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The role of the Mre11–Rad50–Nbs1 complex in double-strand break repair—facts and myths

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ABSTRACT

Homologous recombination (HR) initiates double-strand break (DSB) repair by digesting 5′-termini at DSBs, the biochemical reaction called DSB resection, during which DSBs are processed by nucleases to generate 3′ single-strand DNA. Rad51 recombinase polymerizes along resected DNA, and the resulting Rad51–DNA complex undergoes homology search. Although DSB resection by the Mre11 nuclease plays a critical role in HR in *Saccharomyces cerevisiae*, it remains elusive whether DSB resection by Mre11 significantly contributes to HR-dependent DSB repair in mammalian cells. Depletion of Mre11 decreases the efficiency of DSB resection only by 2- to 3-fold in mammalian cells. We show that although Mre11 is required for efficient HR-dependent repair of ionizing-radiation–induced DSBs, Mre11 is largely dispensable for DSB resection in both chicken DT40 and human TK6 B cell lines. Moreover, a 2- to 3-fold decrease in DSB resection has virtually no impact on the efficiency of HR. Thus, although a large number of researchers have reported the vital role of Mre11-mediated DSB resection in HR, the role may not explain the very severe defect in HR in Mre11-deficient cells, including their lethality. We here show experimental evidence for the additional roles of Mre11 in (i) elimination of chemical adducts from DSB ends for subsequent DSB repair, and (ii) maintaining HR intermediates for their proper resolution.

KEYWORDS: double-strand break repair, double-strand break resection, etoposide, homologous recombination, ionizing radiation, Mre11, non-homologous end joining, topoisomerase

THE CHOICE OF THE TWO MAJOR DSB REPAIR PATHWAYS: HOMOLOGOUS RECOMBINATION AND NONHOMOLOGOUS END JOINING

DNA double-strand breaks (DSBs) are the most dangerous type of DNA damage, as a single unrepaired DSB can trigger apoptosis. DSBs are generated during physiological replication, and are induced by radiotherapy and chemotherapeutic reagents such as the topoisomerase poison. There are two major DSB repair pathways in eukaryotic cells: homologous recombination (HR) and non-homologous end joining (NHEJ).

The choice of the two pathways in *Saccharomyces cerevisiae* is alternative so that DSB resection can inhibit NHEJ, because DSBs containing 3′ single-strand DNA cannot be repaired by canonical NHEJ (Fig. 1A). It has been widely believed that the alternative choice model is also relevant to mammalian cells [1], though the relevance has not yet been demonstrated. The method of examining the precise structure of resected DSB sites is currently available only for meiotic HR in *S. cerevisiae*, but not for mammalian cells [2, 3]. Although DSB resection would strongly inhibit canonical NHEJ, quick initiation of DSB resection does not reduce the overall efficiency of DSB repair in *S. cerevisiae* due to the very small contribution of NHEJ to DSB repair [3]. By contrast, DSB resection from DSB ends (Fig. 1A) would result in a considerable decrease in the overall efficiency of DSB repair in mammalian cells due to the major role of NHEJ in DSB repair.

A structural study of resected DSBs during meiotic HR in *S. cerevisiae* revealed that DSB resection is initiated by a single-strand break (SSB) on the strand to be resected up to 300 bases from the 5′-terminus of the DSB [2] (Fig. 1B). This SSB is subjected to subsequent bidirectional resection, both in the 5′–3′ direction away from the DSB and in the 3′–5′ direction towards the DSB end. The Mre11 nuclease forms a complex with Rad50 and Xrs2 (the yeast ortholog of mammalian Nbs1) [4]. The resulting MRX complex is responsible for the formation of the SSB, followed by the 5′–3′ direction resection in the meiotic HR of *S. cerevisiae*. This model agrees with the *in vitro* nuclease activity of purified Mre11. It

