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Exo1 and Mre11 execute meiotic DSB end resection in the protist *Tetrahymena*

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ABSTRACT

The resection of 5′-DNA ends at a double-strand break (DSB) is an essential step in recombinational repair, as it exposes 3′ single-stranded DNA (ssDNA) tails for interaction with a repair template. In mitosis, Exo1 and Sgs1 have a conserved function in the formation of long ssDNA tails, whereas this step in the processing of programmed meiotic DSBs is less well-characterized across model organisms. In budding yeast, which has been most intensely studied in this respect, Exo1 is a major meiotic nuclease. In addition, it exerts a nuclease-independent function later in meiosis in the conversion of DNA joint molecules into ZMM-dependent crossovers. In order to gain insight into the diverse meiotic roles of Exo1, we investigated the effect of Exo1 deletion in the ciliated protist *Tetrahymena*. We found that Exo1 together with Mre11, but without the help of Sgs1, promotes meiotic DSB end resection. Resection is completely eliminated only if both Mre11 and Exo1 are missing. This is consistent with the yeast model where Mre11 promotes resection in the 3′–5′ direction and Exo1 in the opposite 5′–3′ direction. However, while the endonuclease activity of Mre11 is essential to create an entry site for exonucleases and hence to start resection in budding yeast, *Tetrahymena* Exo1 is able to create single-stranded DNA in the absence of Mre11. Excluding a possible contribution of the Mre11 cofactor Sae2 (Com1) as an autonomous endonuclease, we conclude that there exists another unknown nuclease that initiates DSB processing in *Tetrahymena*. Consistent with the absence of the ZMM crossover pathway in *Tetrahymena*, crossover formation is independent of Exo1.

1. Introduction

Crossing over is a central process in meiosis as it contributes to the generation of novel combinations of genes along chromosomes and, even more importantly, is crucial for the separation of parental chromosomes during the first meiotic division. Crossover (CO) formation begins with programmed DNA double-strand breaks (DSBs) (see Ref. [1]). DSBs are processed to produce single-stranded (ss)DNA overhangs on either side of a break. These ssDNA ends can invade dsDNA for homology sampling and strand exchange. At the same time, ssDNA is required for activation of the ATR-mediated DNA damage checkpoint response and to discourage the potentially deleterious joining of DNA ends by non-homologous end joining [2]. Identification of homology between the invading strand and the template results in formation of DNA joint molecules (JMs) that can be converted into non-CO or CO recombination products by distinct DSB repair pathways (see Ref. [3]). There are different pathways from JMs to COs, and the predominant (class I) COs are dependent on the so-called ZMM group of proteins, including the eponymous Zip1-4, Msh4-5 and Mer3 (see Ref. [4]). They are interfering (i.e., mutually suppressive) and occur in the context of the synaptonemal complex. Other COs (class II) are independent of ZMM proteins and are non-interfering [5]. Organisms differ with respect to the predominant or exclusive use of these pathways (see Ref. [6,7]).

Most, if not all meiotic DSBs are generated by the transesterase Spo11 in a topoisomerase-like reaction (see Ref. [1]). Following break induction, the covalently bound Spo11 has to be removed to provide access to the 5′-strand for further processing. In budding and fission yeast meiosis, this step requires an endonucleolytic nick induced by the Mre11 nuclease [8–11]. Mre11 forms a conserved complex with Rad50 and Nbs1 (Xrs2 in budding yeast), and its nicking activity is regulated by Sae2 (Com1) [12], which is also known as Ctp1 in fission yeast and CtIP in vertebrates. Less is known about the subsequent steps that lead to extensive resection and allow for strand invasion. In the case of mitotic DSB repair, several proteins have been shown to contribute to this step. In budding yeast and in

