Review

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Reprogramming the genome to totipotency in mouse embryos

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Despite investigative interest, the artificial derivation of pluripotent stem cells remains inefficient and incomplete reprogramming hinders its potential as a reliable tool in regenerative medicine. By contrast, fusion of terminally differentiated gametes at fertilization activates efficient epigenetic reprogramming to ensure totipotency of early embryos. Understanding the epigenetic mechanisms required for the transition from the fertilized egg to the embryo can improve efforts to reprogram differentiated cells to pluripotent/totipotent cells for therapeutic use. We review recent discoveries that are providing insight into the molecular mechanisms required for epigenetic reprogramming to totipotency *in vivo*.

Establishing the totipotency of early embryos

Investigations of iPSCs have provided insight into the establishment of pluripotency for potential clinical application. However, genetic and epigenetic abnormalities dampen enthusiasm for their use in regenerative medicine, and the establishment of totipotency presents a better opportunity for this purpose [1]. Unlike pluripotency, totipotency is defined as the ability of cells to differentiate into any cell type and, more stringently, to develop into a complete organism [2]. Although the molecular basis of this capacity remains largely unknown, four processes are designed to generate totipotent cells: (i) fusion of terminally differentiated, haploid sperm and egg into one-cell (1C) diploid zygotes (see Glossary) *in vivo* or by *in vitro* fertilization (IVF); (ii) somatic cell nuclear transfer (SCNT), which relies on the reprogramming activity of eggs; (iii) isolation of transient ESC/iPSC populations with a two-cell (2C)-like embryonic transcriptome [3]; and (iv) *in vivo* generation of iPSCs with totipotent features [4]. While the latter three are of investigative interest, SCNT suffers from inefficiency and transient ESC populations and, *in vivo*, produced iPSCs that lacked the ability to develop into an intact organism. Thus, cleavage stage embryos formed from fused gametes at fertilization provide the most physiological platform to investigate the signature of totipotency and devise strategies for reprogramming terminally differentiated cells.

Despite sharing major regulatory networks, totipotent and pluripotent cells exhibit significant differences such as core histone motility [5] and utilization of regulatory factors for gene expression [6]. Central to developing mouse embryos from differentiated gametes are epigenetic modifications to ensure expression or silencing of genes that define totipotency. Upon fertilization, haploid sperm penetrate into the cytoplasm of haploid eggs to form diploid 1C

Glossary

Active DNA demethylation: an enzymatic process to remove DNA methylation, in contrast to passive DNA demethylation carried out by serial dilutions during cell divisions without DNA methylation activity.
Blastocyst: the 128-cell blastocyst has two distinct cell lineages and a fluid-filled cavity called blastocoel. The outer trophectoderm layer (TE) is the precursor of the placenta and the inner cell mass (ICM) forms the fetus and is the source of embryonic stem cells.
ChIP-seq: ChIP with high-throughput sequencing. It is a widely used genome-wide approach to determine DNA-binding sites of proteins of interest.
Cleavage stage embryogenesis: after fertilization, the 1C embryo undergoes three cell divisions to form eight blastomeres prior to compacting into a morula.
Embryonic genome activation (EGA)/zygotic genome activation (ZGA): after fertilization, embryonic genes are transcriptionally activated by maternal regulators to initiate developmental programs in the early embryo.
Gametes: Mammalian male gametes are small, motile sperm while female gametes are relatively large, nonmotile eggs surrounded by a proteinaceous zona pellucida.
Higher-order chromatin structure: following the formation of nucleosomes, nuclear DNA is further organized topologically into higher-order structures by forming genome-wide physical networks in a nonrandom manner.
Histone variant: noncanonical histones with amino acid sequences distinct from canonical histones. They usually have specific functions. Unlike canonical histones, they can be incorporated into chromatin outside of S-phase and their genes contain introns.
Nano-CAGE: identification of promoter location and gene expression by capturing and quantifying 5′ ends of transcripts using small amounts of total RNA. Combined nano-CAGE and RNA-seq facilitates genome-wide transcriptome studies.
Oogenesis/spermatogenesis: during gametogenesis, oocytes are established by oogenesis in the ovary and sperm by spermatogenesis in the testis. Each gamete undergoes reductive divisions during meiosis to form haploid genomes.
Preimplantation development: embryonic development from 1C zygotes to blastocysts. After this early development, blastocysts hatch from the surrounding zona pellucida and implant on the uterine wall.
Reprogramming: a process that occurs *in vivo* during primordial germ cell development and again during cleavage stage embryogenesis to erase or rewrite epigenetic chromatin marks.
Subcortex: an actin-enriched cytoplasmic layer at the inner aspect of the plasma membrane. The maternal subcortical maternal complex (SCMC) is enriched in the subcortex of eggs and is required for progression beyond two-cell (2C) development.
Syngamy: fusion of gametes at the start embryogenesis. During syngamy, parental pronuclei migrate towards each other and interdigitate their nuclear membranes which breakdown so that chromosomes can congress on the mitotic spindle before the first cleavage division.
Zygote: diploid one-cell (1C) embryo produced at fertilization and is the earliest stage of embryo that develops into a complete organism.

