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Distinct Roles of DMAP1 in Mouse Development[▽]

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DMAP1 (DNMT1-associated protein 1) is a member of the TIP60-p400 complex that maintains embryonic stem (ES) cell pluripotency and a complex containing the somatic form of DNA methyltransferase 1 (DNMT1s). DMAP1 interacts with DNMT1s through a domain that is absent in *Dnmt1$^{V/V}$* mice expressing just the oocyte form (DNMT1o). A *Dmap1*-null allele was generated to study the role of DMAP1 in development. Consistent with the phenotypes of loss of other members of the TIP60-p400 complex, *Dmap1$^{-/-}$* mice died during preimplantation in both *Dnmt1$^{+/+}$* and *Dnmt1$^{V/V}$* backgrounds. Unexpectedly, in the *Dnmt1$^{V/V}$* background, *Dmap1$^{+/-}$* parents produced mainly *Dmap1$^{+/-}$* mice. Most *Dmap1$^{+/+}$* progeny died during midgestation, with loss of DNA methylation on imprinted genes, suggesting that DMAP1 influences maintenance methylation mediated by DNMT1o. In this regard, a DMAP1-DNMT1o complex was detected in ES cells when DNMT1o was stably expressed but not when transiently expressed, indicating a novel interaction between DMAP1 and DNMT1o. These results suggest that DMAP1-DNMT1s and DMAP1-DNMT1o interactions are essential for normal development and that DMAP1-DNMT1o complexes are not readily formed in the embryo. Therefore, DMAP1 mediates distinct preimplantation epigenetic reprogramming processes: TIP60-p400 nucleosome remodeling and DNMT1 maintenance methylation.

A major biological role of chromatin is to control gene expression, through either transcriptional activation or repression. There are many types of chromatin, defined primarily by the organization and composition of nucleosomes, including specific posttranslational modifications of histones within nucleosomes. The significant changes in chromatin that occur during preimplantation development are of particular interest because of their likely association with epigenetic reprogramming, the inheritance of genomic imprints, and the generation of the embryo's stem (ES) cells. To gain insight into this process, we focused on understanding the role of the corepressor DNMT1-associated protein 1 (DMAP1). DMAP1 was initially identified as a protein associated with the N-terminal domain of DNMT1s and was shown to function as a transcriptional corepressor by interacting with histone deacetylase 2 (HDAC2) (26). Later, DMAP1 was found to be a component of the TIP60-p400 histone acetyltransferase complex (14, 28), which is critically important for the maintenance of ES cell pluripotency (8).

Genetic studies using *Dnmt1* mutant mice (11, 12) and immunohistological studies of the intracellular locations of DNMT1 proteins (5, 12) indicate that DNMT1 catalyzes the maintenance of DNA methylation during preimplantation development. The maintenance of CpG methylation patterns is carried out by a combination of maternal (oocyte-derived) and zygotic (embryo-derived) DNMT1 proteins (5). The oocyte synthesizes two DNMT1 proteins: the somatic DNMT1s form of 1,621 amino acids (aa) whose N terminus interacts with DMAP1, and the oocyte form of 1,503 amino acids (DNMT1o) that lacks the known 118-amino-acid DMAP1 interaction domain (5, 18, 26). Both the DNMT1s and DNMT1o proteins coexist in the ooplasm of fully grown mouse oocytes (5). Oocyte-derived DNMT1s maintains methylation patterns during the first two embryonic S phases, whereas DNMT1o functions at the 4th embryonic S phase (4). After the 2-cell stage, zygotic DNMT1s is synthesized, which maintains methylation patterns during the remaining S phases of preimplantation (5, 23).

An important aspect of preimplantation maintenance methylation is its specificity for a subset of gamete-derived DNA methylation patterns. The level of genomic CpG methylation fluctuates during development, with a notable decline from the zygote to the blastocyst stages (3, 19). Differentially methylated domains (DMDs) of imprinted genes maintain (inherit) their gamete-derived methylation patterns during preimplantation development, despite the loss of the bulk of genomic methylation (17, 25). We have postulated that the selective maintenance of DMD methylation during this developmental window is part of a critically important reprogramming process mediated by activities of preimplantation DNMT1 proteins (1, 25). Thus, associations of DMAP1 with DNMT1s and the TIP60-p400 complex suggest that DMAP1 plays an important role in epigenetic reprogramming during preimplantation development. We approached this issue by generating a mouse line with a *Dmap1*-null allele and studying the effects of this allele in wild-type *Dnmt1* and mutant *Dnmt1^{V}* genetic backgrounds.

MATERIALS AND METHODS

Targeted deletion of *Dmap1*. A targeting construct was made by inserting a *loxP* site into an SmaI restriction site in the first intron of *Dmap1* and a *Pgk-neo*^{r} cassette at an engineered EagI site in the intron between exons 10 and 11. The cassette contained a *Pgk-neo*^{r} transcription unit flanked by FLP recombination target (FRT) sites and a 5′ *loxP* site (Fig. 1A). Genotypes of *Dmap1$^{+/+}$*, *Dmap1$^{floxed/+}$* and *Dmap1$^{+/-}$* mice were confirmed by Southern blotting of tail

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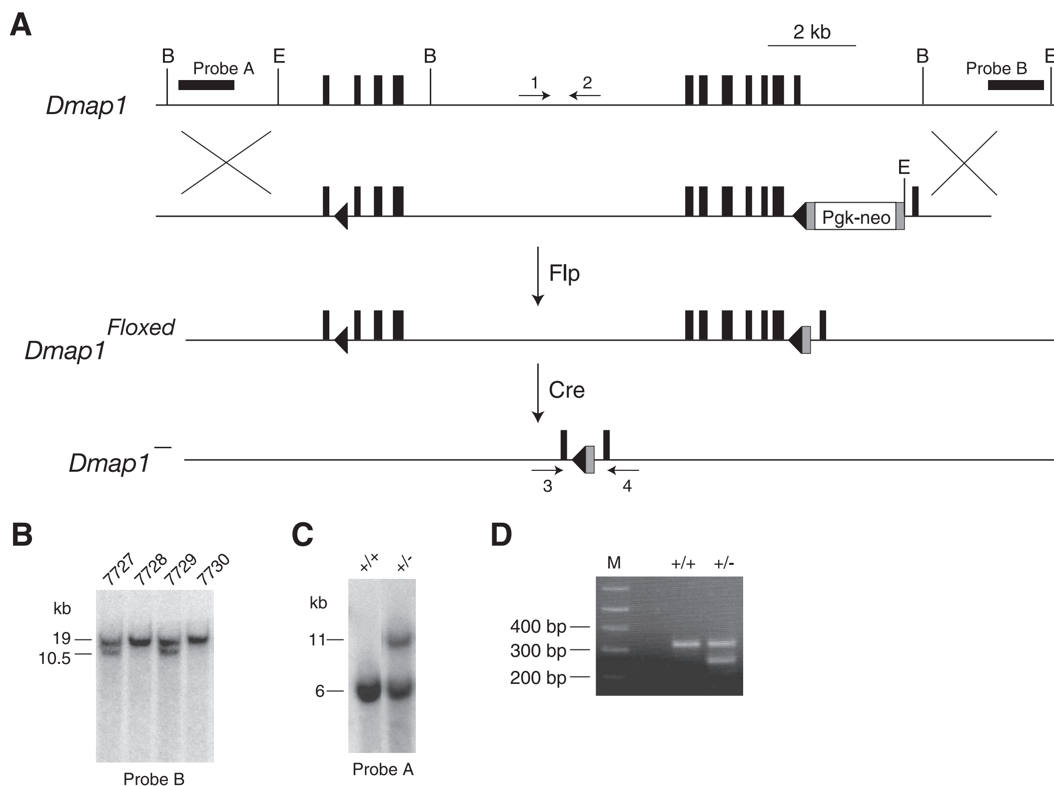

