Regulation of Transposable Elements by TRIM28 Protects the Sex-Determination Program in PGCs

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Recent reports have shown that dynamic TE expression is a hallmark of gonadal somatic cells as they undergo sexualization. Here, we ask whether similar dynamics exist in Primordial Germ Cells (PGCs), the founding cells of the germline. As uncontrolled TE activity represents a threat to the integrity of the germline genome, we hypothesized that any balance between TE function and genomic threat is balanced by TRIM28, an epigenetic scaffolding protein which organizes the deposition of repressive heterochromatin at targeted loci. As TRIM28 is a well-characterized regulator of TEs in pluripotent stem cells (PSCs), we reasoned it may reprise a similar role in mammalian PGCs. To test this hypothesis, we used an *in vivo* PGC-specific conditional TRIM28 knockout (TCKO) model, focusing on PGCs between E10.5 and E14.5, during which they downregulate the early PGC program, exit the indifferent state, and undergo sex determination. We detected TRIM28 in both male and female PGCs and observed that the abundance of TRIM28 was both sex- and stage-dependent. Likewise, we found that in control PGCs the expression of TEs was highly dynamic between E11.5 and E13.5 in both sexes. Upon knockout of TRIM28, we examined expression of TEs in TCKO PGCs at E11.5, E12.5 and E13.5 and found that TEs, especially those of the Long Terminal Repeat (LTR) class, were derepressed. At the chromatin level, ATAC-seq revealed that TRIM28-targeted loci are sex-specific, in line with TE regulation by TRIM28 being required for proper sexualization of PGCs. To assess whether TCKO PGCs could acquire competency for germline differentiation we examined expression of Dazl, an RNA-binding protein thought to facilitate the downregulation of the early PGC program. We found that TCKO PGCs were heterogeneous in their expression of Dazl. Interestingly, we found that male, but not female, TCKO PGCs fail to fully downregulate the early PGC program despite observed deficiencies in Dazl expression. In line with these findings, male TCKO PGCs fail to differentiate into prospermatogonia, while female TCKO PGCs inefficiently progress to meiotic germ cells. Collectively, our results suggest that regulation of TE expression dynamics in the germline is both necessary for proper transition between indifferent and sex-determined PGCs, and that TE regulation during this window is sex-specific. Furthermore, these results suggest that TE reprogramming during sex-determination of PGCs likely licenses the PGC epigenome for gametogenesis.

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