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Keywords: reprogramming; totipotency; epigenetic modification; embryonic genome activation; preimplantation development.

0962-8924/
Published by Elsevier Ltd. http://dx.doi.org/10.1016/j.tcb.2014.09.006

zygotes. This triggers the paternal genome to re-establish nucleosome structures with stored maternal histones, followed rapidly by the formation of the male and female pronuclei. There is minor activation of parental genomes at the 1C stage, but little translation from the *de novo* transcripts. This event has been described both as embryonic genome activation (EGA) and zygotic genome activation (ZGA). The former acronym will be used in this review.

Initially, paternal and maternal chromatin have separate and asymmetric epigenetic profiles. Division of the 1C zygote forms 2C embryos with significantly increased transcription and each of the two blastomeres is totipotent [7]. Newly synthesized embryonic gene products gradually replace maternal factors as regulators of early development. Although a causal relationship has not been established, there is concomitant loss of totipotency of the embryo. For example, single four- and eight-cell (4C, 8C) blastomeres cannot form all the lineages of the embryo without aggregating with carrier blastomeres [8]. Ultimately, embryonic cells differentiate to form either the inner cell mass (ICM, origin of ESCs) or the trophectoderm (TE, precursor of the placenta) as the blastocyst prepares for implantation (Figure 1A).

This review focuses on investigations that define progressive changes in epigenetic reprogramming that affect chromatin dynamics and enable totipotency in the early mouse embryo. Future discoveries that guide chromatin reprogramming technologies will have profound influences on regenerative medicine. Personalized totipotent cells from terminally differentiated cells could provide pools of cells from which specific cell types could be established for the treatment degenerative diseases such as diabetes and Parkinsonism. Evolution has shaped the most efficient means of establishing totipotent cells from terminally differentiated gametes and understanding those molecular mechanisms should provide insight into how to recapitulate them for the therapeutic benefit of patients.

Stored maternal factors

During oogenesis, the volume of the germ cell increases dramatically and provides storage for maternal factors needed to compensate for the absence of transcription during meiotic maturation, ovulation, and early development. At fertilization, each gamete contributes a haploid genome, but the egg is the primary source for gene products (RNA, proteins) vital for the establishment of totipotency and EGA (Figure 1B). These factors are encoded by maternal effect genes, such as genes encoding nucleoplasmin (NPM) 2 [9] and the subcortical maternal complex (SCMC) [10–12]. Their genetic ablation in mice documents the essential roles of both nuclear and cytoplasmic factors in establishing the totipotency of early embryonic cells [13]. The large cytoplasmic volume of the 1C zygote may complicate protein trafficking and recent reports emphasize the importance of actin scaffold and actin flow-driven streaming in supporting the integrity and stability of subcellular structures [14] and redistribution of cytosolic components during genome reprogramming [15].

