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INSIGHT

Chromatin accessibility landscape during mammalian preimplantation development

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Early embryonic development is a key process that results in a blastocyst ready for implantation from a single-cell zygote. During this process, a set of critical biological events takes place to ensure maternal-to-zygotic transition, including zygotic gene activation (ZGA), totipotency to pluripotency transition, and cell lineage differentiation (Du et al., 2022; Xu et al., 2021). Dynamic epigenetic regulations play irreplaceable roles in this process to support embryonic development (Du et al., 2022; Xu et al., 2021). In eukaryotes, DNA wraps on histones to form nucleosomes, which are further organized into a complex chromatin structure. Active regulatory elements usually exist in an open chromatin state, which is nucleosome-free and bound by transcription factors (TFs) and chromatin regulatory factors (Klemm et al., 2019). Li et al. (2025) reported a high-coverage chromatin accessibility landscape of mouse preimplantation embryos using an improved long-read sequencing method, scNanoATAC-seq2. This study tracks stage- and lineage-specific TF activity, X-chromosome activity dynamics, as well as chromatin state of repetitive elements at individual copy resolution.

Several methods have been developed to map the accessible regions in the genome based on their accessible and nucleosome-free nature, such as DNase-seq, NOMe-seq, and ATAC-seq. DNase-seq uses DNase I, NOMe-seq employs the M.CviPI methyltransferase, and ATAC-seq relies on Tn5 transposase to cut or label accessible genomic DNA that is not protected by compact chromatin structures (Minnoye et al., 2021). The sensitivity of these methods has been improved to measure genome-wide chromatin accessibility from a very limited number of cells, such as low-input DNase-seq (liDNase-seq) (Lu et al., 2016), single-cell multi-omics sequencing technology (scCOOL-seq) (Guo et al., 2017), and miniATAC-seq (Wu et al., 2016). These methods have been applied to mouse and human embryos and reveal dynamic chromatin accessibility landscapes that harbor rich epigenetic regulatory information (Gao et al., 2018; Guo et al., 2017; Li et al., 2018; Lu et al., 2016; Wu et al., 2016; Wu et al., 2018). Motif enrichment analyses of accessible regions identify TFs critical for early development, such as NFYA for zygotic gene activation (ZGA) in mice (Lu et al., 2016) and OCT4 in humans (Gao et al., 2018). Allelic chromatin accessibility analyses uncover many parent-of-origin-specific accessible sites, including but not limited to known imprinting-control regions (ICRs) (Lu et al., 2016). Genomic imprinting is the phenomenon of parental-specific gene expression. Because the allelic chromatin accessibility appears before differential gene expression, they represent an additional layer of regulatory mechanism underlying genomic imprinting. This mechanism leads to identification of additional imprinted genes, including a new group of non-canonical imprinted genes controlled by parental-specific H3K27me3 rather than DNA methylation for canonical imprinted genes (Inoue et al., 2017a). Among these non-canonical imprinted genes, *Xist* stands out for its crucial role in X-chromosome inactivation. X-chromosome inactivation in preimplantation embryos in mice has long been known as imprinted X-chromosome inactivation for unknown mechanism that the paternal X-chromosome is inactivated while the maternal one remains active. The maternal H3K27me3 mediated *Xist* imprinting well explains why the paternal X chromosome is inactivated while the maternal X remains active in mouse pre-implantation embryos (Inoue et al., 2017b).

All the methods described above are based on next-generation sequencing (NGS) platform, which produces reads no longer than 150 bp. Such short read-length limits the capability in detection of large-scale structural variations, haplotype phasing, and repetitive regions. To overcome the read-length limitation associated with NGS, a Tn5-based single-cell chromatin accessibility measurement method called scNanoATAC-seq was developed on the Oxford Nanopore Technology (ONT) platform (Hu et al., 2023). This method uses Tn5 transposase with a single adaptor sequence instead of two. Then, PCR amplification using a single primer preferentially amplifies long tagged fragments, enriching DNA fragments of median size around 4–5 kb with at least one end in the accessible chromatin regions. The long read-length enables haplotype phasing, structural variation detection, and mapping within repetitive regions. In the present study, Mengyao Li

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and colleagues improved the protocol to develop scNanoATAC-seq2, which performs nuclear isolation and Tn5 tagging in the same tube (Li et al., 2025). This one-step strategy reduces sample loss and yields more complete single-cell chromatin accessibility maps. Data from scNanoATAC-seq and scNanoATAC-seq2 data largely agree with conventional short-read ATAC-seq (Hu et al., 2023; Li et al., 2025).

Li et al. (2025) applied scNanoATAC-seq2 to single-cell samples from zygote, early 2-cell, later 2-cell, 4-cell, 8-cell, 16-cell, late morula, early and late blastocyst stage embryos in mice, including 11 different cell types and the epiblast (EPI), primitive endoderm (PE), and trophectoderm (TE) lineages. The profiles recapture known knowledge from short-read chromatin accessibility analysis and add new detail. In blastocysts, single-cell resolution highlights TFs potentially important for epiblast (SOX2, OCT4, and KLF2), primitive endoderm (GATA6, SOX17, and HNF1B), and trophectoderm (TEAD4, GATA3, and CDX2). Very interestingly, the long reads also enable haplotype phasing and precise analysis of repetitive regions, revealing features missed by short-read studies.

