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The human Holliday junction resolvase GEN1 rescues the meiotic phenotype of a *Schizosaccharomyces pombe mus81* mutant

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ABSTRACT

A key step in meiotic recombination involves the nucleolytic resolution of Holliday junctions to generate crossovers. Although the enzyme that performs this function in human cells is presently unknown, recent studies led to the identification of the XPG-family endonuclease GEN1 that promotes Holliday junction resolution *in vitro*, suggesting that it may perform a related function *in vivo*. Here, we show that ectopic expression of GEN1 in fission yeast *mus81*Δ strains results in Holliday junction resolution and crossover formation during meiosis.

INTRODUCTION

Homologous recombination (HR) is a key process in chromosome biology. Its capacity to repair double strand breaks (DSBs) in DNA serves to maintain genome integrity, promote correct chromosome segregation during meiosis through the formation of chiasmata, and generate genetic diversity by creating new allelic combinations in the germline. The central intermediate of HR is a four-way DNA junction structure that physically connects the two recombining DNA molecules (1). These structures, known as Holliday junctions (HJs), have to be resolved so that the DNA molecules can segregate at cell division. Resolution is achieved by cleavage of a pair of strands at the junction centre, with the choice of strands determining whether crossover or non-crossover recombinants are made. Crossovers consist of a reciprocal exchange of DNA flanking the site of HJ resolution, and in meiosis, when the recombining molecules are the homologous chromosomes, give rise to chiasmata and allelic reassortment (2–5).

HJ resolution is catalysed by specialized nucleases, examples of which were first identified in bacteria and their phages (6–8). More recently, the Mus81-Eme1 complex, a member of the XPF-family of structure-specific endonucleases, was implicated in HJ resolution in eukaryotes (9,10). However, whilst Mus81-Eme1 can cleave HJs *in vitro*, the manner in which it does so is not representative of a canonical resolvase like *Escherichia coli* RuvC or RusA, which cleave HJs symmetrically to generate nicked linear duplex products that are repairable by DNA ligase (11–13). Moreover, Mus81-Eme1's preferred substrates are HJ precursors (displacement [D] loops and unligated HJs) rather than fully fledged HJs (14–16).

Most recently, two novel HJ resolvases have been identified in humans, which like RuvC and RusA, cleave synthetic HJs symmetrically. These are GEN1 (17,18) and SLX1-SLX4 (19–21). In the case of SLX1-SLX4 genetic data indicate that it plays an important role in promoting the repair of DSBs and interstrand crosslinks (ICLs) (19–21), and in Drosophila the orthologue of SLX4, MUS312, is critical for meiotic crossover formation (22). GEN1's potential involvement in DNA repair and crossover formation has yet to be determined. However, genetic analysis of its orthologue in *Saccharomyces cerevisiae* Yen1 shows how at least one member of this family can play a redundant role with Mus81 in enabling the repair of ICLs and lesions that perturb replication fork progression (M.G. Blanco, J. Matos, U. Rass, S.C. Ip and S.C. West, manuscript submitted for publication). Here, we investigate whether human GEN1 has the ability *in vivo* to promote crossover formation by testing whether it can substitute for Mus81-Eme1 in promoting meiotic recombination in *Schizosaccharomyces pombe*.

MATERIALS AND METHODS

Yeast strains and plasmid construction

S. pombe strains used for this study are listed in Table 1. To express wild-type and mutated versions of GEN1 in fission yeast the following plasmids were constructed: From pDONR221-GEN1(1-527), pCMV6-GEN1(D30A) and pCMV6-GEN1(E134A/E136A), respectively (17), the open reading frames were amplified by PCR with Pfu polymerase (Stratagene, CA, USA) adding a NcoI-site

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Table 1. *Schizosaccharomyces pombe* strains used in this study

Strain	Genotype	Source
MCW1019	*h⁺ rqh1Δ::kanMX6 ura4-D18 leu1-32 his3-D1 arg3-D4*	Lab strain
FO808	*h⁻ ura4-D18 leu1-32 his3-D1 arg3-D4*	Lab strain
MCW1221	*h⁺ ura4-D18 leu1-32 his3-D1 arg3-D4*	Lab strain
MCW1237	*h⁻ mus81Δ::kanMX6 ura4-D18 leu1-32 his3-D1 arg3-D4*	Lab strain
MCW1238	*h⁺ mus81Δ::kanMX6 ura4-D18 leu1-32 his3-D1 arg3-D4*	Lab strain
MCW3200/ALP731	*h⁻ˢᵐᵗ⁰ his3⁺-aim ade6-L469 ura4-D18 his3-D1 arg3-D4*	This study
MCW3202/ALP733	*h⁺ˢ ura4⁺-aim2 ade6-3083 ura4-D18 leu1-32 his3-D1*	This study
FO1267	*h⁻ his3⁺-aim ade6-L469 ura4-D18 leu1-32 his3-D1*	Lab strain
MCW3514/ALP802	*h⁺ˢ mus81Δ::kanMX6 ura4⁺-aim2 ade6-3083 ura4-D18 leu1-32 his3-D1*	This study
MCW3589/ALP822	*h⁻ˢᵐᵗ⁰ mus81Δ::kanMX6 his3⁺-aim ade6-L469 ura4-D18 his3-D1 arg3-D4*	This study
FO1260	*h⁻ mus81Δ::kanMX6 his3⁺-aim ade6-L469 ura4-D18 leu1-32 his3-D1*	Lab strain

