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Mre11 and Exo1 contribute to the initiation and processivity of resection at meiotic double-strand breaks made independently of Spo11

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ABSTRACT

During meiosis DNA double-strand breaks (DSBs) are induced and repaired by homologous recombination to create gene conversion and crossover products. Mostly these DSBs are made by Spo11, which covalently binds to the DSB ends. More rarely in *Saccharomyces cerevisiae*, other meiotic DSBs are formed by self-homing endonucleases such as VDE, which is site specific and does not covalently bind to the DSB ends. We have used experimentally located VDE-DSB sites to analyse an intermediate step in homologous recombination, resection of the single-strand ending 5′ at the DSB site. Analysis of strains with different mutant alleles of *MRE11* (*mre11-58S* and *mre11-H125N*) and deleted for *EXO1* indicated that these two nucleases make significant contributions to repair of VDE-DSBs. Physical analysis of single-stranded repair intermediates indicates that efficient initiation and processivity of resection at VDE-DSBs require both Mre11 and Exo1, with loss of function for either protein causing severe delay in resection. We propose that these experiments model what happens at Spo11-DSBs after removal of the covalently bound protein, and that Mre11 and Exo1 are the major nucleases involved in creating resection tracts of widely varying lengths typical of meiotic recombination.

1. Introduction

The repair of DNA damage is critical to cell survival and the maintenance of genome stability. One important class of damage is DNA double-strand breaks (DSBs) that can arise from various genotoxic sources as well as part of developmental pathways that involve creating genetic change. Two well-studied developmental pathways that involve induction of DSBs are budding yeast mating-type switching and meiosis. The methods of both DSB formation and repair differ in these different developmental pathways. During yeast mating-type switching a single DSB is made in the expressed mating-type locus (MAT) by the HO-endonuclease, which works like a type II restriction endonuclease (RE) [1,2]. Repair of the HO-induced DSB is by gene conversion (GC) using chromosomally linked silent mating-type loci as donors to MAT [1]. During meiosis, ~170 DSBs are induced by Spo11, which acts like a topisomerase II type enzyme, becoming covalently bound to the 5′ end of the DNA [2–4]. Repair is also by homologous recombination utilising sequence on the homologous chromosome as template for non-crossover GC, or GC associated with a crossover (CO). This process is vital for fertility in most organisms, both to bring about homologous chromosome pairing and to mediate the first meiotic chromosome segregation. During the first meiotic division COs collaborate with sister chromatid cohesion to hold homologous chromosomes together as bivalents, until the onset of anaphase.

An essential intermediate step in homologous recombination is resection of the DNA strand ending 5′ on each side for the DSB. Removal of DNA from the strand ending 5′ creates a 3′ overhang of single-stranded DNA (ssDNA) [5]. This ssDNA becomes a substrate for recombination proteins such as RPA and the Rad52 epitasis group [6]. The ssDNA subsequently invades a homologous duplex to create heteroduplex DNA, which is extended by synthesis to replace DNA lost by resection. The length of heteroduplex DNA formed, and therefore the extent of gene conversion is possibly influenced by resection tract length [5,7,8]. How this resection process is initiated and how it progresses for hundreds or even several thousands of bases has been subject to recent detailed reviews [9–12]. Various conserved proteins have been implicated in resection, including some with nuclease activity such as Mre11, Exo1, and Dna2. Some resection proteins are DNA helicases such as Sgs1 and Dna2, while Sae2 regulates Mre11 activity and may also be a nuclease, the resection function of others such as Trm2 is less clear [10–15].

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DSBs could in theory be a direct substrate to the 5′ to 3′ exonuclease activity of Exo1 [16], but other proteins mentioned above clearly play a role. In vegetative budding yeast cells deleted for *EXO1*, resection of HO-induced DSBs can be slower and less extensive than in wild-type cells, but GC can still be efficient [15,17–19]. Singly mutating or deleting other genes such as *SAE2* or *MRE11* can cause similar delay or slowing of resection, though the apparent importance of individual genes varies in different assays [14,15,17,20–22]. Double or multiple mutations including those mentioned above with each other or *sgs1* and *trm2* can exacerbate the repair phenotypes [15,19,23,24]. This suggests there are redundant systems capable of both initiating HO-DSB resection, and progressing it to make sufficient ssDNA substrate for repair by homologous recombination. Recent biochemical data support the view from mitotic cells that Mre11 nuclease activity is not of major importance in resection [22,25–27].

Meiosis presents a special case of DSB repair and the relative importance of different resection proteins might vary significantly from mitosis. A major difference between DSB repair studies in mitosis and meiosis resides with the initiation of HO-induced DSBs versus Spo11-induced DSBs. HO-DSBs are considered 'clean' as there is no modification of the DNA ends. In meiosis initiating resection is complicated by the need to first remove the covalently bound Spo11 from the DSB site. This is dependent on the activity of various proteins including, Mre11, Sae2 and Rad50 (reviewed in [11,28]). Removal of Spo11 is effected by creation of a nearby nick that requires the activity of Mre11 and dissociation of a short Spo11-oligonucleotide complex [29]. As in repair of HO-DSBs, there is evidence that subsequent resection after removal of Spo11 uses multiple proteins including nucleases such as Exo1 and Dna2 [30–33]. The role for Sgs1 in meiotic resection may be significantly reduced compared to in mitosis [31]. Some data are consistent with Exo1 having only a minor role during resection in meiosis [33,34]. Up until now, physical evidence on resection has been gathered from cells lacking *DMC1*, a meiosis specific orthologue of *RAD51* in which hyperresection occurs due to the failure of strand invasion [35]. However, the current accompanying paper and a concurrent study provide both genetic and physical evidence that Exo1 is important during resection in otherwise wild-type meiosis [30,31]. In wild-type cells a wide array of proteins is likely to involve complex collaborations and partial redundancies [9–12].

