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factors that recruit decapping enzymes is one possibility; another is that slower ribosomal elongation could result in a lower rate of initiation from recycled ribosomes reinitiating on circularized transcripts.

Mechanisms aside, these studies indicate that both the onset and the physical process of 5′ → 3′ decay, like other pathways of mRNA surveillance that occur on the ribosome (Shoemaker and Green, 2012), are coupled to translation.

In these genome-scale studies, we continue to see translation through a high-throughput glass, darkly. Debates rage about whether ribosomes pause at some codons, or amino acids, and not others, with seemingly minor differences in growth conditions, sample preparation, and statistical methods yielding incompatible results. Distinguishing biological phenomena from aberrations in the experimental glass remains challenging. What solid ground can the translation field stand on? Very strong signals pop out consistently, such as ribosome pausing at the SecM sequence in bacteria or codon-specific pausing during amino acid starvation (Subramaniam et al., 2014). Weaker signals may be detectable if amplified, such as by prolonging pausing by deletion of release factors (Guydosh and Green, 2014) or addition of artificial amino acid analogs. Details of RNA preparation, including inhibitors, 5′ chemistry, nuclease digestion conditions, and fragment length, may create or defeat artifacts and determine detectable phenomena. Which protocol details can be safely ignored? We do not yet know.

Substantial unexplained variation in read densities generated by high-throughput sequencing makes single-gene profiles difficult to interpret, and any individual peak or trough might be artifactual. Although statistical methods such as "metagene analysis" can reveal signals by aggregating across the transcriptome, any analysis pipeline might mislead and must be validated. It is unclear whether such methods are quantitative. Does a 2-fold increase in some model output correspond to a 2-fold decrease in ribosome elongation? In particular, failing to detect a signal with a particular high-throughput strategy (e.g., codon-specific pausing in ribosome profiling) does not mean the signal is absent. The signal may be detectable by alternative assays or even by alternative analyses of the same data. The arrival of 5PSeq provides valuable checks on the results of other high-throughput methods.

As the serendipitous discovery of sensitive last-ribosome dynamics exemplifies, the accumulation of new and independent methods continues to sharpen our global picture of translation in ways that will inspire future studies—and confidence.

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Forget the Parents: Epigenetic Reprogramming in Human Germ Cells

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Epigenetic reprogramming in the germline resets genomic potential and erases epigenetic memory. Three studies by Gkountela et al., Guo et al., and Tang et al. analyze the transcriptional and epigenetic landscape of human primordial germ cells, revealing a unique transcriptional network and progressive and conserved global erasure of DNA methylation.

Germ cells uniquely transmit the genetic information from one generation to the next and give rise to the totipotent zygote upon fertilization. While the genetic material of the parents is maintained, the epigenome undergoes extensive reprogramming in primordial germ cells (PGCs), the precursors of sperm and oocytes. Despite this pivotal role of PGCs for development and fertility, their specification and epigenetic reprogramming in the human embryo remain relatively

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uncharacterized, and much of our knowledge is based on studies in mice in which reprogramming was first discovered (Hackett et al., 2013; Kobayashi et al., 2013; Seisenberger et al., 2012). Three papers published in this issue of *Cell* (Gkountela et al., 2015; Guo et al., 2015; Tang et al., 2015) chart the transcriptional and epigenetic changes during human PGC development, revealing the remarkable conservation of global erasure of DNA methylation and the distinct gene regulatory network orchestrating epigenetic remodeling and gametogenesis.

Human germ cell development (Figure 1A) begins with the specification of PGCs, which is expected to happen at the onset of gastrulation (developmental week 2) in the posterior epiblast of early postimplantation embryos. During gestation (weeks 3–5), the definitive PGCs migrate from the yolk sac wall through the hindgut to the developing gonads, where they then undergo sex-specific differentiation after week 9. Whereas the male germ cells enter mitotic quiescence synchronously and undergo meiosis after puberty, female human germ cells enter meiosis asynchronously over an extended time period (Gkountela et al., 2013).

