Erase–Maintain–Establish: Natural Reprogramming of the Mammalian Epigenome

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The genetic information is largely identical across most cell types in a given organism but the epigenome, which controls expression of the genome, is cell type– and context-dependent. Although most mature mammalian cells appear to have a stable, heritable epigenome, a dynamic intricate process reshapes it as these cells transition from soma to germline and back again. During normal embryogenesis, primordial germ cells, of somatic origin, are set aside to become gametes. In doing so their genome is reprogrammed—that is, the epigenome of specific regions is replaced in a sex-specific fashion as they terminally differentiate into oocytes or spermatocytes in the gonads. Upon union of these gametes, reprogramming of the new organism's epigenome is initiated, which eventually leads, through pluripotent cells, to the cell lineages required for proper embryonic development to a sexually mature adult. This never-ending cycle of birth and rebirth is accomplished through methylation and demethylation of specific genomic sites within the gametes and pluripotent cells of an organism. This enigmatic process of natural epigenomic reprogramming is now being dissected in vivo, focusing on specific genomic regions—that is, imprinted genes and retrotransposons, where TRIM28 molecular complexes appear to guide the transition from gamete to embryo.

Mammalian reproduction requires union of two highly specialized cells, the egg and sperm, at fertilization, an event that marks the beginning of new life. Epigenetic marks of this newly formed genome are immediately required to establish the cell type–specific gene expression programs required for embryonic growth and development. DNA methylation is an epigenetic modification that leads to repression of specific gene expression, X-chromosome inactivation, and silencing of mobile DNA elements (for review, see Messerschmidt et al. 2014) and affects alternative splicing (Yearim et al. 2015). Establishment and maintenance of stable methylation patterns are thus crucial to execution of a normal developmental program (Okano et al. 1999). The DNA methylome should be, and is, extremely plastic during very early embryogenesis. Its dynamic control is initiated at fertilization during the switch from germ cell to embryo-specific methylation and a reciprocal change manifests during germ cell specification with the switch from a somatic to a germline epigenomic signature (Fig. 1).

Fertilization triggers extensive reprogramming whereby the global DNA hypermethylation of the germ cell–specific epigenomes become, on the whole, demethylated. The paternal genome in the pronucleus of the zygote is rapidly demethylated and reorganized. The methylation of the maternal genome is gradually depleted in a replication-dependent manner over consecutive mitotic divisions (Santos and Dean 2004). Although the precise mechanics of the active process are not completely un-

derstood, TET3-mediated oxidation of 5-methylcytosine (5mC) (Gu et al. 2011; Iqbal et al. 2011; Wossidlo et al. 2011) and restoration of unmethylated cytosines by components of the base excision repair pathway (Hajkova et al. 2010) are involved. These changes to the embryonic genome lead to an open chromatin state, essential for the pluripotency of some cells in the mouse blastocyst's inner cell mass (ICM). Reacquisition of de novo promoter methylation occurs between the time of establishment of the ICM of the blastocyst and the development of the postimplantation epiblast when the cells of the embryo differentiate into the somatic lineages (Borgel et al. 2010).

Resetting the epigenome is also mandatory during primordial germ cell (PGC) development when the methylation marks of the parental epigenome are erased and new gender-specific imprinting patterns are established (Seisenberger et al. 2012). PGCs are specified at E6.5 from a small group of BLIMP1-positive cells located in the somatic proximal postimplantation epiblast (for review, see Magnúsdóttir and Surani 2014). In mouse PGCs 5mC levels gradually decline in a partially TET1/2-dependent manner, as the cells migrate through the hindgut endoderm (E9.5), reach the gonadal anlagen (E11.5), and undergo sexual determination (E13.5) (Fig. 1).

