DNA AND CELL BIOLOGY
Volume 35, Number 10, 2016
© Mary Ann Liebert, Inc.
Pp. 585–590
DOI: 10.1089/dna.2015.3022

Gen1 and *Eme1* Play Redundant Roles in DNA Repair and Meiotic Recombination in Mice

Xiaowen Wang,[1,2,*] Herui Wang,[3,4,*] Bin Guo,[3] Ya Zhang,[1] Yinv Gong,[1] Chi Zhang,[3,5]
Hong Xu,[1] and Xiaohui Wu[1,3]

Resolution of the Holliday junction (HJ) is essential for homologous recombination and DNA repair. In *Saccharomyces cerevisiae*, HJ resolvase Yen1 and the Mus81-Mms4 complex are redundant in DNA damage repair. In cultured mammalian cells, such redundancy also exists between Yen1 ortholog GEN1 and the Mus81-Mms1 ortholog MUS81-EME1. In this report, we further tested if GEN1 and EME1 redundantly affect HJ-related physiological processes in mice. We found that combined homozygous mutations of *Gen1* and *Eme1* led to synthetic lethality during early embryonic stages. Homozygous *Gen1* mutations did not cause DNA repair deficiency in mouse embryonic fibroblast (MEF) cells, but made heterozygous *Eme1* mutant MEFs more sensitive to various DNA-damaging reagents. *Gen1* mutations also reduced the meiotic recombination efficiency in *Eme1* mutant mice. These results suggest that *Gen1* and *Eme1* play redundant roles in DNA repair and meiotic recombination *in vivo*.

Keywords: *Gen1, Eme1,* DNA repair, meiotic recombination

Introduction

A Holliday junction (HJ) is a branched intermediate structure that usually exists in meiosis and DNA recombination repair (Matos and West, 2014). HJs connect two double-stranded DNA molecules together so that they must be resolved to allow the segregation of both molecules (West, 2009). Defective HJ resolution may lead to sterility or increased susceptibility to cancer (Holloway *et al.*, 2008; Matos *et al.*, 2011). Several evolutionary conserved pathways are involved in HJ resolution in eukaryotic organisms.

In *Saccharomyces cerevisiae*, the Yen1 nuclease can symmetrically cut HJ in a manner analogous to the *Escherichia coli* HJ resolvase RuvC (Ip *et al.*, 2008; Rass *et al.*, 2010), whereas the XPF-family heterodimeric endonuclease complex Mus81-Mms4 can asymmetrically cleave HJ (Boddy *et al.*, 2001; Chen *et al.*, 2001). Slx1, another structure-selective endonuclease, is also capable of processing HJs (Fricke and Brill, 2003; Fekairi *et al.*, 2009; Munoz *et al.*, 2009). In addition to these structure-specific nucleases, HJs can be dissolved by the Sgs1-Top3-Rmi1 (STR) complex to produce noncrossover recombinants (Ellis *et al.*, 1995; Wu and Hickson, 2003; Cejka *et al.*, 2010).

In human cells, the Yen1 ortholog GEN1, Mus81-Mms4 ortholgs MUS81-EME1, and the Slx1 ortholog SLX1 can process HJs in similar ways as they do in yeast cells (Ip *et al.*, 2008; Matos and West, 2014). It has been shown that GEN1 forms a dimer that juxtaposes two products in a substrate-like complex, and the chromodomain of GEN1 is indispensable for its DNA recognition and cleavage activities (Lee *et al.*, 2015; Liu *et al.*, 2015). Both MUS81 and SLX1 can be activated by interacting with SLX4, a scaffold protein that serves as an essential docking platform to cooperate with multiple structure-specific endonucleases (Fekairi *et al.*, 2009; Munoz *et al.*, 2009; Svendsen *et al.*, 2009). HJs could also be dissolved by the Bloom's syndrome complex (BLM helicase-topoisomerase IIIα-RMI1/2) to produce noncrossover recombinants (Ellis *et al.*, 1995; Wu and Hickson, 2003; Cejka *et al.*, 2010).