Gkountela et al. (2015), Guo et al. (2015), and Tang et al. (2015) isolate PGCs from 4- to 19-week-old human embryos by fluorescence-activated cell sorting for the surface markers cKIT and/or TNAP and perform RNA sequencing (RNA-seq) (for the transcriptome) and whole-genome bisulfite sequencing (for the methylome). These analyses represent the first comprehensive datasets of early human germ cells in vivo and reveal that DNA methylation is progressively erased genome wide in PGCs to the lowest levels of CpG methylation observed in the human genome to date.

Global methylation levels in early PGCs (weeks 4–7) are already low compared to somatic cells, indicating that the first wave of global DNA demethylation occurs before 4–7 weeks of development. Furthermore, low methylation levels are maintained in female and male PGCs until embryonic week 16 or 19, respectively, implying that global re-methylation occurs later.

A comparison of the methylomes from human and mouse germ cells reveals remarkable parallels, with overall similar DNA demethylation dynamics in human PGCs between developmental week 5 to 19 and those of mice between embryonic day (E) 10.5 to 13.5. This suggests that epigenetic reprogramming of the germline is a fundamental process in mammals, which seems highly conserved. Nonetheless, although all three studies report similar dynamics in the changes of specific genomic features, the exact methylation levels and timelines vary between each other. These differences might be purely a consequence of the difficulties in isolating in vivo human germ cells and slightly different sample preparation but could also point to real biological differences. In contrast to inbred mice, human samples will all be genetically dissimilar, which could perhaps result in slightly different levels and timelines of epigenetic resetting in the germline.

The global demethylation observed in human PGCs leads to a dramatic loss of almost all DNA methylation at CpG islands, transcription start sites, gene bodies, and surrounding intergenic regions, compared to human methylomes from the inner cell mass, somatic cells, or sperm. Nonetheless, in PGCs, gene bodies remain slightly higher methylated than the neighboring intergenic regions. As in the mouse, the loss of CpG methylation does not correlate with changes in gene expression in the human germline, suggesting that erasure of epigenetic memory is a key purpose of demethylation. When looking at the major types of transposable elements, which make up about half of the human genome, the authors find that these follow the trend for the genome average. However, a significant fraction of specific subfamilies show persistent methylation, and in particular, the evolutionarily youngest and currently active retrotransposons are more resistant to global demethylation (again resembling the mouse). As global demethylation of transposable elements does not lead to significant transcriptional activation, other repressive mechanisms must be in place. Whereas mouse germline cells show a persistent enrichment of the repressive chromatin mark H3K27me3 and global loss of H3K9me2 (Seki et al., 2005), human PGCs are depleted for H3K9me2 and H3K27me3 (Figure 1B). Similar to the mouse, a clear punctuated pattern of the stable silencing mark H3K9me3 can be detected in human PGCs at all stages of development, suggesting that H3K9me3 may be the key factor repressing the constitutive heterochromatin in human PGCs.

A hallmark of epigenetic resetting in the germline is imprint erasure (Hackett et al., 2013; Kobayashi et al., 2013; Seisenberger et al., 2012). In contrast to the mouse, hypomethylation of imprints in humans seems to occur before PGCs colonize the genital ridge and is maintained until at least 19 weeks of development (Figure 1B). In mouse PGCs, the inactive X chromosome is reactivated between E8.5 and E12.5, and Tang et al. (2015) and Guo et al. (2015) report X reactivation already in human PGCs prior to 4 weeks of development (Figure 1B). Notably, Gkountela et al. (2015) detect expression of *XIST* noncoding RNA in male and female germline cells at all stages, suggesting that *XIST* may be non-silencing in the human germline.