upstream and a stop codon as well as a BamHI-site downstream of the gene (the FLAG-tags in the original constructs were omitted). These constructs were cloned into the pREP41-3HAN vector (23) (the NcoI–BamHI restriction endonuclease digest removes the N-terminal 3HA-tag entirely), to put GEN1 under the control of the thiamine-repressible *nmt1*-promotor. To express fission yeast Rqh1 from the *nmt1*-promotor the *rqh1,* open reading frame was amplified by PCR using Pfu polymerase from genomic DNA of the *S. pombe* strain MCW1221 adding SalI sites up and downstream of the gene and cloned into pREP41 (24). All resulting plasmids—pGEN1 (wild-type GEN1), pGEN1^{DA} (GEN1 carrying the D30A point mutation), pGEN1$^{EA/EA}$ (GEN1 carrying the E134A and E136A point mutations) and pMW563 (pRqh1)—were sequenced to ensure that no mutations had been introduced.

Plasmids pREP41 (24), pRusA (pMW437, pREP1-NLS-rusA-GFP) (25), pRqh1 (pREP41-Rqh1), pMus81* (pMW592, pREP41-2myc6his-Mus81–Pk-Eme1) (15), pGEN1, pGEN1^{DA} and pGEN1$^{EA/EA}$ were transformed into fission yeast strains MCW1019, FO808, MCW1221, MCW1237 and MCW1238, the resulting strains were used for spot assays and for spore viability testing. For the meiotic recombination assay, the empty vector pREP41 was transformed into the wild-type control strains MCW3202 and FO1267; the *mus81*Δ strains MCW3514 and FO1260 were transformed with pGEN1, pRusA and pMus81* (15).

Culture conditions and genetic methods

Yeast cells were cultured in YES broth and on YES plates, unless they contained plasmids, in which case the cells were grown in EMM2 broth and on EMM2 agar plates containing the required supplements and using the monosodium salt of L-glutamic acid as the nitrogen source instead of NH_4Cl (http://www-rcf.usc.edu/~forsburg/media.html). Sporulation of the crosses MCW3202 × MCW3200 and MCW3514 × MCW3589 were performed on ME agar and for all the crosses with strains containing plasmids on SPAS agar (26). Spot assays (25), determination of spore viability by random spore analysis (26) and the meiotic recombination assay (14,15) have been previously described in detail.

Protein extraction and western blotting

Whole-cell extracts were prepared as detailed before (27) and resolved on 12.5% SDS–PAGE gels, transferred to a Trans-Blot transfer medium membrane (Bio-Rad Laboratories, Hercules, CA, USA) and probed with an anti-GEN1 antibody (raised against the N-terminal 527AA of the protein; dilution 1:1000) or with anti-ß-actin (ab8224; Abcam plc, Cambridge, UK; dilution 1:2000) as a loading control. Blocking of membranes and antibody incubations using the anti-GEN1 antibody were performed in 1× PBS containing 5% skimmed milk, 2.5% BSA, 100 mM L-lysine and 0.1% Tween 20, all washes were done with 0.1% Tween 20 in 1× PBS. For the anti-β-actin immunoblotting 1× TBS containing 5% skimmed milk, 2.5% BSA, 100 mM L-lysine and 0.1% Tween 20 was used for blocking and 1× TBS containing 0.5% skimmed milk, 0.25% BSA, 10 mM L-lysine and 0.1% Tween 20 for the antibody incubations, all washes were done using 0.1% Tween 20 in 1× TBS. Following incubation with an appropriate secondary antibody conjugated to horse-radish peroxidase at a dilution of 1:20 000 the proteins were detected using Amersham ECL Western Blotting Detection Reagents (GE Healthcare UK Ltd., Little Chalfont, UK). Membranes were then exposed to Super RX medical X-ray film (Fujifilm, Tokyo, Japan).

Statistics

Statistical analysis for the recombination data was performed in Excel (Microsoft Office), in Matlab 7 (The MathWorks, UK) and on http://www.physics.csbsju.edu/stats/KS-test.html. First each data set was tested for normal distribution using a one-sample Kolmogorov–Smirnov goodness-to-fit test, rejecting the null hypothesis (H_0; 'data fits a normal distribution') at an α-level of $P < 0.05$. Almost all the data were consistent with a normal distribution and were compared with the appropriate control experiment using a two-tailed, unpaired, two-sample *t*-test, assuming either equal (homoskedastic) or unequal variance (heteroskedastic) depending on the outcome of an *F*-test (H_0 'data sets having an equal variance' rejected at an α-level of $P < 0.05$). One data set did not follow a normal distribution and was therefore tested against the control using a two-sample Kolmogorov–Smirnov test, which is nonparametric and

does not depend on the normal distribution of data sets. The *P*-values are presented in Supplementary Table S2, together with an indication of the type of test used for each comparison. H_0 ('data sets being similar') was rejected at an α-level $P < 0.01$.