Given the size and complexity of the mammalian genome, it is fascinating that demethylation and remethylation events are intricately controlled both temporally and in a genome context-specific manner. How are cer-

Figure 1. Epigenetic reprogramming during the mammalian reproductive cycle. (1) Both male and female germ cells are differentiated cell types and have highly specialized epigenomes. Fertilization of the oocyte by sperm initiates embryonic development. (2) The paternal genome (blue) is rapidly demethylated at early pronuclear stages. (3) The maternal genome (pink) is passively demethylated in a replication-dependent manner. (4) Erasure of germ cell-specific DNA methylation patterns allows the establishment of the pluripotent inner cell mass of the blastocyst. (5) Pluripotent cells begin the acquisition of DNA methylation marks as they begin to differentiate into somatic lineages. (6) PGCs are specified in the proximal postimplantation epiblast. They carry somatic epigenetic features, such as genomic imprints, inactive X-chromosomes, and silenced germ cell/pluripotency genes. (7) These features and potential epimutations are erased during primordial germ cell reprogramming. (8) Male- and female-specific germ cell differentiation includes establishment of paternal and maternal imprints.

tain loci identified to escape the loss of DNA methylation in an environment that favors genome-wide demethylation events? Recent studies of the molecular interactions at specific sites in imprinted genes and in endogenous retroelements (EREs) have afforded a glimpse of this complex process. Because methylation protection of specific DNA regions initiates immediately after fertilization, and during a time of complete transcriptional silence, the molecular agents, either RNA and/or protein, must be in the oocyte—that is, maternally inherited. The role of one such maternal factor, TRIM28, will be used to illustrate the complexity of this process in the mouse.

TRIM28—A MULTIFACETED WORKER IN THE CHROMATIN FIELD

Tripartite motif containing 28/KRAB-domain-associated protein1/transcription intermediary factor-1 β (TRIM28/KAP1/TIF1 β) is a multifunctional protein implicated in processes as diverse as embryonic development, maintenance of pluripotency, imprinting, endogenous and exogenous retroviral silencing, and double-strand break DNA damage response, to name a few. The structure of TRIM28, with its amino-terminal RING, B-box, and coiled-coil (RBCC) domain, central HP1-binding domain, and carboxy-terminal PHD-bromodomains, provides it with the means to interact with multiple transcription factors (TFs) and a variety of chromatin remodelers and epigenetic modifiers (Iyengar and Farnham 2011; Cheng 2014).

TRIM28 itself does not bind to DNA directly but rather relies on DNA-binding TF-mediated recruitment. These are largely members of the Krüppel-associated box

(KRAB) domain zinc-finger proteins (ZFPs), a family of TFs containing more than 200 genes in mice and humans that encode distinct proteins with highly variable DNA-binding domains. KRAB-ZFPs may have evolved from *Prdm9* (*Meisetz*) (Birtle and Ponting 2006) and expanded rapidly by duplication events during evolution. Loss of the original SET domain, accumulation of sequence changes to the ancestral KRAB domain, and appearance of corepressors are responsible for establishing the KRAB-ZFPs as transcriptional repressors. One explanation for the huge expansion and diversification of these TFs lies in their presumed coevolution with EREs to prevent their transcriptional reactivation (Thomas and Schneider 2011).

KRAB-ZFPs contain tandem arrays of C2H2 zinc fingers, each engaging three consecutive nucleotides on one DNA strand and one nucleotide on the opposite strand. The combination of multiple ZNFs within one protein allows KRAB-ZFPs to bind with high specificity and to recognize long stretches of DNA. The amino-terminally encoded KRAB domain is highly conserved throughout the KRAB-ZFP family and mediates interaction with TRIM28.

Numerous other TFs have been reported to interact with and potentially attract TRIM28 to their DNA-binding sites (Fig. 2; Satou 2001; Li et al. 2003; Satou et al. 2004; Wang et al. 2005, 2007; Jin et al. 2007; Tsuruma et al. 2007; Kamitani et al. 2008, 2011; Peng et al. 2009; Seki et al. 2010; Liang et al. 2011; Maruyama et al. 2011; Huang et al. 2013). For most of these, the KRAB-ZFPs included, TRIM28 functions as a co-repressor down-regulating gene transcription by its heterochromatin-inducing activity at its target sites (Iyengar and Farnham 2011; Cheng 2014).

