Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

Single-cell technologies: a new lens into epigenetic regulation in development

Adriano Bolondi[1,2], Helene Kretzmer[1] and Alexander Meissner[1,2,3,4]

The totipotent zygote gives rise to diverse cell types through a series of well-orchestrated regulatory mechanisms. Epigenetic modifiers play an essential, though still poorly understood, role in the transition from pluripotency towards organogenesis. However, recent advances in single-cell technologies have enabled an unprecedented, high-resolution dissection of this crucial developmental window, highlighting more cell-type-specific functions of these ubiquitous regulators. In this review, we discuss and contextualize several recent studies that explore epigenetic regulation during mouse embryogenesis, emphasizing the opportunities presented by single-cell technologies, *in vivo* perturbation approaches as well as advanced *in vitro* models to characterize dynamic developmental transitions.

Addresses
[1] Department of Genome Regulation, Max Planck Institute for Molecular Genetics, 14195 Berlin, Germany
[2] Institute of Chemistry and Biochemistry, Freie Universität Berlin,14195 Berlin, Germany
[3] Broad Institute of MIT and Harvard, 02142 Cambridge, MA, USA
[4] Department of Stem Cell and Regenerative Biology, Harvard University, 02138 Cambridge, MA, USA

Corresponding author: Adriano Meissner (meissner@molgen.mpg.de), Adriano Bolondi (@adrianobolondi), Helene Kretzmer (@helenekretzmer)

Current Opinion in Genetics & Development 2022, **76**:101947

This review comes from a themed issue on **Cell Reprogramming, regeneration and repair**

Edited by **Ophir Klein** and **Ryuichi Nishinakamura**

https://doi.org/10.1016/j.gde.2022.101947

Introduction

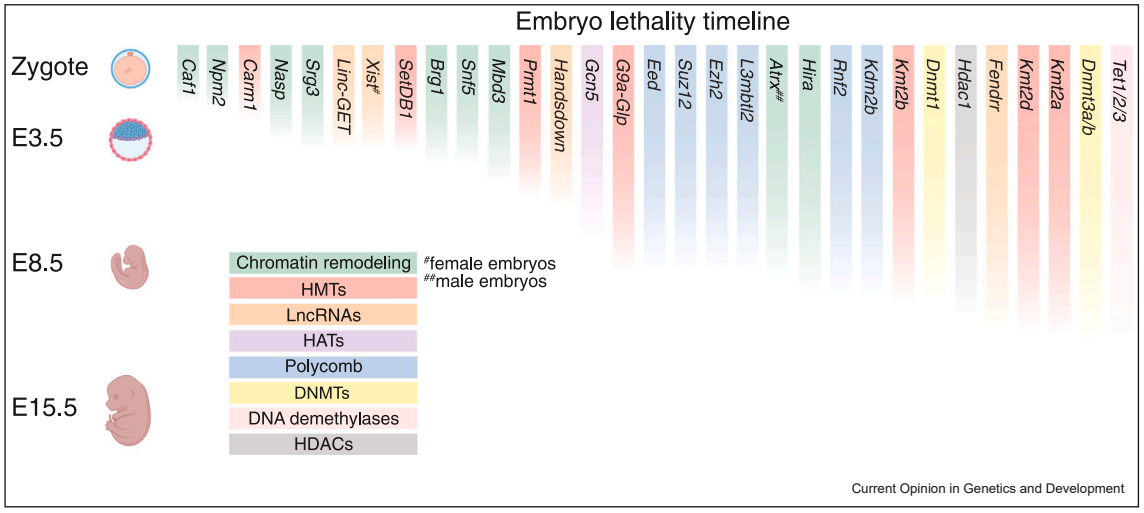
Mammalian embryogenesis is a highly orchestrated process characterized by precisely timed specification events that guide the formation of cellular hierarchies in the developing embryo. These events are in part coordinated by the modulated action of signaling molecules, which can induce genetic and epigenetic responses instrumental for cell state priming and acquisition [1–4]. This highly synergistic interplay of several regulatory layers, including transcription factors, chromatin remodelers, histone modifiers, DNA methyltransferases (DNMTs), and noncoding RNAs (ncRNAs), shapes the dynamic epigenome of the developing embryo. It is well established that epigenetic regulators are central to these processes and many studies over the past decades have demonstrated their essential role in ensuring proper developmental progression [5,6]. For instance, loss of their function during embryogenesis has been associated with cellular miss-specification events, developmental delay or arrest through mouse transgenesis, light microscopy, and next-generation sequencing approaches (Figure 1) [7–10].

Despite the consensus that these ubiquitously expressed regulators are essential, assigning specific roles and understanding the exact cause of lethality in mutant embryos remains largely unanswered. Recent advances in single-cell technologies have now provided an alternative route to classic perturbation and morphological assessment studies and enabled a deeper exploration of early embryogenesis, including improved interpretation of phenotypes associated with loss of function [11]. In this review, we summarize recent insights and technical developments with a focus on epigenetic regulators, their expression profiles and cell-type-specific functions, as well as the associated phenotypic outcomes in embryonic knockouts (KOs). Finally, we close by describing the promise of advanced *in vitro* models of embryogenesis to further explore transcriptional and epigenetic regulator functions with higher throughput.

Epigenetic regulators are essential for early development

Advances in genetic engineering and sequencing technologies from the 1990s onwards have allowed the generation and investigation of phenotypes that emerge in embryos lacking core epigenetic regulators such as Setdb1, Ezh2, Suz12, G9a/Glp (histone modifiers), Brg1, Caf1 (chromatin remodelers), and DNA methyltransferases (DNMTs) [6,8]. While their precise function remained obscure, these studies clearly highlighted the essential role the enzymes and their epigenetic modifications play in early embryonic development (Figure 1) [6–10,12].

Figure 1



Schematic overview of developmental lethality caused by loss of key epigenetic regulators. The timeline covers fertilization until late gestation (embryonic day E15.5) and each bar represents the approximate time of lethality for each mutant based on prior publications [6–8,11]. The frequent early lethality between preimplantation to gastrulation (E6.5-8.5) highlights the essential role of epigenetic regulators during this developmental window. The different colors group classes of regulators are indicated. E, embryonic day. Embryo schematics have been drawn with BioRender.

Nonetheless, linking the observed phenotypes, including embryonic lethality, to specific molecular and cellular functions remains challenging for these global and widely expressed actors. Next-generation sequencing-based technologies such as chromatin immunoprecipitation followed by sequencing, whole-genome bisulfite sequencing, and transcriptome sequencing permit researchers to characterize the molecular effects triggered by epigenetic regulators' disruption, but often only validate the loss of modification status without a clear connection to disrupted cellular or developmental functions. For example, zygotic DNMT1 or DNMT3A and B KO result in a global loss of DNA methylation (DNAme), but it is not yet clear which part of this deregulation is responsible for the observed lethality [10,13]. Two recent studies have investigated the polycomb repressive complexes (PRC)-mediated epigenomic reprogramming during pre-implantation development and showed that, in this context, PRC1 acts upstream of PRC2, guiding the zygotic deposition of H3K27me3 on maternally marked H2AK119ub1 promoters [14,15]. As a result, loss of maternal H2AK119ub1 during zygotic gene activation (ZGA) causes premature developmental gene expression (otherwise targeted by PRC2) and developmental arrest at the blastocyst stage [14].

Using a combination of zygotic disruption and molecular characterization, we recently highlighted that DNAme maintenance *via* DNMT1 and PRC (both PRC1 and PRC2) play a central role in autosomal imprinting in both the embryo proper and the extraembryonic ectoderm (ExE). Notably, the latter relies on an expanded set of imprinting mechanisms, including H3K9 methylation by G9a/GLP for noncanonical imprinting of endogenous retrovirus containing promoters [16]. Below we will further expand on the additional power provided by such an approach when combined with single-cell technologies [11].

Taken together, a large collection of studies highlighted the essential and early developmental function of epigenetic regulators. Molecular characterization moved this further over the past decade and now we are well equipped with new technologies to revisit central questions about their precise function and target regulation.