Yen1 and the Mus81-Mms4 complex play redundant roles in HJ resolution in yeasts. Deletion of *yen1* in *S. cerevisiae* does not affect DNA repair, whereas disruption of both *yen1* and *mus81* caused hypersensitivity to various DNA-damaging reagents (Blanco *et al.*, 2010; Ho *et al.*, 2010; Tay and Wu, 2010). During meiosis, deletion of both *yen1* and *mms4* also caused more severe decrease in chromosome crossover than

[1]Shanghai Kidney Development and Pediatric Kidney Disease Research Center, Children's Hospital of Fudan University, Shanghai, China.
[2]Department of Nephrology, Wuhan Medical and Healthcare Center for Women and Children, Wuhan, China.
[3]State Key Laboratory of Genetic Engineering and National Center for International Research of Development and Disease, Institute of Developmental Biology and Molecular Medicine, Collaborative Innovation Center for Genetics and Development, School of Life Sciences, Fudan University, Shanghai, China.
[4]Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland.
[5]Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado.
*These authors contributed equally to this work.

the single mutations did (Zakharyevich *et al.*, 2012). In addition, ectopic expression of human *GEN1* could overcome HJ defects caused by *mus81* deletion in fission yeasts, resulting in the formation of chromosome crossover (Lorenz *et al.*, 2010). Recent studies in mitotic human cells have shown sequential activation of GEN1 and MUS81-EME1 to eliminate persistently jointed DNA molecules (Matos *et al.*, 2011; Matos and West, 2014). Depletion of both GEN1 and MUS81 in cultured cells from Bloom's syndrome patients resulted in elongated and segmented chromosomes, as well as high levels of cell mortality (Wechsler *et al.*, 2011; Wyatt *et al.*, 2013). These results indicate that the functional redundancy between GEN1 and MUS81-EME1 also exists in mammalian cells. However, little evidence has been provided for the functional redundancy between GEN1 and EME1, making this conclusion remain to be confirmed in mammals.

We have generated *Gen1* and *Eme1* mutations in mice by insertional mutagenesis with the *piggyBac* (PB) transposon. We report synthetic lethality, reduced DNA repair, and decreased meiotic recombination efficiency in mice and cells carrying both mutations. These results suggest a redundant role between *Gen1* and *Eme1* in DNA recombination in mice.

Materials and Methods

Mice

All animal experiments were performed in accordance with protocols approved by the Animal Care and Use Committee of the Institute of Developmental Biology and Molecular Medicine (IDM), Fudan University. Both *Gen1* (081125049-HLA) and *Eme1* (081028120-HLA) mutants were generated by inserting a PB transposon in target genes during the process of a large-scale insertional mutagenesis project on the FVB/NJ background (Ding *et al.*, 2005; Sun *et al.*, 2008). In the $Gen1^{PB}$ allele, the PB insertion was mapped in the second intron (Chr:12.11268138, Ensembl release 54). In the $Eme1^{PB}$ allele, the PB inserts into the third intron (Chr: 11.94510958, Ensembl release 54).

Cell culture and DNA damage assay

Mouse embryonic fibroblasts (MEFs) were isolated from E14.5 embryos and cultured in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin–streptomycin. The assay for sensitivity to DNA damage was performed as previously described (Lei *et al.*, 2012). In brief, MEFs of indicated genotype were plated in three wells of a six-well plate at a density of 5×10^4 per well for 12 h, then treated with CPT (264933; Jingke Chem), Etoposide (E1383; Sigma), MMC (M4287; Sigma), MMS (129925; Sigma), or HU (H8627; Sigma) at indicated doses for 24 h. Surviving cells in each well were counted by FACS after 1-week incubation. Cell survival rate was calculated by dividing survival cell number in each well by mean survival cell number in control group without drug treatment. Statistical analysis was performed by unpaired *t*-test.

Polymerase chain reaction

Genotyping polymerase chain reaction (PCR) was performed with a PB-specific primer (LB2) and two flanking genomic primers (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/dna). PCR and reverse transcription polymerase chain reaction (RT-PCR) primers are listed in Supplementary Table S1.

Measurement of meiotic recombination efficiency

Male *Gen1* mutant mice with mixed FVB/NJ and C57BL/6J background were bred in two steps: $Gen1^{PB/+}$ (FVB/NJ) mice were mated with C57BL/6J wild-type mice to generate $Gen1^{PB/+}$ (FVB/C57); $Gen1^{PB/+}$ (FVB/C57) mice were then mated with $Gen1^{PB/+}$ (FVB/NJ) mice to generate wild-type and $Gen1^{PB/PB}$ mice with mixed background. Combined *Gen1* and *Eme1* mutant mice with mixed background were generated by mating $Gen1^{PB/+}$ mice (FVB/C57) with $Gen1^{PB/+}$; $Eme1^{PB/PB}$ mice (FVB/NJ). Heterozygosity of the single nucleotide polymorphisms (SNPs) in these mice was confirmed using Sanger sequencing after genotyping PCR (Supplementary Table S1). The meiotic recombination efficiency was measured in the offspring of male mice with indicated genotypes and FVB/NJ females by genotyping PCR and Sanger sequencing. Meiotic recombination efficiency = [No. (rs32032816$^{G/G}$; rs32330931$^{A/G}$) + No. (rs32032816$^{C/G}$; rs32330931$^{G/G}$)]/No. (all offspring).