A The TRIM28-complex **B** Interaction Partners of TRIM28 **C** KRAB-ZFPs & Function

Genomic imprinting maintenance		
Mouse/	ZFP57	gDMRs
Human		
exo- and endogenous ERE silencing		
	ZFP809	MuLV
Mouse	ZFP819	IAP, LINE
	Gm6871	LINE
	ZNF93	L1
	ZNF91	SVA
	ZNF282	HTLV
Human	ZNF350	
	ZNF175	HIV
	ZNF10	
others		
Mouse	ZBRK	p21
Human	ZNF160	TLR4
	ZNF689	miRNAs

Figure 2. The TRIM28 complex and interactome. (A) TRIM28 acts as a central scaffolding protein for a multifunctional, large protein complex. It is guided to DNA by interacting transcription factors and attracts in turn: (1) The NuRD histone deacetylation complex (deacetylates H3K9Ac); (2) SETDB1 and G9a histone methyltransferases (trimethylate H3K9me3); (3) DNA methyltransferases (DNMTs) (methylate cytosines). Heterochromatin protein 1 (HP1) recognizes H3K9me3 chromatin and further physically interacts with TRIM28. (B) TRIM28 interaction partners: KRAB-ZFPs and other TFs can target TRIM28 to DNA (*left*) (Satou 2001; Li et al. 2003; Satou et al. 2004; Wang et al. 2005, 2007; Jin et al. 2007; Tsuruma et al. 2007; Kamitani et al. 2008, 2011; Peng et al. 2009; Seki et al. 2010; Liang et al. 2011; Maruyama et al. 2011; Huang et al. 2013). TRIM28 attracts chromatin writers and readers (*right*) (Nielsen et al. 1999; Ryan et al. 1999; Schultz et al. 2001, 2002; Quenneville et al. 2011; Zuo et al. 2012). (C) Few KRAB-ZFP proteins have been studied in detail. ZFP57 plays a conserved role in imprinting maintenance in humans and mice. Diverse ZFPs/ZFNs (mouse/human) are involved in retrovirus silencing and other physiological functions (Okumura et al. 1997; Zheng et al. 2000; Li et al. 2008; Takahashi et al. 2009; Wolf and Goff 2009; Quenneville et al. 2011; Nishitsuji et al. 2012, 2015; Tan et al. 2013; Castro-Diaz et al. 2014; Jacobs et al. 2014; Wolf et al. 2015b).

TRIM28 acts as a scaffolding protein associating with the H3K9me3-writer SETDB1/ESET, the H3K9me3-reader HP1, and the nucleosome remodeling and histone deacetylation complex NuRD (Schultz et al. 2002, 2001). This multisubunit, heterochromatin-inducing molecular complex also serves as a platform for recruiting DNA methyltransferases, which can then induce and maintain DNA methylation in proximity to their target sites (Fig. 2; Quenneville et al. 2011; Zuo et al. 2012).

From a developmental biologist's point of view, it was known for some time that *Trim28* was highly expressed in

the preimplantation embryo and that its zygotic knockout is embryonic lethal in early postimplantation stages (Cammass et al. 2000). *Trim28* was also identified in independent screens for factors required for pluripotency and self-renewal of embryonic stem cells (ESCs) (Fazzio et al. 2008; Hu et al. 2009). However, the precise role of *Trim28* in mammalian cells remained unclear, although it was found to relocate to centromeric heterochromatin upon cell differentiation (Cammass et al. 2002).

