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Review

Dynamic control of endogenous retroviruses during development

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ABSTRACT

Close to half of the human genome encompasses mobile genetic elements, most of which are retrotransposons. These genetic invaders are formidable evolutionary forces that have shaped the architecture of the genomes of higher organisms, with some conserving the ability to induce new integrants within their hosts' genome. Expectedly, the control of endogenous retroviruses is tight and multi-pronged. It is most crucially established in the germ line and during the first steps of embryogenesis, primarily through transcriptional mechanisms that have likely evolved under their very pressure, but are now engaged in controlling gene expression at large, notably during early development.

Contents

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Genetic invasion parallels evolutionary complexity

Close to half of the human genome is derived from retrotransposons replicating by the copy-and-paste mechanism used by exogenous retroviruses such as HIV. These genetic invaders are both essential motors of evolution and threats whose uncontrolled spread would be fatal to their host. Correspondingly, retrotransposons are tightly restricted through mechanisms often also engaged in fighting their exogenous viral counterparts. The RepBase compilation of repetitive sequences is clustered into 6 superfamilies comprising more than 200 groups of retroelements for which consensus sequences are given (or sequence examples for small families) (Blomberg et al., 2009; Jurka, 2000; Jurka et al., 2005; Medstrand et al., 2002). This classification broadly groups retroelements into either long terminal repeat (LTR)-containing (encompassing around 7–8% of human and mouse genomes (Jern and Coffin, 2008; Lander et al., 2001; Waterston et al., 2002) and including simple retrotransposons and endogenous retroviruses (ERVs) that possess an envelope gene), or non-LTR-retrotransposons such as LINE and SINE (long and short interspersed nuclear elements, (Goodier and Kazazian, 2008)), which together constitute 35% of human and mouse genomes (Bannert and Kurth, 2004). By comparison, protein-coding genes account for only 1.5% of the DNA in mammalian genomes (Venter et al., 2001; Waterston et al., 2002). Phylogenetic analyses of reverse transcriptase sequences strongly suggest a common origin for retroelements, likely also related to bacterial and mitochondrial genetic elements that encode a reverse transcriptase and to the eukaryotic telomerase gene.

There is a striking parallel between the accumulation of retroelements and the increasing evolutionary complexity of their host species. For example the genome coverage for repetitive elements runs from 3% in yeast to 6% in worms to 15% in flies to 40% in mice and 44% in humans (reviewed in Kidwell (2002)). Plant genomes are packed with retroelements, occupying 50% of their DNA (SanMiguel et al., 1996), which may strongly influence their modes of gene regulation. Most interestingly, the nature of the retroelements present in these species also changes with evolutionary complexity; LTR and non-LTR-retrotransposons have colonised plants, invertebrates and vertebrates while true endogenous retroviruses (containing an envelope gene) have remained mysteriously restricted to vertebrates (Gifford and Tristem, 2003). The emergence of ERVs (presumed to be remnants of retroviruses that once infected the germ line) coincided with that of adaptive immunity, suggesting that the lympho-tropism of many retroviruses may have played a role in this process (Litman et al., 2010). In turn, this led to the selection of various innate and adaptive retroviral restriction activities. Interestingly, the rise of ERVs occurred simultaneously to that of KRAB-containing zinc finger proteins (KRAB-ZFPs) (Emerson and Thomas, 2009), which likely play a prominent role in their control (Rowe et al., 2010), perhaps leaving RNA interference mechanisms to take care of the evolutionary more ancient LTR and non-LTR retrotransposons. The origin of endogenous retroviruses is still debated. There are both examples of exogenous retroviruses that became endogenized through loss of their envelope gene, some gaining the ability to retrotranspose (Ribet et al., 2008), and of ancient retrotransposons that have captured an envelope and escaped from the cell (Malik et al., 2000; Song et al., 1994) and reviewed in Kim et al. (2004).

In this review, we will focus mostly on ERVs, since more is known about their regulation, and on the highly prevalent (20% of the human genome (Lander et al., 2001)) non-LTR retrotransposon LINE1, L1, even though less is understood of its replication and control. Classification of ERVs (see Table 1 for a summary) is based on their relation to exogenous retroviral families and the nature of their opted tRNA primers serving in their reverse transcription. It should be noted, though that almost identical ERVs can sometimes use different primers due to point mutations in their primer binding site (PBS) (Colicelli and Goff, 1986), just as distantly related viruses can use the same tRNA primer (e.g. MusD and HIV). Although it is known that ERVs are restricted to vertebrates, their relative ages are only now being uncovered (reviewed in Meylan and Trono (2009)): endogenous lentiviruses have recently been discovered in the rabbit and then in the gray mouse lemur, showing that this virus group is likely >14 million years old, more ancient than previously thought (Gifford et al., 2008; Gilbert et al., 2009; Katzourakis et al., 2007; Keckesova et al., 2009), and is capable as other retroviruses of infiltrating the germ line. Complex retroviruses in general are estimated to be >100 million years old as endogenous foamy viruses were recently found in the sloth genome (Katzourakis et al., 2009).

Which retroelements are active today?

Retroelements that the host needs to control actively are those still autonomous for retrotransposition. Today, some 80–100 L1 copies present in the human genome are still active with around 68 distinct among individuals and absent from the human genome reference sequence (Beck et al., 2010). These L1s are thought to account for 0.1% of *de novo* mutations in humans (Maksakova et al., 2006). Also, some rare members of the HERV-K family retain all open reading frames and exhibit polymorphism within the human population, suggesting recent integration events (Belshaw et al., 2005; Macfarlane and Simmonds, 2004; Turner et al., 2001).

In contrast, retrotransposons have remained far more active in rodents, causing close to 10% of spontaneous mutations in inbred strains of mice (Maksakova et al., 2006), while in humans transposition reached a peak around 40 million years ago and then sharply declined (Gifford and Tristem, 2003; Lander et al., 2001; Waterston et al., 2002). This difference may in turn parallel differences in host regulation of cellular genes and ERVs alike (Kunarso et al., 2010). ERVs are correspondingly highly polymorphic amongst mouse strains, attesting to their continuous activity (Takabatake et al., 2008; Zhang et al., 2008). The most active ERVs in the mouse are MusD/ETn (early transposon) elements and intracisternal A-type particle (IAP) elements (Lueders and Kuff, 1977), although only relatively few of them have the fully coding *gag* (group specific antigen) *pro* (polymerase) and *pol* (protease) genes necessary for retrotransposition (around 10 and 300 copies respectively (Dewannieux et al., 2004; Ribet et al., 2004).

Table 1
Classification of endogenous retroviruses.

	Class I	Class II	Class III
Mouse ERV	Murine leukaemia virus (MLV)	MusD, Intracisternal A particles (IAP), Mouse mammary tumour virus (MMTV)	MERV-L
Human HERV	HERV-H (His), HERV-W (Trp)	HERV-K (Lys)	HERV-L (Leu)
Related XRV genus	Gamma RVs, Epsilon RVs	Alpha RVs Beta RVs, Delta RVs Lentiviruses	Spumaviruses

ERV, endogenous retrovirus; HERV, human endogenous retrovirus; XRV, exogenous retrovirus; RV, retrovirus; HERV tRNA primers, used for reverse transcription are stated in brackets.