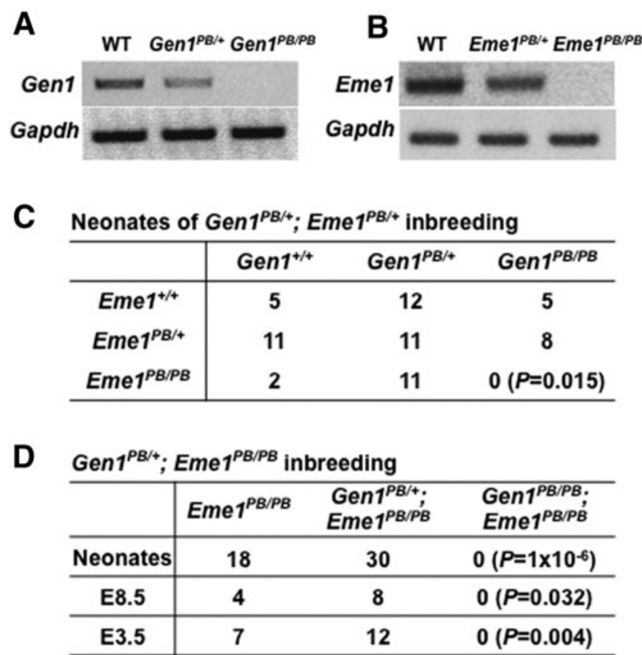
Statistics

Statistical methods were indicated in the figure legend. The threshold for significance was set at $p<0.05$.

Results

Disruption of Gen1 and Eme1 causes synthetic lethality

We have isolated *Gen1* and *Eme1* mutants from a PB insertional mutagenesis project in mice (Sun *et al.*, 2008). The PB transposon was inserted into the second and third intron of *Gen1* and *Eme1*, respectively. Both mutants were viable and fertile. RT-PCR analysis of individual mutant alleles showed that gene expression was reduced or extinguished in MEF cells isolated from heterozygous or homozygous mutants, respectively (Fig. 1A, B). To test if *Gen1* and *Eme1* play redundant roles in mice, we first tried to generate mice carrying both mutations. We have successfully obtained double heterozygous mice ($Gen1^{PB/+}$; $Eme1^{PB/+}$) and $Gen1^{PB/+}$; $Eme1^{PB/PB}$ mutants. However, inconsistent with expected Mendelian distribution, double homozygotes ($Gen1^{PB/PB}$; $Eme1^{PB/PB}$) were observed neither among 65 neonates generated from inbreeding of $Gen1^{PB/+}$; $Eme1^{PB/+}$ mice (Fig. 1C, $p=0.015$), nor among 48 progenies born by $Gen1^{PB/+}$; $Eme1^{PB/PB}$ inbreeding (Fig. 1D, $p=1\times10^{-6}$), indicating prenatal lethality of double homozygotes. To precisely pinpoint the time of embryonic death, embryos from $Gen1^{PB/+}$; $Eme1^{PB/PB}$ intercrosses were dissected at different stages and genotyped by PCR (Fig. 1D). We failed to identify double homozygotes at embryonic day 8.5 (E8.5) (0/12, $p=0.032$), or even at E3.5 (0/19 blastocysts, $p=0.004$), indicating that $Gen1^{PB/PB}$; $Eme1^{PB/PB}$ embryos could not survive through early embryonic development. The synthetic lethality caused by *Gen1* and *Eme1* mutations indicates that both genes are required for embryonic development.



C Neonates of $Gen1^{PB/+}$; $Eme1^{PB/+}$ inbreeding

	$Gen1^{+/+}$	$Gen1^{PB/+}$	$Gen1^{PB/PB}$
$Eme1^{+/+}$	5	12	5
$Eme1^{PB/+}$	11	11	8
$Eme1^{PB/PB}$	2	11	0 (P=0.015)

D $Gen1^{PB/+}$; $Eme1^{PB/PB}$ inbreeding

	$Eme1^{PB/PB}$	$Gen1^{PB/+}$; $Eme1^{PB/PB}$	$Gen1^{PB/PB}$; $Eme1^{PB/PB}$
Neonates	18	30	0 (P=1x10^{-6})
E8.5	4	8	0 (P=0.032)
E3.5	7	12	0 (P=0.004)

FIG. 1. Disruption of *Gen1* and *Eme1* caused synthetic lethality. RT-PCR showed disrupted expression of *Gen1* **(A)** and *Eme1* **(B)** in MEFs bearing homozygous mutations, respectively. Genotyping PCR of neonates **(C, D)** and embryonic day 8.5 (E8.5) offspring **(D)**, and E3.5 blastocysts **(D)** from indicated mice inbreeding detected no double homozygous mutant (*Gen1*$^{PB/PB}$; *Eme1*$^{PB/PB}$), respectively. *p*-Value of Fisher's exact test was labeled in the figure. MEFs, mouse embryonic fibroblasts; PB, *piggyBac*; RT-PCR, reverse transcription polymerase chain reaction; WT, wild-type.