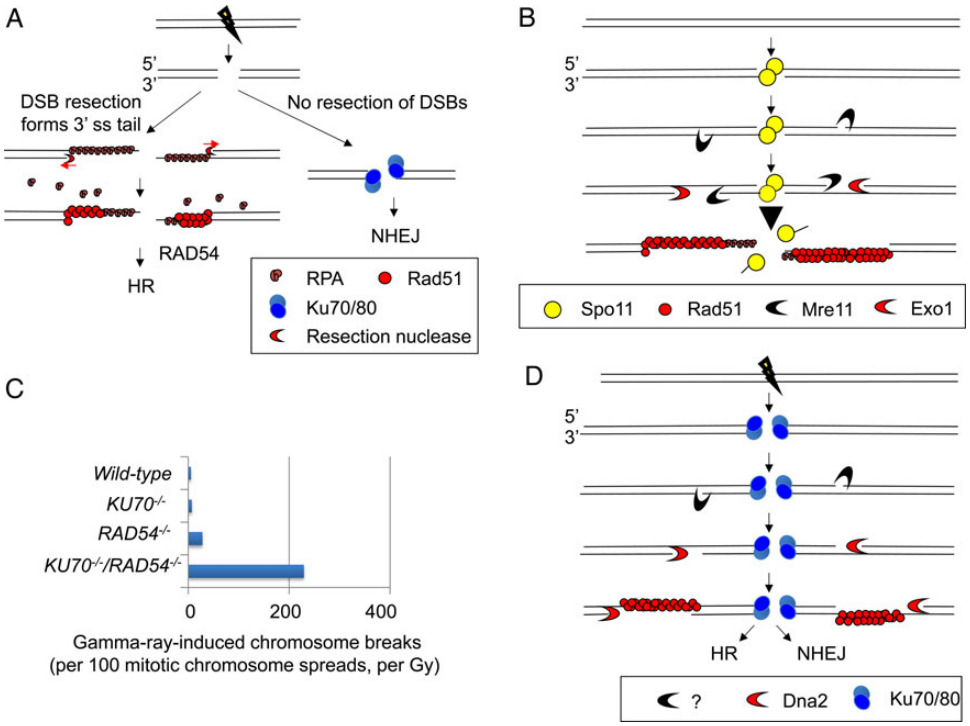


Fig. 1. (A) Double strand break (DSB) repair in *Saccharomyces cerevisiae*. The choice of homologous recombination (HR) or non-homologous end-joining (NHEJ) is determined by the presence or absence of DSB resection, respectively. Rad54 facilitates homology search and strand exchange of resected DSB sites. (B) Bidirectional processing of DSBs during DSB resection for meiotic HR in *S. cerevisiae*. Spo11 protein, a topoisomerase, generates DSBs, where Spo11 is covalently associated with the 5′ end of DSBs leading to formation of blocked ends. Single-strand break formation on the Spo11-associating strand by Mre11 initiates DSB resection, enabling resection in a bidirectional manner, where Exo1 digests in the 5′–3′ direction away from the DSB, and Mre11 digests in the 3′–5′ direction towards the DSB end. (C) Measurement of unrepaired DSBs [6]. We exposed the indicated genotypes of chicken DT40 cells to doses equivalent to LD50% (the dose that reduces cellular survival to 50%) of γ-rays in the G_2 phase, harvested mitotic cells at 3 h, and counted the number of chromosomal breaks in mitotic chromosome spreads. The *x*-axis shows the number of chromosome aberrations per 100 mitotic cells and per Gray. (D) In contrast with meiotic HR in *S. cerevisiae* (B), vertebrate Mre11 has a very minor role in DSB resection for subsequent HR in somatic vertebrate cells. Dna2 plays the dominant role in DSB resection in chicken DT40 cells among the CtIP, Dna2, Exo1 and Mre11 nucleases [12].

remains elusive whether this bidirectional resection also plays a role in mitotic HR in mammalian cells as well as in yeast [5].

Based on findings in chicken DT40 cells (Fig. 1C) [6], we here propose another model for DSB resection (Fig. 1D). If resection from the SSB is carried out only in the 5′–3′ direction away from the DSB, but not in the 3′–5′ direction, DSB ends would be maintained as duplex DNA (Fig. 1D). The absence of homologous single-stranded tails at DSB ends does not significantly interfere with homology search in *S. cerevisiae* [7], while the presence of duplex DNA at DSB ends would ensure efficient repair by canonical NHEJ. The new model shown in Fig. 1D predicts that HR and NHEJ are able to work in parallel, without interfering with each other. In other words, while HR undergoes homology search with the Rad51 recombinase polymerized on resected DSBs, NHEJ is able to efficiently ligate DSB ends.

The new model shown in Fig. 1D is supported by the ionizing radiation sensitivity of DT40 cells deficient in both HR and NHEJ [6] (Fig. 1C). In this study, we completely inactivated canonical NHEJ by disrupting the *KU70* gene, and partially inactivated HR by disrupting the *RAD54* gene. Note that the complete inactivation of HR by disrupting the *RAD51* gene causes cellular lethality associated with severe genome instability, whereas disrupting the *RAD54* gene allows for normal mouse development. Nonetheless, the loss of Rad54 completely inhibits HR-dependent repair of ionizing radiation–induced DSBs, as evidenced by data indicating that the loss of Rad54 reduces cellular tolerance to ionizing radiation in the S/G_2 phases to the tolerance seen in the G_1 phase [6]. Rad54 does not affect DSB resection, but facilitates homology search by resected DSBs associated with polymerized Rad51 (Fig. 1A) [8]. HR is preferentially used over canonical NHEJ for DSB repair in the G_2 phase in DT40 cells, as shown by comparable radiosensitivity between *wild-type* and canonical-NHEJ–deficient $KU70^{-/-}$ cells (Fig. 1C). The alternative choice model (Fig. 1A) predicts that canonical NHEJ could not substitute for abortive HR in $RAD54^{-/-}$ cells because the precedent formation of the 3′ single-strand tail would inhibit canonical NHEJ. However, the radiotolerance of $RAD54^{-/-}$ DT40 cells is considerably higher than that of $KU70^{-/-}/RAD54^{-/-}$ DT40 cells (Fig. 1C), indicating that canonical NHEJ can efficiently ligate

abortive HR intermediates generated in *RAD54*$^{-/-}$ cells. We, therefore, propose that the molecular mechanism for DSB resection in *S. cerevisiae* distinctly differs from that in metazoan cells (compare Fig. 1A and D). The new model agrees with the phenotype of *RAD54*$^{-/-}$ and *KU70*$^{-/-}$/*RAD54*$^{-/-}$ DT40 cells, where canonical NHEJ is able to efficiently repair DSBs, even after polymerization of Rad51 at the DSB sites in *RAD54*$^{-/-}$ cells.

