Repression of Retrotransposonal Elements in Mouse Embryonic Stem Cells Is Primarily Mediated by a DNA Methylation-independent Mechanism∗§

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In defense of deleterious retrotransposition of intracisternal A particle (IAP) elements, IAP loci are heavily methylated and silenced in mouse somatic cells. To determine whether IAP is also repressed in pluripotent stem cells by DNA methylation, we examined IAP expression in demethylated mouse embryonic stem cells (mESCs) and epiblast-derived stem cells. Surprisingly, in demethylated ESC cultures carrying mutations of DNA methyltransferase 1 (Dnmt1), no IAP transcripts and proteins are detectable in undifferentiated Oct4+ ESCs. In contrast, ~3.6% of IAP-positive cells are detected in Oct4− Dnmt1−/− cells, suggesting that the previously observed increase in IAP transcripts in the population of Dnmt1−/− ESCs could be accounted for by this subset of Oct4− Dnmt1−/− ESCs undergoing spontaneous differentiation. Consistent with this possibility, a dramatic increase of IAP mRNA (>100-fold) and protein expression was observed in Dnmt1−/− ESC cultures upon induction of differentiation through the withdrawal of leukemia-inhibitory factor for 6 or more days. Interestingly, both mRNAs and proteins of IAP can be readily detected in demethylated Oct4+ epiblast-derived stem cells as well as differentiated mouse embryoblasts, neurons, and glia upon conditional Dnmt1 gene deletion. These data suggest that mESCs are a unique stem cell type possessing a DNA methylation-independent IAP repression mechanism. This methylation-independent mechanism does not involve Dicer-mediated action of microRNAs or RNA interference because IAP repression is largely maintained throughout embryogenesis (19, 20). In established lines of mouse embryonic stem cells (ESCs) derived from the inner cell mass, IAP elements are already heavily methylated with little detectable IAP expression (21).

When expressed, IAP proteins are non-infectious viral particles that are retained in the cisternae of the endoplasmic reticulum (16). IAP proteins can be transiently detected in preimplantation embryos (17, 18), although DNA methylation of IAP elements is largely maintained throughout embryogenesis (19, 20). In established lines of mouse embryonic stem cells (ESCs) derived from the inner cell mass, IAP elements are already heavily methylated with little detectable IAP expression (21).

Here, we examine expression of IAP proteins in control and demethylated somatic cells, EpiSCs, and mESCs. Using a previously generated antibody against IAP protein (p73) (22) as well as our polyclonal antibody raised against recombinant IAP gag protein (9, 23), we mapped changes in IAP protein levels after Dnmt1 gene deletion in floxed Dnmt1loxP/loxP mouse embryoblasts (MEFs) using virus-mediated cre recombinase. Unexpectedly, we found that IAP mRNA and protein levels are not detected in Oct4+ Dnmt1−/− ESCs but are dramatically increased in demethylated mESC cultures upon in vitro differentiation, suggestive of the presence of DNA methylation-independent repression mechanisms that include de novo (Dnmt3a and -3b) and maintenance methyltransferases (Dnmt1) (1, 2). DNA methylation is known to regulate developmental gene expression, genomic imprinting, X-inactivation, and genomic stability (3–6). Many retrotransposon elements are heavily methylated, and current evidence supports a causal relationship between DNA methylation and repression of retrotransposons (7–9). Intracisternal A particles (IAPs) are murine endogenous retroviral repetitive elements, with an estimation of over 1000 copies across the haploid murine genome (10–12). Germline mutations due to IAP retrotransposition most often occur at intrinsic sites, disrupting gene expression through premature termination, aberrant splicing, or viral LTR-driven transcription (13). Furthermore, insertional mutagenesis of IAP elements is noted in various murine cancer cell lines with subsequent activation of oncogenes or cytokine genes (14, 15).

DNA methylation, an epigenetic modification where a methyl group is covalently added to the cytosine of CpG dinucleotides, is catalyzed by a family of DNA methyltransferases (Dnmts)2 that include de novo (Dnmt3a and -3b) and maintenance methyltransferases (Dnmt1) (1, 2). DNA methylation is known to regulate developmental gene expression, genomic imprinting, X-inactivation, and genomic stability (3–6). Many retrotransposon elements are heavily methylated, and current evidence supports a causal relationship between DNA methylation and repression of retrotransposons (7–9). Intracisternal A particles (IAPs) are murine endogenous retroviral repetitive elements, with an estimation of over 1000 copies across the haploid murine genome (10–12). Germline mutations due to IAP retrotransposition most often occur at intrinsic sites, disrupting gene expression through premature termination, aberrant splicing, or viral LTR-driven transcription (13). Furthermore, insertional mutagenesis of IAP elements is noted in various murine cancer cell lines with subsequent activation of oncogenes or cytokine genes (14, 15).