Gen1 *and* Eme1 *play redundant roles in cellular resistance to DNA damage-induced cell death*

We speculate that the lethality of *Gen1* and *Eme1* double mutants may be due to the redundant roles of GEN1 and the MUS81-EME1 complex in DNA repair in mice. To confirm this hypothesis, we prepared primary MEFs carrying different combinations of *Gen1* and *Eme1* mutations to test their sensitivities to various DNA damaging reagents, such as mitomycin C (MMC), methyl methanesulfonate (MMS), camptothecin (CPT), and Etoposide. We did not observe any significant differences in the survival rates between the wild-type and *Gen1*$^{PB/PB}$ MEFs (Fig. 2A–D).

However, *Gen1*$^{PB/PB}$ mutations significantly increased the sensitivity of *Eme1*$^{PB/+}$ MEFs to DNA-damaging reagents. Compared with that of *Eme1*$^{PB/+}$ MEFs, the survival rate of *Gen1*$^{PB/PB}$; *Eme1*$^{PB/+}$ MEFs dropped by $\sim$12% under the treatment with three different concentrations of CPT or Etoposide (Fig. 2A, B). Although low and high concentrations of MMC treatment did not cause any differences, medial level of MMC (0.67 μg/mL) killed slightly more *Gen1*$^{PB/PB}$; *Eme1*$^{PB/+}$ MEFs than *Eme1*$^{PB/+}$ MEFs (Fig. 2C). The survival rates of *Gen1*$^{PB/PB}$; *Eme1*$^{PB/+}$ and *Eme1*$^{PB/+}$ MEFs are comparable with each other after MMS treatment, both lower than those of the wild-type or *Gen1*$^{PB/PB}$ MEFs (Fig. 2D). Meanwhile, DNA replication stress by hydroxyurea (HU) treatment did not bring any differences between the wild-type and *Gen1*$^{PB/PB}$; *Eme1*$^{PB/+}$ MEFs (Fig. 2E).

Real-time RT PCR showed that *Gen1* mRNA was undetectable in homozygous mutant MEFs (Fig. 2F), whereas $\sim$31% of *Eme1* mRNA still remained in *Gen1*$^{PB/PB}$; *Eme1*$^{PB/+}$ MEFs (Fig. 2G).

These results indicate that *Gen1* and *Eme1* are functionally redundant in the resistance to DNA damage-induced cell death in mammalian cells.

Gen1 *and* Eme1 *play redundant roles in meiotic recombination*

As the intermediate structure of DNA recombination, HJs exist not only in DNA repair, but also in meiosis. We thus checked if *Gen1* and *Eme1* also play redundant roles in meiotic recombination in mice.

We first introduced *Gen1* and *Eme1* mutations into the mixed genetic background of FVB/NJ and C57BL/6J by breeding, then collected $\sim$100 offspring of each of the four genotypes (WT, *Gen1*$^{PB/PB}$, *Eme1*$^{PB/+}$, and *Gen1*$^{PB/PB}$; *Eme1*$^{PB/+}$) to measure the meiotic recombination efficiency by scoring the segregation of SNPs from both ancestral genetic background. After screening >40 SNPs that are not only different in FVB/NJ and C57BL/6J, but also mapped $\sim$25–50 Mbps apart from each other (Supplementary Table S2), we found a pair of Chr. Seven SNPs (rs32032816 and rs32330931) that were both heterozygous in all of the four genotypes so that they could be used to calculate the recombination efficiency (Fig. 3A).

The calculated meiotic recombination efficiency in the offspring of wild-type male mice was 32.22% (29/90). Similar results were obtained in the offspring of *Gen1*$^{PB/PB}$ (33.96%, n = 106) and *Eme1*$^{PB/+}$ (36.89%, n = 103) males. However, the rate was significantly reduced in the offspring of *Gen1*$^{PB/PB}$; *Eme1*$^{PB/+}$ mice. Only 17.48% (18/103, p = 0.0193) of the progeny were identified as recombinants. Thus, disruption of *Gen1* affects meiotic recombination not on the wild type, but on the *Eme1* mutant background (Fig. 3B). Other than this, we did not find obvious differences in the size and gross morphology of the testis in *Gen1*$^{PB/PB}$; *Eme1*$^{PB/+}$ and other mutants (Supplementary Fig. S1A–D). The mating between male mice of each genotype and WT FVB/NJ females resulted in similar litter sizes as well (Supplementary Fig. S1E). Taken together, these results suggest that *Gen1* and *Eme1* also play redundant roles in meiotic recombination.