Parental-specific accessibility can be resolved by leveraging single-nucleotide polymorphisms (SNPs) that distinguish the two parental genomes. In short-read data, this analysis highly depends on the SNP density, while in long-read data, many more reads can be parental-of-origin differentiated due to its ability in haplotype phasing using its long read-length. This capacity allows the method to detect many more allele-specific accessible genes (ASGs) than previous studies, uncovering 325 paternal and 8 maternal ASGs that are potentially non-canonically imprinted (Li et al., 2025). The fate and function of these non-canonical imprinted genes is an interesting direction to be explored in the future.

Long-read sequencing offers a clear advantage in profiling highly repetitive regions. In mammalian genomes, repetitive elements, which account for a large proportion, are used to be considered as "junk DNA". Yet they are now recognized as essential regulators of early embryonic development in both mice and humans, although the specific repetitive elements vary among species. In mice, long interspersed nuclear element-1 (LINE1) and endogenous retrovirus-L (ERVL), are essential regulators of early embryonic development (Xu et al., 2021). Short-read methods struggle to capture epigenetic information in these repetitive regions because short reads are poorly mappable at repetitive sequences. Earlier studies therefore relied on a few representative copies or on averaged signals, obscuring much of the underlying diversity. By contrast, the long-read scNanoATAC-seq2 enables depicting the accessibility of these elements at individual copy resolution (Li et al., 2025). The unprecedented resolution reveals that full-length LINE1 elements are mainly distributed in the heterochromatic B compartment, whereas active copies are enriched in the euchromatic A compartment. Moreover, the individual copy resolution also clearly reveals a positive correlation between the accessibility of individual repeats and the expression of their corresponding target genes, providing strong evidence that ERVL and other repetitive elements act as cis-regulatory elements for ZGA, which is supported by accessible peaks recovered from the low-mappable repetitive regions.

Bulk chromatin accessibility studies reveal imprinted *Xist* expression by maternal H3K27me3 initiates imprinted X-chromosome inactivation in mouse early embryos (Inoue et al., 2017b). Detailed insights into X chromosome inactivation are hindered by bulk analysis, which mixes male and female embryos. scNanoATAC-seq2 analyses overcome this limitation that differentiates embryo sex at the single-cell level. In line with earlier work, the method detects strong paternal-specific *Xist* accessibility in female embryos from the 1- to 8-cell stages (Inoue et al., 2017b), but it declines sharply in the blastocyst (Li et al., 2025). Interestingly, along with the diminish of paternal-specific *Xist* accessibility, an increase in maternal-specific *Tsix* accessibility is seen (Figure 1). The single-cell analysis also reveals that the shift from paternal-specific Xist accessibility to maternal-specific *Tsix* accessibility is evident in both PE and TE, where paternally imprinted X-chromosome inactivation is maintained (Figure 1), while only a minor maternal *Tsix* accessibility bias is seen in EPI (Li et al., 2025). This observation provides an interesting new insight into maintenance of paternally imprinted X-chromosome inactivation in extraembryonic lineages and X-chromosome reactivation in the embryonic lineages just before implantation.

It has been nine years since the first genome-wide map of chromatin accessibility in mouse early embryos. This new long-read study has systematically analyzed the parental-specific chromatin regulation and has provided comprehensive coverage over repetitive regions, greatly advancing our understanding of chromatin regulatory landscape during mammalian pre-implantation development. Several important questions, however, remain open. First, DNase-seq and ATAC-seq, which give similar results in somatic and embryonic stem cells, diverge sharply in 1- to 4-cell embryos of both mice and humans. DNase-seq detects very few accessible sites, while ATAC-seq detects a large number of accessible sites in 2-cell and 4-cell embryos in both mice and humans (Gao et al., 2018; Lu et al., 2016; Wu et al., 2016; Wu et al., 2018). This sharp difference in the number of accessible sites gradually disappeared later in pre-implantation development. In pigs, DNase-seq also reveals a similar pattern of number of accessible sites along development (Zhu et al., 2024), while ATAC-seq data is not available yet. This difference suggests unique chromatin features in mammalian pre-implantation embryos that can be differentiated by DNase-seq and ATAC-seq. Second, both methods also record a weak amplitude of accessibility signals in the zygote regardless of the number of identified peaks, suggesting an as-yet unknown chromatin property at the one-cell stage. Third, the changing accessibility landscape is thought to be driven by transcription factors, yet functional evidence is limited to a short list, including NFYA, OBOX, TPRX, DUX, OCT4, SOX2, NR5A2, TFAP2C, and GABPA. Technologies to validate this in a high-throughput way are not yet available. Fourth, there are a lot of parent-specific accessible sites that appear early but fade as development proceeds. A subset of these sites inherits parental-specific accessibility in an H3K27me3-controlled manner known as non-canonically imprinted. It will be interesting to dissect the mechanism to selectively preserve these non-canonical genomic imprintings. Fifth, initiation and maintenance of imprinted X-chromosome inactivation during mouse embryonic development seem to be controlled by *Xist* domain and *Tsix* domain, respectively. The mechanism underlying this transition remains to be elucidated.