Redundancy in the regulation of DNA methylation

The observation that embryos lacking even single epigenetic regulators, independent of their specific functions, die soon after implantation indicates their crucial and indispensable roles (Figure 1). As may be expected for pathways such as DNAme where multiple and partially redundant enzymes are available, the phenotype can vary. For instance, single mutant embryos for either of the DNMT3s or their antagonistic enzymes, the ten-eleven translocation (TET) methylcytosine dioxygenases, progress much further in development than mutants of the nonredundant DNMT1 maintenance enzyme. The observation that embryos lacking both DNMT3s or all three TET enzymes also die around mid-gestation confirms their crucial but partially

overlapping functions [10,17]. Development in the absence of TET1/2 function, despite generating viable animals, results in genome-wide aberrant cytosine methylation and 5-hydroxymethylation (5hmC) distribution; in particular, germ cells are completely depleted of 5hmC, and embryonic as well as post-natal tissues display a general increase in genome-wide methylation, including at some classes of repetitive elements [18]. Despite this redundancy, some of the regulators appear to also have distinct functions within specific lineages, such as the unique role of DNMT3A in the germline and DNMT3B in shaping the ExE methylome [19,20]. Embryos lacking DNMT3B, but not DNMT3A, lose genome-wide and CpG island-specific methylation in the ExE, without drastic changes in the epigenome of the embryo proper [20].

Of the repressive pathways, DNAme enzymes display a redundancy that can at least buffer some early phenotypes, while both PRCs are required and therefore embryos are susceptible to loss of any individual core subunit (Figure 1). For instance, disruption of either *Ezh2* or *Rnf2* leads to mid-gestation embryonic lethality, indicating a necessary and nonredundant function of these two regulators during mouse development [9,21–24]. Genomic targets of EZH1-containing PRC2 are generally also bound by EZH2-containing PRC2 in F9 cells, and loss of *Ezh1* does not induce changes in chromatin compaction or gene expression [25]. Both *in vitro* and *in vivo*, EZH2 and RNF2 are crucial for PRC2 and PRC1 functions respectively, and can compensate for the loss of their paralogues (EZH1 and RNF1), but not vice versa.

Single-cell atlases provide a new lens on mammalian development

The recent curation of single-cell transcriptomic atlases of mouse embryogenesis has created an opportunity to investigate transcriptional and epigenetic regulator expression dynamics at high resolution across different lineages during pre- [26], peri- [27–29], post-implantation development [11,28,30–35], and organogenesis [36] (Figure 2). As expected, DNMTs show some stage, tissue and cell cycle-dependent expression dynamics. In line with the genome-wide resetting of the methylome, DNMTs are generally lowly expressed during pre-implantation development and Dnmt3b is activated during the late blastocyst stage concomitant with embryo implantation. Although both Dnmt3a and Dnmt3b are expressed at high levels in the embryo, Dnmt3a is only lowly expressed in the E6.5 ExE and, as compared to the epiblast, cannot compensate for the loss of Dnmt3b in this lineage, as described above (Figure 2, E6.5 stage). Other regulators such as *Hdac1*, *Mbd3* and *Nasp*, in contrast, show a more stable expression profile across tissues and developmental stages. Beyond some of the unique patterns, the data highlight the surprisingly ubiquitous expression of most core regulators during gastrulation where cell state diversity increases rapidly (Figure 2, E6.5–8.5 window). The more precise expression dynamics that can be acquired from the analysis of single-cell data are a major advance that represents a driving focus of the community. However, single-cell transcriptomic states alone fail to inform on precise functions of the individual regulators and cannot yet assign specific roles in different lineages or across developmental stages. In the next sections, we describe how genetic perturbations and single-cell readouts provide a path forward.

Interfacing noncoding RNAs and epigenetic regulation in early development

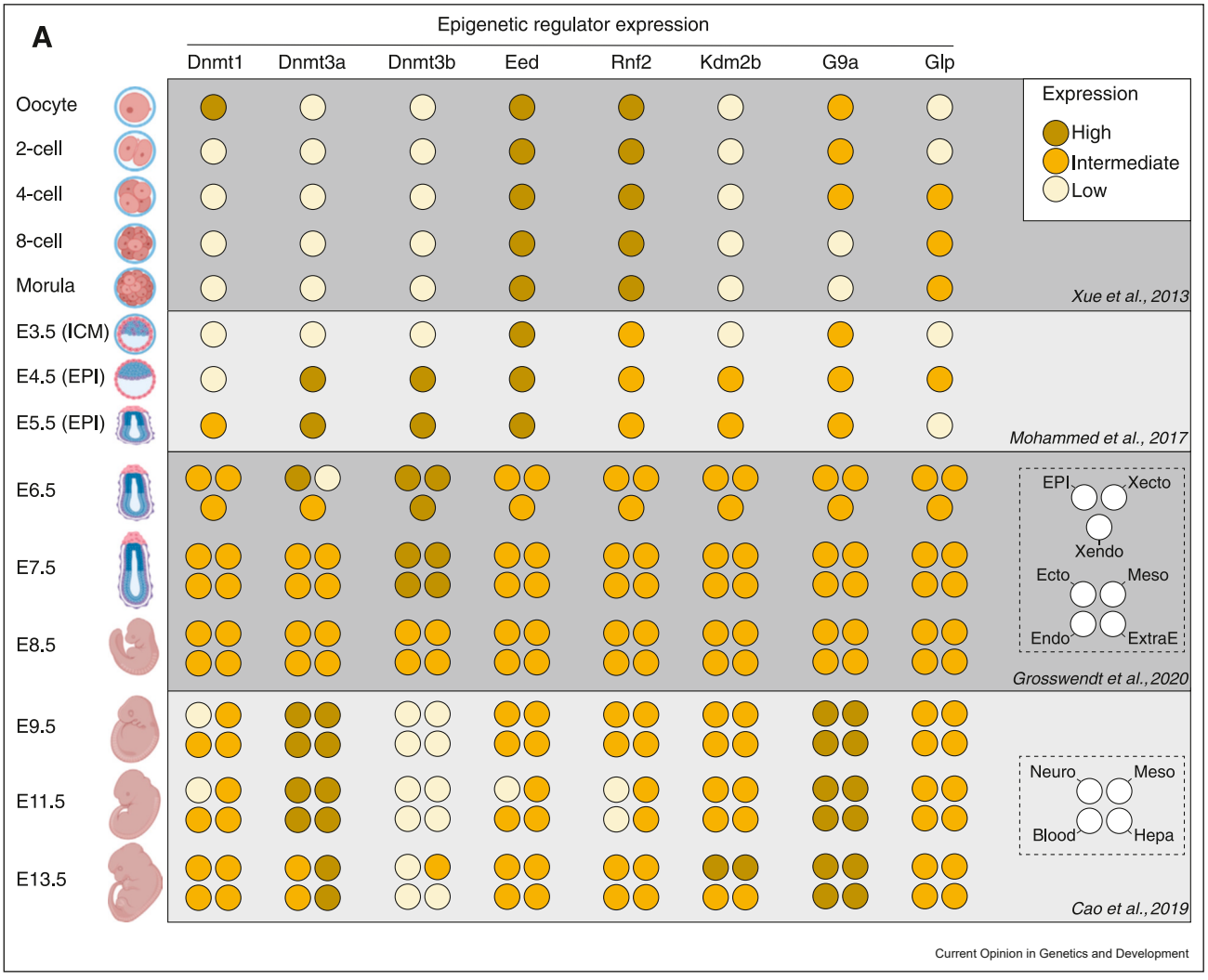
As shown in Figure 1, many ncRNA genes also exhibit early embryonic lethal KO phenotypes comparable to those of core epigenetic regulators. Two studies in this area addressed the regulatory interplay between ncRNAs and epigenetic regulators by combining single-blastomere injection with single-cell gene expression profiling (sc-qPCR), as well as through high-resolution microscopy. Among other interesting findings, these experiments demonstrated the concerted action of *LincGET*, a long ncRNA, and the histone methyltransferase (HMT) CARM1 in the differential induction of ICM and trophectoderm (TE) fates as early as the 2-cell stage embryo (Figure 3a) [37,38]. In *LincGET* expressing blastomeres, CARM1 catalyzes the deposition of histone H3 arginine 26 methylation (H3R26me2) on pluripotency gene promoters such as *Nanog*, *Sox2*, and *Oct4*. This results in their expression, which increases the accessibility of inner cell mass regulatory elements (ICM-RE), and supports the activation of an ICM-specific developmental program (Figure 3a) [38,39].