Retroelements are transcriptionally active in germ cells (Peaston et al., 2004), early embryos and ES cells (Garcia-Perez et al., 2007; Macia et al., 2010). L1 RNA is transcribed in germ cells and then deposited in the zygote where it can undergo retrotransposition (Kano et al., 2009) leading to somatic mosaicism, but usually not to heritable new integrants. MERV-L is specifically activated during zygotic genome activation at the 2-cell stage in mouse embryos and then rapidly repressed by the blastocyst stage (Kigami et al., 2003; Svoboda et al., 2004). MERV-L is an active ERV like IAPs with around 16 copies in the mouse genome that have intact open reading frames (ORFs) and identical LTRs. IAP transcripts and the IAP core protein p73 are carried from the oocyte into early embryos, degraded and then peak again at the blastocyst stage after zygotic expression from the 2-cell stage onwards, until the IAP genome is DNA methylated (Piko et al., 1984; Poznanski and Calarco, 1991; Svoboda et al., 2004). In contrast, MusD/ETn transcripts are abundant in post-implantation embryos (Loebel et al., 2004) and retroelements of the *m*ammalian *a*pparent *L*TR *r*etrotransposon (MaLR) class are again differently regulated, being expressed mainly in mature oocytes (Peaston et al., 2004) (summarised in Figs. 1A + C). Despite the detection of ERV transcription in ES cells, these potently silence newly introduced murine leukeamia virus (MLV), ERVs and L1, likely due to stem cell specific restriction pathways as such silencing does not occur in differentiated cells (Garcia-Perez et al., 2010; Rowe et al., 2010; Teich et al., 1977). It was recently shown that extraembryonic endoderm stem (XEN) cells (see Fig. 1A) are also potent ERV silencers but not trophectoderm stem (TS) cells (Golding et al., 2010). Interestingly, overexpression of IAPs and MERV-L directly affects the pluripotency of ES cells (Ramirez et al., 2006). It is likely then that ERV expression and repression are linked to the control of cellular genes through development (Peaston et al., 2004). Noteworthy, traces of past ERVs in the form of solo LTRs that result from homologous recombination between the two flanking terminal repeats can also conserve a transcriptional and/or repressor activity that may influence the expression of neighbouring cellular genes. Other tissues exhibiting ERV activity are placenta (see next section) and thyroid (de Parseval et al., 2003; Schon et al., 2009; Seifarth et al., 2005). Surprisingly, L1 activity can be detected in neural progenitors in the adult human and mouse brain (Coufal et al., 2009; Muotri et al., 2005).

A fine balance between good and evil

The best illustration of the beneficial potential of ERVs is in the role of syncytins in placental physiology. Syncytins are responsible for the fusogenic ability of cells in the syncytiotrophoblast, and represent the envelope of endogenous retroviruses. In humans, syncytins 1 and 2 are derived from HERV-W and HERV-FRD, respectively, and in the mouse syncytins A and B similarly represent the envelope of endogenized retroviruses (Blaise et al., 2003, 2005; Blond et al., 2000; Dupressoir et al., 2009).

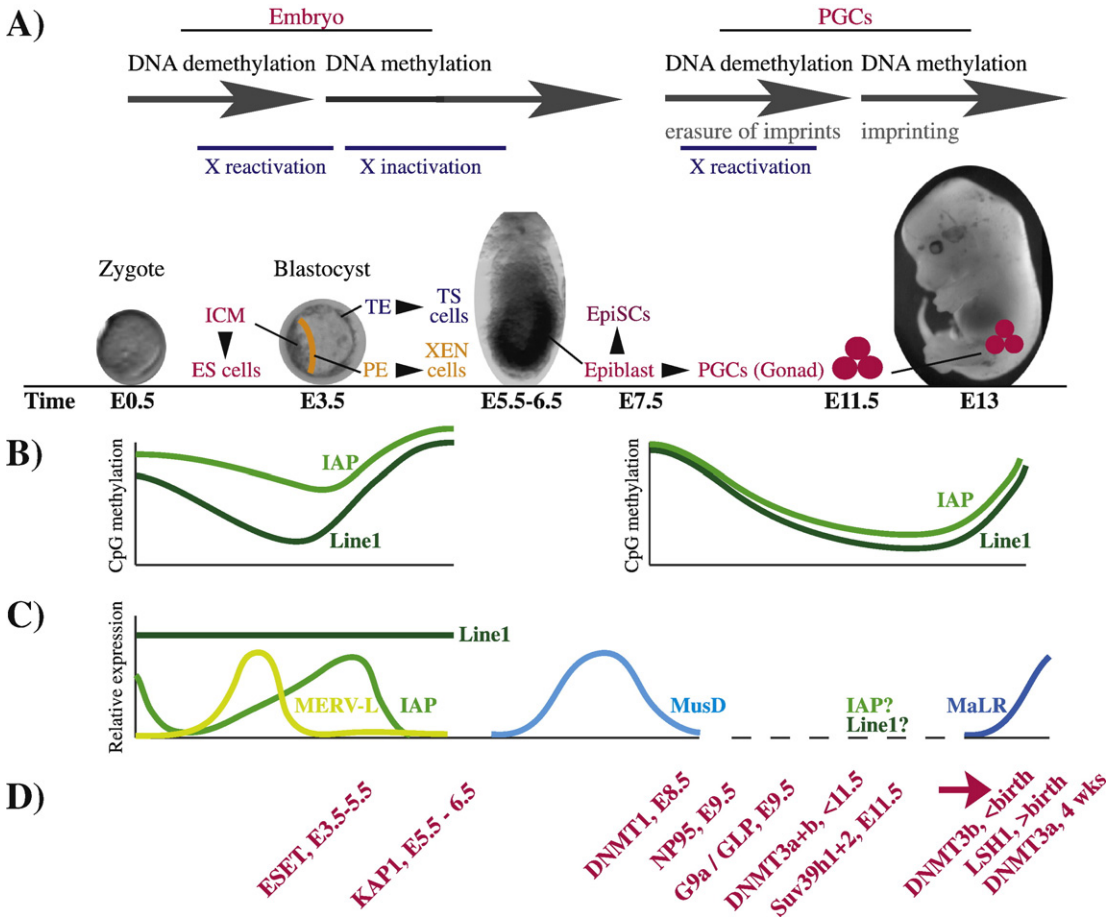

Fig. 1. Resetting ERV silencing during mouse development. (A) Mouse developmental program showing waves of DNA demethylation, X-inactivation and imprinting erasure in early embryos or primordial germ cells (PGCs). A time-line of development is shown along with various cell lines that can be derived and which represent different developmental stages: ICM, inner cell mass; ES, embryonic stem; TE, trophectoderm; TS, trophectoderm stem; PE, primitive endoderm; XEN, extraembryonic endoderm; EpiSCs, epiblast stem cells. We thank Daniel Mesnard for the E5.5 embryo photograph. (B) Differences in DNA methylation between Line1 and IAPs during reprogramming, and between early embryos and PGCs are shown: IAPs reach their lowest level of around 62% by the blastocyst stage, still considerably higher than Line1s that drop to around 25% methylation. See text for details. (C) Broad time-points where stated retroelements peak in their mRNA expression are shown based on available data (see text for details). Comprehensive expression data, though is lacking in PGCs e.g. for IAPs and Line1. (D) Factors known to be important in ERV regulation are shown along with the time at which their knockout is lethal. The arrow indicates lethality beyond the timescale indicated. Additionally, knockout of PIWI-like proteins or Gtsf1 leads to male sterility while loss of Stella affects female fertility. See text for details.

That said, genome analyses reveal that endogenous retroviruses are usually located outside of genes and in antisense orientation to them (Medstrand et al., 2002; Zhang et al., 2008), which considering that integration is random indicates a strong negative selection (Brady et al., 2009). One exception is sense LTRs overlapping 5′ and 3′ untranslated regions particularly in immunoglobulin and KRAB-zinc finger genes suggesting that some genes may harness LTR regulatory mechanisms (Medstrand et al., 2002; van de Lagemaat et al., 2003). In mice, genetic disorders caused by ERVs include diabetes and kinked tail, resulting from IAP insertions in the agouti or axin-fused genes respectively (Duhl et al., 1994; Michaud et al., 1994; Rakyan et al., 2003), as well as limb malformation associated with *Dactylaplasia* mutations caused by MusD insertions (Friedli et al., 2008; Kano et al., 2007). Remarkably, recent evidence suggests that patients with Rett syndrome, due to mutations in MeCP2, are more sensitive to L1 retrotransposition, suggesting a role for this class of retrotransposons in their neurological symptoms (Muotri et al., 2010). For a more extensive description of the effects of ERVs on the genomes of higher species, the reader is directed to recent reviews (Jern and Coffin, 2008; Kurth and Bannert, 2010; Singh et al., 2009).