LOSS OF MRE11 ONLY CAUSES UP TO 2- TO 3-FOLD DECREASE IN DSB RESECTION IN MITOTIC HR IN VERTEBRATE CELLS

The current model of DSB resection in mammalian cells is based on the findings about HR in *S. cerevisiae*, since its molecular mechanism for DSB resection is most precisely defined [2] (Figs 1B and 2A). Mre11 is essential for DSB resection in the meiotic HR of *S. cerevisiae*. Mre11 also plays an important role in DSB resection when HR repairs ionizing radiation–induced DSBs, particularly in the G_2 phase [3]. Extrapolating these findings of *S. cerevisiae*, the vast majority of the manuscripts and reviews have suggested that Mre11 also plays the major role in DSB resection in mammalian cells [9].

It should be noted that the essential role of Mre11 in meiotic HR is irrelevant to some of the mitotic HR in *S. cerevisiae*. For example, the selective inactivation of nuclease activity in Mre11 causes only a modest delay in DSB resection at the DSB site formed by the HO restriction enzyme, which generates 'clean' DSBs containing no abnormal chemical modification [3, 10]. Thus, other nucleases can effectively substitute for the defective nuclease activity of Mre11 in the resection at 'clean' DSBs in *S. cerevisiae*. Moreover,

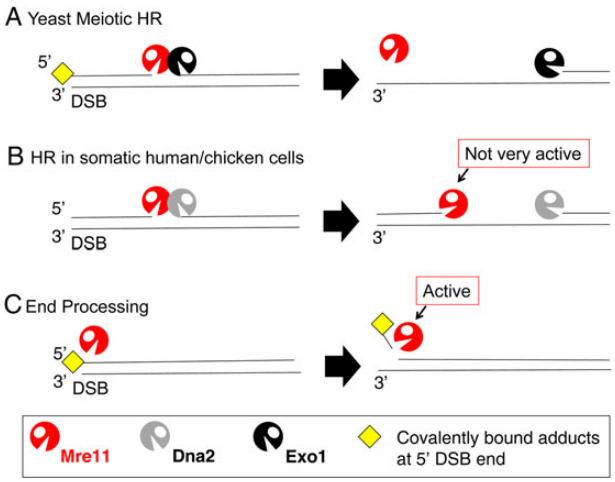


Fig. 2. Differential roles for Mre11 in (A) meiotic HR in *Saccharomyces cerevisiae*, (B) DSB resection for HR in vertebrate cells and (C) the processing of 'dirty' ends containing base damage and covalently associating topoisomerases in *S. cerevisiae* and vertebrate cells. *Saccharomyces cerevisiae* Mre11 actively digests in the 3′–5′ direction towards the DSB end (A). In mammalian cells, Mre11 only contributes a small amount to digestion in the 3′–5′ direction (B), while Mre11 plays an important role in end processing of non-physiological chemical modifications (C).

hypersensitivity of Mre11-nuclease–deficient yeast to ionizing radiation suggests that Mre11 eliminates chemical modifications at 'dirty' DSB sites induced by ionizing radiation before DSB repair by HR [3]. This possibility will be discussed in a later section entitled 'The role of Mre11 nuclease activity in elimination of chemical adducts from DSB ends'.

The importance of the nuclease activity in mammalian Mre11 has been indicated by Buis *et al.* [11], who conditionally generated nuclease-deficient *MRE11*$^{-/H129N}$ and *MRE11*-null mutant (*MRE11*$^{-/-}$) mice. Both mutant mice exhibited a very similar phenotype, including the mortality of cells associated with dramatic genomic instability, hypersensitivity to ionizing radiation, and 2- to 3-fold reduction in the efficiency of DSB resection. The data have been interpreted as compelling evidence for the critical role of Mre11-nuclease-dependent DSB resection in HR. However, this interpretation raises two questions. First, is only 2- to 3-fold reduction in DSB resection solely responsible for a severe defect in HR? Second, does a defect in HR fully explain the dramatic genomic instability? To define the role of Mre11 nuclease activity in DSB resection and genome stability, we conditionally generated *MRE11*$^{-/H129N}$ and *MRE11*$^{-/-}$ cells from the chicken DT40 and human TK6 B cell lines [12]. Note that the TK6 cell line is widely used for evaluating the genotoxicity of industrial chemicals by regulators due to its stable karyotype and phenotype [13]. These *MRE11* mutants retain the capability of performing DSB resection in nearly normal kinetics in both DT40 (Fig. 3A and B) and TK6 (Fig. 3C) cells. The data agree with a recent study directly measuring the length of resected 3′ single-strand overhangs; that study shows only about a 50 to 70% decrease in DSB resection upon depletion of Mre11 [14]. Collectively, the loss of Mre11 causes only up to 2- to 3-fold reduction in the efficiency of DSB resection in vertebrate cells. If DSB resection initiates from a SSB away from a DSB end, the very poor 3′ to 5′ exonuclease activity of Mre11 would allow DSB ends to be maintained as duplex DNA (Fig. 2B).