FIG. 1. Generation of a mouse *Dmap1*$^{-}$ allele. (A) Schematic of targeted-mutagenesis scheme in mouse ES cells used to generate a mouse conditional allele (*Dmap1*^{floxed}), which in turn was used to generate a mouse null allele (*Dmap1*$^{-}$). The targeting plasmid contains a 5′ *loxP* site in the first intron and a 3′ *loxP* site in the last intron of *Dmap1*. *loxP* sites are shown by black triangles and exons by vertical black rectangles. Gray rectangles represent FRT sites. Probes used for Southern blots are indicated by horizontal black rectangles. A mouse line generated with a Pgk-neo^{R}-containing heterozygous ES cell clone was crossed to a CAGGS-*flp* transgenic mouse line (gift from G. Homanics) to remove the Pgk-neo^{R} fragment and generate a *Dmap1*^{floxed} mouse line, which was then crossed to an EIIa-*cre* transgenic line (15) to remove the bulk of the *Dmap1* gene between the 5′ and 3′ LoxP sites. BglII and EcoRI restriction sites are indicated by "B" and "E," respectively. The positions of the oligonucleotides used to genotype mice are indicated by arrows. (B) Southern blot of EcoRI-digested genomic DNA from four offspring (no. 7727 to 7730) derived from a cross between a wild-type mouse and a heterozygous *Dmap1*^{floxed} mouse. The 19-kb band is the wild-type allele, and the 10.5-kb band is the *Dmap1*^{floxed} allele. (C) Southern blot of BglII-digested genomic DNA from a wild-type *Dmap1*$^{+/+}$ mouse and the founder *Dmap1*$^{+/-}$ mouse derived from a cross between a *Dmap1*$^{floxed/+}$ mouse and an EIIA-*cre* transgenic mouse. The 6-kb band is the wild-type allele, and the 11-kb band is the *Dmap1*$^{-}$ allele. (D) Genotypes of a wild-type mouse and a *Dmap1*$^{+/-}$ mouse identify a 320-nucleotide (nt) wild-type allele and a 250-nt *Dmap*$^{-}$ allele in PCR assays by using the oligonucleotide pairs shown in panel A. M, molecular size markers.

DNA digested with either BglII or EcoRI and with the probes indicated (Fig. 1B and C). *Dmap1*$^{+}$ and *Dmap*$^{-}$ alleles were also genotyped by PCR using oligonucleotides 1 (5′CCCCCTCCCTCAAATACTTC3′) and 2 (5′CAGCCATTGA GAGGAAAAGC3′) for the wild-type (WT) allele and oligonucleotides 3 (5′T CCTATCCGTGGGTCTTCAG3′) and 4 (5′GTCAACCCTCTCCTGTCGTC 3′) for the null allele (Fig. 1D).

Mouse lines. The *Dmap1*$^{-}$, *Dnmt1*^{V}, and *TR2 + 3/Igmyc* mouse lines were all maintained in an inbred 129/Sv genetic background. *Dnmt1*$^{+/+}$ and *Dnmt1*^{V} alleles were genotyped as previously described (6). *TR2 + 3/Igmyc* mice were identified by genotyping as previously described (25). All mouse experiments were approved and conformed to the standards of the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Cell lines. *Dnmt1*$^{V/V}$ ES cell lines were generated from blastocysts isolated from crosses between *Dnmt1*$^{V/V}$ mice (6). The R1 mouse ES cell line has been previously described (21). Primary mouse embryonic fibroblasts (MEFs) were established from embryonic day 14.5 (E14.5) embryos.

Expression plasmids. *Dmap1* cDNA amplified from mouse spleen total RNA was cloned in Topo blunt vector (Invitrogen), and the sequence was verified and subcloned into EF1-myc version B expression plasmid (Invitrogen) to stably express DMAP1-MYC fusion peptide in ES cells. An EF1-internal ribosome entry site (IRES)-hyg expression vector constructed by replacing the c-*myc* promoter in pIRES-hyg (Clontech) with PCR-amplified EF1α promoter from EF1-myc vector was used for expression of DNMT1s and DNMT1o proteins. Stable transfections were carried out by electroporation. Transient transfections of ES cells with Lipofectamine 2000 (Invitrogen) gave a transfection efficiency of ~75%. Cell lysates were prepared 48 h after transfection.

DMAP1 immunoprecipitation. Whole-cell lysates prepared using a nondenaturing cell lysis buffer (20 mM Tris HCl [pH 7.5], 137 mM NaCl, 10% glycerol, 1% Nonidet P-40 [NP-40], and 2 mM EDTA) containing protease inhibitor cocktail (Roche) were centrifuged at 12,000 rpm for 20 min at 4°C. The supernatants were transferred to fresh tubes and incubated overnight with anti-MYC antibody conjugated with agarose (Santa Cruz Biotechnology). Protein complexes were pulled down by centrifugation, washed three times with the lysis buffer, and denatured for analysis by immunoblotting.

Immunoblotting. Protein lysates resolved on 5% to 15% gradient polyacrylamide gels were Western blotted and probed with the UPTC21 antibody that detects both DNMT1s and DNMT1o (23), anti-DMAP1 antibody (ab2848; Abcam), and anti-MYC antibody (Covance). Bands on Western blots were quantified with Image J software (http://rsbweb.nih.gov/ij/).

Embryo collection. Blastocysts were obtained from a cross between *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ parents. One-half of the DNA sample from each blastocyst was used to determine the *Dmap1* genotype. The remaining DNA samples from *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ blastocysts were collected into one pool of two blastocysts, and the remaining DNA samples from *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ blastocysts were collected into a separate pool of two blastocysts.

Methylation analysis. Samples of genomic DNA were treated with sodium bisulfite (EZ DNA methylation kit; Zymo Research) and amplified with primers specific to *H19*, *Snurf/Snrpn*, and *Gtl2* differentially methylated domains (DMD)