ERVs are regulated early in development

Retroelements are permanently inactivated during embryonic development so as to exhibit a transcriptionally silent state in adult tissues and in the germ line. Their tight regulation is important to prevent insertional mutagenesis and needs to withstand zygotic genome activation, which takes place at the two-cell stage, shortly after fertilization, as well as the ensuing DNA demethylation that is required for reprogramming (Feng et al., 2010b; Oswald et al., 2000; Reik, 2007) and see Fig. 1A). During this latter process the paternal genome, which is uniquely moulded on protamines instead of histones, undergoes protamine–histone exchange and then suffers a wave of active DNA demethylation (reviewed in Wu and Zhang (2010)) within 4–8 h post-fertilization. The maternal genome, on the other hand, is subjected to passive DNA demethylation through iterative cell divisions, until the blastocyst stage when the embryo becomes newly globally methylated (Feng et al., 2010b). Another wave of genome-wide DNA demethylation takes place later from E10.5–E12.5 in the primordial germ cells ((Hajkova et al., 2002; Lane et al., 2003; Lee et al., 2002; Lees-Murdock et al., 2003) and reviewed in Morgan et al. (2005)) followed by remethylation (by E17.5 in the male germ-line or post birth in the female germ-line). Artificial reprogramming of somatic cells into induced pluripotent stem (iPS) cells (reviewed in Hanna et al. (2010)) also relies on DNA demethylation (Bhutani et al., 2010). DNA methylation is highly relevant to retroelements because it is their best-studied mechanism of control and has even been proposed to have evolved primarily for this purpose (Reik, 2007; Reiss and Mager, 2007; Yoder et al., 1997). Indeed, retroelements are preferentially methylated compared to the genome average in several species, particularly in plants (Feng et al., 2010a), which share many parallels with mammals as well as differences in their mechanisms of reprogramming and retroelement regulation (Feng et al., 2010b; Law and Jacobsen, 2010). However, DNA methylation is only one of the regulatory events controlling ERVs, itself complemented by other silencing pathways, which explains how control is maintained in the face of demethylation and reprogramming. The various pathways involved in ERV repression from cytosine methylation to RNA interference, will now be described in detail.

Cytosine methylation acts from plants to mammals to block transcription

Transcriptional silencing through the methylation of cytosine at CpG dinucleotides is a well-established mechanism of gene regulation in mammals, which interestingly also operates in plants but is absent from *Drosophila* and *Caenorhabditis elegans*. Methylation occurs at CpGs throughout the mammalian genome, except over so-called CpG islands (Feng et al., 2010a). Some non-CpG cytosine methylation also takes place in mammalian ES cells (Lister et al., 2009; Ramsahoye et al., 2000), whereas in plants this specific nucleotide modification is encountered in all sequence contexts. While methylation within promoters is well known to silence transcription, its impact within gene bodies is unknown, although it has been proposed to block transcription from cryptic promoters (Tran et al., 2005). Transposable elements are kept in check by CpG methylation in the model plant *Arabidopsis thaliana* by DNA methyltransferases including Domains Rearranged Methyltransferase 2 (DRM2) and DNA methyltransferase 1 (MET1/DMT1), or in mammalian cells by DNMT3a and 3b (homologues of DRM2) and DNMT1 (the homologue of MET1). Aside from silencing ERVs, such DNA methylation in mammals also controls X chromosome inactivation and genomic imprinting, a process by which one allele for some 80 genes is silenced in a parent-of-origin-specific manner (Kaneda et al., 2004; Sado et al., 2004). DNMT3a and 3b can mediate *de novo* DNA methylation while DNMT1, which has a preference for hemi-methylated DNA, is involved in perpetuating methylation marks during DNA replication (Bestor et al., 1988; Bestor, 1988). DNMT2, on the other hand, is not required for *de novo* or maintenance methylation of viral DNA (Okano et al., 1998) but intriguingly it can methylate a specific cytosine within a transfer RNA, a function conserved from plants and insects to mammals (Goll et al., 2006). Cytosine methylation is, therefore, an ancient epigenetic modification crucial to many aspects of mammalian development and homeostasis; how *Drosophila* has evolved notably to control retroelements without this feature will be remarked upon later.

De novo DNA methyltransferases target specific retroelements

De novo methylation of retroelements occurs after erasure of methylation in cleavage embryos and again in primordial germ cells. These marks must be re-established by DNMT3a, DNMT3b and the related DNMT3-like protein, DNMT3L. DNMT3L does not possess methyltransferase activity itself but it plays a global role in the activation of DNMT3a and DNMT3b (Chedin et al., 2002; Suetake et al., 2004). Both DNMT3a and DNMT3b are required in development: DNMT3b$^{-/-}$ embryos appear normal until E9.5 but die before birth, DNMT3a$^{-/-}$ mice survive development but die at around 4 weeks of age, and double knockouts die before E11.5 (Okano et al., 1999). Endogenous MLV and IAP elements are slightly undermethylated in DNMT3b$^{-/-}$ embryos and this phenomenon is more pronounced in double knockouts but still mild compared to the hypomethylation of IAP elements observed in DNMT1$^{-/-}$ embryos (Okano et al., 1999).

Detailed analysis of fetal prospermatogonia has revealed that SINE-B1 repeats are methylated by DNMT3a, IAP elements and L1 repeats by both DNMT3a and DNMT3b, and satellite repeats by DNMT3b (Kato et al., 2007), while differential methylation at imprinted genes is dependent on DNMT3a in both germ lines (Kaneda et al., 2004; Kato et al., 2007). This suggests there to be some specificity in differential DNMT recruitment to retroelement families, although how DNMTs recognise their targets is an open question. It may be that undiscovered sequence-specific transcription factors control this interaction; presumably such factors would bind to common ERV determinants, though, since retroelements are highly polymorphic even within one family (Zhang et al., 2008). Interestingly, it was revealed that the DNMT3a–DNMT3L complex recognises a specific histone code (see histone methylation section (Ooi et al., 2007) and Fig. 2A) and functions to preferentially methylate CpGs at neat 8–10 bp intervals (Jia et al., 2007); the observation that SINE repeats harbour an overrepresentation of 8 bp spaced CGs might account for some specificity here (Ferguson-Smith and Greally, 2007; Glass et al., 2009).