Tang et al. (2015) further examine the regions that (partially) escape global demethylation and are repeat poor, potentially representing hotspots of transgenerational epigenetic inheritance. Among them, they identify several genes with characteristic trait and disease associations, such as "obesity-related traits," "schizophrenia," and "multiple sclerosis."

Mechanistically, global loss of DNA methylation in mouse PGCs is a consequence of suppression of maintenance and de novo methylation by PRDM14 and activation of active DNA demethylation pathways. Surprisingly, PRDM14 is dispensable for human PGC development (Sugawa et al., 2015) and only expressed at very low levels in the human germline. Perhaps consequently, mRNAs of de novo DNMTs, DNMT1, and UHRF1 are present, but protein levels of DNMT3A and UHRF1 are not detectable by immunofluorescence (IF) in human PGCs, suggesting an alternative mechanism that suppresses maintenance and de novo methylation. TET1 and TET2 are also enriched in human PGCs, and significant levels of 5hmC are detected by IF in early human PGCs. Guo et al. (2015) also perform TAB-seq on male 10 week human PGCs and identify global levels of 1.9% 5hmC in the genome.

Furthermore, the transcriptome of human mitotic PGCs before 10 or 11 weeks

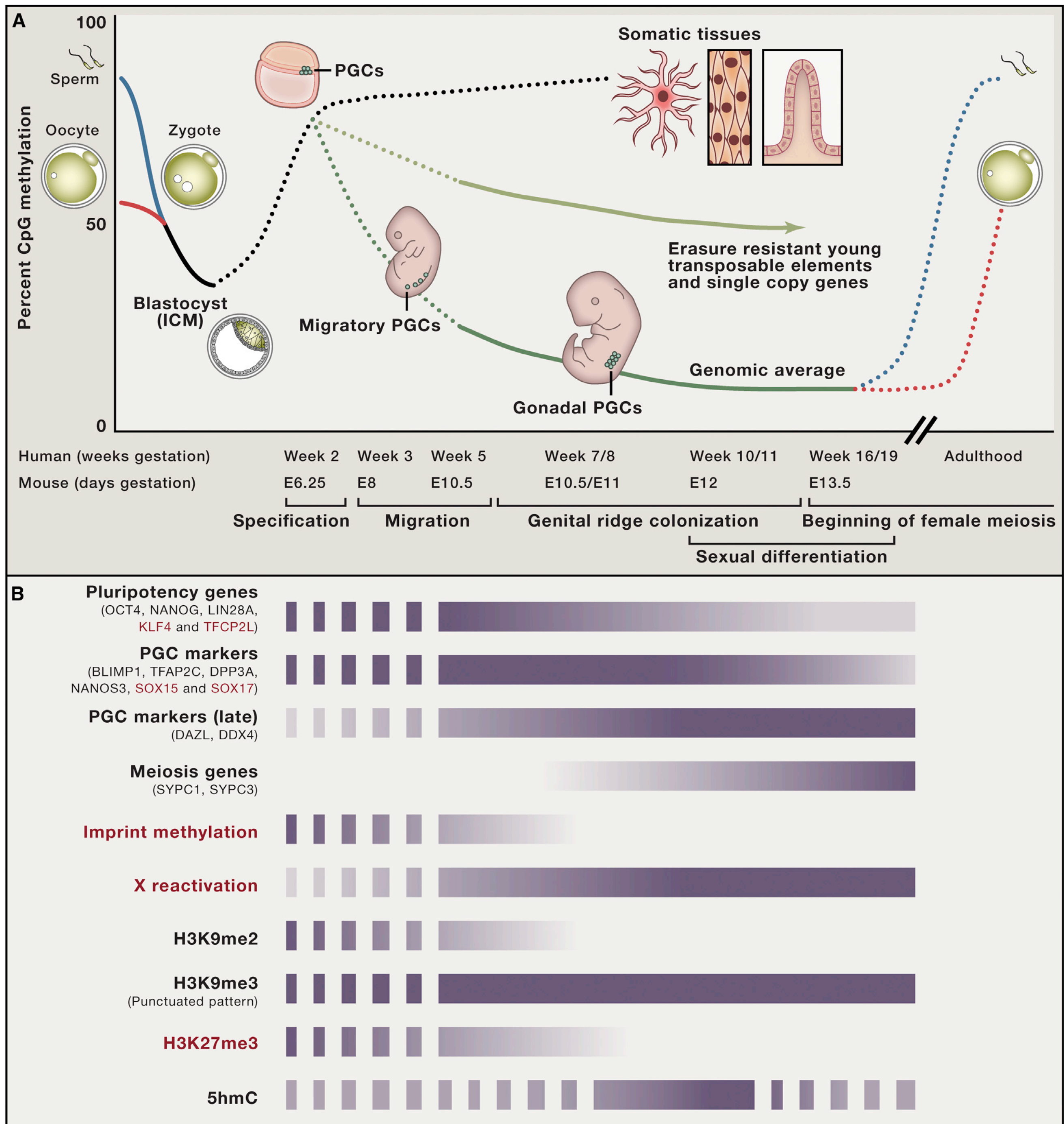

Figure 1. Epigenetic Reprogramming in Human Primordial Germ Cells

(A) After fertilization, the paternal (blue) and maternal (red) genomes undergo global demethylation, resetting the human epigenome for naive pluripotency at the blastocyst stage. Following a yet-uncharacterized phase of de novo methylation in the epiblast, human PGCs are specified in the posterior epiblast (week 2), from where they migrate through the hindgut to the developing genital ridges. During this migratory phase (weeks 3–5), human PGCs must undergo a first wave of global DNA demethylation, including significant loss of methylation at imprint control regions. The methylomes and transcriptomes from human PGCs between 5.5 and 19 weeks of age have now been analyzed. During this phase, DNA methylation is further erased genome wide, restoring germline potency, whereas only a small number of evolutionarily young transposable elements and single copy genes are not completely demethylated and could be potential sites of trans-generational epigenetic inheritance.