Many more such interactions occur throughout development, illustrating the relevance of the RNA-chromatin interface in cell state acquisition.

A multipronged assessment using single-cell multiomic technologies

In general, the opportunity to simultaneously profile multiple regulatory layers can provide additional insights beyond what can be recovered from transcriptomes alone, driving many groups to develop and apply scMultiomics technologies [40–43]. Among other uses, these approaches have enabled the direct association between *cis*-regulatory element activity and gene expression to further define the activity of genetic programs that support cell state transitions during development [40,43]. *Wang* and colleagues developed a technology to simultaneously profile gene expression, DNAme, and chromatin accessibility in the same cell (scNOMeRe-seq) and applied it to mouse early pre-

Figure 2

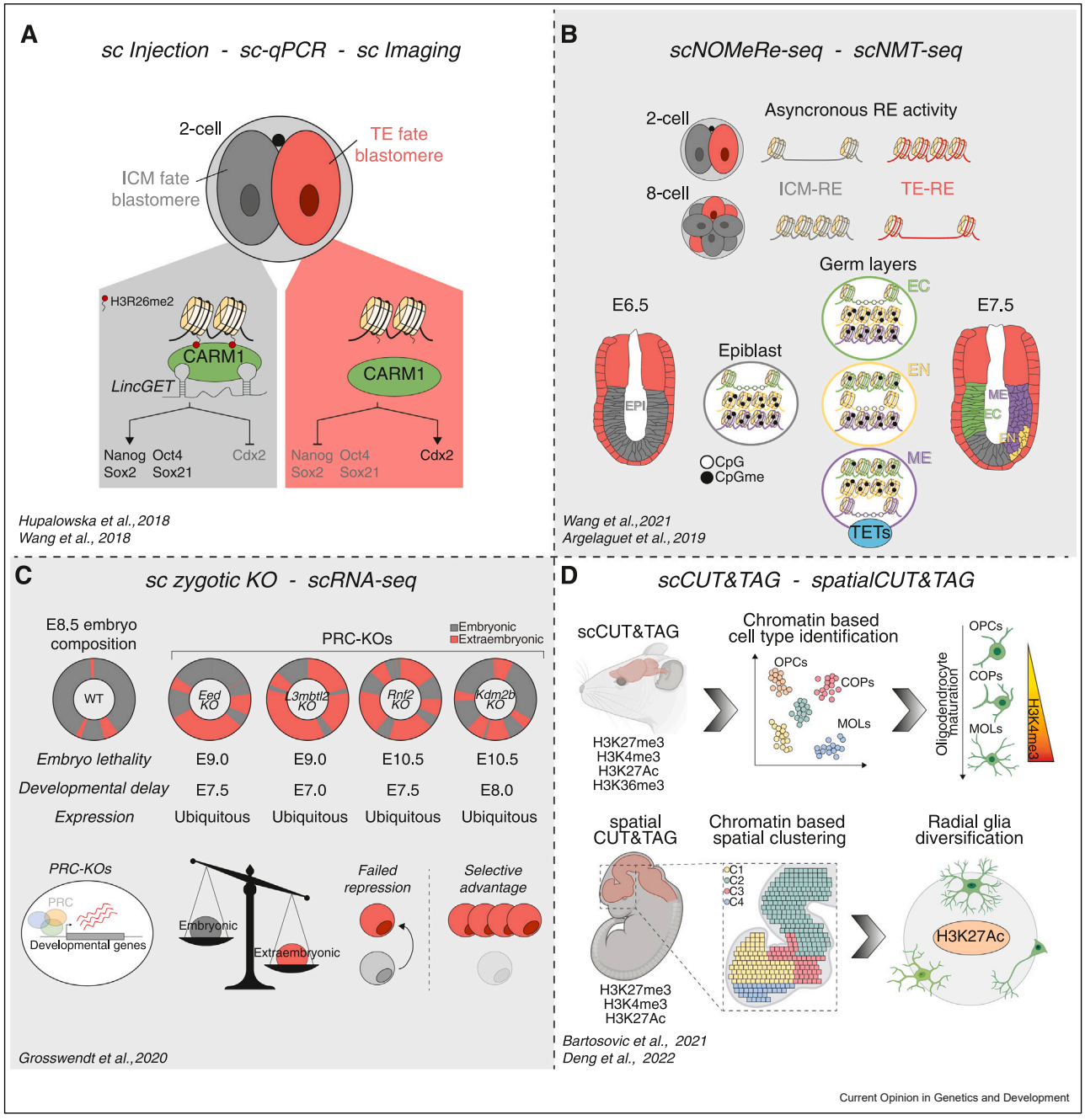


Single-cell-based epigenetic regulator expression in mouse development. Schematic representation of published single-cell expression studies (data from refs [11,26,27,36]). Expression values were normalized to sequencing depth per cell and averaged across lineages and cell types for simplicity. TATA-binding protein (Tbp) expression was used as a reference to compare expression across studies and to allow for classification into low, intermediate or high expression with the transition between intermediate and high being defined at Tbp levels. E, embryonic day; ICM, inner cell mass; EPI, epiblast; Xecto, extraembryonic ectoderm; Xendo, extraembryonic endoderm; Ecto, ectoderm; Meso, mesoderm; Endo, endoderm; ExtraE, extraembryonic; Neuro, neural tissues; Hepa; hepatocytes. Embryo schematics have been drawn with BioRender.

implantation embryos (Figure 3b, upper panel) [43]. This study detailed the stepwise and asynchronous activation of fate-associated *cis*-regulatory elements, with ICM-RE already accessible at the 2-cell stage embryo, while TE-specific regulatory elements become active around the 8-cell stage [43].

As embryos proceed through implantation, cells in the pluripotent epiblast initiate the process of gastrulation, resulting in the generation of the three embryonic germ layers, ectoderm, mesoderm, and endoderm. In this context, another multiomics approach (scNMT-seq) was developed by *Argelaguet* and colleagues to investigate the dynamic epigenomic changes as cells exit from pluripotency and commit to new lineages [40,44]. Similar to the differential activation of lineage-associated elements in the preimplantation embryo, this study found that ectoderm-specific regulatory elements (EC-RE) are accessible and free of methylation in the E6.5 EPI cells, while mesoderm and endoderm-specific REs (ME-RE and EN-RE, respectively) remained inactive. ME-REs and EN-REs are instead gradually demethylated and activated as a response to growth factors within their respective cell populations, which cooccurs with EC-RE compaction and methylation (Figure 3b, lower panel) [40]. Further characterization of these dynamic processes

Figure 3



Single-cell based approaches to study epigenetic regulation during development. **(a)** Single blastomere injection combined with single-cell qPCR and imaging to explore the regulation of early lineage choice by ncRNAs and epigenetic regulators [37,38]. TE, trophectoderm; ICM, inner cell mass; qPCR, quantitative polymerase chain reaction. **(b)** scNOMeRe-seq and scNMT-seq as representative multiomics approaches to explore regulatory elements in early development [40,43]. RE, regulatory elements; TE-RE, trophectoderm-specific regulatory elements; EC, ectoderm; EN, endoderm; ME, mesoderm; E, embryonic day; CpG, unmethylated CpG; CpGme, methylated CpG. **(c)** Single-cell zygotic disruption combined with scRNA-seq profiling in mid-gestational WT and mutant embryos as a novel method to explore phenotypes at a high molecular resolution [11]. Pie charts are generated from data reported in [11]. WT, wild type; PRC, polycomb repressive complex. **(d)** scCUT&TAG and spatialCUT&TAG as examples of single-cell histone modification mapping during embryonic and adult neurogenesis [48,50]. OPCs, oligodendrocyte progenitor cells; COPs, committed oligodendrocyte progenitor cells; MOLs, mature oligodendrocytes; C, spatial cluster. Mouse and embryo schematics have been drawn with BioRender.

in embryoid bodies revealed that the demethylation of ME-REs in mesodermal cells is dependent on TET enzyme function, in line with reported TET activity at somatic enhancers in mouse and human pluripotent stem cells [40,45].