DSB RESECTION BY MRE11 IS DISPENSABLE FOR EFFICIENT HR-DEPENDENT DSB REPAIR

The prominent question is whether only 2- to 3-fold reduction in DSB resection has an impact on the efficiency of HR-dependent DSB repair. A previous study of our laboratory, as well as a genetic study of yeast, consistently indicate that such a small reduction has virtually no impact on the efficiency of HR-dependent DSB repair. Although the selective loss of the Mre11 nuclease activity reduces the resection kinetics of 'clean' DSBs, subsequent HR-dependent DSB repair occurs efficiently in *S. cerevisiae* [3, 10]. We generated the TK6 mutant cells (*CtIP*$^{WT/WT}$ cells), where the amount of CtIP was reduced by five times and the DSB resection efficiency decreased by two times. The resulting mutant is capable of completing HR-dependent DSB repair with normal kinetics (Fig. 3C) and is fully proficient in HR between homologous chromosomes induced by I-*Sce*1 restriction enzyme–mediated DSBs [12]. Thus, 2- to 3-fold reduction in DSB resection does not have a negative impact on the overall efficiency of HR-dependent DSB repair. In summary, DSB resection by Mre11 does not account for the critical role of Mre11 in HR-dependent repair of ionizing radiation–induced DSBs.

If DSB resection by Mre11 has a minor role in DSB resection, an important question is which nuclease plays a critical role in DSB