RESULTS

To determine whether GEN1 is capable of working in a meiotic *in vivo* environment, we made use of the fission yeast *S. pombe*. This is a useful eukaryotic system for validating HJ resolvases *in vivo* because the resolution of HJs, or their precursors, during meiotic recombination relies almost entirely on Mus81-Eme1 (28). Consequently, HJs accumulate in a *mus81*Δ mutant (28). Moreover, it has previously been shown that the ectopic expression of the bacterial HJ resolvase RusA can rescue vegetative and/or meiotic defects associated with HJ accumulation when Mus81-Eme1 or the RecQ-type helicase Rqh1 are deleted (9,15,25,29).

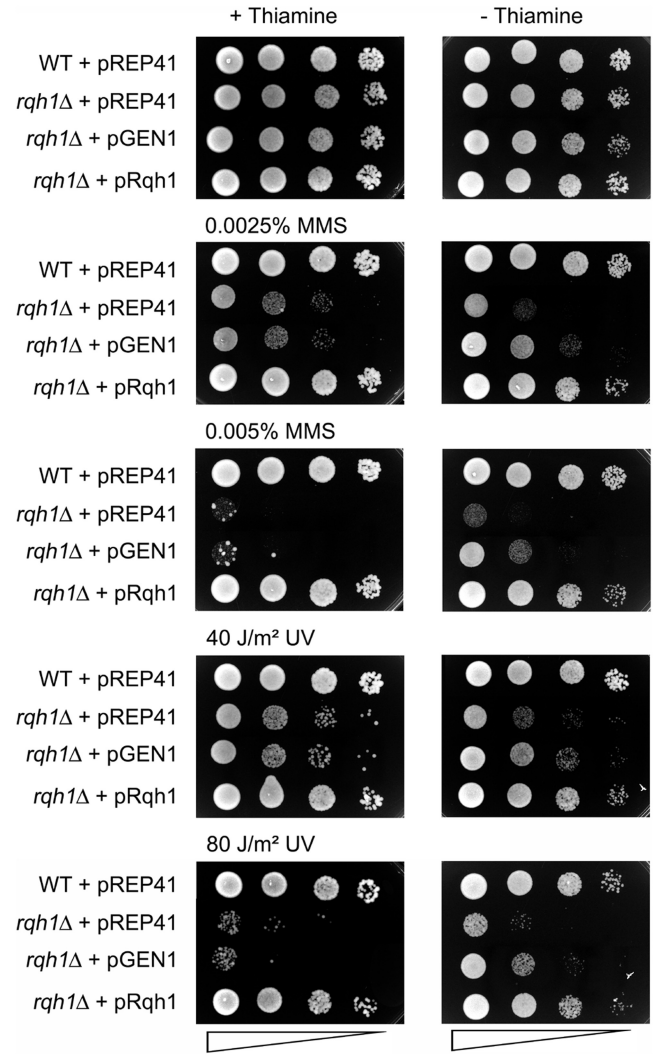

Figure 1. Effect of GEN1 and Rqh1 (expressed from the thiamine-repressible *nmt1*-promotor in pREP41) on genotoxin sensitivities of a *rqh1*Δ (MCW1019) strain. The empty vector pREP41 in the wild-type strain MCW1221 and in MCW1019 serve as controls. The neat spot represents 10^5 cells.

GEN1 can partially rescue the genotoxin sensitivities of *rqh1*Δ and *mus81*Δ mutants

Since RusA partially rescues different genotoxin sensitivities of vegetative yeast cells lacking Mus81 or Rqh1 (25,29), we first tested whether GEN1 could do likewise. Plasmid constructs carrying human GEN1 (amino acids 1-527), NLS-RusA-GFP (25), Rqh1 and 2myc6his-Mus81–Pk-Eme1 (15) under the thiamine-repressible *nmt1*-promotor were transformed into wild type, *rqh1*Δ and *mus81*Δ (see 'Materials and Methods' section). The resulting strains, carrying an empty vector or any of the above plasmids, were tested for sensitivities against the topoisomerase I poison camptothecin (CPT), the alkylating agent methyl methanesulfonate (MMS) and ultraviolet irradiation (UV).