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* This work was supported, in whole or in part, by National Institutes of Health Grant RO1 NS051411 and P01 GM081621 (to G. F.). This work was also supported by California Institute of Regenerative Medicine Grant RC1-0111 (to G. F.) and National Research Service Award 5F31NS051 (to L. K. H.).

§ The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. 1.

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‡ The abbreviations used are: Dnmt, DNA methyltransferase; IAP, intracisternal A particle; ESC, embryonic stem cell; mESC, mouse ESC; EpiSC, epiblast-derived stem cell; MEF, mouse embryoblast; GST, glutathione S-transferase; LIF, leukemia-inhibitory factor; RT, reverse transcription; FISH, fluorescent in situ hybridization; PBS, phosphate-buffered saline; LTR, long terminal repeat; MSCV, murine stem cell virus; GFP, green fluorescent protein; Cre, cre recombinase; En, embryonic day n.
sion mechanism(s) in silencing IAP expression in undifferentiated mESCs.

EXPERIMENTAL PROCEDURES

Antigen Generation and Anti-IAP2 Purification—Sequence from plasmid M1A14 (23) was used to clone the IAP2 fragment (amino acids 250–750) into pGEX4T3 vector (Fig. 1) (9). After isopropyl 1-thio-β-D-galactopyranoside induction, bacteria were harvested, and the IAP2-GST fusion protein was purified with glutathione beads (Amersham Biosciences). After evaluation of the thrombin-cut eluate, antigen was shipped to PickCell Laboratories for rabbit immunoinjection. Rabbit polyclonal serum was tested and evaluated (Rabbit polyclonal serum was tested and evaluated (Rabbit polyclonal serum was tested and evaluated before purification. Briefly, GST-IAP2 fusion protein was adsorbed onto glutathione beads (Roche Applied Science), fixed by 20 mM dimethyl pimelimidate in 200 mM HEPES buffer, pH 8.5, and terminated in 200 mM Tris-HCl buffer, pH 8.3. Antibody bound to IAP2-GST fusion protein was washed with Tris-buffered saline and eluted by 200 mM glycine (pH 2) followed by neutralization with 1 mM Tris-HCl, pH 8.5, buffer to pH 7.4. Eluates were examined by immunoblotting at different dilutions in three independent trials to verify specificity.

Viral Production and Infection—MSCV-Cre-GFP and MSCV-GFP were generated in HEK 293T cells via calcium phosphate transfection. For viral infection, cells were trypsinized and resuspended in 1 ml of cell medium (90% Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum, 1× penicillin/streptomycin, 0.1 mM non-essential amino acid, 0.1 mM β-mercaptoethanol, 505 units LIF/ml) and passaging using trypsin-EDTA. For partial differentiation, mouse ESCs were plated on 0.2% gelatin B-coated plates and coverslips. After 24 h (Day 0 time point), medium was changed to LIF− differentiation medium (90% Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum, 1× penicillin/streptomycin, 1 mM glutamine). Medium was changed every day, with corresponding wells harvested for DNA, RNA, protein, and coverslips over the time course (Days 0–12).

Immunoblotting—The immunoblotting procedure was performed as described previously (29). The original IAP antibody (anti-p73, clone 37.11X from Dr. Kira Lueders) was used at a 1:2000 dilution for Western blots. Anti-glyceraldehyde-3-phosphate dehydrogenase (Abcam; 1:5000) was used as loading control.

RNA Purification and Quantitative RT-PCR—Total RNA was harvested by TRIzol as per the manufacturer’s instructions (Invitrogen). RNA was converted to cDNA using the iScript reaction kit (Bio-Rad). Quantitative RT-PCR was performed using Bio-Rad MyCycler and SYBR Green Supermix (Bio-Rad). Results were normalized to 18 S values expressed as -fold change relative to corresponding control values (30). p values were assessed using Student’s t test (two-tailed, paired). Forward and reverse primers were as follows: IAP forward, 5′-AAG CCC TTT TGT TCC TTT TCA; IAP reverse, GCC CTG TAA TTG GAA TGA GTC CAC TT; 18 S forward, GCC CTG TAA TTG GAA TGA GTC CAC TT; 18 S reverse, CTC CCC AAG ATC CAA CTA CGA GCT TT.