In both the pre and post-implantation cases, the application of these scMultiomics approaches revealed a temporal hierarchy for timed, lineage-specific enhancer activation and subsequent coordination of the respective gene expression programs.

Assigning cell-type-specific functions for global regulators

The ability to combine these technologies with perturbation approaches provides additional opportunities for insights, particularly for epigenetic regulators where their context-specific nature remains ambiguous. For example, we recently demonstrated the utility of combining a zygotic perturbation strategy with an *in vivo* single-cell gene expression readout to explore the developmental outcome and molecular phenotype of embryos lacking core epigenetic regulators [11]. For instance, all the analyzed PRC subunit KO embryos (*Eed*, *L3mbtl2*, *Rnf2*, and *Kdm2b*) show delayed development and unbalanced cellular composition at E8.5, with an over-representation of extraembryonic tissues and under-representation or complete absence of mature embryonic cellular subtypes (Figure 3c) [11]. Interestingly, although transcriptional phenotypes were not seen at E6.5, one could already observe clear epigenetic alterations compared to wild-type controls [11]. In terms of the altered cell type composition, it is possible to envision different models for how these regulators might be acting, including *failed repression of alternative lineages* or *selective advantage of tolerant lineages*. In the former case, PRC subunits suppress one or more lineages that in their absence become active, while in the latter only certain lineages are able to successfully differentiate, proliferate and expand in the absence of PRC-based regulation (Figure 3c). Distinguishing these two possibilities is not trivial, as illustrated by embryos lacking maternal PRC1 (*Pcgf1/6* matKO embryos). These embryos can develop to term, but display placental enlargement (almost doubled the volume as compared to the wild-type placenta) without affecting embryo size [15]. Similarly, *Eed* disrupted embryos show expansion of the allantois as well as primordial germ cell (PGC)-like cells [11], which could arise through either path and would require molecular barcoding or similar genetic lineage tracing strategies to resolve if this phenotype represents a 'default' differentiation state within the epiblast or if instead PGCs and allantois are preferentially tolerant to the loss of Polycomb group regulation [46,47].

These recent advances helped to begin disentenglingthe exact function of PRCs and other regulators to successfully apportion the differentiating embryo into its appropriate lineages.

Single-cell chromatin profiling in the developing brain

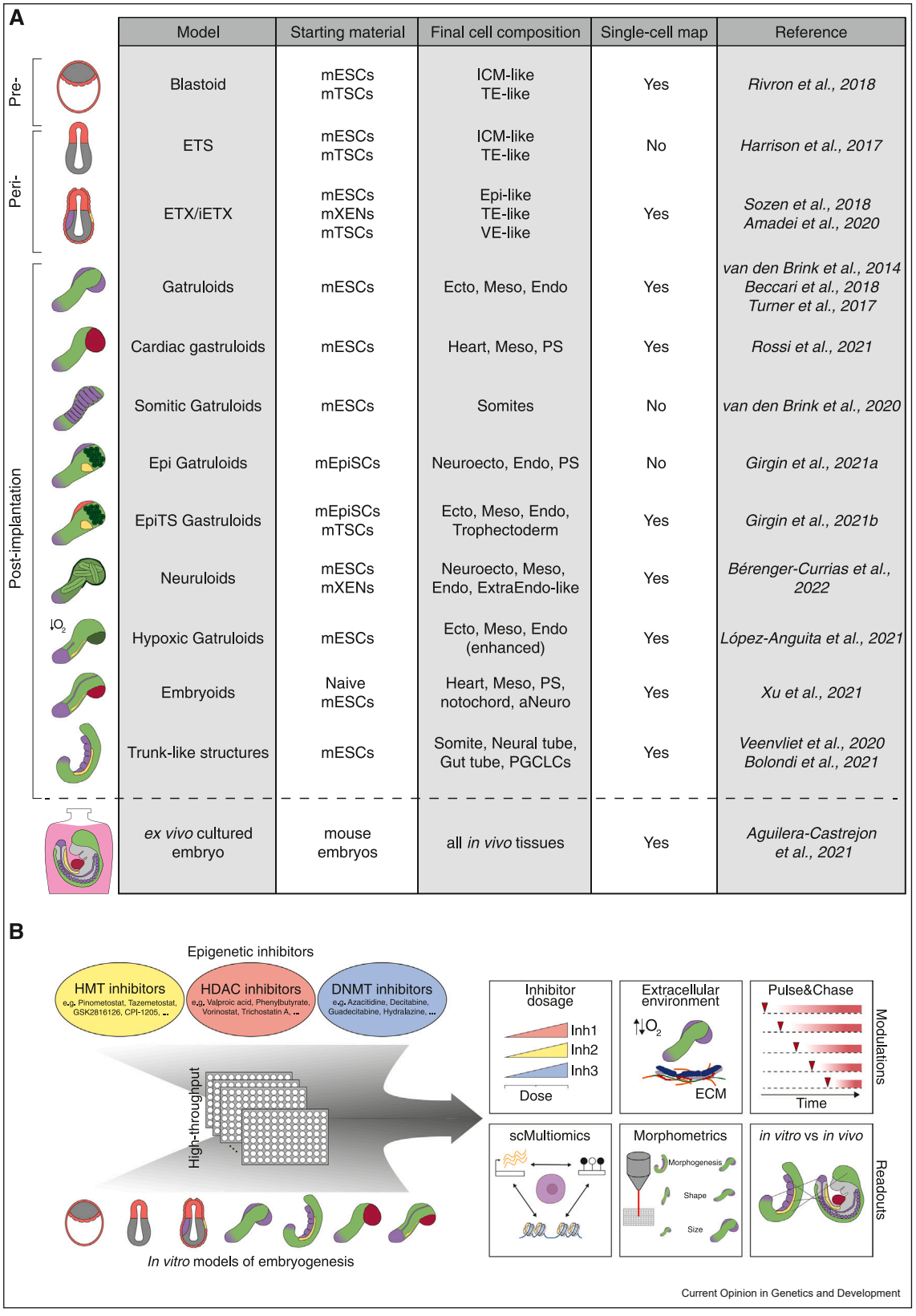
The development of technologies to quantitatively measure histone modifications in individual cells has revealed the presence of "*cell-specific epigenetic signatures*" that can distinguish transcriptionally similar subpopulations based on their histone modifications at precise genomic locations [48,49]. *Bartosovic* and colleagues developed scCUT&TAG to simultaneously profile four histone modifications in individual cells of a post-natal mouse brain. Their single-cell resolution data could then be used to identify distinct chromatin sub-states during oligodendrocyte maturation *in vivo*, revealing an increasing amount of promoter-associated H3K4me3 along the maturation axis from oligodendrocyte progenitor cells to mature oligodendrocytes (Figure 2d, upper panel) [48]. Advances in spatially resolved epigenomic technologies have also facilitated the geographical mapping of diverse cellular epigenetic states in developing tissues [50,51]. For example, a recent study leveraged the scCUT&TAG method to jointly profile three histone modifications in the developing brain of a post-implantation embryo with spatial and near single-cell resolution (spatialCUT&TAG) [50]. The integration of this dataset with conventional scRNA-seq enabled researchers to visualize enhancer activity both genome-wide and within the spatial contexts of the tissue, alongside quantitative measurements of their target gene expression to develop models of gene regulation *in situ*. Their analysis demonstrated the presence of distinct chromatin state zones in the developing brain and identified the acetylation of lysine 27 of histone H3 (H3K27ac) at regulatory elements as a primary epigenetic determinant for radial glia diversification during brain development (Figure 3d, bottom panel) [50].

These examples representrecent technological advances that have provided new means to investigate cell-type-specific epigenetic regulation in both time and space, highlighting complex networks of epigenetic interactions that safeguard cell state identity and transitions in a developing embryo.