Additional insights to TRIM28 function *in vivo* were gained from studies in preimplantation embryos

(Messerschmidt 2012; Messerschmidt et al. 2012). Deletion of maternal *Trim28* alone causes embryonic lethality, despite its reexpression from the paternal allele following embryonic genome activation at the two-cell stage. Intriguingly, the maternal-null embryos show highly variable phenotypes and die at various times throughout gestation, albeit at a much later time than when the molecular deficiency of TRIM28 first occurs. The pleiotropic defects displayed by these embryos are indicative of accumulated, random combinations of epimutations incompatible with life (Messerschmidt et al. 2012; Lorthongpanich et al. 2013).

We now know that TRIM28 is essential for proper and successful epigenetic reprogramming through at least two fundamental functions—that is, maintaining imprinting and silencing retrotransposons.

THE TRIM28-COMPLEX FUNCTION IN GENOMIC IMPRINTING

Genomic imprinting, or the establishment and maintenance of monoallelic expression of particular genes in a parent-of-origin-specific manner, is at the heart of the developmental failure observed in genetically bimaternal (gynogenote) and bipaternal (androgenote) embryos. The observation that the incomplete development of embryos derived from gynogenotes is largely embryo-like, whereas that of androgenotes is mostly of extraembryonic-like tissues, showed that the maternal and paternal genomes are functionally nonequivalent (McGrath and Solter 1984; Surani et al. 1984). Imprinting implies that the maternal and paternal genomes are differentially marked at specific genetic loci but does not involve changes in the DNA sequence. These epigenetic marks regulate gene expression, are mitotically heritable, and are set during female and male gametogenesis, respectively. Imprinted genes are often clustered in specific sites of the genome and within these clusters they can be coordinately regulated by differentially methylated regions at the so-called imprinting control regions (ICRs). Methylation at ICRs ultimately controls gene expression in *cis* by multiple mechanisms. Many imprinting clusters express noncoding RNAs (ncRNAs) that can further regulate genes within the cluster in *trans*. Collectively, epigenetic modification ensures that maternally imprinted genes are expressed from the maternally transmitted allele and silenced on the paternal allele, and vice versa (Hanna and Kelsey 2014; Peters 2014).

Parent-specific DNA methylation patterns at the ICRs are instituted during gametogenesis, part of a process requiring erasure of the inherited parental marks, so that imprints can be established sex-specifically. These parental imprints are protected and preserved postfertilization and stabilized throughout embryonic and adult life, as proper levels of the product of imprinted genes are essential to embryonic development, or are involved in adult cell and tissue homeostasis. For this reason, specialized mechanisms must be put in place to protect such sites from erasure during the wave of demethylation in the

early embryo. Genomic imprints are therefore paradigmatic of the subtle epigenetic fine-tuning required during genomic reprogramming. Although the genomic imprinting cycle of “erasure—establishment—maintenance and erasure again” has been postulated and shown for some time, the molecular mechanisms behind imprint maintenance involving the TRIM28 complex were only recently unraveled.

Demethylation in the preimplantation embryos is achieved by a combination of active and passive erasure of the methylation from 5mC (for review, see Messerschmidt et al. 2014). To allow for efficient methylation removal, the maintenance DNA methyltransferase-1 (DNMT1) is largely excluded from the nuclei of the cleavage-stage embryos (Howell et al. 2001; Ratnam et al. 2002; Branco et al. 2008; Hirasawa et al. 2008). However, this compartmentalization and nuclear exclusion cannot be complete, as genetic deletion of both maternal and zygotic *Dnmt1* results in imprinting defects, the result of complete ICR demethylation (Hirasawa et al. 2008). DNMT1 is canonically targeted to the replication fork in mitotic cells by its cofactor NP95. Because the drastically reduced DNMT1 levels and the global DNA methylation maintenance function of this complex make it unlikely to maintain imprints in embryos, a noncanonical targeting mechanism of DNMT1 had been proposed.