TABLE 1. Genotypes of offspring from crosses of *Dnmt1* and *Dmap1* parents

Cross	Parent genotype	Developmental stage[a]	*Dmap1* genotype			Total no. of mice
			+/+	+/−	−/−	
1	$Dmap1^{+/-}$, $Dnmt1^{+/+}$ × $Dmap1^{+/-}$, $Dnmt1^{+/+}$	Adult	21	62	0	83
		E10.5	6	17	0	23
		Blastocyst	9	20	0	29
		8-cell	10	17	0	27
2	$Dmap1^{+/-}$, $Dnmt1^{V/V}$ × $Dmap1^{+/-}$, $Dnmt1^{V/V}$	Adult	4[b]	74	0	78
		E9.5	5[b]	63	0	68
		E8.5	1	8	0	9
		E7.5	3	21	0	24
		Blastocyst	6	18	0	24
		8-cell	2	8	0	10
3	$Dmap1^{+/-}$, $Dnmt1^{V/V}$ × $Dmap1^{+/+}$, $Dnmt1^{V/V}$	Adult	0[b]	21	0	21
4	$Dmap1^{+/+}$, $Dnmt1^{V/V}$ × $Dmap1^{+/-}$, $Dnmt1^{V/V}$	Adult	0[b]	23	0	23
5	$Dmap1^{+/-}$, $Dnmt1^{V/V}$ × $Dmap1^{+/+}$, $Dnmt1^{+/+}$	Adult	7	9	0	16
6	$Dmap1^{+/+}$, $Dnmt1^{+/+}$ × $Dmap1^{+/-}$, $Dnmt1^{V/V}$	Adult	5	9	0	14
7	$Dmap1^{+/-}$, $Dnmt1^{V/V}$ × $Dmap1^{+/+}$, $Dnmt1^{V/+}$	Adult	0[b]	9	0	9
8	$Dmap1^{+/+}$, $Dnmt1^{V/+}$ × $Dmap1^{+/-}$, $Dnmt1^{V/V}$	Adult	6	5	0	11
9	$Dmap1^{+/-}$, $Dnmt1^{V/+}$ × $Dmap1^{+/+}$, $Dnmt1^{V/V}$	Adult	4	7	0	11
10	$Dmap1^{+/+}$, $Dnmt1^{V/V}$ × $Dmap1^{+/-}$, $Dnmt1^{V/+}$	Adult	4	7	0	11

[a] "Adult" indicates mice genotyped at 3 weeks after birth.
[b] Significant deviation from expected ratio of $Dmap1^{+/+}$ to $Dmap1^{+/-}$ offspring.

and the imprinted *TR2 + 3/Igmyc* transgene sequences (1, 25). Methylation levels of *Snurf/Snrpn* and *Gtl2* DMDs were estimated with combined bisulfite restriction analysis (COBRA) assays (1). The level of methylation on *H19/Igf2* DMD and the *TR2 + 3/Igmyc* transgene was determined by sequencing individual cloned DNA fragments amplified from the bisulfite-treated DNA (1, 25).

RESULTS

Homozygous $Dmap1^{-/-}$ embryos show a lethal embryonic phenotype. To explore the function of the DMAP1 protein in embryonic development, we generated a mouse line with a null allele of the mouse *Dmap1* gene. We first generated mice with a conditional allele of *Dmap1* ($Dmap1^{floxed}$) in which *loxP* sites were placed in the first and last introns of the *Dmap1* gene. By crossing $Dmap1^{floxed}$ mice with mice carrying an EIIa-*cre* transgene (15), a mouse line carrying an allele lacking nine exons coding for amino acids 1 to 448 of the 468-aa DMAP1 protein was produced (Fig. 1). As shown in Table 1, homozygous $Dmap1^{-/-}$ neonatal mice from crosses between $Dmap1^{+/-}$ parents were not recovered, and heterozygous and wild-type mice were recovered at an approximate ratio of 2:1 (cross 1), indicating that homozygous $Dmap1^{-/-}$ mice died during embryonic development, whereas the development of heterozygous $Dmap1^{+/-}$ mice is normal. To define the age of embryonic lethality, we determined the genotypes of E10.5 embryos, E3.5 blastocysts, and 8-cell embryos from crosses between heterozygous $Dmap1^{+/-}$ parents. No homozygous mutant $Dmap1^{-/-}$ 8-cell embryos or blastocysts were identified (cross 1). We conclude that the DMAP1 protein is required for preimplantation development.

DMAP1 is required for development even in the absence of its known DNMT1 interaction domain. The DMAP1 interaction domain was mapped to the first 118 aa of DNMT1s, and DNMT1o was suggested not to interact with DMAP1 (26). We previously showed that $Dnmt1^{V/V}$ mice that produce a mutant form of DNMT1s lacking this interaction domain (equivalent to DNMT1o) are viable and fertile (6), indicating that the DMAP1 interaction domain is not required for normal development. Here we were interested in knowing whether loss of DMAP1 would have no consequences in a $Dnmt1^{V/V}$ background. Heterozygous $Dmap1^{+/-}$ mice in a $Dnmt1^{V/V}$ background ($Dmap1^{+/-}$, $Dnmt1^{V/V}$) were crossed, and progeny were genotyped at multiple stages. As shown in Table 1 (cross 2), none of the progeny (examined as early as the 8-cell stage) were $Dmap1^{-/-}$, indicating that the $Dnmt1^{V/V}$ background did not suppress or modify in any discernible way the lethality of homozygous $Dmap1^{-/-}$ mice. We conclude that DMAP1 serves an essential function in preimplantation that is independent of its specific interaction with DNMT1s.

Contrary to expectations, we also did not recover mice with the $Dmap1^{+/+}$ genotype at the expected frequency from crosses between $Dmap1^{+/-}$, $Dnmt1^{V/V}$ mice (Table 1, cross 2). When the progeny of these parents were genotyped at different stages of development, we observed that the $Dmap1^{+/+}$ embryos died around 9.5 days of gestation. Because the progeny of the same genotype ($Dmap1^{+/+}$, $Dnmt1^{V/V}$) are viable when the parents are $Dmap1^{+/+}$, $Dnmt1^{V/V}$, we examined whether $Dmap1^{+}$, $Dnmt1^{V}$ gametes from $Dmap1^{+/-}$, $Dnmt1^{V/V}$ mice are compromised. When the genotypes of the opposite parents

are *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$, these crosses also resulted in only *Dmap1*$^{+/-}$ mice (Table 1, crosses 3 and 4), indicating that the *Dmap1*$^{+}$, *Dnmt1*^{V} gametes from *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ parents are not equivalent to those from the *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ mice in their ability to support the development of *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ embryos. This observed transmission ratio distortion indicates that interactions between *Dmap1* and *Dnmt1* alleles are complex.

To further investigate the effects of *Dmap1* and *Dnmt1* interactions during development, we conducted a series of crosses in which the *Dnmt1*^{V} allele was replaced by the wild-type *Dnmt1* allele. In the first set of experiments, *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ mice were crossed with wild-type mice (Table 1, crosses 5 and 6). These crosses yielded both *Dmap1*$^{+/+}$ and *Dmap1*$^{+/-}$ mice in the expected ratios, suggesting that in *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ mice, both *Dmap1*$^{-}$ and *Dmap1*$^{+}$ gametes are viable and postzygotic expression of a *Dnmt1*$^{+}$ allele is sufficient to suppress the lethality in *Dmap1*$^{+/+}$ mice obtained from *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ parents. Second, we conducted reciprocal crosses in which one of the parents was *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ and the other was *Dmap1*$^{+/+}$, *Dnmt1*$^{V/+}$ (Table 1, crosses 7 and 8). *Dmap1*$^{+/+}$ mice were obtained when the *Dnmt1*$^{+}$ allele was from the mother (cross 8) but not from the father (cross 7). When the *Dmap1*$^{+/+}$ progeny from cross 8 were genotyped for *Dnmt1* alleles, there was a 1:1 distribution of both *Dnmt1*$^{+}$ and *Dnmt1*^{V} alleles. This suggests that a zygotic *Dnmt1*$^{+}$ allele is not required for normal development of the *Dmap1*$^{+/+}$ progeny, and the presence of a *Dnmt1*$^{+}$ allele in the mother seems to be sufficient to support development. In these mothers, both DNMT1o and DNMT1s proteins that are expressed in the germ line are deposited in the *Dmap1*$^{+}$, *Dnmt1*^{V} oocytes, and transmission of maternal DNMT1s is able to fully rescue the *Dmap1*$^{+/+}$ genotype in the offspring. In conclusion, *Dmap1*$^{+/+}$ offspring can be recovered at the expected frequencies from a homozygous *Dnmt1*$^{V/V}$ carrier if the offspring inherit a *Dnmt1*$^{+}$ allele from the opposite parent or, alternatively, if the female parent is heterozygous *Dnmt1*$^{V/+}$.