In some yeast strains there are rare meiotic DSBs created by nucleases that do not covalently bind to the DNA. One example is the *VMA1* derived homing endonuclease, VDE, which is related to the HO-endonuclease [36]. In cells heterozygous for the activity of VDE, it creates a site-specific DSB in the *TFP1/VMA1* open reading frame [37]. This VDE-DSB is repaired by gene conversion utilising the homologue, which already contains the VDE coding sequence inserted within the *TFP1* gene and expressed as an intein (reviewed in [38]). The gene conversion activity that propagates the VDE intein is sensitive to mutations in the meiosis specific recombinases such as *DMC1*, as well as resection genes *SAE2*, *MRE11* and *EXO1* [39,40]. Here we report on the resection roles of Mre11 and Exo1 during meiosis in repair of DSBs induced by VDE. We studied VDE-induced DSBs rather than Spo11-induced DSBs because the frequency of breaks is much higher (up to approximately 100% of chromosomes), and because VDE does not covalently bind to the DNA. Measuring repair and resection at a VDE-DSB facilitates discovery of potential Mre11 nuclease roles downstream of the early function that liberates Spo11, and has already been used to correctly predict that Sae2 function is required beyond removal of Spo11 [32,41]. The VDE-DSB also has the advantage of being site specific, allowing a more detailed analysis of resection distances closer than would be easily done at Spo11-DSBs, which are scattered over a 200–300 base pair region [3].

Mre11 has five domains predicted to have phospho-esterase activity (see [42]). Various site-specific mutations of *MRE11* have been isolated that reduce its apparent nuclease function. We compared two of these, *mre11-58S* (*mre11-H213Y*; a mutation in the fourth phospho-esterase domain [20]) and *mre11-H125N*; a mutation in the third phospho-esterase domain [22]. These alleles are known to have different DSB repair phenotypes during mating-type switching. Cells expressing *mre11-H125N* are proficient for resection and HO-DSB repair, while cells expressing *mre11-58S* are delayed for HO-DSB repair and sensitive to IR [20,22,23,26,27]. The *mre11*-58S allele was created to separate the importance of H213 for function, following isolation of the allele *mre11-58*, which contains two amino acid changes (H213Y and L225I; [20,43]). Evidence from co-immunoprecipitation experiments suggests that the Mre11-58 protein has a defect in maintaining the Mre11-Rad50-Xrs2 (MRX) complex in vegetative cells [44]. The proposed complex forming defect of Mre11-58 could explain the more severe mitotic DSB-repair phenotype of *mre11-58S* cells versus *mre11-H125N* cells. In meiosis Mre11 is required for Spo11-DSB formation, while both *mre11* alleles used allow formation of Spo11-DSBs, neither stimulate Spo11-DSB resection [20,22]. Like deletion of MRX complex members, mutations of *XRS2* that inhibit its ability to interact with Mre11 prevent Spo11-DSB formation [45,46]. Thus, perhaps *in vivo* the Mre11-58S protein forms an MRX complex that is easily disrupted in co-immunoprecipitation experiments. If true, then function of both phospho-esterase domains is likely to be required for normal resection during meiosis.

Analysis of Exo1 function is of importance because genetic reports have implicated Exo1 in meiotic resection, as gene conversion tracts tend to be shorter in *exo1Δ* cells [7,33,34]. However, physical analyses of Exo1 function in meiosis have, until very recently [30,31], been undertaken in strains mutant for *dmc1* and unable to strand invade the homologue. Spo11-DSBs in such cells hyperresect, and it is not clear whether the influence of Exo1 on hyperesection is representative of its influence on more normal meiotic resection tracts. We have previously found that in *dmc1* cells there is an imbalance in the availability of repair proteins, which causes pleiotropic effects avoided in both this study and the accompanying paper [30,47].

Here we show that cells expressing either of the two alleles of *mre11* have different resection phenotypes at VDE-DSBs, which were not predicted by either the mitotic data or apparent complex forming abilities. Cells expressing *mre11-H125N* have a severe defect in VDE-DSB resection, while those expressing *mre11-58S* have nearly wild-type VDE-DSB resection. VDE-DSBs in cells lacking Exo1 function were severely delayed for repair, with a reduced evidence of long resection tracts being created as intermediates or reflected in the repair products. Our data support the view that nuclease function of both Mre11 and Exo1 are important for both the initiation of resection at VDE-DSBs and in creating long resection tracts in *DMC1* cells.

2. Materials and methods

2.1. Strains

The diploid SK1 strains [48] are listed in Supplementary Table 1. The strains containing the VDE-DSB1 and VDE-DSB2 inserts were made as previously described [41,47]. The *EXO1* open reading frame was replaced by the KanMX gene as described [39]. An integrative plasmid containing the *mre11-H125N* (pSM438) was a gift from Lorraine Symington. The plasmid was linearised for integration using the restriction endonuclease AflII, transforments were isolated by selection for URA3. Following further selection on 5-FOA to isolate recombinants deleted for the URA3, vector and MRE11 repeated sequences, presence of the mutation was confirmed by sensitivity