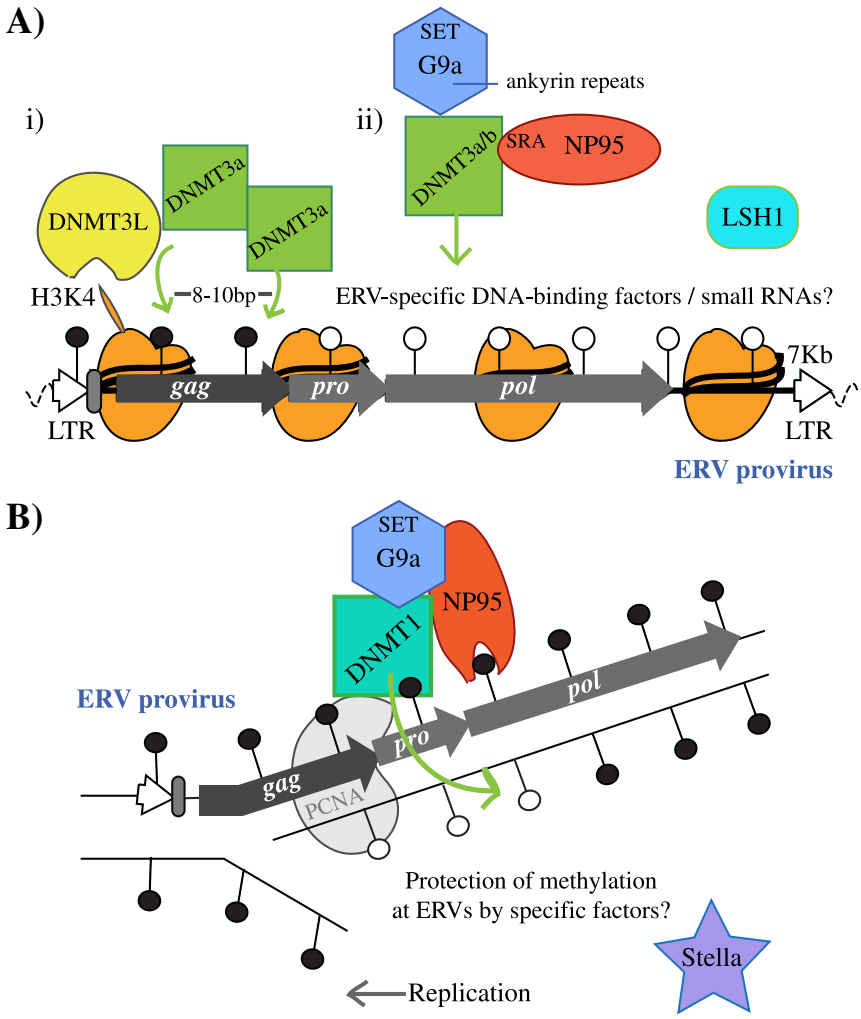

Fig. 2. DNA methylation of ERVs in development. (A) *De novo* methylation: (i) binding of a DNMT3L–DNMT3a complex to unmethylated H3K4 tails ensures some specificity of *de novo* methylation also thought to act best at 8–10 bp intervals, at which CpGs are often spaced apart in ERVs (Glass et al., 2009; Jia et al., 2007; Ooi et al., 2007). (ii) NP95 interacts with regulatory domains of DNMT3a and DNMT3b through its SRA and ubiquitin-like domains (Meilinger et al., 2009) and with G9a (Kim et al., 2009) that also interacts with DNMT3a and DNMT3b (Epsztejn-Litman et al., 2008; Feldman et al., 2006). This complex is required for the initiation of silencing that is followed by *de novo* methylation (Meilinger et al., 2009) that occurs on newly introduced MLV (Dong et al., 2008), but whether there are specific factors that bridge this complex to ERVs is not known. The helicase LSH1 is also required for methylation of newly introduced MLV by an unknown mechanism (Zhu et al., 2006). (B) Maintenance methylation: DNMT1 (or the maternal DNMT1o in cleavage embryos) is recruited to replication forks through binding proliferating cell nuclear antigen (PCNA), G9a and NP95, which itself recognises hemimethylated DNA and directs DNMT1 to methylate the symmetrical CpG on the newly synthesized DNA strand. Loss of NP95 leads to diffused localisation of DNMT1 and IAP and L1 demethylation and expression in embryos and ES cells (Achour et al., 2008; Bostick et al., 2007; Sharif et al., 2007). Histones are shown in orange and unfilled vs. filled lollipops represent unmethylated vs. methylated CpGs respectively.

DNA methyltransferase 1 protects methylation at ERVs

Maintenance methylation plays a crucial role in ERV silencing in development as illustrated by the embryonic lethality of DNMT1-null embryos at E8.5 accompanied by 50–100 fold elevated transcript levels of IAPs (Walsh et al., 1998). This importance of DNMT1 in ERV regulation may be explained by data revealing IAP sequences to behave differently from other retroelements like L1 in their relative resistance to global demethylation during pre-implantation development. Indeed, IAPs remain heavily DNA methylated during this period (Fig. 1B) similar to imprinted genes (Howlett and Reik, 1991; Lane et al., 2003). The role of DNMT1 in this protection from demethylation was demonstrated by crossing DNMT1 mutant mice with viable yellow (A^{vy}) mice that harbour an IAP insertion in their agouti locus (Gaudet et al., 2004). In these mice, an IAP has inserted 100 kb upstream of the agouti gene in the opposite orientation and in its hypomethylated state it drives constitutive ectopic expression of *agouti* leading to yellow fur, obesity and tumours (Duhl et al., 1994; Michaud et al., 1994). In turn, the methylation state of the maternal IAP, which is partially inherited in embryogenesis (Morgan et al., 1999), silences the cryptic promoter within the 3′LTR and allows agouti expression from the hair-cycle promoters along with normal coat colour. Therefore the coat colour of these mice provides a direct read-out of the methylation status of the IAP. In this way, it was demonstrated that the short form of DNMT1 (DNMT1o) that is produced in oogenesis and abundant during pre-implantation development plays a crucial role in the maintenance of IAP methylation; depletion of this DNMT isoform during oogenesis led to a shift in the coat colour of litters from 4% yellow to 41% yellow (Gaudet et al., 2004). The role of DNMT1 in maintaining methylation patterns in cleavage embryos is further supported by another study showing that maternal and zygotic DNMT1 is sufficient to maintain methylation at most imprinted regions in pre-implantation embryos (Hirasawa et al., 2008). In this work, inactivation of maternal or zygotic DNMT1 (but not DNMT3a or DNMT3b) during early embryogenesis correlated with a dramatic loss of methylation imprints. Like-wise, DNMT1 was also shown to maintain methylation imprints in ES cells (Okano et al., 1999). How some retroelements such as IAPs are specifically targeted for DNMT1-mediated maintenance methylation in the face of global demethylation is unknown.

Also unexplained is the finding that DNMT1, although highly expressed in preimplantation embryos, is primarily cytoplasmic and its nuclear translocation at the eight-cell stage paradoxically coincides with rapid 5meC decline (Carlson et al., 1992). This further suggests that other regulatory factors likely including IAP sequence-recognising zinc finger proteins (see below) and methyl-CpG binding proteins (Reese et al., 2007) are important in controlling DNA methylation at ERVs.

A KRAB zinc finger protein in imprinting

It has recently been reported that, for many imprinted genes, the KRAB zinc finger protein ZFP57 is necessary to protect imprints from demethylation during early development (Li et al., 2008), presumably through the recruitment of DNMT1 (Hirasawa et al., 2008). Imprinted genes exhibit monoallelic expression due to heritable parent-of-origin-dependent DNA methylation, which is established in germ cells through the action of DNMT3a and DNMT3L (Kaneda et al., 2004). The predominant role of ZFP57 appears to be in maintaining methylation at multiple imprinting control regions (ICRs) in early embryos and complete loss of both maternal and zygotic ZFP57 leads to loss of imprints, gene dysregulation and lethality around midgestation. Furthermore, in humans, mutations in this protein are associated with hypomethylation at ICRs and transient neonatal diabetes (Mackay et al., 2008), stressing the importance of imprinting in development. Interestingly, mouse ZFP57 was also shown to be necessary for the reacquisition of maternal imprints at one locus. This is in line with previous data showing that the KRAB domain is able to recruit KRAB-associated protein 1 (KAP1, discussed later) and lead to *de novo* DNA methylation specifically during the first few days of embryogenesis (Wiznerowicz et al., 2007). So far, it is not known how ZFP57 recognises its ICR targets and loss of this factor does not affect methylation at IAP or L1 repeats, implying that either only certain loci are affected or that ERVs may be protected by (a) related factor(s)(Li et al., 2008).

Stella/PGC7 guards DNA methylation at ERVs in early embryos

DNA methylation is also under the control of a maternally expressed factor Stella/PGC7/Dppa3 that is carried into preimplantation embryos and acts to protect the maternal genome from demethylation specifically at IAP elements and some imprinted regions (Nakamura et al., 2007). In contrast, L1 and some other imprinted genes are not affected by loss of Stella. It should be noted, however, that LINE control in early embryos seems less dependent on DNA methylation, since in this setting these elements exhibit around 30% DNA methylation, compared with up to 90% for IAPs (Lane et al., 2003; Nakamura et al., 2007 and see Fig. 1b). Stella-null females display reduced fertility and their deficient embryos rarely survive until the blastocyst stage (Payer et al., 2003). Interestingly, IAP methylation levels in stella deficient oocytes are indistinguishable from controls indicating that stella acts only following fertilization to maintain methylation at these ERVs in preimplantation development (Nakamura et al., 2007).

LSH1 preferentially controls methylation at ERVs over single copy genes

A SNF2 chromatin-remodelling factor known as lymphoid-specific helicase 1 (LSH1) (with a homolog DDM1 in plants) is integral to genome stability since LSH1 knockout embryos suffer a striking widespread DNA demethylation of retroelements including IAP, L1 and SINE B1 (Dennis et al., 2001), and die shortly after birth. Interestingly, cellular gene expression is largely unaffected while retroelements lose repressive histone marks (Yan et al., 2003) and become overexpressed showing some specificity of LSH1 repression (Huang et al., 2004). IAP elements also become demethylated and derepressed in female germ cells lacking LSH1 (De La Fuente et al., 2006). Furthermore, LSH1 was documented to bind retroelement DNA in embryonic cells by chromatin immunoprecipitation (Huang et al., 2004), and to be required for DNMT3a and 3b methylation of newly introduced MLV as well as endogenous genes such as Oct4 (Zhu et al., 2006).