Discussion

GEN1, MUS81-EME1, and SLX1 are three HJ resolvases that could process persistent HJs in mammalian cells (Boddy *et al.*, 2001; Chen *et al.*, 2001; Fricke and Brill, 2003; Ip *et al.*, 2008; Fekairi *et al.*, 2009; Munoz *et al.*, 2009; Rass *et al.*, 2010). However, their contributions to HJ-mediated processes *in vivo* have not been well defined. In this study, we found that a *Gen1* mutation alone did not affect DNA repair or meiotic recombination in mice. It caused synthetic lethality, nevertheless, when combined with *Eme1* mutations at an early embryonic stage. In addition, the combination of *Gen1*$^{PB/PB}$; *Eme1*$^{PB/+}$ mutations makes MEFs more sensitive to DNA damage and mice less capable of meiotic recombination. These results indicate that *Gen1* and *Eme1* are functionally redundant in DNA repair and meiotic recombination in mice.

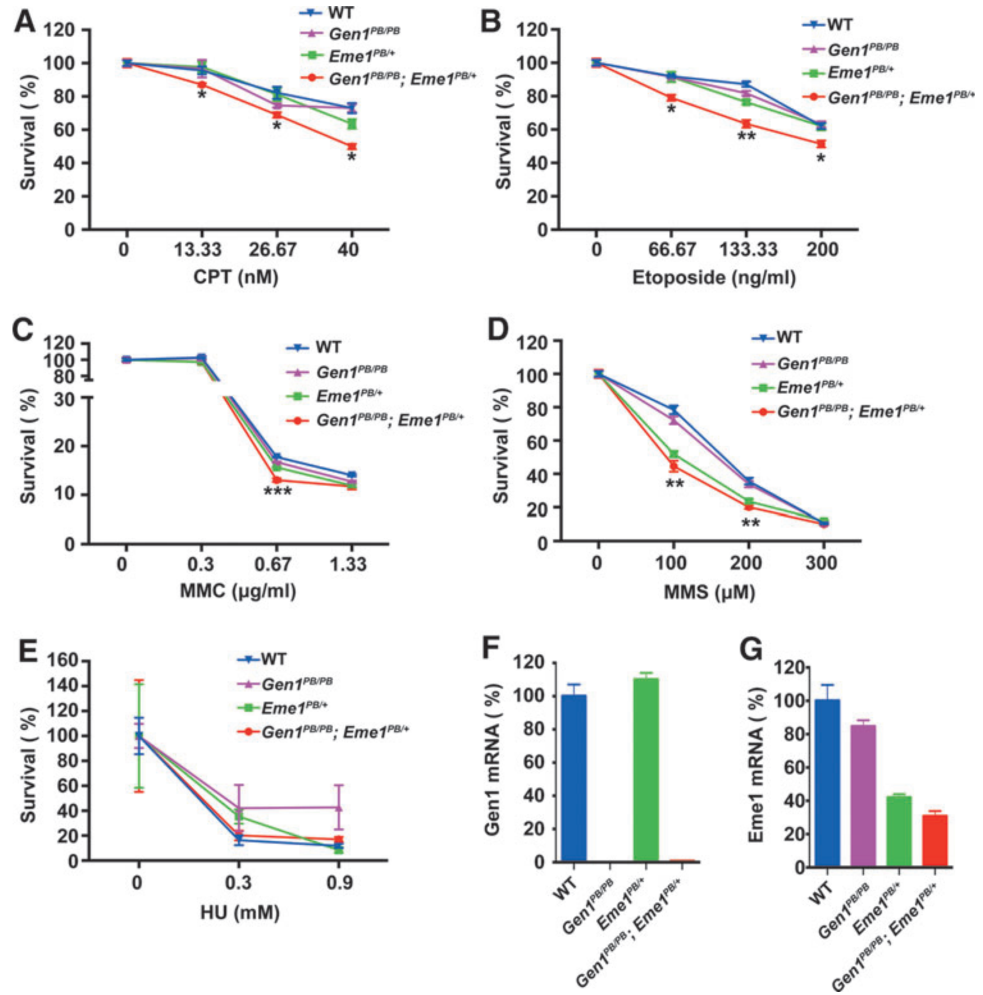


FIG. 2. *Gen1* mutation increased the sensitivity of $Eme1^{PB/+}$ MEFs to DNA-damaging reagents. WT, $Gen1^{PB/PB}$, $Eme1^{PB/+}$, and $Gen1^{PB/PB}$; $Eme1^{PB/+}$ MEF cells were subjected to doses of CPT **(A)**, Etoposide **(B)**, MMC **(C)**, MMS **(D)**, and HU **(E)** as indicated. The survival rate of the cells was quantified 1 week later. Compared with $Eme1^{PB/+}$ MEF cells, $Gen1^{PB/PB}$; $Eme1^{PB/+}$ MEF cells were more sensitive to CPT **(A)**, Etoposide **(B)**, and MMC **(C)**. **(F)** Real-time RT PCR results of *Gen1* in the four cell lines. **(G)** Real-time RT-PCR results of *Gen1* in the four cell lines. Data are presented as mean ± SEM. *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$; unpaired *t* test. CPT, camptothecin; HU, hydroxyurea; MMC, mitomycin C; MMS, methyl methanesulfonate.

Compared with those of the *Mus81* and *Eme1* mutants, the phenotypes related to DNA recombination are milder in *Gen1* mutants. Homozygous deletion of *Eme1* caused hypersensitivity to MMC and other DNA-damaging reagents in embryonic stem cells and MEFs (Abraham *et al.*, 2003). $Mus81^{-/-}$ mice exhibited significant meiotic defects such as the depletion of mature epididymal sperm and partial failure of DNA double-strand breaks repair (Holloway *et al.*, 2008). Thus, GEN1 likely plays a supporting role in DNA repair and meiosis recombination in mice. This may also explain why $Gen1^{PB/PB}$; $Eme1^{PB/+}$ MEFs showed only a mild additional effect on drug sensitivity when compared with $Eme1^{PB/+}$ MEFs, in which substantial amount of *Eme1* expression could still be detected (Fig. 2G).