Expression of GEN1 partially rescued the sensitivities of *rqh1*Δ against UV and MMS (Figure 1) and *mus81*Δ against MMS and CPT (Figure 2, Supplementary

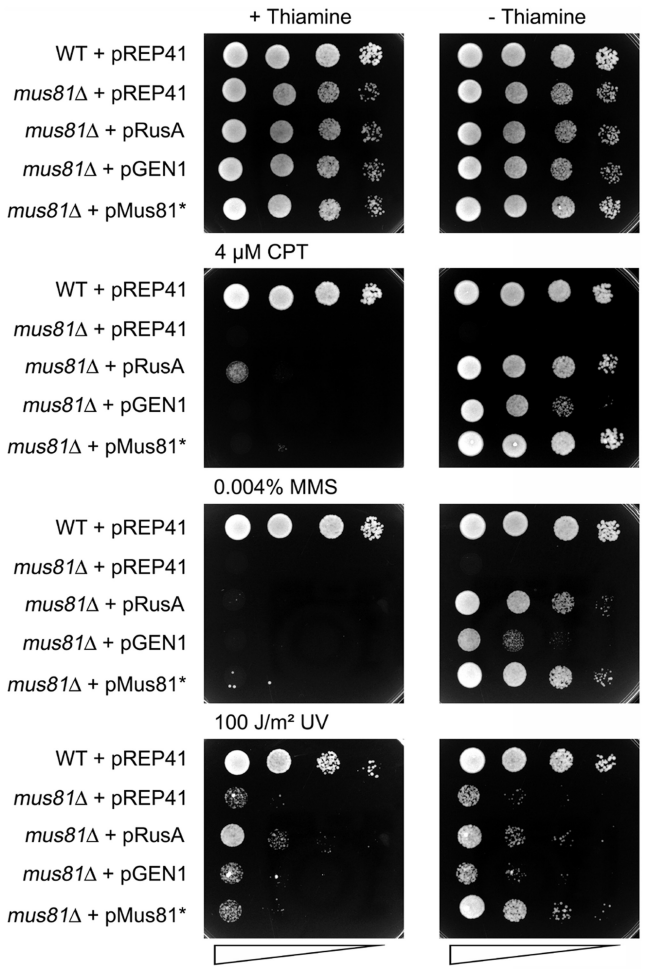

Figure 2. Effect of RusA (expressed from the thiamine-repressible *nmt1*-promotor in pREP1), GEN1 and Mus81-Eme1 (expressed from the thiamine-repressible *nmt1*-promotor in pREP41) on genotoxin sensitivities of a *mus81*Δ (MCW1238) strain. The empty vector pREP41 in the wild-type strain MCW1221 and in MCW1238 serve as controls. The neat spot represents 10^5 cells.

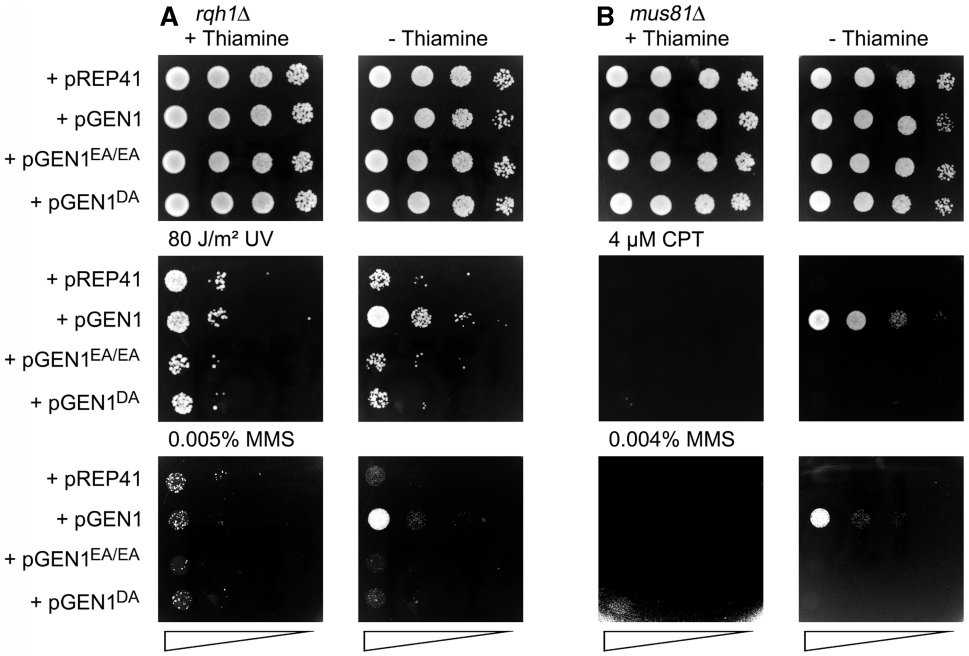

Figure 3. Effect of wild-type and nuclease-defective GEN1 (expressed from the thiamine-repressible *nmt1*-promotor in pREP41) on genotoxin sensitivities of (**A**) *rqh1*Δ (MCW1019) and (**B**) *mus81*Δ (MCW1238) strains. pREP41 serves as the empty vector control. The neat spot represents 10^5 cells.