In vitro models of embryogenesis as an expanded toolbox

To complement ongoing *in vivo* efforts, many groups have developed *in vitro* models, including two-dimensional (2D) differentiation systems from mouse embryonic stem cells (mESCs) [6]. Recently, more advanced stem cell-based *in vitro* models of

Figure 4


A
Pre-
Peri-
Post-implantation
Model
Starting material
Final cell composition
Single-cell map
Reference
Blastoid
mESCs
mTSCs
ICM-like
TE-like
Yes
Rivron et al., 2018
ETS
mESCs
mTSCs
ICM-like
TE-like
No
Harrison et al., 2017
ETX/iETX
mESCs
mXENs
mTSCs
Epi-like
TE-like
VE-like
Yes
Sozen et al., 2018
Amadei et al., 2020
Gatruloids
mESCs
Ecto, Meso, Endo
Yes
van den Brink et al., 2014
Beccari et al., 2018
Turner et al., 2017
Cardiac gastruloids
mESCs
Heart, Meso, PS
Yes
Rossi et al., 2021
Somitic Gatruloids
mESCs
Somites
No
van den Brink et al., 2020
Epi Gatruloids
mEpiSCs
Neuroecto, Endo, PS
No
Girgin et al., 2021a
EpiTS Gastruloids
mEpiSCs
mTSCs
Ecto, Meso, Endo, Trophectoderm
Yes
Girgin et al., 2021b
Neuruloids
mESCs
mXENs
Neuroecto, Meso, Endo, ExtraEndo-like
Yes
Bérenger-Currias et al., 2022
↓O2
Hypoxic Gatruloids
mESCs
Ecto, Meso, Endo (enhanced)
Yes
López-Anguita et al., 2021
Embryoids
Naive mESCs
Heart, Meso, PS, notochord, aNeuro
Yes
Xu et al., 2021
Trunk-like structures
mESCs
Somite, Neural tube, Gut tube, PGCLCs
Yes
Veenvliet et al., 2020
Bolondi et al., 2021
ex vivo cultured embryo
mouse embryos
all in vivo tissues
Yes
Aguilera-Castrejon et al., 2021
B
Epigenetic inhibitors
HMT inhibitors
e.g. Pinometostat, Tazemetostat, GSK2816126, CPI-1205, ...
HDAC inhibitors
e.g. Valproic acid, Phenylbutyrate, Vorinostat, Trichostatin A, ...
DNMT inhibitors
e.g. Azacitidine, Decitabine, Guadecitabine, Hydralazine, ...
High-throughput
In vitro models of embryogenesis
Inhibitor dosage
Inh1
Inh2
Inh3
Dose
Extracellular environment
↓↑O2
ECM
Pulse&Chase
Time
Modulations
scMultiomics
Morphometrics
Morphogenesis
Shape
Size
in vitro vs in vivo
Readouts
Current Opinion in Genetics and Development

Advanced *in vitro* models of embryogenesis and their possible implementation for epigenetic studies. **(a)** Table summarizing the pre- [54], peri- [55–57], and post-implantation [58–66,72,81,85] mouse *in vitro* models of embryogenesis developed to date as well as a recently reported *ex vivo* embryo culture [86]. For each model, information regarding the starting and final cellular composition and the presence of an available single-cell map has been provided. **(b)** Schematic outline of conceptual designs for high-throughput investigation of epigenetic and other regulators using *in vitro* models of embryogenesis combined with inhibitors compound screens.

embryogenesis have been optimized [52,53], and their detailed characterization continues to reveal high cellular and molecular similarity with the pre- [54], peri- [55–57], and post-implantation embryo developing *in utero* (Figure 4a) [58–67]. Unlike comparatively inaccessible mouse embryos, these *in vitro* models enable streamlined application of chemical perturbation to affect lineage trajectories with greater experimental control, allowing researchers to direct these structures into different cell identities [61,64,66]. In parallel, chemical compound libraries to selectively inhibit epigenetic regulator function have been developed and used in high-throughput toxicology screens in 2D culture [68,69], 3D adult organoids [70], or *in vivo* [71].

The ability to combine these approaches is providing conceptual evidence for the opportunity to explore the functions of different regulator classes at higher throughput as well as with greater replicate numbers and across more diverse cellular systems (Figure 4b). The use of different preimplantation and postimplantation models offers the unique opportunity to dissect early and late dependencies on epigenetic regulator function for cell identity acquisition. Moreover, the ability to modulate inhibitor treatment combination, timing, and dosage allows for the quantitative investigation of dependencies and synergistic functions. Finally, the accessible nature of these models also allows for easy modification of the extracellular environment at the metabolic (e.g. via oxygen deprivation) or mechanical levels (e.g. modulating extracellular matrix stiffness and modular components) [66,72]. These investigations are furthering our appreciation of the direct crosstalk between metabolism and epigenetic regulation [73]. For example, hypoxic conditions can inhibit the action of several epigenetic regulators, including TETs and several histone demethylases, resulting in DNA and histone hypermethylation that appears to influence cellular identities and potential [74–76]. Interestingly, gastruloids developing in hypoxia display an enhanced and more embryo-like lineage representation, indicating a direct effect of oxygen deprivation on early differentiation events [72].

Taken together, the modularity, accessibility, tractability, and scalability of these new *in vitro* models of embryogenesis offer a toolkit to complement *in vivo* studies, and could provide a powerful new lens to explore long-standing questions regarding the specific functions of epigenetic and environmental cues in regulating cell identities.

Conclusion

The recent development and increased accessibility of high-resolution single-cell technologies offer unprecedented avenues to study early embryogenesis. This new lens will greatly facilitate the interpretation of the complex and dynamic epigenetic regulation during early cell state transitions.

As outlined above, many epigenetic regulators contribute to cell identity and fate choice. However, they often appear to act in a tissue-specific fashion despite being broadly expressed in most of the cell types present in a developing embryo. How is cell-type-specific epigenetic regulation then achieved, and what commonalities are shared across the responses seen in diverse progenitor states? How can broadly expressed regulators identify and act on cell-specific targets in the genome? Recent studies have shown that the expression of sequence-specific co-factors can drive tissue-specific epigenetic regulation by tethering them to defined genomic locations [77–79]. The highly tissue-specific nature of long long ncRNAs may also support this fine-tuned regulation [7]. The resulting network implies the presence of ubiquitously expressed epigenetic effectors (such as DNMTs, PRCs, TETs, SWI/SNF) combined with often lowly expressed, tissue-specific co-factors (such as transcription factors, long ncRNAs, and post-translational modifiers) that act as a scaffold to provide the cell-type-specific positional information necessary to acquire diverse cell identities during development.

Given their accessibility and scalability, *in vitro* models of embryogenesis offer the potential for high-throughput readouts of broad chemical screens, offering an exciting toolkit to accelerate our understanding of epigenetic regulator function in the establishment of cell identities. Finally, the recent implementation of human *in vitro* models of embryogenesis faithfully recapitulating critical stages of pre- [80,81] and postimplantation [82–84] development may help overcome ethical limitations associated with human embryo research and offer a unique path to understand how conserved epigenetic principles are across mammals as well as to more directly address aspects of these findings as they relate to reproductive and fetal health.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgements

We would like to thank all members of the Meissner Lab for fruitful discussions and in particular Zachary D. Smith for critical reading of the manuscript. A.M. is supported by the Max Planck Society.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest.

1. Arnold SJ, Robertson EJ: **Making a commitment: cell lineage allocation and axis patterning in the early mouse embryo**. *Nat Rev Mol Cell Biol* 2009, **10**:91-103.

2. Arzate-Mejia RG, Valle-Garcia D, Recillas-Targa F: **Signaling epigenetics: novel insights on cell signaling and epigenetic regulation**. *IUBMB Life* 2011, **63**:881-895.

3. Bardot ES, Hadjantonakis AK: **Mouse gastrulation: coordination of tissue patterning, specification and diversification of cell fate**. *Mech Dev* 2020, **163**:103617.