Increased sensitivity of *Gen1* and *Eme1* double mutants to DNA damaging reagents may be a result of multiple cellular events. It has been shown that codepletion of MUS81 and GEN1 in human fibroblasts led to severe chromosome segregation defects (Wyatt *et al.*, 2013). MUS81- and GEN1-codepleted HeLa cells also showed genome instability, which is exemplified by impaired replication fork movement and S-phase progression, endogenous checkpoint

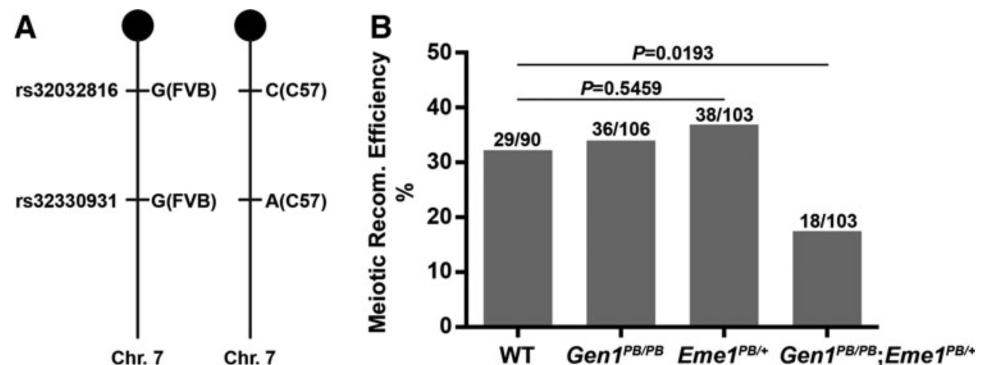


FIG. 3. Decreased meiotic recombination efficiency in $Gen1^{PB/PB}$; $Eme1^{PB/+}$ mice. **(A)** Brief illustration shows the genotype of two SNPs on FVB/NJ and C57BL/6J background, respectively. **(B)** Meiotic recombination efficiency of WT, $Gen1^{PB/PB}$, $Eme1^{PB/+}$, and $Gen1^{PB/PB}$; $Eme1^{PB/+}$ mice. Recombinant pups/total pups is labeled above each column. *p* Value was calculated by Fisher's exact test. SNPs, single nucleotide polymorphisms.

activation, chromosome segmentation, and multinucleation (Sarbajna *et al.*, 2014). These events may also contribute to the synthetic lethality observed in *Gen1*$^{PB/PB}$; *Eme1*$^{PB/PB}$ embryos, which may experience DNA damages caused by environment during development.

Meanwhile, mutant embryos may die of HJ processing deficiency during mitosis. DNA replication is known to produce HJs (Petermann and Helleday, 2010). Failure of the resolution of replication-induced HJs could cause delayed or arrested mitosis in mammalian cells, leading to cell death in the absence of exogenous DNA damage (Garner *et al.*, 2013). Depletion of GEN1 in human cells expressing mutant SLX4 that no longer binds and activates MUS81 would result in dysfunctional mitosis and cell death under normal culturing conditions (Garner *et al.*, 2013; Sarbajna *et al.*, 2014). Double mutations of GEN1 and EME1 may cause cell death through similar mitotic defects.