IAP2 Fluorescent in Situ Hybridization (FISH) and Northern Analysis—IAP2 (amino acids 250–700) sequence was PCR-amplified from the MIA14 plasmid gel purified (Promega). 100 ng of IAP2 template was used to generate a Cy3-labeled cDNA probe using the Bioprime kit (Roche Applied Science) as per the manufacturer’s instructions. FISH was carried out as previously described (31). The same amplified region was used to create a Northern probe. Northern blot analysis was performed using the standard procedure in our previous publication (29).

Immunostaining—Coverslips were picked and fixed in 4% paraformaldehyde for 15 min and washed three times (5 min each) with 1× PBS. Blocking and permeabilization were carried out for 1 h with 0.5% Triton X-100 in PBS plus 10%...
normal goat serum. Primary antibody diluted in PBS plus 1% normal goat serum and 0.1% Triton X-100 was added to cover slips and incubated overnight at room temperature. Primary antibodies used were the following: anti-IAP2 (rabbit polyclonal, 1:1000) and anti-Oct 3/4 (Santa Cruz Biotechnology, Inc.; mouse monoclonal, 1:20). Anti-H4K20 3me (Millipore; mouse monoclonal, 1:500) was incubated at room temperature for 1 h. Cover slips were washed, and secondary antibody (Jackson Immunoresearch) diluted in PBS plus 1% normal goat serum and 0.1% Triton X-100 was added for 1 h.

Section immunohistochemistry was performed as described previously (32). The original anti-IAP (p73) antibody was diluted (1:500) in PBS containing 5% normal goat serum, 3% bovine serum albumin, and 0.25% Triton X-100 overnight at room temperature. Our IAP2 antibody was used at 1:1000 in PBS plus 5% normal goat serum and 0.25% Triton X. The next day, the sections were washed in 1× PBS three times for 10 min each, and then secondary antibody (Jackson Immunoresearch) was applied for 2 h at room temperature.

Confocal Fluorescence—Images were taken at 63 magnification using Leica confocal software on a Leica TCS-SP MP Confocal and Multiphoton Inverted Microscope (Heidelberg, Germany) equipped with an argon laser (488-nm blue excitation, JDS Uniphase), a 561-nm (green) diode laser (DPSS, Melles Griot), a 633-nm (red) helium-neon laser, and a two-photon laser setup consisting of a Spectra-Physics Millenia X 532-nm green diode pump laser and a Tsunami Ti-Sapphire picosecond pulsed infrared laser tuned at 768 nm for UV excitation.

RESULTS

IAP Antiserum Detects the gag Protein Encoded in IAP Elements, Which Is Expressed in Demethylated Fibroblast Cells—Given that IAP repeats are heavily methylated by Dnmt1, we asked whether IAP protein could be detected in DNA demethylation models. The original p73 antiserum also recognizes other proteins containing partial products of IAP coding regions (23) (Fig. 1B). For specific recognition of IAP gag protein product, we created a recombinant protein fusing GST to a partial fragment of IAP gag protein (IAP2, amino acids 251–699) (Fig. 1A). Once purified over IAP2-GST columns, the original serum yielded a clean p73 band via immunoblot in tissue possessing over 95% Dnmt1−/− cells in the central nervous system (32) (Fig. 1B). The purified serum was partially blocked by preadsorption against IAP2 peptide fragment, indicating the specificity of antiserum (data not shown).
used this recombinant IAP2 protein fragment to generate a separate polyclonal antibody for both immunoblotting and immunostaining assays to detect IAP protein expression (Fig. 1B). To examine the onset of IAP protein reactivation after Dnmt1 gene deletion, Dnmt12lox/2lox MEFs were infected with retrovirus containing cre recombinase fused to green fluorescent protein (MSCV-Cre-GFP) (33). Significant genomic demethylation was detected after 4 days postinfection via Southern blotting for the IAP repeat probe (Fig. 2A). Because DNA methylation levels dropped after the deletion of Dnmt1, we found reactivation of IAP protein translation in infected cells starting 5 days postinfection via immunoblot (Fig. 2B). When individual cells were examined for IAP immunoreactivity, IAP protein was detectable in a minority of infected cells as early as 4 days postinfection (Fig. 2C). IAP expression was restricted to the Cre-GFP-infected cell population (Fig. 2C, arrows). Thus, the reactivation of IAP protein expression is tightly associated with the onset of genomic DNA demethylation caused by the deletion of Dnmt1.