4. Ho L, Crabtree GR: **Chromatin remodelling during development**. *Nature* 2010, **463**:474-484.

5. Hemberger M, Dean W, Reik W: **Epigenetic dynamics of stem cells and cell lineage commitment: digging Waddington's canal**. *Nat Rev Mol Cell Biol* 2009, **10**:526-537.

6. Surani MA, Hayashi K, Hajkova P: **Genetic and epigenetic regulators of pluripotency**. *Cell* 2007, **128**:747-762.

7. Andergassen D, Rinn JL: **From genotype to phenotype: genetics of mammalian long non-coding RNAs *in vivo***. *Nat Rev Genet* 2021, **23**:229-243.

8. Meissner A: **Epigenetic modifications in pluripotent and differentiated cells**. *Nat Biotechnol* 2010, **28**:1079-1088.

9. O'Carroll D, Erhardt S, Pagani M, Barton SC, Surani MA, Jenuwein T: **The polycomb-group gene Ezh2 is required for early mouse development**. *Mol Cell Biol* 2001, **21**:4330-4336.

10. Okano M, Bell DW, Haber DA, Li E: **DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development**. *Cell* 1999, **99**:247-257.

11. •• Grosswendt S, Kretzmer H, Smith ZD, Kumar AS, Hetzel S, Wittler L, Klages S, Timmermann B, Mukherji S, Meissner A: **Epigenetic regulator function through mouse gastrulation**. *Nature* 2020, **584**:102-108.

The authors combined zygotic perturbation and single-cell RNA-sequencing to assay the role of key epigenetic factors in regulating cell diversity in mouse embryos and reveal a dominant role of the PRC prior to gastrulation.

12. Fu X, Zhang C, Zhang Y: **Epigenetic regulation of mouse preimplantation embryo development**. *Curr Opin Genet Dev* 2020, **64**:13-20.

13. Haggerty C, Kretzmer H, Riemenschneider C, Kumar AS, Mattei AL, Bailly N, Gottfreund J, Giesselmann P, Weigert R, Brandl B, *et al.*: **Dnmt1 has *de novo* activity targeted to transposable elements**. *Nat Struct Mol Biol* 2021, **28**:594-603.

14. Chen Z, Djekidel MN, Zhang Y: **Distinct dynamics and functions of H2AK119ub1 and H3K27me3 in mouse preimplantation embryos**. *Nat Genet* 2021, **53**:551-563.

15. Mei H, Kozuka C, Hayashi R, Kumon M, Koseki H, Inoue A: **H2AK119ub1 guides maternal inheritance and zygotic deposition of H3K27me3 in mouse embryos**. *Nat Genet* 2021, **53**:539-550.

16. • Andergassen D, Smith ZD, Kretzmer H, Rinn JL, Meissner A: **Diverse epigenetic mechanisms maintain parental imprints within the embryonic and extraembryonic lineages**. *Dev Cell* 2021, **56**:2995-3005.e2994.

In this work, the authors provide a comprehensive analysis of the diverse epigenetic mechanism that control parent-specific imprints in the embryonic and extraembryonic compartments.

17. Dawlaty MM, Breiling A, Le T, Barrasa MI, Raddatz G, Gao Q, Powell BE, Cheng AW, Faull KF, Lyko F, Jaenisch R: **Loss of Tet enzymes compromises proper differentiation of embryonic stem cells**. *Dev Cell* 2014, **29**:102-111.

18. Dawlaty MM, Breiling A, Le T, Raddatz G, Barrasa MI, Cheng AW, Gao Q, Powell BE, Li Z, Xu M, *et al.*: **Combined deficiency of Tet1 and Tet2 causes epigenetic abnormalities but is compatible with postnatal development**. *Dev Cell* 2013, **24**:310-323.

19. Kaneda M, Okano M, Hata K, Sado T, Tsujimoto N, Li E, Sasaki H: **Essential role for *de novo* DNA methyltransferase Dnmt3a in paternal and maternal imprinting**. *Nature* 2004, **429**:900-903.

20. Smith ZD, Shi J, Gu H, Donaghey J, Clement K, Cacchiarelli D, Gnirke A, Michor F, Meissner A: **Epigenetic restriction of extraembryonic lineages mirrors the somatic transition to cancer**. *Nature* 2017, **549**:543-547.

21. del Mar Lorente M, Marcos-Gutierrez C, Perez C, Schoorlemmer J, Ramirez A, Magin T, Vidal M: **Loss- and gain-of-function mutations show a polycomb group function for Ring1A in mice**. *Development* 2000, **127**:5093-5100.

22. Ezhkova E, Lien WH, Stokes N, Pasolli HA, Silva JM, Fuchs E: **EZH1 and EZH2 cogovern histone H3K27 trimethylation and are essential for hair follicle homeostasis and wound repair**. *Genes Dev* 2011, **25**:485-498.

23. Piunti A, Shilatifard A: **The roles of Polycomb repressive complexes in mammalian development and cancer**. *Nat Rev Mol Cell Biol* 2021, **22**:326-345.

24. Voncken JW, Roelen BA, Roefs M, de Vries S, Verhoeven E, Marino S, Deschamps J, van Lohuizen M: **Rnf2 (Ring1b) deficiency causes gastrulation arrest and cell cycle inhibition**. *Proc Natl Acad Sci USA* 2003, **100**:2468-2473.

25. Margueron R, Li G, Sarma K, Blais A, Zavadil J, Woodcock CL, Dynlacht BD, Reinberg D: **Ezh1 and Ezh2 maintain repressive chromatin through different mechanisms**. *Mol Cell* 2008, **32**:503-518.

26. Xue Z, Huang K, Cai C, Cai L, Jiang CY, Feng Y, Liu Z, Zeng Q, Cheng L, Sun YE, *et al.*: **Genetic programs in human and mouse early embryos revealed by single-cell RNA sequencing**. *Nature* 2013, **500**:593-597.

27. Mohammed H, Hernando-Herraez I, Savino A, Scialdone A, Macaulay I, Mulas C, Chandra T, Voet T, Dean W, Nichols J, *et al.*: **Single-cell landscape of transcriptional heterogeneity and cell fate decisions during mouse early gastrulation**. *Cell Rep* 2017, **20**:1215-1228.

28. Nowotschin S, Setty M, Kuo YY, Liu V, Garg V, Sharma R, Simon CS, Saiz N, Gardner R, Boutet SC, *et al.*: **The emergent landscape of the mouse gut endoderm at single-cell resolution**. *Nature* 2019, **569**:361-367.

29. Scialdone A, Tanaka Y, Jawaid W, Moignard V, Wilson NK, Macaulay IC, Marioni JC, Gottgens B: **Resolving early mesoderm diversification through single-cell expression profiling**. *Nature* 2016, **535**:289-293.

30. de Soysa TY, Ranade SS, Okawa S, Ravichandran S, Huang Y, Salunga HT, Schricker A, Del Sol A, Gifford CA, Srivastava D: **Single-cell analysis of cardiogenesis reveals basis for organ-level developmental defects**. *Nature* 2019, **572**:120-124.

31. Ibarra-Soria X, Jawaid W, Pijuan-Sala B, Ladopoulos V, Scialdone A, Jorg DJ, Tyser RCV, Calero-Nieto FJ, Mulas C, Nichols J, *et al.*: **Defining murine organogenesis at single-cell resolution reveals a role for the leukotriene pathway in regulating blood progenitor formation**. *Nat Cell Biol* 2018, **20**:127-134.

32. La Manno G, Siletti K, Furlan A, Gyllborg D, Vinsland E, Mossi Albiach A, Mattsson Langseth C, Khven I, Lederer AR, Dratva LM,

et al.: **Molecular architecture of the developing mouse brain**. *Nature* 2021, **596**:92-96.

33. Mittnenzweig M, Mayshar Y, Cheng S, Ben-Yair R, Hadas R, Rais Y, Chomsky E, Reines N, Uzonyi A, Lumerman L, *et al*.: **A single-embryo, single-cell time-resolved model for mouse gastrulation**. *Cell* 2021, **184**:2825-2842.e2822.

