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Unwind and transcribe: chromatin reprogramming in the early mammalian embryo

Steffen Biechele[1,2], Chih-Jen Lin[1,2], Paolo F Rinaudo[1] and Miguel Ramalho-Santos[1,2]

Within the first few days of life, the unipotent gametic genomes are rapidly reprogrammed to support emergence of pluripotent cells in the early mammalian embryo. It is now appreciated that this crucial stage of development involves dramatic changes to chromatin at multiple levels, such as DNA methylation, histone modifications, histone mobility, and higher-order chromatin organization. Technological advances are beginning to allow genome-wide views of this chromatin reprogramming, and provide new approaches to functionally dissect its regulation. Here we review recent insights into the dynamic chromatin environment of the early mouse embryo. New data challenge long-held assumptions, for example, with regards to the asymmetry of DNA methylation of the parental genomes or the onset of functional zygotic genome activation. We discuss how impaired chromatin reprogramming can lead to early embryonic lethality, but might also have delayed effects that only manifest later in embryogenesis or postnatally, potentially influencing the propensity for adult-onset diseases.

Addresses
[1] Center for Reproductive Sciences, Department of Obstetrics, Gynecology and Reproductive Sciences, 35 Medical Center Way, University of California, San Francisco, CA 94143, USA
[2] Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, 35 Medical Center Way, University of California, San Francisco, CA 94143, USA

Corresponding author: Ramalho-Santos, Miguel (mrsantos@ucsf.edu)

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Introduction

A key goal of contemporary biology is to understand the mechanisms that underlie cellular potency, defined as the ability to give rise to different cell types. The highest level of cellular potency is the totipotency of the zygote and early blastomeres of the mammalian preimplantation embryo. This is rapidly followed by pluripotency, which defines the state of the peri-implantation epiblast cells that are no longer able to differentiate into extra-embryonic tissues, but can give rise to any tissue of the embryo proper and thus the organism after birth. Although the instructive roles of transcription factors and signaling pathways in these processes have been investigated and described extensively (reviewed in [1]), an increasing body of data suggests that reprogramming of chromatin states plays an important role in allowing, buffering and/or instructing transitions in cellular potency.

The ability to observe preimplantation development *ex vivo* and the extensive tools available for genetic and genomic studies make the mouse an excellent model system to dissect chromatin reprogramming in the early mammalian embryo. Moreover, a limited number of studies can be carried out in human embryos, which can also be cultured *ex vivo* from the zygote to the blastocyst stage. This research represents a pivotal intersection of basic and applied research, as understanding of the epigenetic reprogramming of the gametes towards totipotency and the establishment of pluripotency will be instructive both for reprogramming-based regenerative medicine applications as well as Assisted Reproductive Technologies (ART) in humans. Here we review recent advances in chromatin reprogramming during early mammalian development.

Epigenetic reprogramming of gametic genomes

H3.3 incorporation in nucleosome assembly and transcription

Very soon after a sperm cell enters the oocyte, a dramatic protamine-to-histone exchange takes place in the paternal genome. Recent studies in flies reveal that histone chaperones (TAP/p32, NAP-1, NLP) act to remove protamines from the sperm genome [2]. Maternal Histone 3.3 (H3.3), a histone variant that does not depend on DNA replication to be incorporated and is associated with active transcription, is the main type of H3 incorporated into the paternal genome [3,4], but its functional role in the mouse had remained unclear. Recent work from our lab and others shows that incorporation of H3.3 by its chaperone Hira is not involved in protamine removal, but is indispensable for nucleosome assembly and subsequent DNA replication in the male genome [5,6••], as well as for ELYS-mediated nuclear pore complex assembly [7].