**IAP Protein Immunostaining Marks Demethylated Cells and Cultured Neuroblastoma Cells at a Single Cell Resolution**—We next asked whether IAP protein could be used to detect demethylated cells at a single cell resolution in the developing nervous system after conditional Dnmt1 deletion in neural precursors. Using our anti-IAP2, IAP immunoreactivity was restricted to the zone of Dnmt1 deletion as dictated by the expression pattern of Emx1-Cre and Nestin-Cre (Fig. 3, A and B) (9, 32). In the Emx1-Cre-driven deletion of Dnmt1, no immunoreactivity for IAP was seen in control regions of striatum, thalamus, brain stem, or cerebellum, where DNA methylation is maintained (data not shown).

The neuroblastoma cell line N2a, which is known to transcribe certain IAP-LTR-containing genes (34, 35), shows strong anti-IAP2 immunoreactivity in the cytoplasm with a characteristic juxtanuclear staining pattern for viral A particles (Fig. 3C). Endogenous IAP protein levels are strongly reactivated in N2a cultured cells, which correlates with the known hypomethylation of IAP elements in the
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A

DAPI OCT4 IAP MERGE

WT

Dnmt1 -/-

B

IAP qRT-PCR: Time course of LIF withdrawal

Relative Expression (compared to vehicle)

Days -LIF:

0 1 2 3 4 5 6 7 8 9

J1 (+/+)

cc (Dnmt1 -/-)

C

J1 ESCs Dnmt1-/- ESCs

Days -LIF 3 4 5 6 3 4 5 6 6 8 10 12 6 8 10 12

IAP

GAPDH

D

Days -LIF 0 DIV 4 DIV 6 DIV 8DIV

Oct4 DAPI

IAP DAPI

E

J1 Day 0 -LIF c/c Day 0 -LIF

c/c Day 5 -LIF

IAP2 DAPI

F

IAP mRNA induction in Dnmt1 -/- mESCs (normalized to wildtype band)

Days -LIF:

Fold Induction

2 4 6 8

Fold increase:

3.3 14.8 206.7 197.9
N2a genome (35). Thus, IAP immunoreactivity is a very useful tool to locate demethylated cells in a variety of DNA demethylation models.

Demethylated Embryonic Stem Cells Do Not Contain Detectable IAP mRNAs and Proteins yet Exhibit Dramatic IAP Induction upon Differentiation—Dnmt1<sup>−/−</sup> mESCs possess less that 22% normal genomic methylation levels and exhibit increased expression of IAP mRNAs (27, 28, 36). Surprisingly, when we performed Western blot analysis to assay IAP proteins, we could not detect IAP proteins in lysate from undifferentiated Dnmt1<sup>−/−</sup> mESC cultures (Fig. 4A). When we used IAP FISH to detect mRNA signal in the undifferentiated Dnmt1<sup>−/−</sup> mESCs, we did not visualize increased IAP mRNA levels (Fig. 4A). We then examined the time course of IAP immunoreactivity in demethylated mESCs after in vitro differentiation. Upon LIF withdrawal in the absence of feeder cells, Dnmt1<sup>−/−</sup> mESCs undergo a dramatic increase in IAP transcription and translation (Fig. 4, B and C). Quantitative RT-PCR reveals a 10-fold increase in IAP transcript levels 5 days after LIF withdrawal in Dnmt1<sup>−/−</sup> cells. Moreover, a similar profile of mRNA expression is detected using FISH labeling and Northern blot analysis specific for IAP2 (Fig. 4, E and F). After 6 days of LIF withdrawal, IAP protein is detectable in total cell lysate (Day 6 long exposure; Fig. 4C). By Day 9 of the LIF withdrawal time course, we see robust protein expression (Fig. 4C) and a corresponding 103 ± 29-fold relative increase of IAP transcription in Dnmt1<sup>−/−</sup> cultures (p < 0.0001; Fig. 4B). Z-stack confocal microscopy through individual colonies revealed IAP immunoreactivity present only in cells with low or no Oct3/4 immunoreactivity throughout the time course (Fig. 4D). Over the course of LIF withdrawal, the differentiated population increases dramatically (Fig. 4D), and only after a significant increase in this population was the threshold for immunoblot detection reached.