To more precisely define the developmental requirements for interactions between *Dmap1* and *Dnmt1* alleles, we crossed *Dmap1*$^{+/-}$, *Dnmt1*$^{V/+}$ mice with *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ mice (Table 1, crosses 9 and 10). Unlike the previous crosses (crosses 7 and 8), both male and female parents had the same ability to produce *Dmap1*$^{+/+}$ offspring at expected ratios. Moreover, when *Dmap1*$^{+/+}$ progeny were genotyped further for the presence of *Dnmt1* alleles, we observed an equal representation of both *Dnmt1*$^{+}$ and *Dnmt1*^{V} alleles among the *Dmap1*$^{+/+}$ progeny, suggesting that, as was the case in cross 8, there was no absolute requirement for the continued presence of a *Dnmt1*$^{+}$ allele for the survival of *Dmap1*$^{+/+}$ progeny produced from these parents. A comparison between crosses 3 and 9 or 4 and 10 indicated that the *Dmap1*$^{+}$, *Dnmt1*^{V} gametes from *Dmap1*$^{+/-}$, *Dnmt1*$^{V/+}$ parents are capable of supporting development, whereas the gametes of the same genotype from *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ parents were compromised. Taking the results shown in Table 1 together, we observed that *Dmap1*$^{+}$, *Dnmt1*^{V} gametes produced from the *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ parent were defective in supporting the development of *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ progeny. This defect can be corrected by the presence of a *Dnmt1*$^{+}$ allele during gametogenesis or early

Dmap1^{+} ● Dnmt1^{+}
Dmap1^{-} ○ Dnmt1^{V}
Known DMAP1 interaction domain

Cross	Dmap1 genotypes ●●	Dmap1 genotypes ●○	Dmap1 genotypes ○○
1	21	62	0
2	4	74	0
3	0	21	0
4	0	23	0
5	7	9	0
6	5	9	0
7	0	9	0
8	6	5	0
9	4	7	0
10	4	7	0

FIG. 2. Summary of genotypes of adult offspring from crosses of *Dmap1* and *Dnmt1* parents. The data are derived from Table 1, and crosses 1 to 10 correspond to the crosses labeled in Table 1. The symbols correspond to different *Dmap1* and *Dnmt1* alleles (top). Statistically significant deviations from the expected number of homozygous *Dmap1*$^{+/+}$ offspring are highlighted in red.

embryogenesis. This conclusion is indicated in the schematic shown in Fig. 2.

Imprinted DNA methylation in *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ embryos. Abnormalities in the inheritance of genomic imprints during preimplantation can lead to defects in development and death of postimplantation embryos (12). Therefore, we examined the methylation pattern of imprinted genes in progeny of *Dmap*$^{+/-}$, *Dnmt1*$^{V/V}$ parents. We first compared the methylation of the endogenous *H19*/*Igf2* DMD, which is normally methylated on the paternal allele, in *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ and *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ blastocysts, a time before the onset of *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ lethality. There was a small, but detectable reduction in *H19*/*Igf2* DMD methylation in *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ blastocysts compared to *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ blastocysts (Fig. 3A).

To further evaluate the integrity of DMD methylation in *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ embryos, we compared the levels of maternal methylation of *Snurf*/*Snrpn* DMD and paternal methylation of *Gtl2* DMD between *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ and *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ E9.5 embryos. As shown in Fig. 3B, both DMDs in all six embryos examined showed both methylated and unmethylated DMD alleles, with no obvious and consistent differences in *Snurf*/*Snrpn* and *Gtl2* methylation between the two different embryonic genotypes. We conclude from this