34. Pijuan-Sala B, Griffiths JA, Guibentif C, Hiscock TW, Jawaid W, Calero-Nieto FJ, Mulas C, Ibarra-Soria X, Tyser RCV, Ho DLL, *et al*.: **A single-cell molecular map of mouse gastrulation and early organogenesis**. *Nature* 2019, **566**:490-495.

35. Qiu C, Cao J, Martin BK, Li T, Welsh IC, Srivatsan S, Huang X, Calderon D, Noble WS, Disteche CM, *et al*.: **Systematic reconstruction of cellular trajectories across mouse embryogenesis**. *Nat Genet* 2022, **54**:328-341.

36. Cao J, Spielmann M, Qiu X, Huang X, Ibrahim DM, Hill AJ, Zhang F, Mundlos S, Christiansen L, Steemers FJ, *et al*.: **The single-cell transcriptional landscape of mammalian organogenesis**. *Nature* 2019, **566**:496-502.

37. • Hupalowska A, Jedrusik A, Zhu M, Bedford MT, Glover DM, Zernicka-Goetz M: **CARM1 and paraspeckles regulate pre-implantation mouse embryo development**. *Cell* 2018, **175**:1902-1916.e1913.
Using a set of single-cell technologies, this study characterized the role of the HMT CARM1 in the induction of the ICM/TE lineages during the first days of mouse development.

38. Wang J, Wang L, Feng G, Wang Y, Li Y, Li X, Liu C, Jiao G, Huang C, Shi J, *et al*.: **Asymmetric expression of LincGET biases cell fate in two-cell mouse embryos**. *Cell* 2018, **175**:1887-1901.e1818.

39. Torres-Padilla ME, Parfitt DE, Kouzarides T, Zernicka-Goetz M: **Histone arginine methylation regulates pluripotency in the early mouse embryo**. *Nature* 2007, **445**:214-218.

40. •• Argelaguet R, Clark SJ, Mohammed H, Stapel LC, Krueger C, Kapourani CA, Imaz-Rosshandler I, Lohoff T, Xiang Y, Hanna CW, *et al*.: **Multi-omics profiling of mouse gastrulation at single-cell resolution**. *Nature* 2019, **576**:487-491.
This paper reports chromatin accessibility, DNAme and RNA expression in the same individual cell, revealing the regulation of the step-wise lineage decisions resulting in development of the three germ layers.

41. Gaiti F, Chaligne R, Gu H, Brand RM, Kothen-Hill S, Schulman RC, Grigorev K, Risso D, Kim KT, Pastore A, *et al*.: **Epigenetic evolution and lineage histories of chronic lymphocytic leukaemia**. *Nature* 2019, **569**:576-580.

42. Gu H, Raman AT, Wang X, Gaiti F, Chaligne R, Mohammad AW, Arczewska A, Smith ZD, Landau DA, Aryee MJ, *et al*.: **Smart-RRBS for single-cell methylome and transcriptome analysis**. *Nat Protoc* 2021, **16**:4004-4030.

43. Wang Y, Yuan P, Yan Z, Yang M, Huo Y, Nie Y, Zhu X, Qiao J, Yan L: **Single-cell multiomics sequencing reveals the functional regulatory landscape of early embryos**. *Nat Commun* 2021, **12**:1247.

44. Clark SJ, Argelaguet R, Kapourani CA, Stubbs TM, Lee HJ, Alda-Catalinas C, Krueger F, Sanguinetti G, Kelsey G, Marioni JC, *et al*.: **scNMT-seq enables joint profiling of chromatin accessibility DNA methylation and transcription in single cells**. *Nat Commun* 2018, **9**:781.

45. Charlton J, Jung EJ, Mattei AL, Bailly N, Liao J, Martin EJ, Giesselmann P, Brandl B, Stamenova EK, Muller FJ, *et al*.: **TETs compete with DNMT3 activity in pluripotent cells at thousands of methylated somatic enhancers**. *Nat Genet* 2020, **52**:819-827.

46. Bowling S, Sritharan D, Osorio FG, Nguyen M, Cheung P, Rodriguez-Fraticelli A, Patel S, Yuan WC, Fujiwara Y, Li BE, *et al*.: **An engineered CRISPR-Cas9 mouse line for simultaneous readout of lineage histories and gene expression profiles in single cells**. *Cell* 2020, **181**:1410-1422.e1427.

47. Chan MM, Smith ZD, Grosswendt S, Kretzmer H, Norman TM, Adamson B, Jost M, Quinn JJ, Yang D, Jones MG, *et al*.: **Molecular recording of mammalian embryogenesis**. *Nature* 2019, **570**:77-82.

48. Bartosovic M, Kabbe M, Castelo-Branco G: **Single-cell CUT&Tag profiles histone modifications and transcription factors in complex tissues**. *Nat Biotechnol* 2021, **39**:825-835.

49. Rotem A, Ram O, Shoresh N, Sperling RA, Goren A, Weitz DA, Bernstein BE: **Single-cell ChIP-seq reveals cell subpopulations defined by chromatin state**. *Nat Biotechnol* 2015, **33**:1165-1172.

50. • Deng Y, Bartosovic M, Kukanja P, Zhang D, Liu Y, Su G, Enninful A, Bai Z, Castelo-Branco G, Fan R: **Spatial-CUT&Tag: spatially resolved chromatin modification profiling at the cellular level**. *Science* 2022, **375**:681-686.
This paper presents a method to assess spatially distinct as well as cell-type-specific histone modifications during embryonic organogenesis and the in the post-natal brain, which provide a valuable tool to map epigenetic marks in unpreceded resolution.

51. Liu Y, Yang M, Deng Y, Su G, Enninful A, Guo CC, Tebaldi T, Zhang D, Kim D, Bai Z, *et al*.: **High-spatial-resolution multi-omics sequencing via deterministic barcoding in tissue**. *Cell* 2020, **183**:1665-1681.e1618.

52. Shahbazi MN, Siggia ED, Zernicka-Goetz M: **Self-organization of stem cells into embryos: a window on early mammalian development**. *Science* 2019, **364**:948-951.

53. Veenvliet JV, Lenne PF, Turner DA, Nachman I, Trivedi V: **Sculpting with stem cells: how models of embryo development take shape**. *Development* (24) 2021, **148**:dev192914.

54. Rivron NC, Frias-Aldeguer J, Vrij EJ, Boisset JC, Korving J, Vivie J, Truckenmuller RK, van Oudenaarden A, van Blitterswijk CA, Geijsen N: **Blastocyst-like structures generated solely from stem cells**. *Nature* 2018, **557**:106-111.

55. Amadei G, Lau KYC, De Jonghe J, Gantner CW, Sozen B, Chan C, Zhu M, Kyprianou C, Hollfelder F, Zernicka-Goetz M: **Inducible stem-cell-derived embryos capture mouse morphogenetic events *in vitro***. *Dev Cell* (3) 2021, **56**:366-382.e369.

56. Harrison SE, Sozen B, Christodoulou N, Kyprianou C, Zernicka-Goetz M: **Assembly of embryonic and extraembryonic stem cells to mimic embryogenesis *in vitro***. *Science* (6334) 2017, **356**:eaal1810.

57. Sozen B, Amadei G, Cox A, Wang R, Na E, Czukiewska S, Chappell L, Voet T, Michel G, Jing N, *et al*.: **Self-assembly of embryonic and two extra-embryonic stem cell types into gastrulating embryo-like structures**. *Nat Cell Biol* 2018, **20**:979-989.

58. Beccari L, Moris N, Girgin M, Turner DA, Baillie-Johnson P, Cossy AC, Lutolf MP, Duboule D, Arias AM: **Multi-axial self-organization properties of mouse embryonic stem cells into gastruloids**. *Nature* 2018, **562**:272-276.

59. Bérenger-Currias NM M, Semrau S, Adegeest E, van den Berg PR, Feliksik M, Hochane M, Idema T, Tans SJ, Mircea M: **A gastruloid model of the interaction between embryonic and extra-embryonic cell types.** *J Tissue Eng* 2022, **13**:20417314221103042, , https://doi.org/10.1177/20417314221103042

60. Girgin MU, Broguiere N, Hoehnel S, Brandenberg N, Mercier B, Arias AM, Lutolf MP: **Bioengineered embryoids mimic post-implantation development *in vitro***. *Nat Commun* 2021, **12**:5140.