NP95, an essential DNA methyltransferase cofactor

The SET and RING finger-associated (SRA) protein, NP95 (nuclear protein of 95 kDa), also called UHRF1 or ICBP90, was recently defined as essential for global and local DNA methylation (Bostick et al., 2007; Sharif et al., 2007), after its plant homologue V1M1 was assigned a similar role. This conserved cofactor operates to maintain CpG methylation in Arabidopsis and zebrafish genomes as well as in the mouse (Feng et al., 2010a). Although DNMT1 has a natural binding affinity for hemi-methylated DNA that facilitates the deposition of 5meC marks on the newly replicating DNA strand, it depends on NP95 for its function (see Fig. 2B), and loss of NP95 leads to loss of methylation on retrotransposons and imprinted genes. Specifically, methylation marks are lost on IAP and L1 at E9.5, a time point at which both of these retroelements should normally be fully methylated. This correlates with overexpression of IAP (around 8-fold) and to a lesser extent LINE and SINE transcripts. Consistent with this, NP95-null embryos fail to develop beyond around E9.5 like DNMT1-null embryos (see Fig. 1D). Interestingly NP95 targeting to DNA is dependent on its prior methylation (Bostick et al., 2007; Sharif et al., 2007), but paradoxically NP95 seems also involved in *de novo* methylation in ES cells (Meilinger et al., 2009) (see Fig. 2A). Indeed, NP95$^{-/-}$ cells behave similarly to DNMT3a/b$^{-/-}$ cells in that they cannot methylate newly introduced DNA (in this case the cytomegalovirus promoter). NP95 interacts through its SRA and ubiquitin-like domains with the regulatory regions of DNMT3a and DNMT3b and this interaction is necessary for promoter silencing that precedes DNA methylation (Meilinger et al., 2009). Intriguingly, NP95 can also associate with the histone methyltransferase G9a (Kim et al., 2009), another important player in the methylation of DNA (see Fig. 2).

G9a acts at the centre of histone and DNA methylation cross talk

G9a is one of 6 histone methyltransferases (HMTases) with H3K9 catalytic activity present in mammalian cells; it is involved in depositing H3K9me1 and H3K9me2 marks, yet curiously exerts a potent role in controlling DNA methylation in ES cells, which it performs independently of its HMTase activity (Dong et al., 2008; Tachibana et al., 2008). This is in contrast to the H3K9 HMTase KRYPTONITE in plants, the requirement of which for DNA methylation depends entirely on its methylation of histones (Freitag and Selker, 2005; Jackson et al., 2002). In G9a$^{-/-}$ ES cells, H3K9me3 marks remain intact at retrotransposons while DNA methylation is dramatically reduced (e.g. at MLV, IAP elements and L1 (Dong et al., 2008; Tachibana et al., 2008). The same is true for GLP$^{-/-}$ cells, presumably because this closely related HMTase forms a complex with G9a. Complementation with catalytically inactive mutants of G9a partially rescues DNA methylation indicating its HMTase function to be dispensable. It is rather the recruitment of DNMT3a that is affected and accordingly G9a$^{-/-}$ cells are impaired in their ability to *de novo* methylate newly integrated MLV vectors. Surprisingly, expression of retroelements is only mildly affected in G9a$^{-/-}$ cells compared to DNMT1$^{-/-}$ ES cells and to a lesser extent DNMT3a/b$^{-/-}$ cells, which display a slight overexpression of IAP elements. This implies that either the underlying DNA methylation is sufficient to retain retroelements in a silent state or that their transcription is still blocked likely through histone methylation.

Histone methylation complements DNA methylation

Histone N-terminal tails serve as targets for a wide range of dynamic chemical modifications including methylation, acetylation, phosphorylation and ubiquitination. Gene expression is then modulated through interactors transiently recruited by the resulting chromatin structure. This important layer of gene regulation attributed to histone modification may explain how, for example, *Drosophila* can retain control (albeit less effectively (Yoder et al., 1997) of its retroelements in spite of the absence of cytosine methylation at CpG dinucleotides and why DNMT3a/b double knockout ES cells can still silence newly integrating MLV (Pannell et al., 2000). Indeed, MLV silencing in embryonic carcinoma cells is well established to occur within 2 days, while LTR methylation is not present until 8–14 days post transduction (Gautsch and Wilson, 1983; Kempler et al., 1993; Niwa et al., 1983) (and see Fig. 3). This initial silencing rather involves histone modifications.

Here, we will focus on histone methylation because of its established role in retroelement silencing and its known link with DNA methylation. Indeed, one example of cross-talk between histone and DNA methylation is in the embryo: the maternal genome inherits its epigenetic memory from the oocyte in its histone code and when *de novo* methylation takes place, it is dependent on DNMT3L binding to unmethylated histone 3 lysine 4 sites and not to CpG islands that are enriched for H3K4 methylation (Jia et al., 2007; Ooi et al., 2007). More generally, genome-wide maps of histone and DNA methylation have shown the two to be intricately linked (Meissner et al., 2008; Mohn et al., 2008; Okitsu and Hsieh, 2007; Weber et al., 2007), with methylation of lysine 9 or 27 of histone 3 being positively correlated while lysine 4 H3 methylation is anticorrelated.

ERVs are silenced by histone 3 lysine 9 methyltransferases

Histone 3 lysine 9 marks are observed at LTR elements in ES cells (Martens et al., 2005; Mikkelsen et al., 2007) and could potentially be written by six HMTases, including the above mentioned G9a, most of which also directly or indirectly recruit DNMT proteins (Epsztejn-Litman et al., 2008; Fuks et al., 2003; Lehnertz et al., 2003; Li et al., 2006). Each member is critical at a distinct time during development as indicated by gene disruption studies in mice: double knockout of Suv39h1 and Suv39h2, which trimethylate H3K9 at satellite repeats, severely impairs viability, although embryos develop normally until E12.5 (Lehnertz et al., 2003; Peters et al., 2001). Knockout of G9a or GLP, which catalyse H3K9 di and trimethylation in euchromatin, is lethal at E9.5 (Tachibana et al., 2002; Tachibana et al., 2005). Finally, knockout of ESET (SETDB1), which mediates H3K9 di and trimethylation is lethal at E3.5–E5.5 (Dodge et al., 2004). The most interesting of these in terms of ERV regulation is ESET because it is required precisely when ERVs become inactivated (Jahner et al., 1982) and correspondingly, ESET$^{-/-}$ ES cells lose transcriptional control of

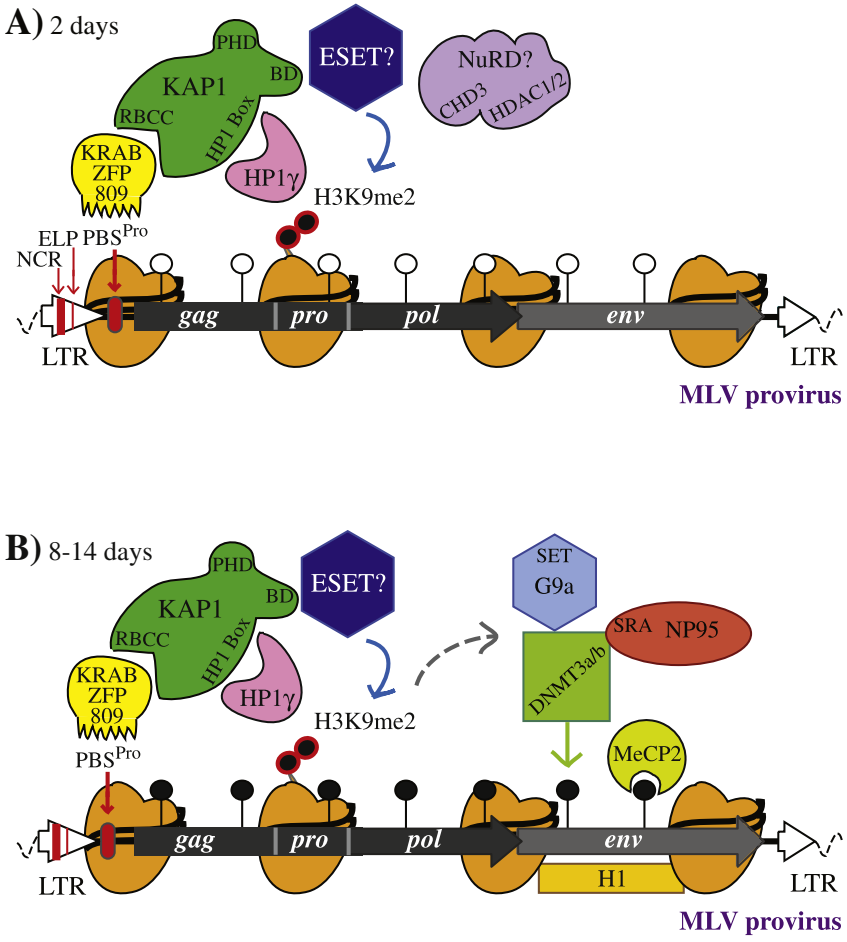

Fig. 3. MLV is potently silenced in embryonic cells. (A) Silencing is rapid and precedes DNA methylation: *Cis*-acting sequences inducing silencing include the NCR (negative control region) and an ELP-binding site, but the most potent is the PBS that recruits a ZFP809–KAP1 complex and HP1γ leading to H3K9me2 (Wolf et al., 2008a; Wolf and Goff, 2007, 2009). KAP1 may also recruit ESET and the NuRD complex as shown for other contexts (Matsui et al., 2010; Schultz et al., 2001). (B) MLV is sustained in a silent state through multiple mechanisms: DNA methylation is progressive following silencing and reaches around 80% after 8–14 days. In EC cells, stably silent MLV proviruses are enriched for KAP1 (Wolf and Goff, 2007), DNA methylation, MeCP2 and histone H1 (Pannell et al., 2000). Silencing and DNA methylation of introduced MLV is also dependent on DNMT3a/b, G9a and NP95, which may be attracted to ERVs through histone methylation or unknown factors (Meilinger et al., 2009).