Detection of IAP Protein Expression in Demethylated EpiSCs—To determine if the DNA methylation-independent mechanism in suppression of IAP elements is unique to pluripotent stem cells, such as mouse ESCs, we derived Dnmt1<sup>lox/lox</sup> EpiSCs and examined the effect of DNA demethylation on IAP expression. EpiSCs are derived from the epiblast of the post-implantation embryo at E5.5, express pluripotent stem cell markers, such as Oct4 and Nanog, and form cells of the three germ layers in vitro and in vivo (25, 26). However, distinct differences in morphology, culture conditions, and gene expression profiles set EpiSCs apart from mESCs. Because EpiSCs exhibit up-regulation of endodermal and ectodermal markers when compared with mESCs, EpiSCs are regarded as more lineage-committed in developmental pathways.

Using lentivirus to deliver cre recombinase, we monitored infected Dnmt1<sup>lox/lox</sup> EpiSCs for IAP protein reactivation. By 2 days postinfection, IAP protein was dramatically up-regulated in Oct3/4-positive cells (Fig. 5). IAP signal remained high in the infected (GFP<sup>+</sup>) EpiSCs and their derivatives in early
passages. However, demethylated EpiSCs do not survive pas-
saging and behave more like demethylated MEF cells (33).

Examine Potential DNA Methylation-independent IAP Rep-
pression Mechanism(s) in Demethylated mESCs—Dnmt1−/−
mESCs appear to possess an alternative mechanism unique to
embryonic stem cells to silence IAP elements in the absence of
DNA methylation. Inhibition of histone deacetylases shifts chro-

FIGURE 6. Inhibition of histone deacetylases and proteosome activities does not lead to IAP protein expression in undifferentiated Dnmt1−/− mESCs. A, HDAC inhibitor treatments of Dnmt1−/− mESCs in the presence of LIF were performed to question whether blocking histone deacetylation, thus promoting the active chromatin conformation, in Dnmt1−/− mESCs would lead to IAP protein expression in undifferentiated cells. Confocal images show that HDAC inhibitors do not reactivate IAP protein expression in Dnmt1−/− mESCs, suggesting that an alternative repressive mechanism is involved. Scale bar, 25 μm. B, cell counts show that HDAC inhibitors increase the number of IAP-positive colonies, which correlates with the known differentiating effect of HDAC inhibitors in cell culture. C, after 8 h of treatment with the proteosome inhibitor MG132, IAP protein was not visualized in Oct3/4-expressing Dnmt1−/− mESCs (confocal photomicrograph; scale bar, 25 μm). Error bars, S.E. DAPI, 4',6-diamidino-2-phenylindole.

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either wild type or demethylated mESCs after MG132 treatment, perinuclear stain can be occasionally seen in few Oct3/4 are expressed. Strong IAP immunostaining is seen in differentiating (Oct3/4-negative) cells, whereas a weak colocalization of Oct3/4 and IAP immunostaining was seen in differentiating cells (Oct3/4-negative). After treatment with the proteosome inhibitor MG132 for 8 h, no increase in IAP immunoreactivity.

Because IAP reactivation a reliable indicator of global changes in transcription factors or lead to an inactive form of chromatin at target loci (40). As the time course of Dnmt1 conditional deletion in MEF cells indicates, IAP protein expression is tightly correlated with genomic DNA methylation levels, making IAP protein reactivation a reliable indicator of global changes in IAP expression in mESCs.

DISCUSSION

Host cell defensive strategies have evolved to counteract the deleterious consequences of IAP element reactivation. The best documented mechanism, DNA methylation of LTR promoters, can directly impede access of transcription factors or lead to an inactive form of chromatin at target loci (40). As the time course of Dnmt1 conditional deletion in MEF cells indicates, IAP protein expression is tightly correlated with genomic DNA methylation levels, making IAP protein reactivation a reliable indicator of global changes in DNA methylation levels in individual cells. Previous tools for examining global DNA hypomethylation levels relied on evaluating DNA or RNA extracted from a population of cells. Although these techniques are sensitive, no evaluation could be made for global DNA methylation changes in individual cells within tissue. As shown in Fig. 3, IAP immunohistochemistry allows for recognition of DNA demethylation at a cellular level in different tissue types. In cultured N2a mouse neuroblastoma cells, a robust reactivation of IAP protein is present. Similarly, in hypomethylated neural lineage cells, IAP protein can be used to trace the demethylation status at the individual cell level.
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Thus, IAP antibodies are valuable reagents to detect demethylated cells in the murine system.