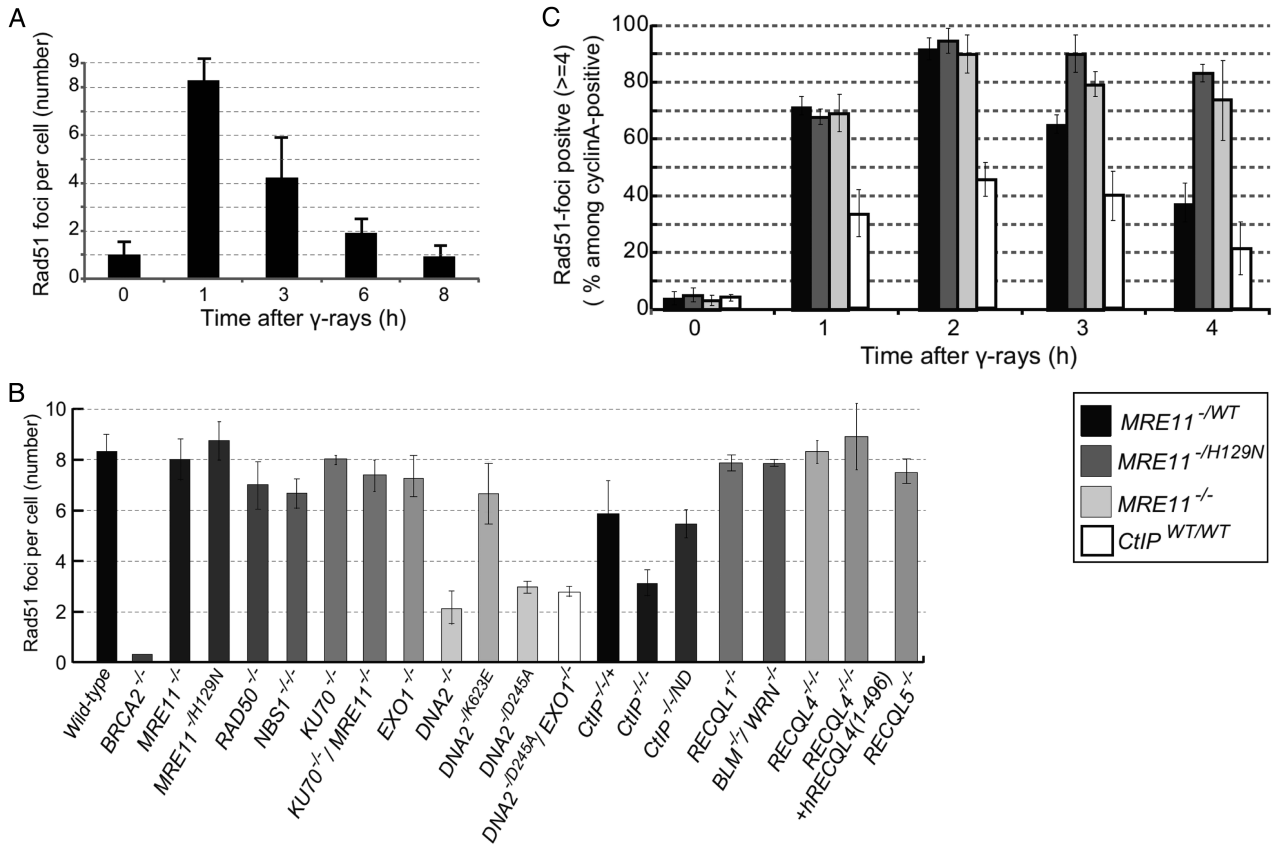


Fig. 3. (A) Rad51-focus formation of *wild-type* chicken DT40 cells at the indicated time after exposure to 2 Gy ionizing radiation [12]. The average number of Rad51 foci per cell was calculated from at least 100 cells. Note that we counted all foci, without discriminating faint foci from prominent foci. Error bars were plotted for standard deviation [11] from three independent experiments. (B) The average number of Rad51 foci per cell having the indicated genotypes of DT40 cells. *MRE11*$^{-/H129N}$ and *DNA2*$^{-/D245A}$ are deficient in the nuclease activities of Dna2 and Mre11, while *DNA2*$^{-/K623E}$ is deficient in the helicase activity of Dna2. Cells were analyzed 1 h after the ionizing irradiation. Error bars are shown as in (A). (C) TK6 cell lines carrying the indicated genotypes were exposed to 0.5 Gy γ-rays at time zero. Mre11 null (*MRE11*$^{-/-}$) and Mre11-nuclease-deficient (*MRE11*$^{-/H129N}$) mutants show normal kinetics of DSB resection, but delayed resolution of HR intermediates in comparison with heterozygous mutant (*MRE11*$^{-/WT}$) cells, which show the same phenotype as *wild-type*.

resection and HR? Four nucleases (CtIP, Dna2, Exo1 and Mre11) contribute to DSB resection in both *S. cerevisiae* and mammalian cells. Short-range DSB resection, removing up to a few hundred nucleotides from the 5′ strand by Mre11 and Sae2 (the CtIP ortholog), is followed by long-range DSB resection, more than 10 kb in length, by Dna2 and Exo1 in *S. cerevisiae* [15, 16]. The short-range resection is sufficient for efficient HR. To measure the relative contribution of the four nucleases to DSB resection, we generated isogenic DT40 mutants deficient in individual nucleases. We assessed DSB resection by measuring Rad51 focus formation at 1 h after ionizing radiation (Fig. 3A). Only CtIP and Dna2, but not Exo1 or Mre11, are required for efficient DSB resection (Fig. 3B) [12, 17]. Nuclease-deficient *DNA2*$^{-/D245A}$ as well as null-mutant *DNA2*$^{-/-}$ cells show a significant defect in DSB resection, indicating Dna2 contributes to DSB resection as a nuclease. In contrast, mammalian CtIP has the non-catalytic role in DSB resection, and does not work as a nuclease in HR [18]. We showed that the non-catalytic role played by CtIP is essential for DSB resection by recruiting Dna2 to DSB sites [17]. The modest contribution of Exo1 to DSB resection agrees with the normal development of Exo1-deficient mice, despite the embryonic mortality of various HR-deficient mice [19]. Collectively, Dna2 plays the dominant role in DSB resection required for efficient HR among the four nucleases in the chicken DT40 cell line [17] (Fig. 2B). Given the very minor contribution of Dna2 to DSB resection for efficient HR in *S. cerevisiae*, the relative contribution of the four nucleases to DSB resection and HR is very different between *S. cerevisiae* and the DT40 cells.

THE ROLE OF MRE11 NUCLEASE ACTIVITY IN ELIMINATION OF CHEMICAL ADDUCTS FROM DSB ENDS

Although human *MRE11*$^{-/H129N}$ as well as *MRE11*$^{-/-}$ TK6 cells retain the capability of efficiently performing DSB resection, these mutants show delayed resolution of Rad51 focus formation after ionizing irradiation (Fig. 3C). Similarly, although chicken *MRE11*$^{-/-}$ DT40 cells efficiently perform DSB resection, the cells show a severe

defect in repairing ionizing radiation–induced DSBs by HR [20, 21]. These observations indicate that Mre11 plays important roles in DSB repair other than DSB resection. We hereafter discuss two roles played by Mre11: (i) removal of chemical adducts from DSB ends, and (ii) maintenance of Holliday junction HR intermediates.