MusD and IAP elements (Matsui et al., 2010), (see section on KAP1) and, curiously have been reported to have an ability to form trophoblast-like cells (Bilodeau et al., 2009; Yuan et al., 2009). Another histone methylation mark associated with ERVs is H4K20me3, which is highly enriched at ETn/MusD and IAP elements and overlaps with H3K9me3 (Kourmouli et al., 2004; Martens et al., 2005; Mikkelsen et al., 2007; Schotta et al., 2004). Intriguingly, H3K27 methylation has also been reported to overlap with H3K9 methylation in ES cells (Mikkelsen et al., 2007), in support of data implicating polycomb group proteins in ERV silencing (Golding et al., 2010; Leeb et al., 2010), a pathway implicated in plants in preventing re-replication and expression of transposon DNA (Jacob et al., 2010).

Although the functional consequences of H3K9me are not completely understood, DNA and histone methyl marks together lead to the assembly of more compact chromatin that further attracts effectors of gene silencing; heterochromatin proteins (HP1), for example, recognise an interface created by H3K9methylation (Bannister et al., 2001; Lachner et al., 2001). Also, silent chromatin is bound by the linker histone H1 (Pannell et al., 2000) that allows contraction of nucleosomes. An important question, though, is still which factors represent correlates of repression and which act as the controllers?

Histone deacetylation suppresses ERV transcription

Histone deacetylation is another key histone modification involved in silencing viral genes and is well known to take place on histone 3 and histone 4 tails at lysine residues (Chen and Townes, 2000; Lorincz et al., 2000; Schubeler et al., 2000). Treatment of cells with the HDAC inhibitor TSA reactivates virus-like 30S elements, a subgroup of LTR-retrotransposons. Interestingly, this process is greatly enhanced if the MAP kinase pathway is activated to deposit a histone 3 serine 10 phosphorylation mark. This indicates that this retroelement is regulated by a dual histone mark of phosphoacetylation (Brunmeir et al., 2010). Crosstalk can also occur between DNA methylation and histone deacetylation because the methyl-CpG-binding protein MeCP2 interacts with deacetylase complexes (Jones et al., 1998; Nan et al., 1998). Histone methylation is further linked to histone deacetylation, illustrated by the histone demethylase LSD1/KDM1A that demethylates the active marks H3K4 mono and dimethylation (Shi et al., 2004) and is in turn connected to HDAC1 and HDAC2 (You et al., 2001). Histone deacetylation further plays a key role in silencing IAP elements (Rowe et al., 2010), as well as introduced L1 constructs in embryonic cells (Garcia-Perez et al., 2010).

Higher order dimensions of ERV silencing

Transcriptional repression is often maximised in specific nuclear subdomains. Imprinted clusters are found in such higher order repressive nuclear compartments, the formation of which depends on polycomb group proteins (Terranova et al., 2008). Interestingly, the creation of a silent nuclear compartment in X-inactivation has recently been reported to involve both silent and active L1s resident on the X-chromosome (Chow et al., 2010). Silent L1s are thought to mediate the assembly of this compartment, while active L1s may mediate the RNA-directed spread of silencing.

As well, it has been observed from yeast to humans that repression of blocks of genes is broadly achieved by targeting them to the nuclear periphery (Dekker, 2008; Fraser and Bickmore, 2007; Towbin et al., 2009), although this concept remains largely unexplored in terms of ERV regulation. Alternatively, some retrotransposons like gypsy are already targeted to silent nuclear compartments during integration under the influence of the chromodomain present in their integrase (Gao et al., 2008). In contrast, mammalian ERVs tend to integrate, like their exogenous counterparts, near active genes (Brady et al., 2009; Schroder et al., 2002; Zhang et al., 2008). The more frequent detection of ERVs outside of genes or running in antisense orientation to them is thus the result of a counter-selection.

A recent study classified chromatin into five distinct types in *Drosophila* embryonic cells due to their different profiles of associated proteins as defined by DamID technology (Filion et al., 2010). This method involves fusing your protein of interest to a DNA adenine methyltransferase (Dam) so that an adenine-methylation footprint will act as a stable readout for binding sites of the selected protein (van Steensel et al., 2001). Three repressive types of chromatin were identified this way and one of these, designated as black chromatin, covered half the genome and intriguingly exerted the strongest repressor activity, yet lacked classical heterochromatin markers. It also encompassed the longest domains that could extend to over 100 kb in size. Whether this compartment applies to mammalian cells and is most enriched for certain retroelements is unknown but if so, such long distinct domains could explain another layer of ERV silencing as well as how, for example, IAP elements can influence cellular genes 100 kb distant from them (Duhl et al., 1994; Michaud et al., 1994; Morgan et al., 1999).

Finally, differentiation itself is known to exert an extra level of ERV repression as has also been described for MLV; even MLV-derived vectors modified to escape initial repression in ES cells through removal of *cis*-acting sequences ultimately suffer silencing when the cells undergo differentiation (Niwa et al., 1983), while no such phenomenon is observed if these vectors are introduced in already differentiated cells (Teich et al., 1977). An attractive hypothesis would be that, during differentiation, ERVs are pulled into LOCKs (large organised chromatin K9 modifications), which are silent chromatin blocks of up to 2.9Mb that spread to cover around 31% of the genome in differentiated cells, compared to 4% in undifferentiated ES cells (Wen et al., 2009). These LOCKs are covered with G9a-dependent H3K9me2, thought to be anchored to the nuclear membrane and are tissue specific to silence genes not needed in a particular cell type. Whether LOCKs explain ERV silencing during differentiation and whether they are retained in LOCKs depending on where they are integrated or through an ERV-directed mechanism is unknown.

Targeted silencing in embryogenesis by KAP1 and its KRAB-ZFP partners

As discussed, there is a plethora of data that show a clear correlation between DNA methylation or certain histone marks and a transcriptionally inactive state of viral genes, as well as crosstalk between the two (reviewed in Cedar and Bergman (2009)). On the other hand, very little is understood concerning how retroelements are specifically targeted for writing and maintaining methylation marks at their DNA and histones. One pathway that links specificity to silencing machinery involves the KAP1 corepressor and its DNA binding partners, the KRAB-zinc finger proteins, which together constitute the largest family of transcription factors in mammals (Huntley et al., 2006). The importance of this regulatory pathway is illustrated by the early embryonic lethality of the KAP1 knockout mouse at E5.5–E6.5 (Cammas et al., 2000) as well as lethality in ES cells following widespread activation of hundreds of genes (Rowe et al., 2010).