Although progress has been made in understanding the complexity of reprogramming to totipotency, the paucity of biological materials in mice has hampered efforts to

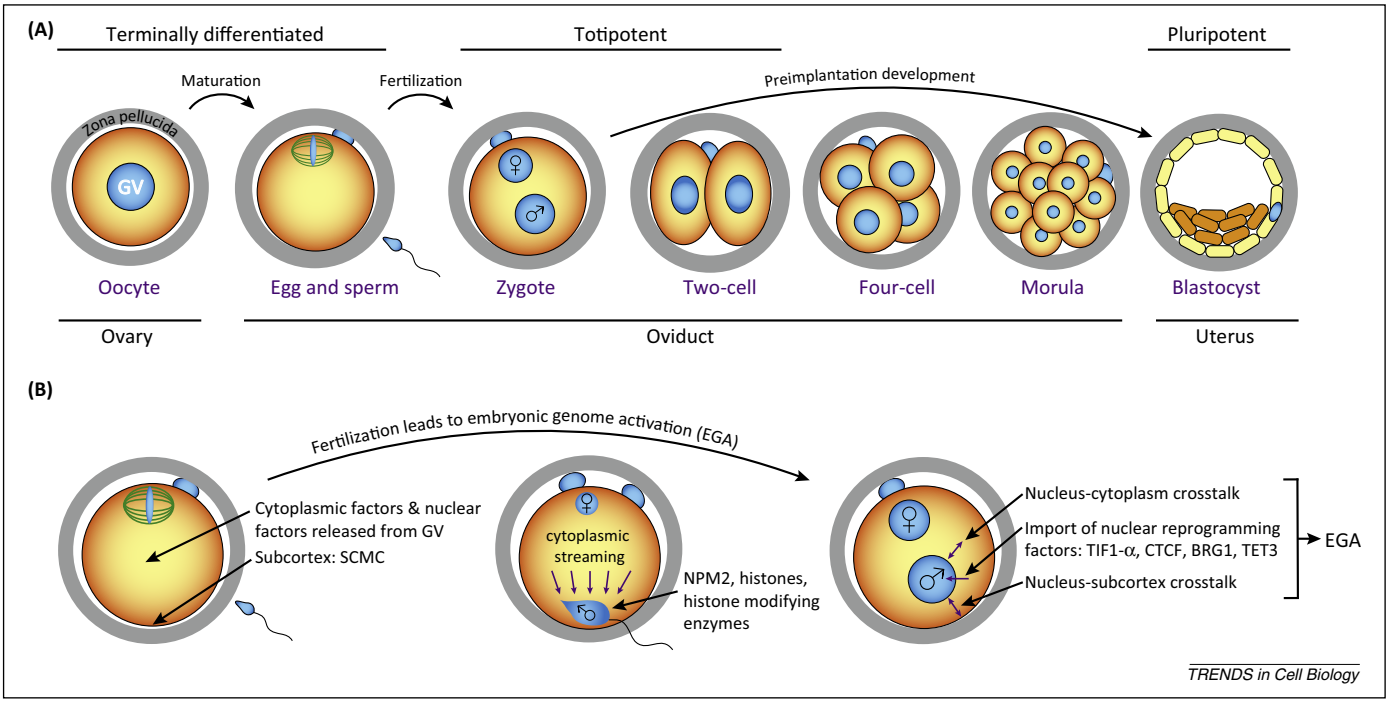

Figure 1. Mouse preimplantation development and maternal-to-embryonic transition. **(A)** After ovulation from the ovary into the oviduct, terminally differentiated mature eggs (surrounded by the extracellular zona pellucida) are fertilized by sperm to establish totipotent zygotes that divide during preimplantation development to become blastocysts prior to implantation in the uterus at embryonic day 4.5. **(B)** Upon fertilization, stored maternal factors activate the embryonic genome to trigger maternal-to-embryonic transition that results in formation of a totipotent zygote. Specifically, parental genomes are reorganized by chromatin remodeling factors with the aid of cytoplasmic streaming, and form pronuclei that import nuclear reprogramming factors and crosstalk with other cellular compartments. Abbreviations: BRG1, a chromatin remodeling protein; CTCF, CCCTC-binding factor; GV, germinal vesicle; NPM2, nucleoplasmin protein 2; SCMC, subcortical maternal complex; TET3, ten-eleven translocation protein 3; TIF-α, transcription intermediary factor 1 α.