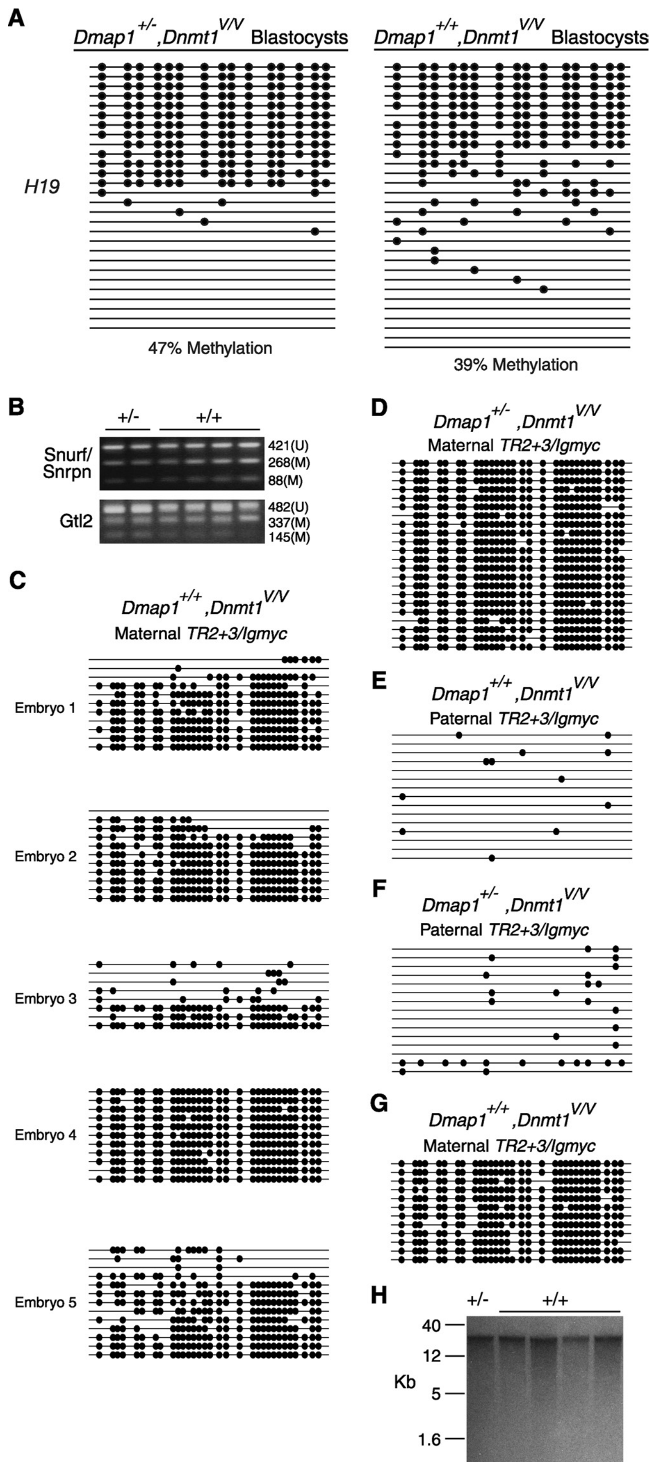

FIG. 3. Methylation of imprinted DNA sequences in $Dmap^{+/-}$, $Dnmt1^{V/V}$ and $Dmap^{+/+}$, $Dnmt1^{V/V}$ embryos derived from $Dmap1^{+/-}$, $Dnmt1^{V/V}$ mice. (A) *H19*/*Igf2* DMD methylation in two pools of blastocysts. Each horizontal line is an *H19*/*Igf2* DMD allele whose methylation was determined by bisulfite genomic sequencing (1). Filled circles represent methylated CpG dinucleotides, and the absence of circles indicates unmethylated CpGs. (B) COBRA of *Snurf*/*Snrpn* and *Gtl2* DMD sequences from E9.5 embryos. *Snurf*/*Snrpn* PCR products were digested with BstUI and *Gtl2* PCR products with TaqI. Fragments representing methylated (M) or unmethylated (U) DNA and their sizes in nucleotides are indicated. +/−, $Dmap1^{+/-}$, $Dnmt1^{V/V}$; +/+, $Dmap1^{+/+}$, $Dnmt1^{V/V}$. (C) Bisulfite sequencing of the maternal *TR2 + 3*/*Igmyc* transgene in five different $Dmap1^{+/+}$, $Dnmt1^{V/V}$ E9.5 embryos from crosses between $Dmap1^{+/-}$, $Dnmt1^{V/V}$ parents. (D) Methylation of the maternal *TR2 + 3*/*Igmyc* transgene in a $Dmap1^{+/-}$, $Dnmt1^{V/V}$ embryo from a cross between $Dmap1^{+/-}$, $Dnmt1^{V/V}$ parents. (E and F) Methylation of the paternal *TR2 + 3*/*Igmyc* transgene in a $Dmap1^{+/+}$, $Dnmt1^{V/V}$ and a $Dmap1^{+/-}$, $Dnmt1^{V/V}$ embryo, both from a cross between crosses between $Dmap1^{+/-}$, $Dnmt1^{V/V}$ parents. (G) Methylation of the maternal *TR2 + 3*/*Igmyc* transgene in a $Dmap1^{+/+}$, $Dnmt1^{V/V}$ E9.5 embryo from a cross between a $Dmap1^{+/-}$, $Dnmt1^{V/+}$ female and a $Dmap1^{+/+}$, $Dnmt1^{V/V}$ male mouse. (H) Southern blot of HpaII-digested DNA hybridized with an IAP probe (1). DNA was obtained from one E9.5 $Dmap1^{+/-}$, $Dnmt1^{V/V}$ embryo (D) and four E9.5 $Dmap1^{+/+}$, $Dnmt1^{V/V}$ embryos (1 to 4 in panel C).

that for the DMDs tested here, allele-specific DMD methylation is largely intact.

Because the COBRA assay provides at best a semiquantitative measurement of methylation on specific DNA sequences, it is possible that significant differences in imprinted methylation between $Dmap1^{+/-}$, $Dnmt1^{V/V}$ and $Dmap1^{+/+}$, $Dnmt1^{V/V}$ embryos would not be detected. For instance, a partial loss of methylation from the normally methylated parental allele would not be easily detectable in the presence of the normally unmethylated, opposite parental allele. To better assess imprinted methylation, we examined methylation of the *TR2 + 3*/*Igmyc* transgene in E9.5 embryos. *TR2 + 3*/*Igmyc* is an imprinted transgene in which *Igf2r* DMD2 sequences were incorporated into the imprinted *RSVIgmyc* transgene (4, 24, 25). Importantly, the parental origin of *TR2 + 3*/*Igmyc* can be unambiguously determined in crosses between a *TR2 + 3*/*Igmyc* hemizygous carrier and a nontransgenic mouse, and in wild-type strain backgrounds, there are strict patterns of methylation, with the maternal *TR2 + 3*/*Igmyc* highly methylated and the paternal *TR2 + 3*/*Igmyc* unmethylated (4). As shown in Fig. 3C, four out of five $Dmap1^{+/+}$, $Dnmt1^{V/V}$ E9.5 embryos from crosses between $Dmap1^{+/-}$, $Dnmt1^{V/V}$ parents showed deficiencies in maternal *TR2 + 3*/*Igmyc* methylation. In comparison, $Dmap1^{+/-}$, $Dnmt1^{V/V}$ E9.5 embryos from $Dmap1^{+/-}$, $Dnmt1^{V/V}$ crosses showed complete methylation of maternal *TR2 + 3*/*Igmyc* alleles (Fig. 3D). Paternally inherited *TR2 + 3*/*Igmyc* alleles in both types of E9.5 embryos were unmethylated (Fig. 3E and F). The maternal *TR2 + 3*/*Igmyc* allele was also completely methylated in a $Dmap1^{+/+}$, $Dnmt1^{V/V}$ adult from a cross between a $Dmap1^{+/-}$, $Dnmt1^{V/+}$ female and a $Dmap1^{+/+}$, $Dnmt1^{V/V}$ male (Fig. 3G); there is no evidence of a decrease in viability in $Dmap1^{+/+}$, $Dnmt1^{V/V}$ offspring of this cross (cross 9) and its reciprocal (cross 10) (Table 1). No differences in methylation between $Dmap1^{+/-}$, $Dnmt1^{V/V}$ and $Dmap1^{+/+}$, $Dnmt1^{V/V}$ E9.5 embryos were seen at intracisternal A particle (IAP) retrotransposon loci (Fig. 3H). Collectively, the findings indicate that there are defects in imprinted DMD methylation in $Dmap1^{+/+}$, $Dnmt1^{V/V}$ embryos. Such defects are reminiscent of the preimplantation imprinting defects observed in embryos derived from $Dnmt1^{\Delta 1o/\Delta 1o}$ female mice (12) and likely account for the lethality of $Dmap1^{+/+}$, $Dnmt1^{V/V}$ mice derived from $Dmap1^{+/-}$, $Dnmt1^{V/V}$ parents.

DNMT1s and DNMT1o proteins physically associate with DMAP1 with different affinities. The ability of $Dmap1^{+}$, $Dnmt1^{V}$ gametes to support embryonic development varies

