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Epigenome regulation during germ cell specification and development from pluripotent stem cells

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Germ cells undergo epigenome reprogramming for proper development of the next generation. The realization of germ cell derivation from human and mouse pluripotent stem cells offers unprecedented opportunity for investigation of germline development. Primordial germ cells reconstituted *in vitro* (PGC-like cells [PGCLCs]) show progressive dilution of genomic DNA methylation, tightly linked with chromatin remodeling, during their specification. PGCLCs can be further expanded by plane culture, allowing maintenance of the gene-expression profiles of early PGCs and continuance of the DNA methylation erasure, thereby establishing an epigenetic 'blank slate'. PGCLCs undergo further epigenome regulation to acquire the male or female fates. These findings will provide a foundation for basic germ cell biology and for in-depth evaluations of *in vitro* gametogenesis.

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Introduction

Germ cells are the origin of individuals and the carrier of hereditary information and genetic diversity, and undergo epigenome reprograming for proper development of the next generation. Mouse primordial germ cells (mPGCs) have been efficiently reconstituted (mPGC-like cells [mPGCLCs]) from pluripotent stem cells (mPSCs) [1, [5••]]. This achievement has led to additional breakthroughs in *in vitro* germ cell reconstitution, including gametogenesis from mPGCLCs [3•,4••], expansion of mPGCLCs in plane culture [5••], entry of the female pathway and meiosis under a defined condition [6•], and induction of human PGCLCs (hPGCLCs) [7–9]. Together, these achievements provide unprecedented opportunities for the experimental investigation of mammalian germ cell development, including regulation of the epigenome reprogramming, and will contribute to our knowledge of human biology, heredity, and reproductive medicine [10]. In this review, we will discuss the recent advances in this field.

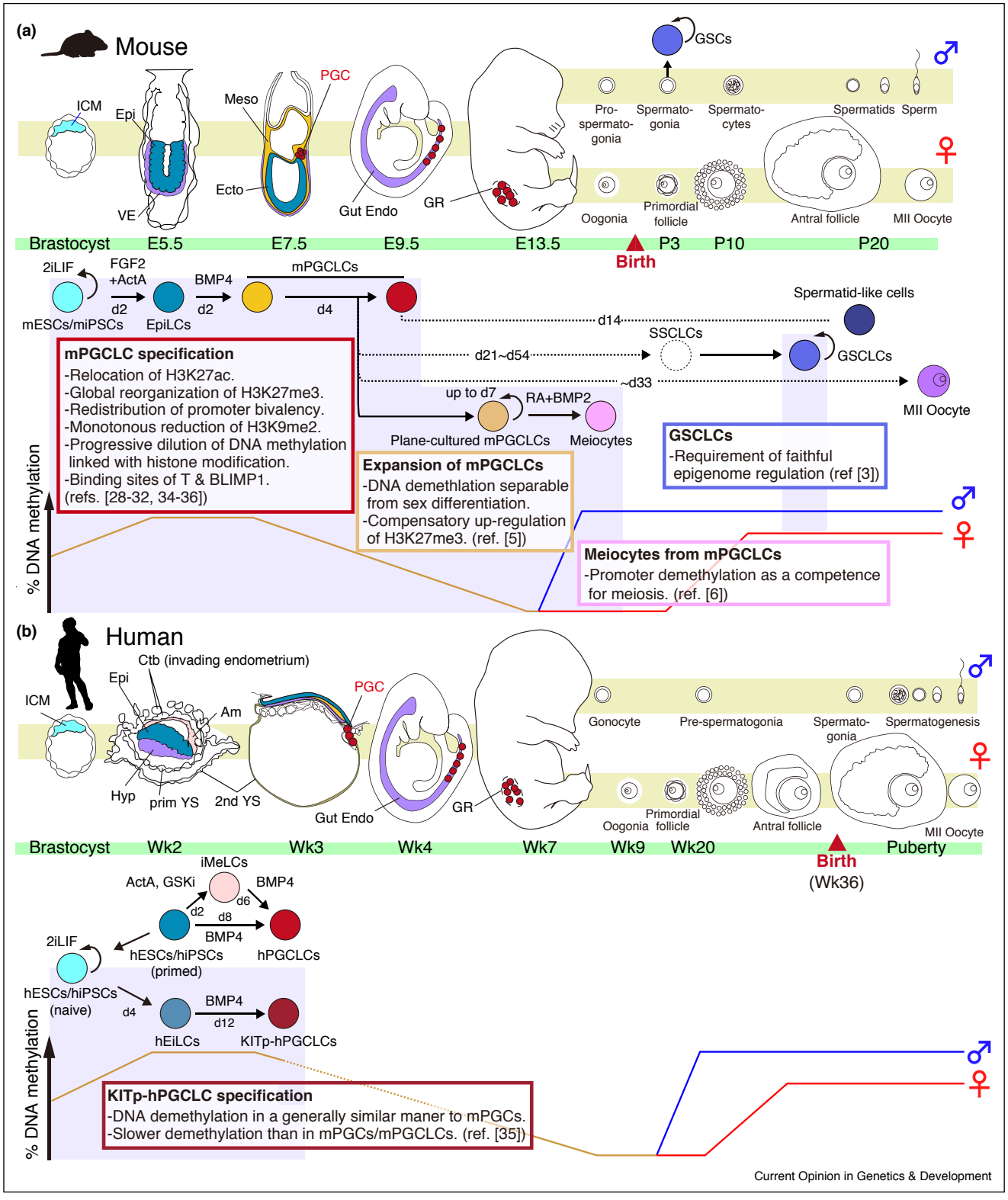
In vitro reconstitution of mouse germ cell development