61. Girgin MU, Broguiere N, Mattolini L, Lutolf MP: **Gastruloids generated without exogenous Wnt activation develop anterior neural tissues**. *Stem Cell Rep* 2021, **16**:1143-1155.

62. Rossi G, Broguiere N, Miyamoto M, Boni A, Guiet R, Girgin M, Kelly RG, Kwon C, Lutolf MP: **Capturing cardiogenesis in gastruloids**. *Cell Stem Cell* 2021, **28**:230-240.e236.

63. Turner DA, Girgin M, Alonso-Crisostomo L, Trivedi V, Baillie-Johnson P, Glodowski CR, Hayward PC, Collignon J, Gustavsen C, Serup P, *et al*.: **Anteroposterior polarity and elongation in the absence of extra-embryonic tissues and of spatially localised signalling in gastruloids: mammalian embryonic organoids**. *Development* 2017, **144**:3894-3906.

64. van den Brink SC, Alemany A, van Batenburg V, Moris N, Blotenburg M, Vivie J, Baillie-Johnson P, Nichols J, Sonnen KF, Martinez Arias A, van Oudenaarden A: **Single-cell and spatial transcriptomics reveal somitogenesis in gastruloids**. *Nature* 2020, **582**:405-409.

65. van den Brink SC, Baillie-Johnson P, Balayo T, Hadjantonakis AK, Nowotschin S, Turner DA, Martinez Arias A: **Symmetry breaking, germ layer specification and axial organisation in aggregates of mouse embryonic stem cells**. *Development* 2014, **141**:4231-4242.

66. •• Veenvliet JV, Bolondi A, Kretzmer H, Haut L, Scholze-Wittler M, Schifferl D, Koch F, Guignard L, Kumar AS, Pustet M, *et al*.: **Mouse embryonic stem cells self-organize into trunk-like structures with neural tube and somites**. *Science* 2020, **370**:eaba4937.
This study is the first report of simultaneous generation of a neural tube, a gut tube and somites in a mESC-based self-organized *in vitro* model of embryogenesis.

67. Xu PF, Borges RM, Fillatre J, de Oliveira-Melo M, Cheng T, Thisse B, Thisse C: **Construction of a mammalian embryo model from stem cells organized by a morphogen signalling centre**. *Nat Commun* 2021, **12**:3277.

68. Scheer S, Ackloo S, Medina TS, Schapira M, Li F, Ward JA, Lewis AM, Northrop JP, Richardson PL, Kaniskan HU, *et al*.: **A chemical biology toolbox to study protein methyltransferases and epigenetic signaling**. *Nat Commun* 2019, **10**:19.

69. Wu Q, Heidenreich D, Zhou S, Ackloo S, Kramer A, Nakka K, Lima-Fernandes E, Deblois G, Duan S, Vellanki RN, *et al*.: **A chemical toolbox for the study of bromodomains and epigenetic signaling**. *Nat Commun* 2019, **10**:1915.

70. Ostrop J, Zwiggelaar RT, Terndrup Pedersen M, Gerbe F, Bosl K, Lindholm HT, Diez-Sanchez A, Parmar N, Radetzki S, von Kries JP, *et al*.: **A semi-automated organoid screening method demonstrates epigenetic control of intestinal epithelial differentiation**. *Front Cell Dev Biol* 2020, **8**:618552.

71. Wu B, Pan X, Chen X, Chen M, Shi K, Xu J, Zheng J, Niu T, Chen C, Shuai X, Liu Y: **Epigenetic drug library screening identified an LSD1 inhibitor to target UTX-deficient cells for differentiation therapy**. *Signal Transduct Target Ther* 2019, **4**:11.

72. López-Anguita N, Gassaloglu S, Stötzel M, Typou M, Virta I, Hetzel S, Buschow R, Koksal B, Atilla D, Maitschke-Rajasekharan R, *et al*.: **Hypoxia induces a transcriptional early primitive streak signature in pluripotent cells enhancing spontaneous elongation and lineage representation in gastruloids**. *BiorXiv* 2021,, https://doi.org/10.1101/2021.07.21.452906

73. Dai Z, Ramesh V, Locasale JW: **The evolving metabolic landscape of chromatin biology and epigenetics**. *Nat Rev Genet* 2020, **21**:737-753.

74. Batie M, Frost J, Frost M, Wilson JW, Schofield P, Rocha S: **Hypoxia induces rapid changes to histone methylation and reprograms chromatin**. *Science* 2019, **363**:1222-1226.

75. Chakraborty AA, Laukka T, Myllykoski M, Ringel AE, Booker MA, Tolstorukov MY, Meng YJ, Meier SR, Jennings RB, Creech AL, *et al*.: **Histone demethylase KDM6A directly senses oxygen to control chromatin and cell fate**. *Science* 2019, **363**:1217-1222.

76. Thienpont B, Steinbacher J, Zhao H, D'Anna F, Kuchnio A, Ploumakis A, Ghesquiere B, Van Dyck L, Boeckx B, Schoonjans L, *et al*.: **Tumour hypoxia causes DNA hypermethylation by reducing TET activity**. *Nature* 2016, **537**:63-68.

77. Dixon G, Pan H, Yang D, Rosen BP, Jashari T, Verma N, Pulecio J, Caspi I, Lee K, Stransky S, *et al*.: **QSER1 protects DNA methylation valleys from *de novo* methylation**. *Science* 2021, **372**.

78. Hota SK, Rao KS, Blair AP, Khalilimeybodi A, Hu KM, Thomas R, So K, Kameswaran V, Xu J, Polacco BJ, *et al*.: **Brahma safeguards canalization of cardiac mesoderm differentiation**. *Nature* 2022, **602**:129-134.

79. Macrae TA, Ramalho-Santos M: **The deubiquitinase Usp9x regulates PRC2-mediated chromatin reprogramming during mouse development**. *Nat Commun* 2021, **12**:1865.

80. Kagawa H, Javali A, Khoei HH, Sommer TM, Sestini G, Novatchkova M, Scholte Op Reimer Y, Castel G, Bruneau A, Maenhoudt N, *et al*.: **Human blastoids model blastocyst development and implantation**. *Nature* 2022, **601**:600-605.

81. Yu L, Wei Y, Duan J, Schmitz DA, Sakurai M, Wang L, Wang K, Zhao S, Hon GC, Wu J: **Blastocyst-like structures generated from human pluripotent stem cells**. *Nature* 2021, **591**:620-626.

82. Moris N, Anlas K, van den Brink SC, Alemany A, Schroder J, Ghimire S, Balayo T, van Oudenaarden A, Martinez Arias A: **An *in vitro* model of early anteroposterior organization during human development**. *Nature* 2020, **582**:410-415.

83. Olmsted ZT, Paluh JL: **Co-development of central and peripheral neurons with trunk mesendoderm in human elongating multi-lineage organized gastruloids**. *Nat Commun* 2021, **12**:3020.

84. Sanaki-Matsumiya M, Matsuda M, Gritti N, Nakaki F, Sharpe J, Trivedi V, Ebisuya M: **Periodic formation of epithelial somites from human pluripotent stem cells**. *Nat Commun* 2022, **13**:2325.

85. Bolondi A, Haut L, Gassaloglu SI, Burton P, Kretzmer H, Buschow R, Meissner A, Herrmann BG, Veenvliet JV: **Generation of mouse pluripotent stem cell-derived trunk-like structures: an *in vitro* model of post-implantation embryogenesis**. *Bio Protoc* 2021, **11**:e4042.

86. Aguilera-Castrejon A, Oldak B, Shani T, Ghanem N, Itzkovich C, Slomovich S, Tarazi S, Bayerl J, Chugaeva V, Ayyash M, *et al*.: **Ex utero mouse embryogenesis from pre-gastrulation to late organogenesis**. *Nature* 2021, **593**:119-124.