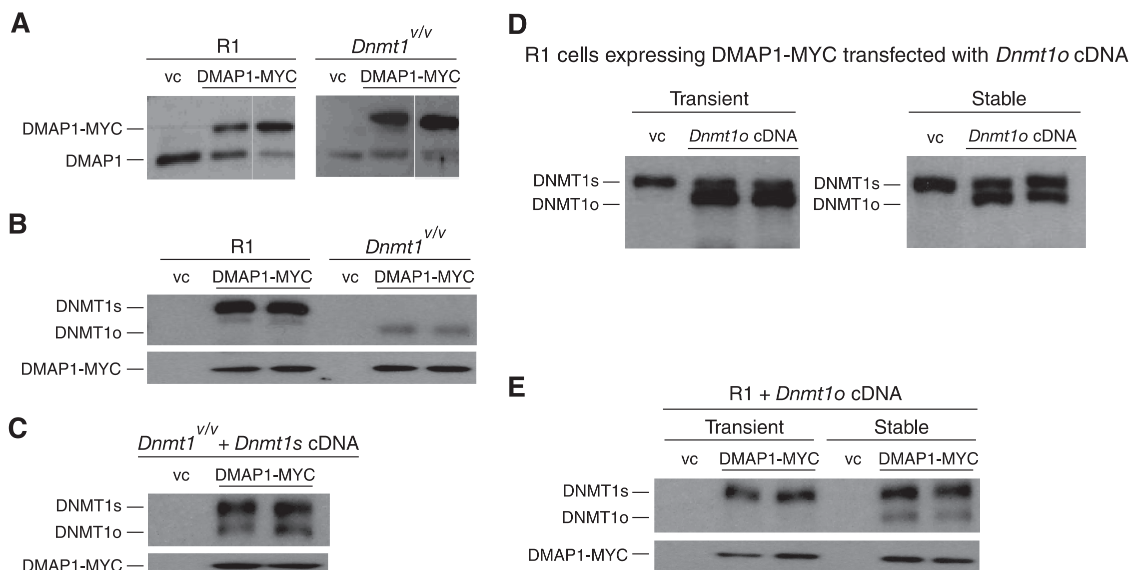

FIG. 4. Physical interactions between DMAP1 and DNMT1 proteins. (A) Stable expression of DMAP1-MYC in WT R1 and *Dnmt1*$^{V/V}$ ES clones. Both endogenous DMAP1 (M_r, 52.9) and the exogenous DMAP1-MYC (M_r, 55.9) proteins were detected by anti-DMAP1 antibody. vc, ES cell clone with stably integrated empty expression vector. (B) DMAP1 is present in a complex with both DNMT1 and DNMT1o. Immunoprecipitates from clones stably expressing DMAP1-MYC were probed with UPTC21 to detect DNMT1o (M_r, 165,000) and DNMT1s (M_r, 190,000) proteins (top) and with anti-DMAP1 antibody (bottom). (C) DMAP1 binds preferentially to DNMT1s. Lysates from *Dnmt1*$^{V/V}$ ES cell clones stably expressing DMAP1-MYC DNMT1s proteins were immunoprecipitated with anti-MYC, resolved on SDS-PAGE gels, and probed with UPTC21 and anti-DMAP1 antibodies. (D) Levels of DNMT1o in R1 cells after transient transfection (left panel) and stable transfection (right panel) with DMAP1-cDNA expression plasmid. (E) Detection of DMAP1-DNMT1o complex in ES cells stably expressing DNMT1o. Immunoprecipitates from the cell types shown in panel D were probed with UPTC21 and anti-DMAP1 antibodies.

based on their parental genotypes. Gametes from *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ parents are less capable of supporting development than those from *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ parents. This suggests that DNMT1o might functionally interact with DMAP1 during gametogenesis. To measure physical associations between DMAP1 and DNMT1 proteins, we first expressed an epitope (MYC)-tagged version of DMAP1 in wild-type and *Dnmt1*$^{V/V}$ ES cell lines derived from *Dnmt1*$^{V/V}$ blastocysts. As shown in Fig. 4A, ES cell clones expressing exogenous DMAP1-MYC protein were isolated. To determine if DMAP1 interacts with DNMT1o, we immunoprecipitated DMAP1. As shown in Fig. 4B, both DNMT1s and DNMT1o formed complexes with DMAP1-MYC. However, the amount of DNMT1o protein associated with DMAP1 was approximately 5 times lesser than the amount of DNMT1s protein associated with DMAP1. To directly compare the association of DNMT1s and DNMT1o with DMAP1, we stably transfected a *Dnmt1s* cDNA construct into *Dnmt1*$^{V/V}$ ES cells expressing DMAP1-MYC. As shown in Fig. 4C, both DNMT1o and DNMT1s can be immunoprecipitated with DMAP1-MYC. Although the level of expression of DNMT1s is similar to the level of endogenous DNMT1o in these transfected cells, the ratio of coimmunoprecipitated DNMT1s to DNMT1o was approximately 5:1 (Fig. 4C). Collectively, these findings suggest that although the strongest physical association between DNMT1 and DMAP1 is through the previously described 118-amino-acid DMAP1 interaction domain (26), other regions of DNMT1 enable formation of a complex with DMAP1.

To further investigate the nature of the interaction between DMAP1 and DNMT1o, we compared the levels of DMAP1-DNMT1o complexes formed in R1 ES cells expressing DNMT1o for a short time (transiently transfected) to those in R1 cells expressing DNMT1o for a long time (stably transfected). We detected higher levels of DNMT1o in transiently transfected cells than in stably transfected cells (Fig. 4D). However, DMAP1-DNMT1o complexes were detected in only the stably transfected cells (Fig. 4E). These findings are consistent with the notion that physical interactions between DMAP1 and DNMT1o proteins did not readily occur.

In addition to ES cells, we examined the relationship between DMAP1 and DNMT1 protein levels in somatic cells. As shown in Fig. 5A, in primary mouse embryonic fibroblasts (MEFs) obtained from wild-type and *Dmap1*$^{+/-}$ heterozygous embryos, the concentration of DMAP1 protein was proportional to the number of wild-type alleles, such that wild-type MEFs have roughly twice the concentration of DMAP1 as *Dmap1*$^{+/-}$ MEFs. Moreover, the level of DNMT1s protein in *Dmap1*$^{+/+}$ MEFs was approximately twice that seen in *Dmap1*$^{+/-}$ MEFs. The same positive correlation between DMAP1 and DNMT1 protein levels was evident in *Dnmt1*$^{+/+}$ spleens, but not in *Dnmt1*$^{V/V}$ spleens (Fig. 5B). These observations suggest that the concentration of DNMT1s depends on the concentration of DMAP1, possibly because DNMT1s is stabilized in the DMAP1-DNMT1s complexes through its known DMAP1 interaction domain. The absence of correlation between DMAP1 and DNMT1o concentrations in spleen cells may be due to the absence of requirement for stabilization through the DMAP1-DNMT1o interaction or simply to an innately stable DNMT1o protein.