Figure S1). However, it was unable to rescue the UV sensitivity of a *mus81*Δ mutant (Figures 2, Supplementary Figure S1). The degree of rescue by GEN1 was not as complete as by complementation of the mutants with plasmid-expressed Rqh1/Mus81-Eme1 (Figures 1 and 2, Supplementary Figure S1) and in the case of *mus81*Δ it was also not as great as achieved with RusA expression (Figure 2, Supplementary Figure S1). GEN1's ability to only partially rescue the genotoxin sensitivities of *mus81*Δ and *rqh1*Δ is not a consequence of any overt toxicity associated with its expression, because in a wild-type it causes only a modest increase in UV sensitivity (Supplementary Figure S2). Overall, these data establish that GEN1 can at least partially substitute for Rqh1 and Mus81 in promoting resistance to genotoxins.

GEN1's nuclease activity is required to rescue the genotoxin sensitivities of *rqh1*Δ and *mus81*Δ mutants

To determine whether GEN1's catalytic activity is needed for its rescue of *mus81*Δ and *rqh1*Δ genotoxin sensitivities, we repeated the above experiments using plasmids that express nuclease-dead mutants of GEN1 ($GEN1^{E134A/E136A}$ and $GEN1^{D30A}$). As shown in Figure 3, neither mutant protein can rescue the genotoxin sensitivities of *rqh1*Δ or *mus81*Δ strain. These data suggest that it is GEN1's HJ resolution activity that is critical for its ability to rescue *mus81*Δ and *rqh1*Δ, which in turn is consistent with the idea that unresolved HJs accumulate in these mutants.

GEN1 can rescue the meiotic defects of a *mus81*Δ mutant

Having established that GEN1 can partially compensate for the lack of Rqh1 and Mus81 in the tolerance/repair of DNA damage, we next looked to see whether it could rescue the meiotic defects of a *mus81*Δ mutant. In *S. pombe,* the deletion of *mus81* causes a very severe meiotic phenotype, where the meiotic nuclear division cannot proceed due to unresolved HJs (9,14,28). The resulting asci, despite holding up to four spores, very often contain only a single DNA mass, and therefore many of the spores are completely devoid of DNA (Figure 4A). Consequently spore viability is very low (Figure 4B). Expression of GEN1 from the *nmt1*-promotor ameliorates both the chromosome segregation defect and the poor spore viability of a *mus81*Δ mutant just as well as does RusA or Mus81-Eme1 (Figure 4, Supplementary Table S1). As with its rescue of genotoxin sensitivities, suppression of *mus81*Δ's meiotic defects depends on GEN1's nuclease activity because neither $GEN1^{E134A/E136A}$ nor $GEN1^{D30A}$ expression provided any amelioration (Figure 4, Supplementary Table S1).

The resolution of HJs and/or their precursors by Mus81-Eme1 generates crossover recombinants during meiosis. To see if GEN1 is capable of producing crossovers as well, the wild-type pGEN1 construct was transformed into *mus81*Δ strains carrying a meiotic recombination reporter substrate in which gene conversion is measured at the *ade6* locus using the *ade6-3083* hotspot (30) and crossing over is assessed by two flanking auxotrophic markers, *ura4-aim2* and *his3-aim* (Figure 5A). Similar to previous data at *ade6-M26,* loss of *mus81* reduces crossover formation between *ura4-aim2* and *his3-aim* by at least 4-fold (Figure 5B) (14). This can be rescued back to wild-type levels by expression of GEN1, RusA or Mus81-Eme1 (Figure 5C). In wild-type fission yeast, there is a general bias to resolve meiotic gene