Insights into how DNMT1 might be targeted specifically to ICRs came from studies in vivo and in vitro. We were able to show that loss of maternal *Trim28* alone can result in dramatic misexpression of imprinted genes in midgestation embryos (Messerschmidt 2012; Messerschmidt et al. 2012, 2014; Lorthongpanich et al. 2013). This observed deregulation was observed across multiple imprinting clusters with the deregulation following a pattern predicated on a loss-of-ICR-methylation scenario. Indeed, partial, severe ICR demethylation, predominantly at the paternally imprinted H19/IGF2 locus, was found in these embryos. All tested imprinted loci, however, displayed variable degrees of methylation loss, arguing for (1) a global mechanism and (2) a partial rescue of the defect by paternal *Trim28* expression (Messerschmidt et al. 2012; Lorthongpanich et al. 2013). Indeed, single-cell analysis of DNA methylation in eight-cell-stage embryos revealed that not all blastomeres are affected equally; hence, the pleiotropic defects observed are likely a consequence of epigenetic mosaicism in the mutant embryos. Interestingly, maternal/zygotic-null *Trim28* embryos develop to apparently normal blastocysts and implant (DM Messerschmidt, unpubl.); thus, the complete knockout does not show a phenotype during preimplantation embryogenesis. However, it remains to be seen whether loss of both maternal and paternal *Trim28* will result in complete eradication of ICR methylation in preimplantation embryos.

TRIM28 is physically bound to known ICRs in midgestation embryos, arguing for a direct recognition and maintenance mechanism (Messerschmidt et al. 2012). ICRs in these embryos are co-occupied by ZFP57 and as a consequence of the binding of the TRIM28 complex, histones were H3K9me3 modified (Messerschmidt et al.

2012). ZFP57 is a TRIM28-interacting KRAB-ZFP and both maternal and zygotic *Zfp57* are necessary for the proper maintenance of parental imprints in blastocysts (Li et al. 2008). Investigations in ESCs showed ZFP57 recognizes a CpG-containing consensus TGCCGC sequence, highly conserved at ICRs (Quenneville et al. 2011). However, TRIM28 binding to hypomethylated sites was lost in embryos (Messerschmidt et al. 2012) leading us to suggest that ZFP57 recognizes the methylated cytosine. Indeed, ZFP57 shows much higher affinity to its methylated target sequence in vitro (Quenneville et al. 2011; Liu et al. 2012). Thus, sequence-specific and methylation-dependent binding to ICRs offers an elegant solution to the convoluted undertaking of (1) recognizing specialized regions within the genome and (2) maintaining differential DNA methylation at a given locus. It also explains the lack of paternal rescue in the maternal *Trim28*-null embryo because preexisting DNA-methylated sites, inherited from the gametes, are fundamental to the ZFP57-mediated TRIM28-complex binding. If a particular CpG is demethylated under maternal-null circumstances, reexpressed paternal TRIM28 cannot be targeted to it and cannot restore DNA methylation. Similarly, reexpression of ZFP57 in *Zfp57*-deleted ES cells cannot restore methylation to sites that have lost it (Zuo et al. 2012). Finally, this mode of interaction offers a plausible model for the noncanonical targeting of the dramatically reduced levels of DNMT1 to ICRs in preimplantation embryos; both maintenance DNA-methyltransferase and the de novo DNA-methyltransferases (DNMT3A/B) are bona fide interaction partners of TRIM28 (Quenneville et al. 2011; Zuo et al. 2012).

The highly specialized role and mode of chromatin interaction of ZFP57 with specific genomic sequences, be they methylated or not, is an exquisite example of the functional diversity acquired by the KRAB-ZFPs. Further, the multiple interactions made possible through the TRIM28 scaffolding complex not only clarify the specificity of epigenetic reprogramming but also accentuate the importance of the balance between erasure of most parental epigenetic features versus the careful maintenance of a few stable epigenetic sites.

THE TRIM28 COMPLEX IN RETROTRANSPOSON REGULATION

Mammalian cells face a formidable challenge in dealing with potentially damaging endogenous retroviruses (ERVs) and retrotransposons, collectively termed endogenous retroviral elements (EREs). These elements make up ~40% of the mammalian genome, highlighting the magnitude of this task (Lander et al. 2001; Mouse Genome Sequencing Consortium et al. 2002).