Hypomethylated mESCs appear to possess an alternative mechanism for IAP silencing beyond the DNA methylation-mediated pathway. Interestingly, EpiSCs, which are also pluripotent yet differ from mESCs in both transcriptional networks and epigenetic modifications (25, 26), show strong IAP protein reactivation after Dnmt1 deletion. Thus, a methylation-independent repressional mechanism is unique to mESCs. Further analysis of transcriptional and epigenetic profiles may highlight this unique repressional mechanism in mESCs.

It can be potentially argued that DNA methylation directed by de novo methyltransferases in Dnmt1−/− mESCs silences IAP elements. We evaluated IAP protein expression levels in Dnmt1/Dnmt3al/Dnmt3b-deficient mESCs (TKO cells) (28). Under standard mESC culture conditions, the IAP-positive TKO cell population was not Oct4-positive (supplemental Fig. 1). Because TKO cells have less than 2% of the normal methylation levels (28), we argue that mESCs possess an alternative mechanism in the absence of DNA methylation that mediates IAP gene silencing.

We have considered the involvement of both transcriptional and translational mechanisms controlling IAP expression in demethylated mESCs. It is possible that redundant repressive mechanisms are present in mESCs that include DNA methylation and histone modifications, yet HDAC inhibitors did not induce IAP expression in demethylated Dnmt1−/− mESCs (Fig. 6). Furthermore, preliminary results showed that deficiency of either PRC2 (eed−/− mESCs) or PRC1 (Bmi1−/− brain tissues) alone did not relieve IAP protein repression (data not shown). To definitively ascertain PcG complex involvement, deficiency of both polycomb complexes and DNA methylation in mESCs may be needed to trigger IAP protein expression.

Small RNA-mediated silencing pathways have been reported to regulate retrotransposons. Presently, contradictory reports regarding Dicer involvement in IAP silencing appear in the literature. One group observed increased transcription from centromeric repeats, L1s, and IAPs (39); however, other groups state that mammalian DICER and Elf2c2 (Ago2) have no roles in maintaining genomic methylation in mESCs, with direct evidence that the RISC complex does not repress IAP expression post-transcriptionally (41, 42). Our double deletion model in mESCs reveals no large induction of IAP protein reactivation, arguing that Dicer-mediated transcriptional silencing is not strongly targeting IAP repression. Although we observed weak IAP immunostaining in a few Dnmt1−/−; Dicer−/− cells, this could be due to the fact that Dicer mutation dramatically increases Oct3/4 levels in mESCs (39, 41). The appearance of a few Oct3/4 immunopositive cells weakly co-stained for IAP could be explained by longer turnover of Oct3/4 protein; thus, cells undergoing early stages of spontaneous differentiation would retain residual Oct3/4 protein.

While this manuscript was in preparation, two recent publications demonstrated that KAP1/Trim28 repressor protein coupled with histone lysine 9 methyltransferase KMT1E (ESET/SETDB1) is involved in repressed IAP gene transcription in mESCs (43, 44). These studies revealed a novel role of repressive histone modifications as an alternative transcriptional repressive mechanism independent of DNA methylation. Interestingly, in the absence of all three Dnmts in mESCs, Matsui et al. (44) reported that the KAP-repressive complex remains associated with IAP retrotransposon elements. Furthermore, KAP1/Trim28 acts synergistically with DNA methylation to repress IAP transcription (43). However, neither of the two groups have examined whether IAP proteins are present in KAP1−/− mESCs. In our study, we found that IAP gene transcription is only moderately increased when compared with differentiated Dnmt1−/− cells. Furthermore, IAP protein is not detected in demethylated mESCs, consistent with an additional inhibitory mechanism that blocks IAP expression in undifferentiated mESCs.

Based on the findings of this current study, we conclude that IAP protein expression can be used to detect DNA hypomethylation at the cellular level in murine cells ranging from epiblast-derived stem cells to adult neurons. Both IAP transcription and protein translation can be detected upon DNA hypomethylation in lineage-committed cells. However, in undifferentiated mESCs, there exists a compensatory mechanism(s) to repress retrotransposon elements independent of DNA methylation. The need for an alternative mechanism further highlights the importance of maintaining genomic stability to prevent insertional mutations in fast replicating mESCs.

Acknowledgments—We thank Dr. Kira Lueders for the gifts of the p73 antibody and the MIA14 plasmid and Thuc Le for editing of the manuscript.

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