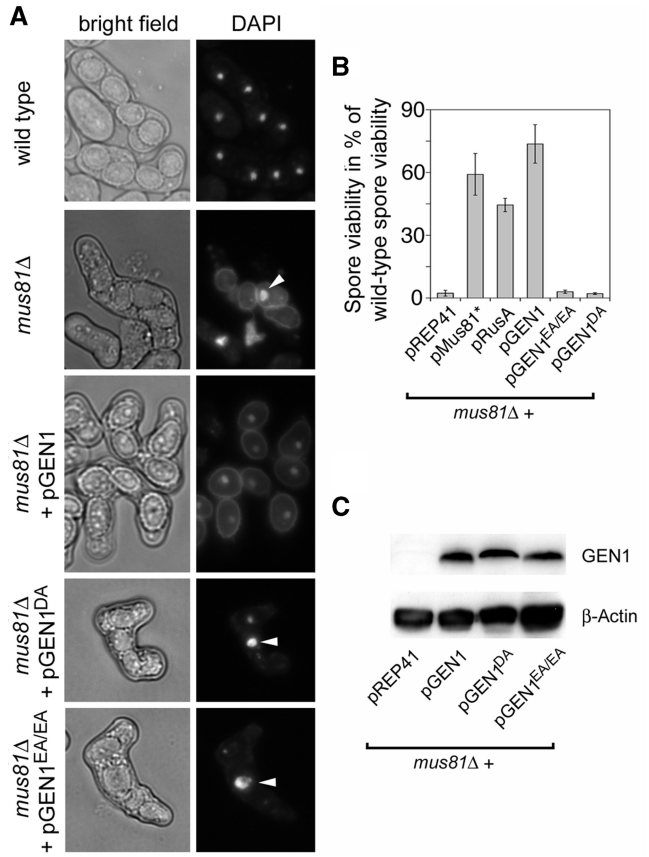

Figure 4. Effect of expression of RusA, as well as wild-type and nuclease-defective GEN1 on *mus81*Δ during meiosis. (**A**) Typical examples of asci from crosses of wild-type and *mus81*Δ strains carrying plasmids as indicated. The DNA is stained with DAPI and arrowheads indicate non-segregated DNA. (**B**) Restoration of spore viability in a *mus81*Δ mutant by pMus81*, pRusA and pGEN1. See also Supplementary Table S1. (**C**) Western blot showing protein levels of wild-type and nuclease-defective GEN1 in cultures of *mus81*Δ strains carrying the respective plasmids, as indicated, grown for 20 h without thiamine. β-actin serves as loading control.

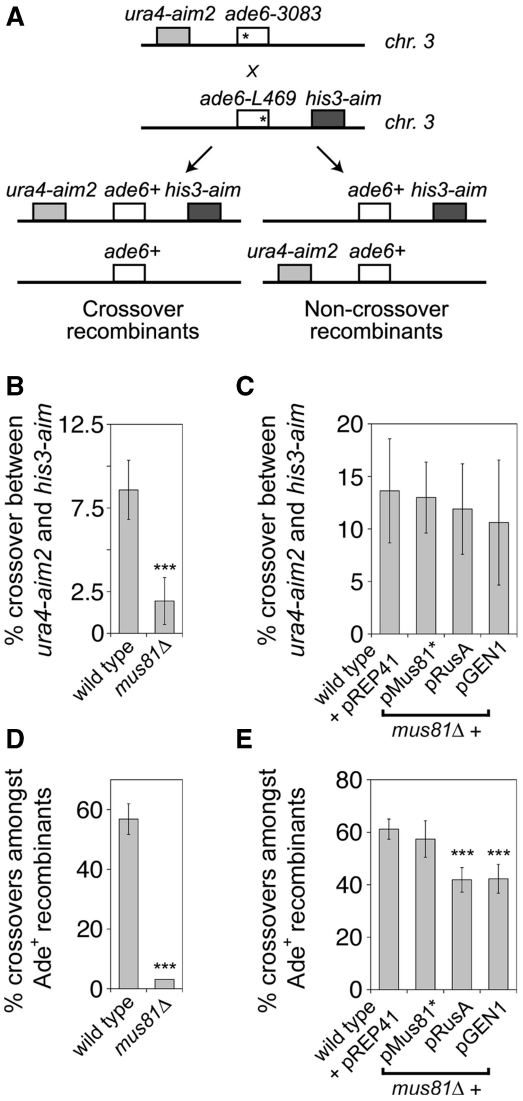

Figure 5. Effect of expression of RusA and wild-type GEN1 on meiotic recombination in a *mus81*Δ background. (**A**) Schematic representation of the meiotic recombination assay and the possible recombinants associated with gene conversions at *ade6* (note that crossover recombinants in the *ura4-aim2–his3-aim* interval can also arise without an associated gene conversion event at *ade6*). Asterisks indicate the position of point mutations in the *ade6*$^-$ alleles. (**B** and **C**) Percentage of crossovers between *ura4-aim2* and *his3-aim* in the indicated strains. (**D** and **E**) Percentage of crossovers between *ura4-aim2* and *his3-aim* associated with a gene conversion at *ade6-3083* that produces an Ade$^+$ prototroph. Data in (**B–E**) are mean values from at least 10 independent crosses with error bars being standard deviations about the mean. Statistical significance at $***P < 0.001$ against the wild-type control within each chart (for details see Supplementary Table S2).

conversion events into crossover recombinants. As such >55% of gene conversions at *ade6* are associated with a crossover of the flanking markers (Figure 5D). Importantly, unlike the wild type, or the *mus81*Δ mutant complemented by Mus81-Eme1 expressed from a plasmid, expression of GEN1 or RusA results in crossing over in only ~40% of gene conversion events (Figure 5E), which is essentially the same as that determined previously for RusA at the *ade6-M26* hotspot (15). Presumably, both GEN1 and RusA resolve the HJs that accumulate in a *mus81*Δ mutant without bias for either crossover or non-crossover recombinant, whereas Mus81-Eme1's ability to act on HJ precursors enables it to establish a crossover bias (3). The reason that ~40% of gene conversion events are associated with a crossover rather than 50% is probably because ~20% of gene conversion events occur via synthesis-dependent strand annealing (SDSA) (see 'Discussion' section). These data highlight an important difference between canonical HJ resolvases, like RusA and GEN1, and Mus81-Eme1 in dealing with recombination junctions.