Aberrant activation of EREs can impact the host on two major levels. For one, certain retrotransposons remain capable of active retrotransposition that can lead to random insertional mutagenesis following replicative transposition into the host's genome. This must be prevented

at all costs, particularly in the germline. Most EREs, however, have lost their ability to replicate but still their remnants are important targets of the host's silencing machinery. Loss of silencing at such sites can result in drastic, localized chromatin changes and impact on certain cellular genes whose expression may be under the influence of neighboring, ERE-derived promoters/enhancers. In certain contexts, ERE control over gene regulation and mobility is of beneficial effect to the host. A case in point is the developmentally regulated expression of nonconventional chimeric transcripts driven by transposon-derived 5' sequences that can encode new proteins with potentially novel functions (Peaston et al. 2004). Temporary loss of a silencing mechanism can also explain the stage-specific expression of different long terminal repeat (LTR) retrotransposons during the oocyte-to-embryo transition (Peaston et al. 2004). As it now stands, most EREs are continuously repressed and the first line of defense against their expression is DNA methylation-based silencing, substantially contributing to the overall hypermethylated state of the mammalian genome.

One insight into the molecular mechanism controlling retrotransposon silencing during embryonic development came from the discovery that *Dnmt1*-deficient embryos reactivate all major classes of the intracisternal A-type particles (IAPs) (Walsh et al. 1998). Later, DNMT3L was shown to protect against spurious transcriptional reactivation of long interspersed nuclear elements 1 (LINE1) and IΔ1 IAP retrotransposons in male germ cells (Bourc'his and Bestor 2004). Extensive research has shown that during mammalian reproduction, when DNA methylation patterns are globally modified and removed, other mechanisms serve as a barrier to ERE activation. The small RNAs (piRNAs) seem to predominate in the germline but in the embryo piRNAs play a minor role to that of the KRAB-ZFP TFs, which mediate formation of heterochromatin.

One transacting repressor complex specific to pluripotent cells, and present in embryos, was shown to be important for ERV silencing. Initially termed binding factor A, this 1.3-MDa multisubunit complex contains TRIM28 as a repressor subunit (Reik 2007) and the KRAB-ZFP809 that serves as its sequence-specific DNA-binding partner (Wolf and Goff 2009). TRIM28 links the DNA methylation and KRAB-ZFP repressive machineries as shown by tethering a doxycycline-controllable KRAB domain to a lentiviral construct in vivo (Wiznerowicz et al. 2007). Here, a green fluorescent protein (GFP) reporter gene was found to be expressed only if recruitment of the repressive KRAB-ZFP was prevented by adding doxycycline in a narrow time window right after fertilization (between E0.5 and E3.5). Conversely, GFP silencing was permanently established if the KRAB-containing fusion protein bound its target sequence very early in embryogenesis.

ERVs are classified based on their sequence similarities. Class I and particularly class II ERVs such as IAPs and MusD are drastically deregulated in mouse ESCs lacking TRIM28. Also, treatment of *Trim28* knockout-

derived ESCs with 5-azacytidine (a DNMT inhibitor) has a synergistic effect on ERV activation. However, concurrent reactivation is accompanied by depletion of the H3K9me3 histone modification of the IAP and MusD sequence. DNA methylation-independent silencing in ESC has also been described, which requires TRIM28 and SETDB1 but does not rely on de novo or maintenance methylation because *Dnmt1/3a/3b* triple-knockout ESCs do not display the massive derepression of murine leukemia virus (MLV), IAP, and MusD observed in *Setdb1*- or *Trim28*-deficient cells (Matsui et al. 2010; Rowe et al. 2010).