mPGCs are specified in the posterior epiblast at the onset of gastrulation around embryonic day (E) 6.0, in response to bone morphogenetic protein 4 (BMP4) [11,12], and subsequently migrate into the hindgut endoderm (~E7.75), colonize the genital ridges (~E10.5), and start sex differentiation (~E12.5) (for review, see [13]). mPGCs undergo epigenome reprogramming, including progressive demethylation of genomic DNA, imprint erasure, elevation of histone H3 lysine 27 tri-methylation (H3K27me3) (polycomb repressive complex 2 [PRC2]), and reduction of H3K9 di-methylation (H3K9me2) (G9A/GLP repressive complex) [14,15]. At E13.5, gonadal germ cells bear the lowest level of genomic DNA methylation in the life cycle (~5% 5-methylcytosine [5mC]) [14,16–18] (Figure 1).

On the basis of a key finding in regard to the signaling principle of mPGC specification [19], mPSCs, cultured with an MEK inhibitor, a GSK3 inhibitor, and LIF (2iLIF) [20], were induced into epiblast-like cells (EpiLCs), and, by cytokines including BMP4, floating aggregates of EpiLCs were further induced into mPGCLCs, which contributed to both spermatogenesis and oogenesis, and to fertile offspring [1,2]. More recently, the entire process of oogenesis was reconstituted by combining mPGCLCs and a culture system for the fully *in vitro* maturation of oocytes [4••,21].

The gene-expression dynamics during mPGCLC induction precisely recapitulate mPGC specification [1]; an early mesoderm-like program is transiently activated followed by robust repression, and, concomitantly, the germ cell program is swiftly acquired [22]. Accordingly, at day 6 (d6) of induction, mPGCLCs acquire transcriptome and epigenetic features highly similar to those of the E9.5, migrating mPGCs.

Figure 1



Mouse and human germ cell development and epigenome events revealed in the reconstitution systems. **(a)** A schematic representation of mouse germ cell development is shown (top). *In vitro* reconstituted cells are represented with colored, filled circles at the corresponding developmental stages. Days taken for the reconstitution are indicated. Lists of key epigenome events revealed using the reconstituted germ cells are enclosed in colored squares and are overlaid on the shadowed areas, which indicate the *in vitro* processes with epigenome analyses reported. The colors of