KRAB-ZFPs (Krüppel-associated box domain-zinc finger proteins) are encoded in the hundreds by the mouse and human genomes (Emerson and Thomas, 2009; Hamilton et al., 2006) and they target specific DNA sequences through their zinc finger motifs, while recruiting KAP1 (KRAB-associated protein 1, also known as tripartite motif-containing protein 28, TRIM28 or transcription intermediary factor beta, TIF1b) with their conserved KRAB domain. KAP1 then acts as a scaffold for a silencing complex that involves the histone methyltransferase ESET, heterochromatin proteins in the HP1 family and the NuRD histone deacetylase complex (Ayyanathan et al., 2003;

Cammas et al., 2004; Ivanov et al., 2007; Satou et al., 2001; Schultz et al., 2002, 2001; Sripathy et al., 2006; Underhill et al., 2000; Zeng et al., 2008), although remarkably, the in vivo targets of KAP1 are largely unknown. Several KAP1 target genes have recently been characterised (Chang et al., 2009; O'Geen et al., 2007; Riclet et al., 2009; Takahashi et al., 2009; Wolf et al., 2008a; Wolf and Goff, 2007, 2009) and among these, one striking example is the MLV primer binding site (PBS) that is complementary for proline tRNA, a previously well-characterised embryonic cell specific *cis*-acting repressor (Akgun et al., 1991; Barklis et al., 1986; Feuer et al., 1989; Haas et al., 2003; Linney et al., 1984; Loh et al., 1987, 1990; Niwa et al., 1983; Petersen et al., 1991; Teich et al., 1977; Tsukiyama et al., 1989; Vernet and Cebrian, 1996; Yamauchi et al., 1995). The 18 nucleotide-long MLV PBS^{Pro} is targeted by a KRAB-ZFP809–KAP1 complex in a highly sequence-specific fashion, single point mutations sufficing to prevent this event (Wolf and Goff, 2007, 2009)(see Fig. 3). Interestingly, an independent PBS sequence, complementary for Lys1, 2 tRNA that is used by some other retroviruses including visna, spuma and Mason–Pfizer monkey virus also mediates repression (Modin et al., 2000; Yamauchi et al., 1995) through KAP1 (Wolf et al., 2008b) and presumably a distinct KRAB-ZFP.

We previously found that KAP1 plays a role in *de novo* DNA methylation of a KRAB-controlled lentiviral vector precisely when (E3.5–E8.5) ERVs normally become silenced (Wiznerowicz et al., 2007). This prompted us to evaluate the potential role of KAP1 in resetting ERVs in a transcriptionally silent state during embryogenesis. We found that conditional knockout of KAP1 in murine embryonic stem cells led to the overexpression of IAP elements (by around sixty fold) as well as MusD, MERVL, MERVK and to a lesser extent L1 (Rowe et al., 2010). We focused our further studies on IAPs because they are particularly active (Dewannieux et al., 2004; Zhang et al., 2008) and known to cause spontaneous mutations in laboratory mice. It was recently demonstrated that IAPs, which no longer express an envelope and bud in the endoplasmic reticulum, originated from full-length retroviruses that once infected the germ line (Ribet et al., 2008). We found that IAPs are highly overexpressed (up to 1000×) in $KAP1^{-/-}$ early embryos, specifically in the epiblast where KAP1 is normally enriched. In contrast, IAPs are not re-activated by disruption of KAP1 in embryonic fibroblasts (Rowe et al., 2010). This is consistent with the lower (twenty times less) level of KAP1 protein in these cells and the notion that once silenced in development, such genomic viruses remain controlled in adult tissues by DNA methylation that is stably inherited (Martens et al., 2005; Meissner et al., 2008; Mikkelsen et al., 2007).

Additionally, we found KAP1 to be associated with IAP proviruses by chromatin immunoprecipitation studies, particularly at the 5′UTR region, and that such sequences could mediate KAP1-dependent repression of a reporter in ES cells. Knockout of KAP1 led to loss of histone methylation and deacetylation marks consistent with a model whereby active genomic viruses are controlled through direct docking of a KAP1-containing repressor complex that resets them in a silent state during development by histone modifications (Rowe et al., 2010). This data also suggests that neighbouring genes could be regulated through KAP1 binding to ERV platforms, especially since it was found that KAP1 can mediate long-range repression (Groner et al., 2010). We postulate that this ERV-targeting transcriptional control has been co-opted to regulate cellular gene expression during early development.

These results were corroborated by the demonstration that IAPs and MusD are dramatically upregulated in ES cells deleted for ESET, the histone methyltransferase that was suspected to act downstream of KAP1 (Matsui et al., 2010). By comparison, only a mild reactivation of these retroelements was observed in other HMTase knockout ES cells or upon ESET deletion in embryonic fibroblasts, consistent with the demonstration of ESET binding to proviruses and the presence of histone marks specifically in ES cells (Matsui et al., 2010). Class III ERVs and non-LTR retroelements were less affected by disruption of this pathway. Interestingly, it was also shown that in DNMT triple knockout cells, ESET and KAP1 binding is preserved as well as H3K9me3, explaining why ERV silencing is largely maintained in these cells (Matsui et al., 2010; Tsumura et al., 2006) and during embryogenesis when DNA methylation is reprogrammed. DNA methylation and KAP1 act synergistically to silence ERVs (Rowe et al., 2010) and furthermore, when ESET is removed, DNA methylation is eventually lost at an introduced MSCV vector presumably due to loss of histone methylation (Matsui et al., 2010), showing that a KAP1-ESET complex is necessary to establish a silent transcriptional state in early embryos. It seems that, during development, the key mechanism to silence ERVs is H3K9me3 while DNA methylation is often present but dispensable, whereas in differentiated cells, DNA methylation is key while H3K9me3 is absent at ERVs (Mikkelsen et al., 2007) (summarised in Fig. 4).

These studies imply that a series of sequence-specific KRAB-ZFPs most likely act in early embryogenesis to bridge KAP1 to ERV sequences. They remain to be formally identified, but it is likely that a significant fraction of the over 250 KRAB-ZFPs found to be expressed in ES cells were originally selected to target ERVs (Rowe et al., 2010). Indeed, phylogenetic analyses have revealed that KRAB-ZFP genes appeared with the first tetrapods, and have since then been under strong positive selection (Emerson and Thomas, 2009) and subject to rapid expansion (Waterston et al., 2002), adding fuel to the hypothesis that viruses within our genome have acted as moving targets in relentless genetic conflict with zinc finger proteins necessary for their control.

Importantly, aside from partnering KRAB-ZFPs in repression, KAP1 has pleiotropic functions perhaps controlled by its different post-translational modifications (Ivanov et al., 2007; Li et al., 2007, 2010; Ziv et al., 2006), some of which may be shared with its TIF1 *Drosophila* homolog, Bonus (Beckstead et al., 2005), which presumably doesn't interact with KRAB-ZFPs, that are absent from *Drosophila* only having undergone rapid expansion in mammals (Birtle and Ponting, 2006; Emerson and Thomas, 2009; Tadepally et al., 2008; Urrutia, 2003).

Small RNAs silence ERVs in the germ line: expression drives repression

In plants it is well established that somewhat paradoxically, transcription is needed to drive gene silencing through small RNA-mediated *de novo* DNA methylation (reviewed in Bourc'his and Voinnet (2010); Law and Jacobsen (2010)). By analogy, the mammalian genome may undergo expression following methylation erasure in order to reset DNA methylation patterns on retroelements during development, so that silencing becomes stabilized in adult tissues. Such control of retroelements by small RNAs appears a relevant approach to regulate diverse families of highly polymorphic mobile elements by relying on their own sequences to target silencing machinery. A piwi-interacting RNA (piRNA) pathway that acts in germ cells is well described in *Drosophila* (Aravin et al., 2001): piRNAs are 24–30 nt long, derived from repeat-rich clusters and their processing is dicer-independent. Instead, antisense, piRNAs bind Piwi or Aubergine proteins that direct cleavage of sense mRNA from retroelements, which produces sense piRNAs that bind Ago3, which directs cleavage of antisense mRNAs, leading to ping-pong amplification of silencing (Aravin et al., 2007a; Brennecke et al., 2007; Gunawardane et al., 2007). Furthermore, *Drosophila* Piwi associates with HP1 and chromatin at H3K9me3 sites also implicating piRNAs in transcriptional gene silencing of repeats (Brower-Toland et al., 2007). Similar germ line-specific Piwi-like proteins termed Miwi, Mili and Miwi2 are also found in the mouse (Girard et al., 2006). In Mili mutant oocytes, IAP transcripts are elevated by 3.5 fold (Watanabe et al., 2008) and, interestingly, loss of Mili and Miwi2 in the male germ line leads to sterility, L1 activation and impaired *de novo* CpG methylation