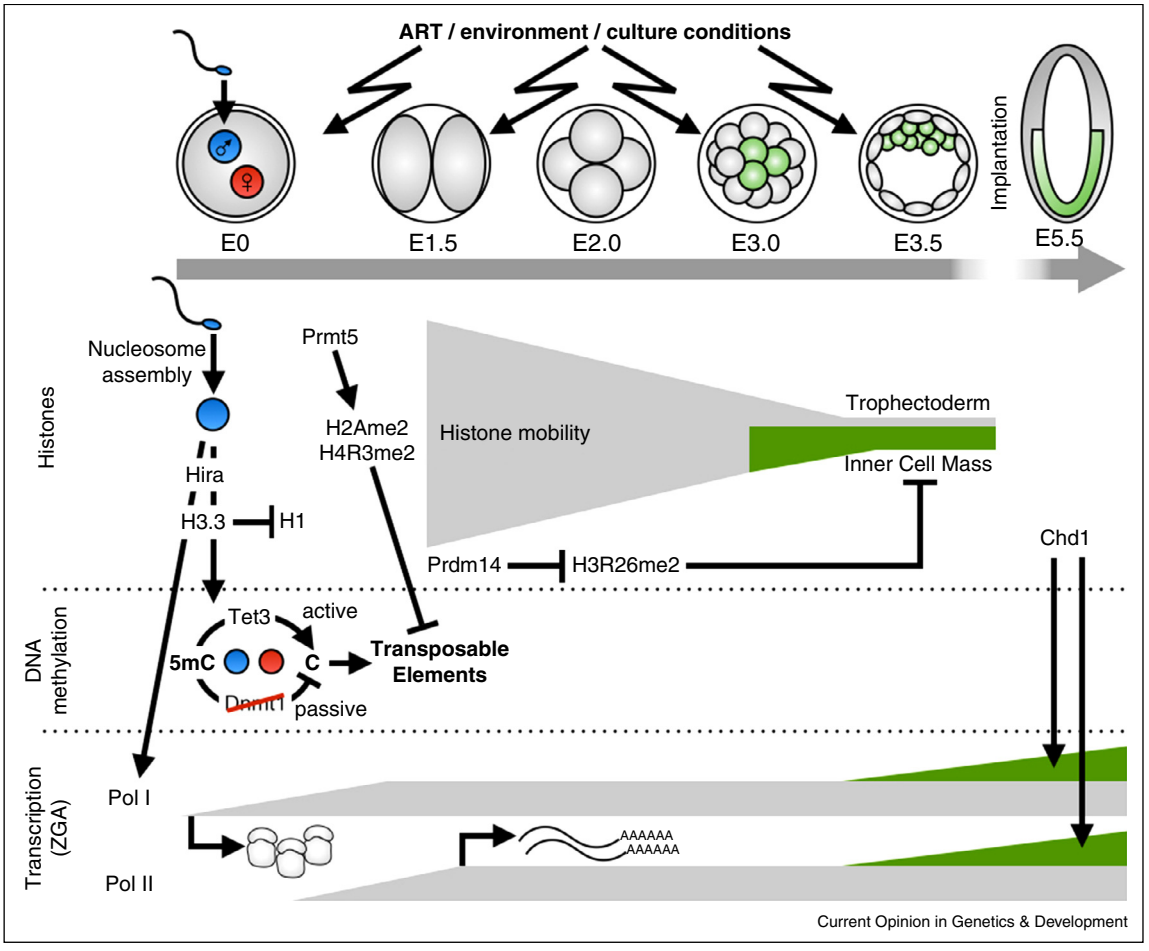
An unexpected finding of our studies is that Hira-mediated H3.3 incorporation is required for RNA Polymerase I

(Pol I) transcription in both the maternal and the paternal pronuclei, which in turn is essential for zygote development [6••]. These results indicate that the female pronucleus is not a passive 'bystander' as previously assumed, but rather undergoes active reprogramming. Moreover, these data challenge a decades-old dogma that transcription of the zygotic genome in mouse is minor and not required for the development to the 2-cell stage [8,9]. Phenotypic consequences of zygotic Pol II inhibition only become obvious beyond the 2-cell stage [10], but Pol I function is strictly required for the transition to the two cell stage [6••]. Thus, Zygotic Genome Activation (ZGA) can be considered to begin at the zygote stage with the transcription of rRNA by Pol I, in preparation for the translation of mRNAs transcribed by Pol II at the 2-cell stage (Figure 1). Although these findings are exciting, they raise several new questions. Further studies should focus on (1) the characterization of the genome-wide location of H3.3 in the parental genomes, (2) the mechanisms by which H3.3-containing nucleosomes support DNA replication and rRNA transcription, and (3) the kinetics of ribosomal RNA biosynthesis and translation in the zygote.

High resolution views of DNA demethylation in early mouse embryo

Around the same time that the parental genomes are reprogrammed at the nucleosomal level to support development, they also undergo DNA demethylation on a remarkable genome-wide scale. Previous low resolution studies based primarily on immunofluorescence staining suggested that the paternal genome undergoes rapid active DNA demethylation via Tet3-mediated oxidation of 5-methyl-cytosine (5mC) to 5-hydroxymethyl-cytosine (5hmC) [11–14]. On the other hand, the female genome was thought to undergo passive DNA demethylation by dilution of 5mC with each DNA replication in the

Figure 1



Summary of key recent advances in epigenetic reprogramming during early mouse development at the level of histones, DNA (de)methylation, and transcription. Paternal and maternal pronuclei are indicated in blue and red, respectively. Pluripotent cells are indicated in green. See text for details.