(B) The chart summarizes the main transcriptional and epigenetic characteristics of human germ cell development and also highlights key differences between human and mouse PGCs (marked in red).

is, in general, stable without prominent differences between male and female PGCs and similar to human ICM cells but distinct from older PGCs. This suggests that, after 10 or 11 weeks of age, the germline cells transition to another state, and single-cell RNA-seq analysis of individual PGCs by Guo et al. (2015) also show strong heterogeneity in 17 week female PGCs, supporting the observation that female germ cells enter meiosis asynchronously in humans. Late female PGCs also start to express markers of meiosis (e.g., SYPC1 and SYPC3), whereas mitotically arrested late male PGCs already express a significant number of genes related to spermatogenesis and sexual reproduction. The comparison of human and mouse PGCs show that PGCs share a core transcriptome of key germ cell genes (e.g., BLIMP1, TFAP2C, DAZL, and DPP3A) and pluripotency genes (e.g., OCT4, NANOG, and LIN28A) with some notable differences, including lack of ESRRB, SOX2, or SOX3 expression in human PGCs and strong expression of naive pluripotency genes KLF4 and TFCP2L. While the PGC-specific modules also included SOX17, which has recently been reported to be a critical specifier for human PGCs (Irie et al., 2015), Guo et al. (2015) report that SOX15 is expressed more homogenously and at a much higher level specifically in early PGCs, suggesting a possible key functional role for human PGC development (Figure 1B).

In conclusion, these studies provide detailed maps of the transcriptional and epigenetic events that are fundamental for resetting genomic potential, erasing epigenetic memory, and establishing the human germline. This knowledge will help to better understand the epigenetic regulation of human development, and future work might identify potential biological differences underlying the discrepancies in overall methylation levels and timelines between the three studies. Whether such differences suggest that the extent of reprogramming and potentially transgenerational epigenetic inheritance could be regulated in mammals is an intriguing question for future work.

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