Although Mre11-nuclease–deficient *S. cerevisiae* shows only a modest delay in DSB resection at the HO restriction enzyme–induced DSB sites in asynchronous populations, the mutant yeast is hypersensitive to ionizing radiation [3, 22]. Likewise, in the fission yeast, *Schizosaccharomyces pombe* (*S. pombe*), the Mre11 nuclease plays an important role in the repair of ionizing irradiation–induced DSBs, but is not required for resection at an HO-induced DSB [23]. The data suggests that Mre11 may eliminate chemical modification induced by ionizing radiation before DSB repair by HR as well as NHEJ. This idea is further supported by the data that Mre11 nuclease activity of *S. pombe* is involved in the removal of both Topoisomerase I (TopI) and TopII from 3′ and 5′ DNA ends, respectively, *in vivo* [24]. TopII resolves DNA catenanes by catalyzing the transient formation of double-strand DNA breakage, enabling intact DNA to pass through the DSB and re-ligating the DSB [25]. During such transient DSB formation, Top2 becomes covalently bound to the 5′-DNA end of the breakage, forming TopII-DNA cleavage complex (TopIIcc) intermediates. A chemotherapeutic agent, etoposide, strongly stabilizes TopIIcc, leading to the formation of DSBs [26]. Mre11-nuclease–deficient *S. pombe* shows a more significant accumulation of TopIIcc than *wild-type* cells, following treatment with etoposide. Moreover, Mre11 promotes NHEJ of etoposide-induced DSBs in G_1 in mammalian cells [27]. These observations indicate that the incision activity of Mre11 eliminates TopII adducts from DSB ends (Fig. 2C) prior to the repair of the DSB by NHEJ. Note that the incision activity is likely to be independent of the role of Mre11 in DSB resection (Fig. 2B), since the incision can occur in the G_1 phase, when DSB resection is inhibited.

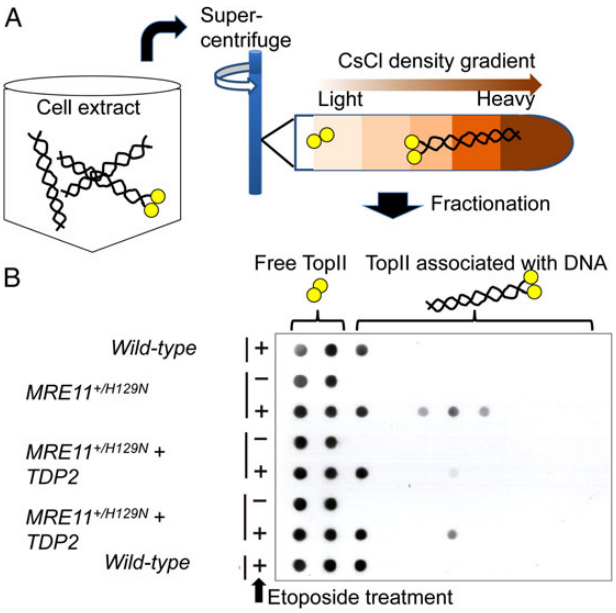
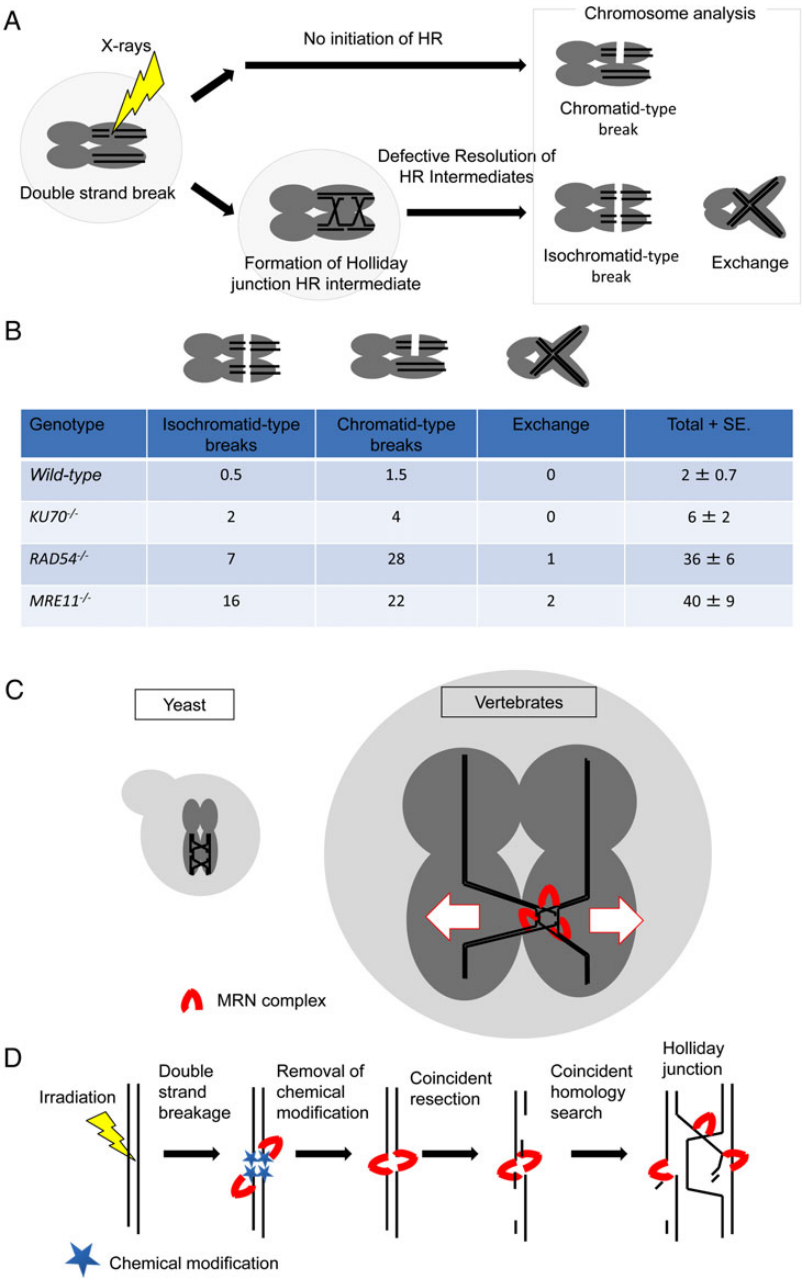