DISCUSSION

We have investigated whether GEN1 has the ability to resolve HJs *in vivo* by testing it in a heterologous system under three different conditions where HJs are expected, or have been shown, to accumulate. In the first case, we asked whether GEN1 could rescue the hypersensitivity of a *rqh1*Δ mutant to two different genotoxins, UV and MMS, both of which are known to cause lesions in

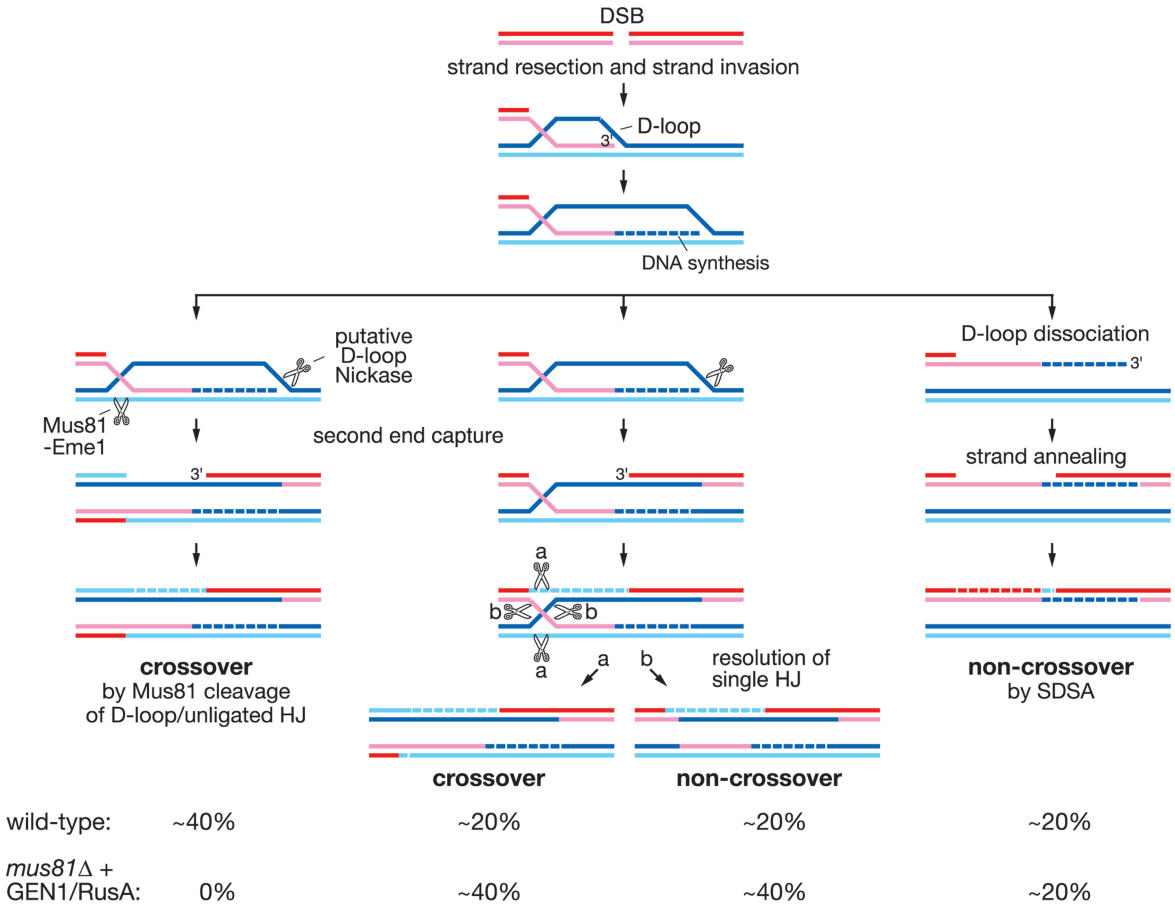

Figure 6. Pathways of meiotic interhomologue DSB repair in *S. pombe*. Here, we have invoked the existence of a D-loop nickase to account for the fact that mostly single HJs accumulate in a *mus81*Δ mutant (28). However, in the absence of such an activity, Mus81-Eme1 could perform essentially the same cleavage following second end capture (14). Regardless of whether single and/or double HJs accumulate in a *mus81*Δ mutant, both GEN1 and RusA appear to be able to resolve them efficiently into either crossover or non-crossover products. The bottom panel shows the percentage of interhomologue DSB repair events at the *ade6* locus that could proceed via each of the postulated pathways to account for the observed crossover values in wild-type and GEN1/RusA-rescued *mus81*Δ mutant cells.