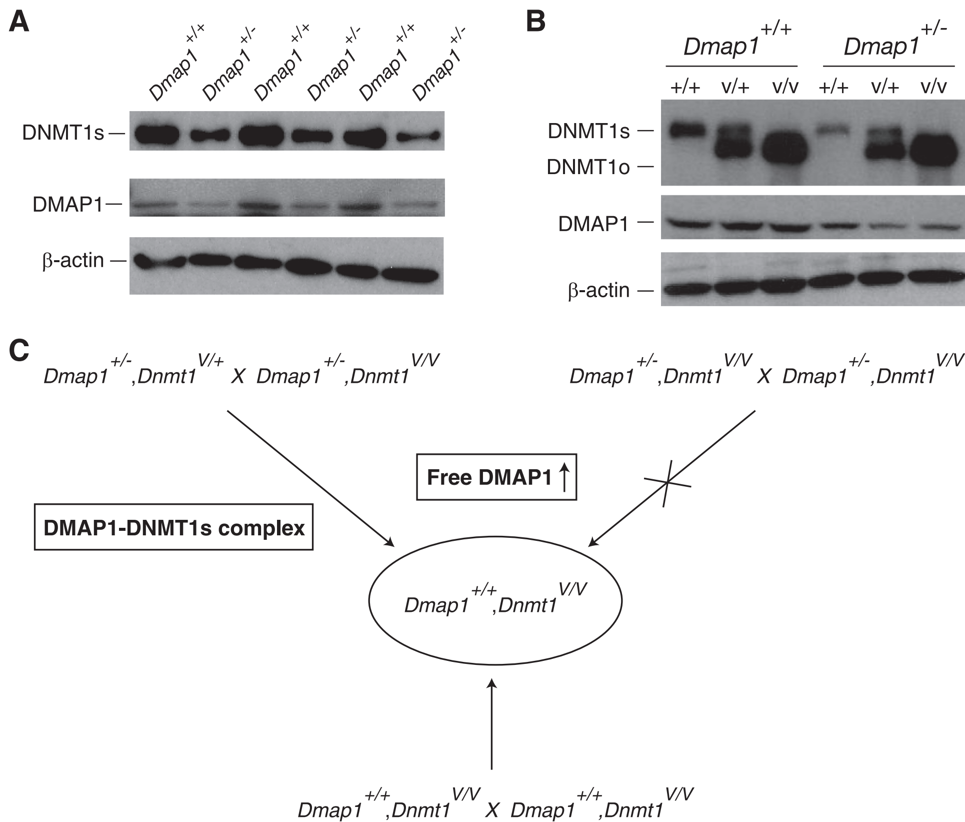

FIG. 5. Relationships between *Dmap1* and *Dnmt1* in mutant mice. (A) Immunoblots showing expression of DMAP1 and DNMT1s proteins in three *Dmap*$^{+/+}$, *Dnmt1*$^{+/+}$ and three *Dmap1*$^{+/-}$, *Dnmt1*$^{+/+}$ mouse embryonic fibroblast (MEF) lines. The concentration of β-actin protein was measured to ensure that approximately equal amounts of cellular protein were loaded in each lane. (B) Immunoblots showing expression of DMAP1 and DNMT1s proteins in spleens from six different mice. Genotypes are indicated at the top, with *Dnmt1* genotypes listed below *Dmap1* genotypes. +/+, *Dnmt1*$^{+/+}$; V/+, *Dnmt1*$^{V/+}$; V/V, *Dnmt1*$^{V/V}$. The concentration of the β-actin protein was measured to normalize the amount of protein loaded in each lane. (C) Effects of parental *Dmap1* and *Dnmt1* genetic backgrounds on outcome of *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ offspring. Three different crosses are shown, all of which result in *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ embryos. Despite identical genotypes, *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ embryos derived from crosses between *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ mice are rarely recovered.

DISCUSSION

During our investigations into the roles of DNMT1 proteins and their interactions with the DMAP1 protein, we identified two *Dmap1* lethal genotypes, *Dmap1*$^{-/-}$ embryos from crosses between *Dmap1*$^{+/-}$ heterozygous parents, and *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ embryos from crosses between *Dnmt1*$^{+/-}$, *Dnmt1*$^{V/V}$ parents. *Dmap1*$^{-/-}$ embryos die prior to the 8-cell stage of preimplantation development, whereas most *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ embryos die around midgestation. We conclude that the cause of embryonic death in *Dmap1*$^{-/-}$ embryos is different from the cause of death in *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ embryos, indicating two distinct and essential DMAP1-mediated processes.

The early preimplantation lethality of *Dmap1*$^{-/-}$ embryos is most likely due to inactivation of the TIP60-p400 complex, which functions in DNA repair and transcriptional regulation (28, 29). An RNA interference (RNAi) screen performed in mouse ES cells showed that reduction of the expression of individual components of the complex, including DMAP1, caused a loss of characteristic ES cell morphology and activation of genes associated with cell differentiation (8). Specifically, downregulation of DMAP1 expression induced by the stable expression of a *Dmap1* short hairpin RNA (shRNA) induced a flattening of the normal ES colony morphology and loss of expression of genes associated with ES cell pluripotency. Genes encoding different components of the TIP60-p400 complex are expressed not only in ES cells, but also in early mouse embryos, and embryos lacking *Tip60* or *Trrap*, two components of the complex, die before implantation (9, 10). We illustrated that the lack of another component of the complex, *Dmap1*, also led to preimplantation lethality as early as the 8-cell stage, possibly by the severe disruption to transcriptional control in preimplantation stages. In contrast to *Dmap1*$^{-/-}$ embryos, *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ embryos perish because of epigenetic defects, as shown by the decreased methylation of DMDs of both maternally and paternally imprinted genes (Fig. 3 and 4). Although we cannot formally exclude a role of the TIP60-p400 complex in DMD methylation, the lack of DNMT1 in the purified TIP60-p400 complex (2, 7) indicates that DMAP1 interacts with DNMT1 in a distinct complex. Thus, DMAP1-dependent functions of the TIP60-p400 complex appear to be independent of the DMAP1 function associated with DNMT1 proteins.

How might maintenance methylation be disrupted in

Dmap1$^{+/+}$, *Dnmt1*$^{V/V}$ embryos but not in *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ embryos derived from *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ parents? Based on the genetic evidence, we propose that in the absence of DMAP1-DNMT1s complex, a transgenerational change in the level of DNMT1s-free DMAP1 (free DMAP1) is lethal in embryogenesis. In support of this hypothesis, we show three crosses producing *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ embryos (Fig. 5C). The expected numbers of *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ offspring were recovered in two crosses—one in which the parents were both *Dmap1*$^{+/+}$ and the other in which one of the parents carried a wild-type *Dnmt1*$^{+}$ allele. In the case of the *Dmap*$^{+/+}$ parents, there is no change in the level of free DMAP1, whereas in the case of the cross in which one parent carries a *Dnmt1*$^{+}$ allele, the inheritance of DMAP1-DNMT1s complex from this parent enables the embryo to survive the transgenerational change in the level of free DMAP1. In crosses between *Dmap1*$^{+/-}$, *Dnmt1*$^{V/V}$ mice, there is no DNMT1s-DMAP1 complex and the *Dmap1*$^{+/+}$, *Dnmt1*$^{V/V}$ embryos experience a sudden increase in free DMAP1 and do not survive. A plausible mechanism whereby free DMAP1 could lead to embryonic death is through interference with the normal preimplantation functions of DMAP1-DNMT1s and DMAP1-DNMT1o complexes. For example, free DMAP1, which is slow in forming a complex with DNMT1o, as suggested by Fig. 4, would interfere with the resident DMAP1-DNMT1o complex, possibly by occupying the sites of maintenance methylation and blocking access of preformed DMAP1-DNMT1o complex to them. If these sites include DMDs of imprinted genes, DMD methylation would be irreversibly lost, and this would show up as a loss of DMD methylation later in embryogenesis. Alternatively, free DMAP1 may have an indirect effect on DNMT1o maintenance methyltransferase activity. In this regard, free DMAP1 might influence TIP60-p400 activity in preimplantation, which in turn would lead to abnormalities in chromatin and effects on DNMT1o activity.

Associations of DMAP1 with both DNMT1s and DNMT1o suggest the possibility that the interaction with DMAP1 is needed for a DNMT1 protein's enzymatic function. In addition to the 4-fold-lower avidity of DNMT1o for DMAP1 seen in ES cells (Fig. 4), *Dnmt1*$^{V/V}$ mice have a roughly 4-fold-higher level of DNMT1o protein compared to the level of DNMT1s in the wild-type mouse (6). Thus, the concentrations of DMAP1-DNMT1 complexes would be the same in wild-type and *Dnmt1*$^{V/V}$ mice and would predict that all or most DMAP1 protein is in complex with either DNMT1s or DNMT1o. Additional support for this notion is the direct correlation between the *Dmap1* allelic dose or the cellular DMAP1 concentration and the level of DNMT1s in MEFs and in spleens from mice with different *Dmap1* and *Dnmt1* genotypes (Fig. 5). Thus, neither DNMT1s nor DNMT1o would be expected to function independently of an interaction with DMAP1 protein. This notion could explain the observation that reduction of DMAP1 increases gene expression, possibly because of a reduction in DNA methylation (22, 26).