Fig. 4. Accumulation of topoisomerase II adduct (TopIIcc) in human TK6 cells heterozygous for nuclease-deficient Mre11 (*MRE11*$^{+/H129N}$). (A) Schematic of the *in vivo* TopIIcc measurement by immunodetection with anti-TopII antibody. A 200 μg genomic DNA sample was subjected to sedimentation by CsCl-gradient ultracentrifugation. Genomic DNA of *wild-type* TK6 cells that had been treated with 10 μM etoposide for 2 h was included as a control in every ultracentrifugation. The treatment reduced the survival of *wild-type* cells by ∼3% relative to untreated cells. Individual fractions were blotted to nylon filters, followed by western blot using anti-TopII antibody. The remaining two fractions include free TopII (yellow circle), while the other fractions include TopII covalently associated with genomic DNA. (B) Western blot analyses of TopII in the indicated TK6 cells that had been treated with 10 μM etoposide (+) or DMSO (−) for 2 h. '+*TDP2*' indicates overexpression of a *TDP2* transgene. The Tdp2 enzyme eliminates TopII adducts from DSB ends.

To directly test whether the nuclease activity of Mre11 is involved in elimination of TopII from DSB ends, we measured the amount of TopIIcc (TopII covalently associated with genomic DNA), following exposure of cells to etoposide (Fig. 4A). We separated TopIIcc from free TopII by subjecting the cellular extract to cesium-chloride-gradient ultracentrifugation, followed by fractionation and western blot analysis with anti-TopII antibody. TopIIcc is seen only in cells treated with etoposide (Fig. 4B). The heterozygous mutation of the nuclease activity (*MRE11*$^{+/H129N}$) significantly increased the amount of TopIIcc. Moreover, overexpression of tyrosyl-DNA-phosphodiesterase 2 (*TDP2*), an enzyme eliminating covalently bound TopII, normalized the amount of TopIIcc [28]. The prominent phenotype of the heterozygous mutant (*MRE11*$^{+/H129N}$) cells suggests the important role of the Mre11 nuclease activity in elimination of the TopII adduct, as yeast Mre11 is essential for removing the Spo11 topoisomerase from DSB ends (Fig. 1B). The dependency of DSB resection on Mre11 is significantly higher at 'dirty' DSBs induced by ionizing radiation than at 'clean' DSBs generated by restriction enzymes [3]. These observations support the notion that the nuclease activity of mammalian Mre11 is required for processing various forms of blocked DSB ends, including those containing damaged nucleotides and topoisomerase adducts, for subsequent DSB repair by NHEJ as well as HR. We propose that Mre11 contributes to cellular tolerance to ionizing radiation through processing of DSB ends (Fig. 2C) rather than DSB resection (Fig. 2B).

THE ROLE OF MRE11 IN HR AT A STEP AFTER THE FORMATION OF HOLLIDAY JUNCTION HR INTERMEDIATES

Nuclease-dead Mre11 mutants have a significantly milder phenotype during mitosis than do null-Mre11 mutants in *S. cerevisiae* [3, 29]. Likewise, nuclease-deficient Mre11 mutant DT40 cells are able to slowly proliferate, although the null-Mre11 mutant DT40 cells are lethal [12]. However, the non-catalytic functioning of Mre11 in HR has not yet been defined. This functioning might depend on the capability of the MRN complex to tether two DNA strands, since the complex has a structure similar to that of cohesion and condensin [30]. Although human *MRE11*$^{-/-}$ as well as *MRE11*$^{-/H129N}$ TK6 cells initiate DSB



Genotype	Isochromatid-type breaks	Chromatid-type breaks	Exchange	Total + SE.
Wild-type	0.5	1.5	0	2 ± 0.7
KU70$^{-/-}$	2	4	0	6 ± 2
RAD54$^{-/-}$	7	28	1	36 ± 6
MRE11$^{-/-}$	16	22	2	40 ± 9