Conclusion

In summary, GEN1 and MUS81-EME1 are functionally redundant in mice to ensure the elimination of HJs during both mitotic DNA damage repair and meiotic recombination. Given that both MUS81-EME1 and SLX1 interact with the scaffold protein SLX4 to resolve HJs together in mammalian cells, it would be reasonable to speculate that GEN1 and SLX1 are also functionally redundant in these processes *in vivo*.

Acknowledgments

The authors thank Dr. Kai Lei, Zhisheng Ye, and Xiaoqiang Zhu for helpful discussion, and Guicheng Wang, Yanyan Nie, Xiaorong Huang, and Zhiyan Xia for technical assistance. This study was supported by grants from the National Natural Science Foundation of China (81270763), Chinese Hi-Tech Research and Development Project (2014AA021104), Chinese Key Projects for Basic Research (2011CB944001), Shanghai Municipal Government (15XD1500500 and 12431900100), and the Research Fund of the State Key Laboratory of Genetic Engineering, Fudan University.

Disclosure Statement

No competing financial interests exist.

References

Abraham, J., Lemmers, B., Hande, M.P., Moynahan, M.E., Chahwan, C., Ciccia, A., Essers, J., Hanada, K., Chahwan, R., Khaw, A.K., *et al.* (2003). Eme1 is involved in DNA damage processing and maintenance of genomic stability in mammalian cells. EMBO J **22,** 6137–6147.

Blanco, M.G., Matos, J., Rass, U., Ip, S.C., and West, S.C. (2010). Functional overlap between the structure-specific nucleases Yen1 and Mus81-Mms4 for DNA-damage repair in *S. cerevisiae*. DNA Repair (Amst) **9,** 394–402.

Boddy, M.N., Gaillard, P.H., McDonald, W.H., Shanahan, P., Yates, J.R., 3rd and Russell, P. (2001). Mus81-Eme1 are essential components of a Holliday junction resolvase. Cell **107,** 537–548.

Cejka, P., Plank, J.L., Bachrati, C.Z., Hickson, I.D., and Kowalczykowski, S.C. (2010). Rmi1 stimulates decatenation of double Holliday junctions during dissolution by Sgs1-Top3. Nat Struct Mol Biol **17,** 1377–1382.

Chen, X.B., Melchionna, R., Denis, C.M., Gaillard, P.H., Blasina, A., Van de Weyer, I., Boddy, M.N., Russell, P., Vialard, J., and McGowan, C.H. (2001). Human Mus81-associated endonuclease cleaves Holliday junctions in vitro. Mol Cell **8,** 1117–1127.

Ding, S., Wu, X., Li, G., Han, M., Zhuang, Y., and Xu, T. (2005). Efficient transposition of the piggyBac (PB) transposon in mammalian cells and mice. Cell **122,** 473–483.

Ellis, N.A., Groden, J., Ye, T.Z., Straughen, J., Lennon, D.J., Ciocci, S., Proytcheva, M., and German, J. (1995). The Bloom's syndrome gene product is homologous to RecQ helicases. Cell **83,** 655–666.

Fekairi, S., Scaglione, S., Chahwan, C., Taylor, E.R., Tissier, A., Coulon, S., Dong, M.Q., Ruse, C., Yates, J.R., 3rd, Russell, P. *et al.* (2009). Human SLX4 is a Holliday junction resolvase subunit that binds multiple DNA repair/recombination endonucleases. Cell **138,** 78–89.

Fricke, W.M., and Brill, S.J. (2003). Slx1-Slx4 is a second structure-specific endonuclease functionally redundant with Sgs1-Top3. Genes Dev **17,** 1768–1778.

Garner, E., Kim, Y., Lach, F.P., Kottemann, M.C., and Smogorzewska, A. (2013). Human GEN1 and the SLX4-associated nucleases MUS81 and SLX1 are essential for the resolution of replication-induced holliday junctions. Cell Rep **5,** 207–215.

Ho, C.K., Mazon, G., Lam, A.F., and Symington, L.S. (2010). Mus81 and Yen1 promote reciprocal exchange during mitotic recombination to maintain genome integrity in budding yeast. Mol Cell **40,** 988–1000.

Holloway, J.K., Booth, J., Edelmann, W., McGowan, C.H., and Cohen, P.E. (2008). MUS81 generates a subset of MLH1-MLH3-independent crossovers in mammalian meiosis. PLoS Genet **4,** e1000186.

