be dependent or independent of the regulation of the nuclear expression of LncRNAs such as *LincGET* and *Neat1*? Also, recent studies have revealed that Alu repeats in LncRNAs, which is a typical short interspersed nuclear elements (SINEs) of a retrotransposon, increasing RNA nuclear localization [8]. This resonates with the two studies here where both LncRNAs (*LincGET* and *Neat1*) are found to be enriched with repeat elements [5, 6]. The transposable elements have been shown to have a surge transcription during mammalian early embryo development, and they are essential for embryonic genome activation [9]. The repeat elements enriched *LincGET* and *Neat1* may also participate in this process in addition to biasing cell fate, and their function may simply represent the tip of the iceberg among thousands of other LncRNAs found in the preimplantation embryos, which apparently deserve further investigations. Finally, it is worth noting that in both Zhou's and Zernicka-Goetz's studies, they used the antisense oligonucleotides or locked nucleic acid to study the function of *Neat1* and *LincGET* in the embryos and found the embryo arrested in early stages. Yet, previous studies on *Neat1* knockout mice found that they are viable and showed only subfertility [10], and the *LincGET* knockout mice are not available now. Thus, the precise function of these LncRNAs may need further scrutinization by using different approaches.

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