In addition to DMAP1, what other proteins comprise a DMAP1-DNMT1 complex, and which of these is required for enzymatic function of DNMT1 in cells? Rountree et al. (26) showed that the noncatalytic amino terminus of DNMT1s interacts with histone deacetylase 2 (HDAC2) as well as with DMAP1. They identified the interaction between DNMT1s and DMAP1 in a 2-hybrid screen using the first 125 amino acids of DNMT1s as the bait protein. Their approach to identifying DMAP1 suggested that DMAP1 interacts with DNMT1s via a domain not present in the oocyte-derived DNMT1o protein. DNMT1s and DMAP1 exist as a complex during the entirety of S phase, whereas HDAC2 is associated with the DNMT1-DMAP1 complex during late S phase. In the same study, DMAP1 was shown to have intrinsic repressive activity and was also associated with the corepressor TSG101 (26, 27). Subsequent reports added further support to the notion that DMAP1 is a corepressor (13, 16, 20). Regardless of the precise transcriptional function of DMAP1, it is clear that DMAP1 and DNMT1s directly physically interact via a DNMT1s-specific N-terminal domain. This direct interaction presumably accounts for the higher avidity of the DMAP1-DNMT1s interaction (approximately a 4-fold difference in avidity based on the results of immunoprecipitation experiments). Whether DMAP1 directly interacts with DNMT1o or whether the interaction is due simply to coexistence within a complex needs to be investigated further.

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REFERENCES

1. **Borowczyk, E., K. N. Mohan, L. D'Aiuto, M. C. Cirio, and J. R. Chaillet.** 2009. Identification of a region of the DNMT1 methyltransferase that regulates the maintenance of genomic imprints. Proc. Natl. Acad. Sci. U. S. A. **106:**20806–20811.
2. **Cai, Y., et al.** 2003. Identification of new subunits of the multiprotein mammalian TRRAP/TIP60-containing histone acetyltransferase complex. J. Biol. Chem. **278:**42733–42736.
3. **Chaillet, J. R., T. F. Vogt, D. R. Beier, and P. Leder.** 1991. Parental-specific methylation of an imprinted transgene is established during gametogenesis and progressively changes during embryogenesis. Cell **66:**77–83.
4. **Cirio, M. C., et al.** 2008. DNA methyltransferase 1o functions during preimplantation development to preclude a profound level of epigenetic variation. Dev. Biol. **324:**139–150.
5. **Cirio, M. C., et al.** 2008. Preimplantation expression of the somatic form of Dnmt1 suggests a role in the inheritance of genomic imprints. BMC Dev. Biol. **8:**9.
6. **Ding, F., and J. R. Chaillet.** 2002. In vivo stabilization of the Dnmt1 (cytosine-5)-methyltransferase protein. Proc. Natl. Acad. Sci. U. S. A. **99:**14861–14866.
7. **Doyon, Y., W. Selleck, W. S. Lane, S. Tan, and J. Côté.** 2004. Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans. Mol. Cell. Biol. **24:**1884–1896.
8. **Fazzio, T. G., J. T. Huff, and B. Panning.** 2008. An RNAi screen of chromatin proteins identifies Tip60-p400 as a regulator of embryonic stem cell identity. Cell **134:**162–174.
9. **Gorrini, C., et al.** 2007. Tip60 is a haplo-insufficient tumor suppressor required for an oncogene-induced DNA damage response. Nature **448:**1063–1067.
10. **Herceg, Z., et al.** 2001. Disruption of TRRAP causes early embryonic lethality and defects in cell cycle progression. Nat. Genet. **29:**206–211.
11. **Hirasawa, R., et al.** 2008. Maternal and zygotic Dnmt1 are necessary and sufficient for the maintenance of DNA methylation imprints during preimplantation development. Genes Dev. **22:**1607–1616.
12. **Howell, C. Y., et al.** 2001. Genomic imprinting disrupted by a maternal-effect mutation in the Dnmt1 gene. Cell **104:**829–838.
13. **Kang, B. G., et al.** 2007. Corepressor MMTR/DMAP1 is involved in both histone deacetylase 1- and TFIIH-mediated transcriptional repression. Mol. Cell. Biol. **27:**3578–3588.
14. **Koizumi, T., et al.** 2010. Depletion of Dnmt1-associated protein 1 triggers DNA damage and compromises the proliferative capacity of hematopoietic stem cells. Int. J. Hematol. **91:**611–619.
15. **Lakso, M., et al.** 1996. Efficient in vivo manipulation of mouse genomic sequences at the zygote stage. Proc. Natl. Acad. Sci. U. S. A. **93:**5860–5865.

16. **Lee, G. E., J. H. Kim, M. Taylor, and M. T. Muller.** 2010. DNA methyltransferase 1 associate protein (DMAP1) is a co-repressor that stimulates DNA methylation globally and locally at sites of double strand break repair. J. Biol. Chem. **285:**37630–37640.
17. **Li, E.** 2002. Chromatin modification and epigenetic reprogramming in mammalian development. Nat. Rev. Genet. **3:**662–673.
18. **Mertineit, C., et al.** 1998. Sex-specific exons control DNA methyltransferase activity in mammalian germ cells. Development **125:**889–897.
19. **Monk, M., M. Boubelik, and S. Lehnert.** 1987. Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. Development **99:**371–382.
20. **Muromoto, R., et al.** 2004. Physical and functional interactions between Daxx and DNA methyltransferase 1-associated protein, DMAP1. J. Immunol. **172:**2985–2993.
21. **Nagy, A., J. Rossant, R. Nagy, W. Abramour-Newerly, and J. C. Roder.** 1993. Derivation of completely cell-culture derived mice from early-passage embryonic stem cells. Proc. Natl. Acad. Sci. U. S. A. **90:**8424–8428.
22. **Negishi, M., et al.** 2007. Bmi1 cooperates with Dnmt1-associated protein 1 in gene silencing. Biochem. Biophys. Res. Commun. **353:**992–998.
23. **Ratnam, S., et al.** 2002. Dynamics of Dnmt1 methyltransferase expression and intracellular localization during oogenesis and preimplantation development. Dev. Biol. **245:**304–314.
24. **Reinhart, B., M. Eljanne, and J. R. Chaillet.** 2002. Shared role for differentially methylated domains of imprinted genes. Mol. Cell. Biol. **22:**2089–2098.
25. **Reinhart, B., A. Paoloni-Giacobino, and J. R. Chaillet.** 2006. Specific differentially methylated domain sequences direct the maintenance of methylation at imprinted genes. Mol. Cell. Biol. **26:**8347–8356.
26. **Rountree, M. R., K. E. Bachman, and S. B. Baylin.** 2000. DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. Nat. Genet. **25:**269–277.
27. **Ruland, J., et al.** 2001. p53 accumulation, defective cell proliferation, and early embryonic lethality in mice lacking tsg101. Proc. Natl. Acad. Sci. U. S. A. **98:**1859–1864.
28. **Sapountzi, V., I. R. Logan, and C. N. Robson.** 2006. Cellular functions of TIP60. Int. J. Biochem. Cell Biol. **38:**1496–1509.
29. **Squatrito, M., C. Gorrini, and B. Amati.** 2006. Tip60 in DNA damage response and growth control: many tricks in one HAT. Trends Cell Biol. **16:**433–442.