Fig. 5. (A) Model for generation of isochromatid-type breaks following ionizing irradiation at the G_2 phase. The irradiation generates DSBs in one of two sister chromatids. Defective initiation of HR leads to chromatid-type breaks in mitotic chromosome spreads. Formation of HR intermediates such as Holliday junctions followed by failure of their resolution could generate isochromatid-type breaks, where two sister chromatids are broken at the same sites due to a defect in local chromosome condensation at HR intermediates. (B) The numbers of the indicated chromosome aberrations in the mitotic chromosome spreads after ionizing irradiation at the G_2 phase. DT40 cells carrying the indicated genotypes were exposed to 0.3 Gy γ-rays and harvested at 3 h for chromosome analysis. The numbers shown are per 100 mitotic cells. (C) The spatial distance between two sister chromatids is much bigger in mammalian cells than in yeast. Following DNA replication, dynamic chromosome condensation significantly separates two sister chromatids. Accordingly, HR intermediates are constantly pulled in opposite directions, as shown by the arrows. The MRN complex may stabilize HR intermediates by tethering DNA strands. (D) Model for the repair of ionizing radiation–induced DSBs. Ionizing radiation causes 'dirty DSBs' to associate with chemical modifications at DSB ends. The nuclease activity of Mre11 plays the major role in eliminating chemical modifications, particularly from 5′ ends. *Saccharomyces cerevisiae* Mre11 coordinates initiation of DSB resection at the two ends of individual DSBs [3]. We propose that Mre11 maintains HR intermediates for their proper resolution.

resection with nearly normal kinetics, these mutants showed a significant delay in resolution of ionizing radiation–induced Rad51 foci (Fig. 3C). The data suggest a role for Mre11 in a late step of HR as well as in processing the ends of 'dirty' DSBs.

There is no reliable phenotypic assay for analyzing HR intermediates in mammalian cells or mitotic yeast. However, phenotypic analyses of meiotic HR in *S. cerevisiae* most significantly contribute to our understanding of the precise molecular mechanism for resolution of HR intermediates [31]. DT40 cells provide a unique phenotypic analysis for evaluating resolution of HR intermediates. HR plays the dominant role in repairing ionizing radiation–induced DSBs during the G_2 phase in DT40 cells. Thus, we can assess the efficiency of HR-dependent DSB repair by exposing G_2-phase cells to γ-irradiation and measuring the number of chromosome aberrations in the subsequent M phase [32]. Moreover, morphological observation of mitotic chromosomes allows for identifying abortive HR intermediates (Fig. 5A).

In *RAD54*$^{-/-}$ cells, where the efficiency of homology search and strand exchange is reduced [8], γ-irradiation of G_2-phase cells significantly increased the number of chromatid-type breaks (Fig. 5B). Remarkably, the loss of Mre11 caused marked increases in the number of isochromatid-type breaks as well as chromatid-type breaks. Thus, a significant fraction of the γ-irradiation–induced DSBs in one of the sister chromatids is converted to chromosome breaks in both sister chromatids. These observations indicate that Mre11 can contribute to HR-dependent repair of DSBs, even after the formation of HR intermediates containing pairs of sister chromatids. We propose that Mre11 is required for the stable maintenance of HR intermediates for their proper resolution.

A critical difference in HR between yeast cells and vertebrate cells is a spatial distance between donor sequences in intact sister chromatids and recipient homologous sequences in broken sister chromatids. The distance is much smaller in yeast in comparison with mammalian cells (Fig. 5C) [33, 34]. Moreover, dynamic condensation of sister chromatids may constantly increase their distance after DNA replication in mammalian cells. Accordingly, HR intermediates containing two sisters may be pulled in opposite directions, leading to dissociation of the intermediates. The following two mechanisms may prevent the dissociation of the HR intermediates. Extensive strand exchange between two sister chromatids leads to the formation of stable structure such as the double Holliday junction [31]. Moreover, the tethering by MRN may further stabilize HR intermediates and promote the completion of DSB repair by HR. This MRN-dependent mechanism might be relevant to mitotic HR in *S. cerevisiae*, since yeast Mre11 also has a prominent non-catalytic role in genome maintenance [3, 29].

CONCLUSION

The genetic study of yeast species provides a framework for examining molecular mechanisms underlying HR in mammalian cells. The genetic study of yeast, but not mammalian cells, allows for examining the structure of various HR intermediates. In addition, since HR is the most complex DNA repair reaction, involving more than 100 molecules (including histone-modifying enzymes [4]), neither the biochemical study nor genetic study of mammalian cells allows for dissecting the role of individual factors in various HR reactions. Accordingly, the functions of individual mammalian HR factors have been postulated by the function of their yeast ortholog proteins. A large number of researchers of mammalian Mre11 seem to have interpreted their own data by extrapolating known functions of *S. cerevisiae* Mre11. However, although mammalian Mre11 does contribute to DSB resection, its limited contribution does not necessarily account for the very severe defect in HR-dependent repair of ionizing radiation–induced DSBs [11, 14]. The present data suggest that the severe defect reflects the important role of Mre11 in DSB repair aside from DSB resection: (i) processing of DSB ends and (ii) maintenance of HR intermediates. This conclusion is also supported by genetic studies of *S. cerevisiae* indicating (i) the greater contribution of Mre11 to repair of 'dirty' DSBs than 'clean' DSBs, and (ii) a severer phenotype of the null-Mre11 mutant compared with that of the nuclease-deficient Mre11 mutant [3]. Future studies will clarify the roles of mammalian Mre11 in the processing of DSB ends and the maintaining of HR intermediates (Fig. 5D).

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