Ip, S.C., Rass, U., Blanco, M.G., Flynn, H.R., Skehel, J.M., and West, S.C. (2008). Identification of Holliday junction resolvases from humans and yeast. Nature **456,** 357–361.

Lee, S.H., Princz, L.N., Klugel, M.F., Habermann, B., Pfander, B., and Biertumpfel, C. (2015). Human Holliday junction resolvase GEN1 uses a chromodomain for efficient DNA recognition and cleavage. Elife 4, e12256.

Lei, K., Zhu, X., Xu, R., Shao, C., Xu, T., Zhuang, Y., and Han, M. (2012). Inner nuclear envelope proteins SUN1 and SUN2 play a prominent role in the DNA damage response. Curr Biol **22,** 1609–1615.

Liu, Y., Freeman, A.D., Declais, A.C., Wilson, T.J., Gartner, A., and Lilley, D.M. (2015). Crystal structure of a eukaryotic GEN1 resolving enzyme bound to DNA. Cell Rep **13,** 2565–2575.

Lorenz, A., West, S.C., and Whitby, M.C. (2010). The human Holliday junction resolvase GEN1 rescues the meiotic phenotype of a *Schizosaccharomyces pombe* mus81 mutant. Nucleic Acids Res **38,** 1866–1873.

Matos, J., Blanco, M.G., Maslen, S., Skehel, J.M., and West, S.C. (2011). Regulatory control of the resolution of DNA recombination intermediates during meiosis and mitosis. Cell **147,** 158–172.

Matos, J., and West, S.C. (2014). Holliday junction resolution: regulation in space and time. DNA Repair (Amst) **19,** 176–181.

Munoz, I.M., Hain, K., Declais, A.C., Gardiner, M., Toh, G.W., Sanchez-Pulido, L., Heuckmann, J.M., Toth, R., Macartney, T., Eppink, B. *et al.* (2009). Coordination of structure-specific nucleases by human SLX4/BTBD12 is required for DNA repair. Mol Cell **35,** 116–127.

Petermann, E., and Helleday, T. (2010). Pathways of mammalian replication fork restart. Nat Rev Mol Cell Biol **11,** 683–687.

Rass, U., Compton, S.A., Matos, J., Singleton, M.R., Ip, S.C., Blanco, M.G., Griffith, J.D., and West, S.C. (2010). Mechanism of Holliday junction resolution by the human GEN1 protein. Genes Dev **24,** 1559–1569.

Sarbajna, S., Davies, D., and West, S.C. (2014). Roles of SLX1-SLX4, MUS81-EME1, and GEN1 in avoiding genome instability and mitotic catastrophe. Genes Dev **28,** 1124–1136.

Sun, L.V., Jin, K., Liu, Y., Yang, W., Xie, X., Ye, L., Wang, L., Zhu, L., Ding, S., Su, Y. *et al.* (2008). PBmice: an integrated database system of piggyBac (PB) insertional mutations and their characterizations in mice. Nucleic Acids Res **36(Database issue)**, D729–D734.

Svendsen, J.M., Smogorzewska, A., Sowa, M.E., O'Connell, B.C., Gygi, S.P., Elledge, S.J., and Harper, J.W. (2009). Mammalian BTBD12/SLX4 assembles a Holliday junction resolvase and is required for DNA repair. Cell **138,** 63–77.

Tay, Y.D., and Wu, L. (2010). Overlapping roles for Yen1 and Mus81 in cellular Holliday junction processing. J Biol Chem **285,** 11427–11432.

Wechsler, T., Newman, S., and West, S.C. (2011). Aberrant chromosome morphology in human cells defective for Holliday junction resolution. Nature **471,** 642–646.

West, S.C. (2009). The search for a human Holliday junction resolvase. Biochem Soc Trans **37,** 519–526.

Wu, L., and Hickson, I.D. (2003). The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature **426,** 870–874.

Wyatt, H.D., Sarbajna, S., Matos, J., and West, S.C. (2013). Coordinated actions of SLX1-SLX4 and MUS81-EME1 for holliday junction resolution in human cells. Mol Cell **52,** 234–247.

Zakharyevich, K., Tang, S., Ma, Y., and Hunter, N. (2012). Delineation of joint molecule resolution pathways in meiosis identifies a crossover-specific resolvase. Cell **149,** 334–347.

Address correspondence to:
Xiaohui Wu, PhD
Institute of Developmental Biology and Molecular Medicine
Fudan University
Shanghai 200433
China

E-mail: xiaohui_wu@fudan.edu.cn

Hong Xu, MD
Department of Nephrology and Rheumatology
Children's Hospital of Fudan University
Shanghai 201102
China

E-mail: hxu@shmu.edu.cn

Received for publication July 25, 2015; received in revised form May 29, 2016; accepted June 5, 2016.