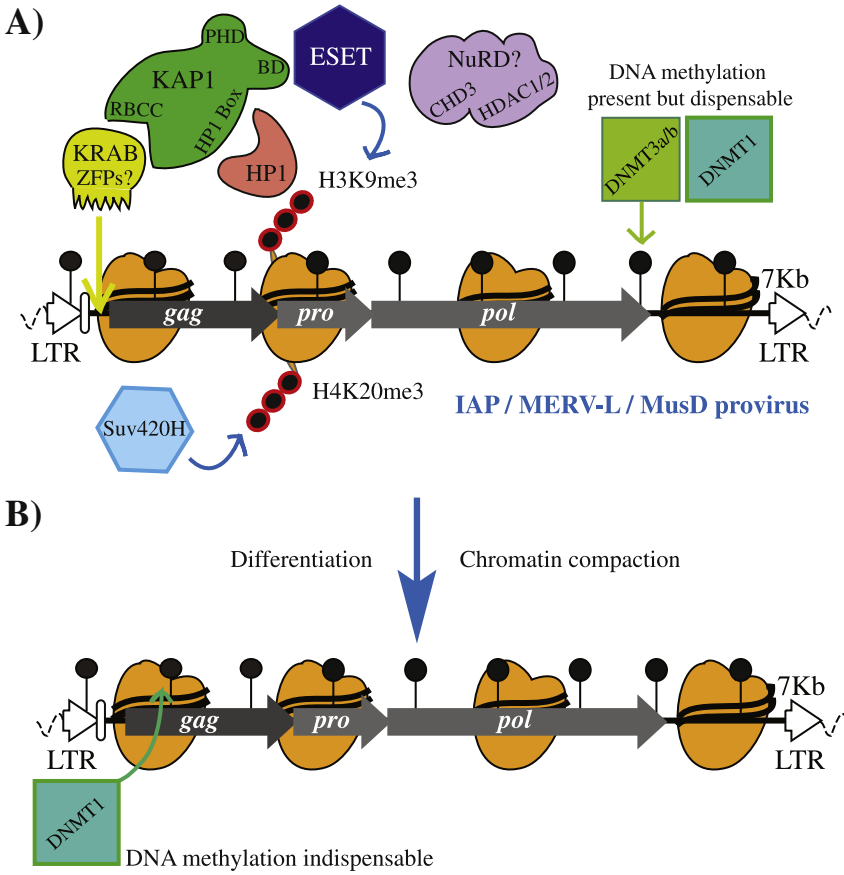

Fig. 4. Silencing of ERVs from ES cells and early embryos to adult tissues. (A) ERV control in ES cells where chromatin is in a dynamic state: Several active ERVs are enriched in ES cells for KAP1 (particularly at the 5′UTR), HP1, ESET, H3K9me3 and H4K20me3 (Matsui et al., 2010; Rowe et al., 2010). It is not known if KRAB-ZFPs or the NuRD complex play a role in this pathway as shown for other contexts (Schultz et al., 2001; Wolf and Goff, 2009). These proviruses are also all highly DNA methylated, but this mechanism is not the main regulator in ES cells but rather H3K9me3. (B) Differentiation brings another layer of ERV control where chromatin is set in a fixed state: KAP1 and H3K9me3 are lost at ERVs in differentiated cells and DNA maintenance methylation becomes crucial (Matsui et al., 2010; Rowe et al., 2010; Walsh et al., 1998). This process involves chromatin reorganisation including into large silent domains where ERVs may be retained (Wen et al., 2009).

of IAPs and L1 (Aravin et al., 2006; Aravin and Bourc'his, 2008; Aravin et al., 2008, 2007b; Carmell et al., 2007; Deng and Lin, 2002; Kuramochi-Miyagawa et al., 2008).

Dicer-dependent small interfering RNAs (siRNAs) that are produced from long double-stranded RNAs also cooperate to suppress retroelements during development: dicer knockout ES cells display elevated levels of IAPs and L1 and similarly, dicer-depleted early embryos suffer an increase in IAPs and MERV-L that give rise to both sense and antisense transcripts owing to their bidirectional promoters (Svoboda et al., 2004). Dicer knockout in mouse oocytes leads to an increase particularly in some retrotransposons (e.g. retrotransposon LTR 10 was 5-fold increased) (Tam et al., 2008; Watanabe et al., 2008). L1 siRNAs have even been detected in cell lines (Yang and Kazazian, 2006).

A network of restriction pathways

Although restriction factors probably now act mainly to counter somatic infections of exogenous retroviruses (reviewed in Wolf and Goff (2008)), they may also act to prevent amplification of ERVs. For example, APOBEC3s reduce L1, Alu, IAP and MusD activity (Esnault et al., 2005) and reviewed in Chiu and Greene (2008) and Turelli and Trono (2005), and may act as important post-transcriptional ERV blockers in early embryos and germ cells where they are expressed. L1 may also be blocked by DNA-repair mechanisms (Gasior et al., 2008). Trex prevents accumulation of reverse-transcribed DNA from L1 and IAPs (Stetson et al., 2008) and reviewed in (Goodier and Kazazian, 2008). L1 is controlled within a germ cell-specific organelle composed of the mouse protein Mael, the disruption of which leads to L1 derepression (Soper et al., 2008). Several other germ cell expressed genes are important in spermatogenesis with Tex19.1 specifically repressing the class II ERV, MMERVK10C (Ollinger et al., 2008) and the zinc finger protein Gtsf1 acting on L1 and to a lesser extent IAPs (Yoshimura et al., 2009). Since many retroelements possess bidirectional promoters they may even inhibit themselves by antisense transcription. The immune system too likely selectively clears any cells harbouring ERVs due to their presentation of novel ERV antigens or due to as yet unidentified cell-type specific intracellular sensors that detect retroviral and possibly ERV capsids (Manel et al., 2010).

Most interestingly, endogenous envelopes or Gag proteins themselves can confer resistance to exogenous retroviral infection. The best example of this is the restriction factor Fv1 that resembles a MERV-L Gag protein and blocks MLV after reverse transcription (Best et al., 1996) likely through a capsid interaction similar to that effected by human TRIM5alpha on the capsids of MLV and other viruses including those of ancient retroviruses (Huthoff and Towers, 2008; Kaiser et al., 2007). The expression of ERVs in stem cells may thus protect the host from further assaults.

Control beyond development

ERVs are generally regulated during development (Fig. 1) and accordingly the expression of many ERV controllers such as DNMT3a/b, and DNMT1 is predominant in germ cells, early embryos and ES cells (Carlson et al., 1992; Howlett and Reik, 1991). Furthermore, microarray atlas data (Su et al., 2004), indicate Stella, LSH1, KAP1,

DNMT3L, NP95, ESET, Suv39h1, Suv39h2, HDAC1, Mili, Miwi, and Mael to also be predominantly expressed in embryos, ovary, testes or thymus. It therefore follows that, in differentiated cells, H3K9me3 and H4K20me3 are lost at ERVs (Martens et al., 2005; Matsui et al., 2010; Mikkelsen et al., 2007) presumably because they are no longer needed due to independent silencing mechanisms (Wen et al., 2009). However interestingly, retroviral silencing is not strictly stem cell-specific as there are some reports of immediate or progressive silencing of introduced retroviruses for example in early passage mouse embryonic fibroblasts (MEFs) and bone marrow stromal cells (Haas et al., 2003). This implies that active ERV silencing may take place in specific adult tissues and may even relate to HIV latency (reviewed in (Trono et al., 2010)); while this process may depend on the genomic context of certain integrants, there are certain cell types prone to latency and in turn tissue-specific factors that play an active role in inducing latency. Such novel latency-related factors will no doubt be discovered in the coming years.

Dysregulation of ERVs

ERVs have long been suspected to play roles in cancer, autoimmunity or immune suppression, which could result from loss of their regulation (reviewed in (Balada et al., 2009; Moyes et al., 2007)). Importantly, it still remains to be determined if ERV silencing is properly maintained during reprogramming of somatic cells to induced pluripotent stem (iPS) cells (Okita et al., 2007; Takahashi and Yamanaka, 2006; Wernig et al., 2007). Good vs. bad iPS clones (those that are germ line competent vs. not) may even reflect differences in ERV expression. Interestingly, silent blocks of chromatin termed LOCKS (see above) are found to be lost in cancer cell lines, explaining an increase in genome plasticity in these cells and perhaps also the subsequent overexpression of ERVs (Ono et al., 1987; Schiavetti et al., 2002), the antigens of which might prove beneficial as tumour vaccines.

Conclusions

The present review described our growing understanding of the complex regulatory networks responsible for inactivating the expression of retroelements during early development. Converging recent data additionally suggests that development itself is in fact controlled by temporal and spatial expression and repression of retroelements, and that some cellular genes crucial during this period are co-regulated with ERVs and related entities. This concept still remains quite unexplored, as do the identities of the stem cell-specific factors thought to target gene silencing to ERVs, LINEs and other retrotransposons. Improving our understanding of these factors and networks and of their impact on the genome will facilitate the comprehension not only of the early phases of mammalian development but also of pathologies that may stem from their dysregulation, such as autoimmunity and cancer.

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