DNA that perturb replication fork progression and promote Rad51-dependent recombination (31–33). In *S. cerevisiae,* the RecQ-type helicase Sgs1 has been implicated in processing Rad51-dependent DNA junctions that can form between sister chromatids following replication fork perturbation (31,32,34,35). Such processing is thought to include HJ branch migration, which when combined with topoisomerase III(α) activity can result in the dissolution of double HJs (36). In the absence of this activity, HJs can accumulate and impede chromosome segregation (34). This may account for the high frequency of aberrant mitoses in a *rqh1*Δ mutant following replication fork stalling (37). Importantly, RusA partially rescues this chromosome segregation defect in *S. pombe*, together with associated genotoxin sensitivities, presumably by resolving HJs that would otherwise physically prevent sister chromatid separation (25). We suspect that the amelioration of *rqh1*Δ's UV and MMS sensitivities by GEN1 expression is similarly associated with the resolution of HJs that would otherwise impede chromosome segregation and cause cell death.

In the second case, we showed that, like RusA, GEN1 can partially rescue the hypersensitivity of a *mus81*Δ mutant to MMS and CPT. Mus81-Eme1 can cleave quite a wide range of substrates *in vitro*, including 3′ flaps, forked DNAs, and four-way DNA junctions including fully ligated HJs (9,14–16,29,38). Exactly which of these substrates is targeted *in vivo* to promote the repair and/or tolerance of DNA damage is still a matter of conjecture (39). However, at least in the case of replication fork breakage induced by CPT, a strong argument can be made for Mus81-Eme1 being required for resolving the single four-way DNA junction (D-loop/unligated HJ/ligated HJ) that is formed during the repair of the broken fork by Rad51 (29,39,40). Moreover, the fact that both RusA and GEN1 have a preference for cleaving HJs *in vitro* (17,41), makes it likely that their partial suppression of *mus81*Δ mutant genotoxin sensitivity is directly as a result of this ability *in vivo*.

The third, and most compelling, case for GEN1 being able to resolve HJs *in vivo* is its ability to promote crossover formation during meiosis in a *mus81*Δ mutant. In *S. pombe,* there is strong evidence that Mus81-Eme1 is the principal enzyme responsible for resolving meiotic recombination intermediates (9,14,28,42). Moreover, it resolves these intermediates with a marked bias for

generating crossover recombinants. HJs are essentially symmetrical structures, and can therefore be cleaved by HJ resolvases in either orientation to generate equal numbers of crossover and non-crossover products. This lack of cleavage bias was recently shown to hold true in *E. coli* where each of the known resolution systems (i.e. RecG-, RuvABC- and RusA-dependent) produced approximately equal proportions of crossovers and non-crossovers during DSB repair (43). What guides Mus81-Eme1 to deliver junction resolution with a bias for crossover recombinants is not certain; however, one simple model is that it resolves D-loops and unligated HJs, which are the precursors of fully ligated HJs (14). These structures contain a 5′ DNA end at or close to the junction centre that directs Mus81 cleavage to a specific strand, which, in the context of DSB repair, generates only crossovers (14) (Figure 6). Clearly not all gene conversion events in *S. pombe* are associated with a crossover, which implies that resolution does not always proceed via Mus81-Eme1-mediated cleavage of D-loops/unligated HJs. It is likely that some DSB repair occurs via SDSA, which is a form of HR that does not necessitate HJ formation and resolution and generates only non-crossovers (Figure 6). It is also possible that some D-loops/unligated HJs evade early processing and mature into fully fledged HJs that are cleaved by Mus81-Eme1 without a bias for generating crossovers (15) (Figure 6).

In contrast to Mus81-Eme1, RusA does not utilize a DNA end to guide strand cleavage of four-way DNA junctions, and therefore appears to have no intrinsic mechanism for generating a crossover bias (14,15). We suspect the same is true for GEN1. Moreover these enzymes exhibit preferences for specific nucleotide sequences being present at the junction centre for efficient strand cleavage (12,17). This property makes it more likely that the four-way junction has migrated and become a fully fledged HJ before being resolved by them (6). As such both RusA and GEN1 should deliver equal numbers of crossover and non-crossover recombinants in a *mus81*Δ mutant. Therefore, the fact that both of them produce ~40% crossovers amongst Ade^+ convertants, suggests that overall ~80% of interhomologue recombination events at this locus proceed via junction resolution (Figure 6). We presume that the other ~20% are SDSA events. These data also imply that in a wild-type Mus81-Eme1 is responsible for processing ~80% of interhomologue recombination intermediates. So the fact that it generates only ~60% of crossovers amongst Ade^+ convertants suggests that ~40% of events may involve HJ cleavage generating equal numbers of crossovers and non-crossovers as proposed previously (15) (Figure 6).

In summary, we have provided evidence that GEN1 can work as a HJ resolvase *in vivo*. Moreover, we have shown that in a meiotic environment it can generate crossovers, and thereby is a potential candidate for performing this function in humans. However, like RusA, and other canonical HJ resolvases, GEN1 does not appear to have an intrinsic mechanism for producing a crossover bias during DSB repair. So if HJs in humans are resolved predominantly into crossover recombinants, like in *S. cerevisiae* (44,45), additional factors would be needed to ensure that GEN1 delivers such a bias.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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