It appears that certain redundant mechanisms function to ensure efficient ERV silencing; the preferred mechanism depends on the class and evolutionary history of the particular element (ancient vs. young). It has been suggested that the repressive KRAB-ZFP machinery likely coevolved together with the ERVs as a host mechanism to restrict their horizontal and vertical transmission (Wolf et al. 2015a,b). Surprisingly, only a limited number of KRAB-ZFPs have been shown to regulate specific ERV classes in mice. Such examples include the regulation of MLV by ZFP809 described earlier, of IAPs and LINE1 by ZFP819, and of LINE1 by Gm6871 (Fig. 2). It remains to be seen whether and which KRAB-ZFPs are still actively involved exclusively in retrotransposon silencing and whether many have evolved to undertake “endogenous” host gene regulatory functions, such as ZFP57.

ERV repression by the TRIM28 complex has been scantily studied in vivo. Class II ERVs, particularly IAP elements, are known to be massively activated in zygotic *Trim28* knockout blastocysts and periimplantation embryos (Rowe et al. 2010). During oocyte growth, these elements are generally repressed (Peaston et al. 2004)

but *ZP3-cre*-mediated removal of maternal *Trim28* in the growing oocyte does not result in IAP or MusD derepression nor in the ectopic activation of any other retroviral element tested here (Fig. 3). Thus, *Trim28*-independent mechanisms, in collaboration with DNA methylation and possibly piRNAs, may act to silence ERVs in the mouse egg.

As early as the two-cell stage, when the embryonic genome is activated, a veritable explosion of ERV transcription takes place. Predominantly class III mouse transcript (MT) and ERV-like elements (ERVL) and class II elements (IAPs) are transcribed (Kigami et al. 2003; Peaston et al. 2004). This massive activation of ERVs gives the two-cell-stage mouse embryo a characteristic transcriptome that is recapitulated in a small subfraction (1%) of ESCs (Kigami et al. 2003; Macfarlan et al. 2012). It is unclear whether this wave of transcriptional activation is required for proper development or is only a by-product of epigenetic reprogramming in the embryo.

Mouse (Mu) ERVL transcription is regulated by chromatin modifications, particularly H3K4me3. Removal of LSD1/KDM1, an H3K4me3 demethylase, results in MuERVL activation and embryonic arrest at gastrulation (Macfarlan et al. 2012). We find loss of maternal *Trim28* does not impact MuERVL expression or that of any other class III or class I ERV tested. Also LINE and short interspersed nuclear element (SINE) expression remained unchanged (Fig. 3). Remarkably, despite the massive endogenous activation of class II element observed at the two-cell stage, loss of maternal *Trim28* causes a hyperactivation of IAPs and MusD (Fig. 3). The dynamics of this regulation and to what extent this overexpression, with all its repercussions, contributes to the maternal-deletion phenotype of *Trim28* are unclear and warrant further investigation.

Figure 3. ERV expression in maternal-null *Trim28* and wild-type oocytes and embryos. Long terminal repeat (LTR) and non-LTR retrotransposon expression was tested in (A) MII oocytes and (B) two-cell-stage embryos in the presence and absence of maternal *Trim28* (white and black bars, respectively). Although oocytes show no changes in ERV expression, two-cell-stage maternal-null *Trim28* embryos show elevated expression of the LTR class II elements, IAP and MusD.

CONCLUSION

Establishment of a unique epigenome is the basis for correct functioning of each cell in a multicellular organism. Mechanistically we can envision the existence of two types of epigenome. The dynamic or transitory epigenome is unique to each cell and tissue type, and it is driven by classical TFs. It is malleable and reprogrammable as showed by the phenomena of cloning, transdifferentiation, and induced pluripotent stem (iPS) cell derivation. In the stable epigenome, as represented by imprinted genes and retroelements, silencing is based predominantly on stable genomic modifications, which are mitotically inherited and change only at the specific stages of the life cycle of the organism. Establishment and control of the stable epigenome has likely evolved through an evolutionary process we are just beginning to decipher. Considering the importance of a stable epigenome to normal development, significant efforts should be undertaken to understand its establishment and maintenance.

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