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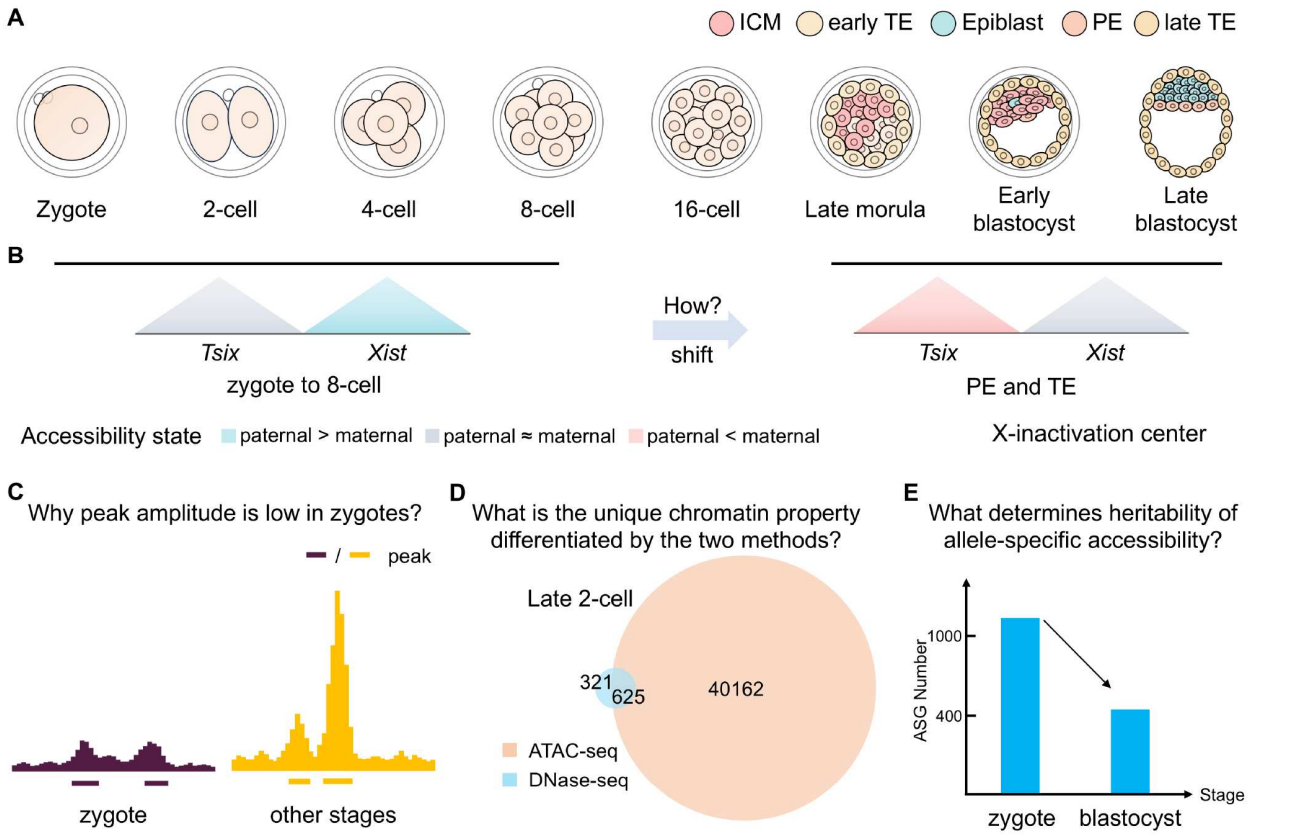

Figure 1. Remaining questions about chromatin regulation of mouse preimplantation development. A, Stages of mouse preimplantation development. B, New study reveals a shift of allele-specific chromatin accessibility for imprinted X-chromosome inactivation between the *Xist* and *Tsix* domains. The mechanism is an interesting theme to explore. C, Both ATAC-seq and DNase-seq record a weak amplitude of accessibility signals in the zygote, suggesting an as-yet unknown chromatin property at the one-cell stage. D, DNase-seq and ATAC-seq, which give similar results in most cells. However, in 1- to 4-cell mammalian embryos, DNase-seq detects much fewer accessible peaks compared to ATAC-seq, suggesting unique chromatin features in mammalian pre-implantation embryos that can be differentiated by DNase-seq and ATAC-seq. E, A lot of parental-specific accessible sites appear early but fade as development proceeds. What determines the heritability of a subset of these sites is interesting to be studied.

Early embryonic development, which transforms a single-cell zygote into an implantation-ready blastocyst, is driven by a complex epigenetic program. Charting chromatin accessibility provides direct insight into this program. A full understanding will require integrating chromatin accessibility maps with profiles of chromatin modifications and with the underlying transcriptional network. Continued advances in epigenomic technologies will pave the way to fully understand early embryonic development.

Compliance and ethics

The authors declare that they have no conflict of interest.

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