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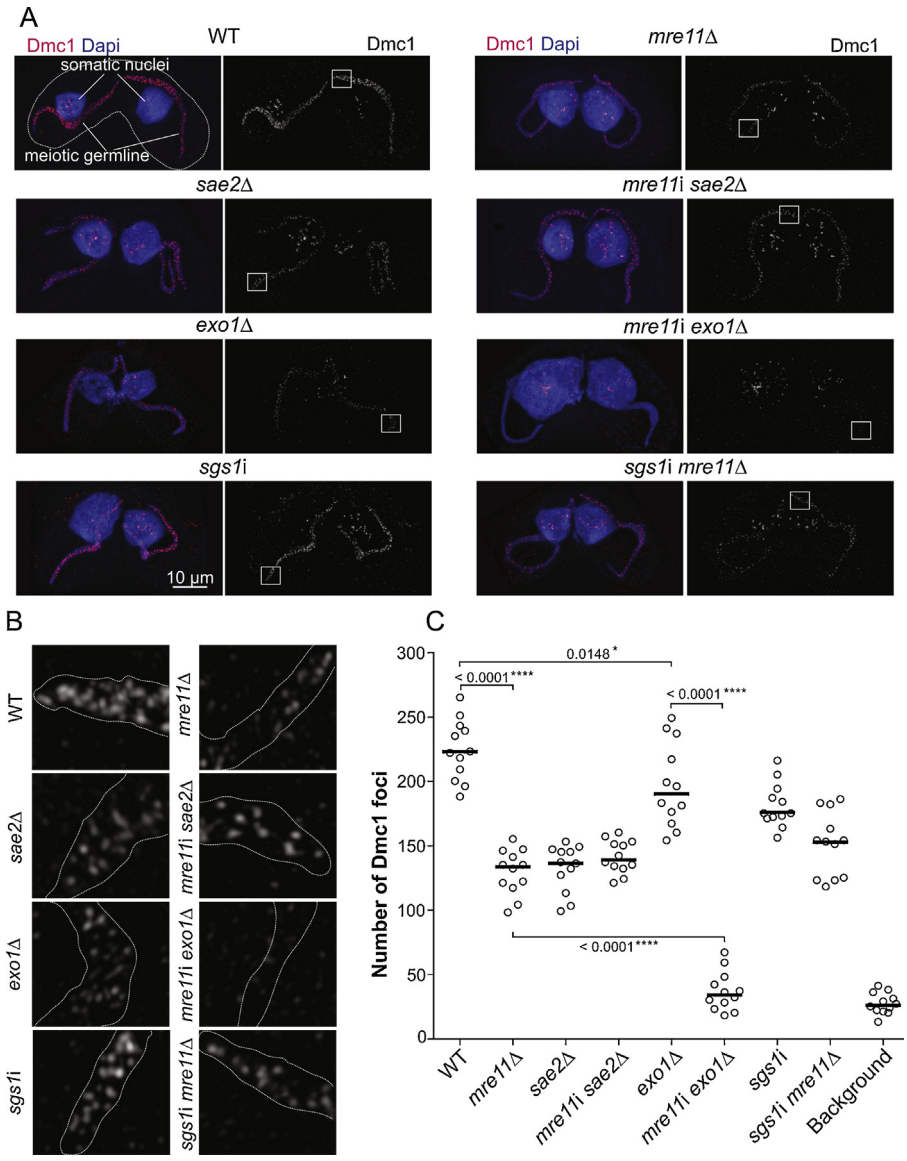

Fig. 1. Dmc1 localization to spread nuclei of the wild type and mutants. (A) Dmc1 is virtually absent in the elongated prophase nuclei of the *mre11*i *exo1*Δ mutant. The foci in the non-meiotic somatic nuclei are caused by crossreaction of the antibody with Rad51, which does not localize to the elongated meiotic nuclei, as previously reported [25]. (B) Magnified details from the boxed areas in (A). (C) Numbers of Dmc1 foci. Signals that occupied the DAPI-stained area of the meiotic nucleus were counted as Dmc1 foci. Dmc1 foci scored in *me11*i *exo1*Δ resemble background staining. The level of signal background due to unspecific precipitation of the antibody was determined by counting signals in the chromatin-free (DAPI-negative) area of a cell that was equal to the area occupied by the meiotic nucleus in this cell. Bars represent medians, and *p*-values (Mann–Whitney *U* test) are given.

mammals, Exo1 and Sgs1-Dna2 work redundantly to produce long tracts of ssDNA. In meiosis, so far, only Exo1 has been shown to play a role in extensive resection (see Ref. [2]). According to the budding yeast resection model, Exo1 can access the nick generated by Mre11 and use its 5′–3′ exonucleolytic activity to resect the 5′ end. In addition, Mre11 itself, via its 3′–5′ exonuclease activity, extends the gap in the opposite direction towards the break. Thus, Mre11′s endo- and exo-nucleolytic activity ensures the release of short Spo11-associated oligonucleotides, and Exo1 catalyses the formation of long 3′ ssDNA ends [13]. In addition, Exo1 helps to transform JMs into class I COs independently of its nucleolytic activity [14,15].

The enzyme or enzymes which may execute meiotic DSB end resection in fission yeast are unknown. Fission yeast Exo1 is required for mismatch corrections formed during meiotic recombination, but it has no significant role in crossing over [16], which may be due to the fact that in fission yeast class I COs are absent [17]. In mouse meiosis, where the class I pathway predominates, CO formation is strongly reduced in the absence of EXO1. However, the nuclease activity of mouse EXO1 is dispensable for meiosis [18,19]. Conversely, studies in *C. elegans* suggest a direct role of EXO-1 in promoting 5′ end resection but not crossing over [20,21]. This resection function likely acts in parallel with MRE-11 and its cofactor COM-1 (Sae2) and, perhaps, another nuclease. *Arabidopsis* possesses two *EXO1* paralogs [22], and double mutants are fully fertile (Karel Riha and Max Rössler, pers. commun.), which suggests that they play only a minor if any role in meiosis in this organism.

To gain understanding of the role of Exo1 in meiotic DSB processing in a broad range of organisms, we studied the evolutionarily distant ciliated protist *Tetrahymena thermophila*. *Tetrahymena* cells possess two nuclei, a polyploid somatic nucleus and a diploid ($2n = 10$) germline nucleus. Only the latter undergoes meiosis as part of the facultative sexual cycle. A striking feature of *Tetrahymena* meiosis is the immense elongation of the prophase nucleus to about twice the length of the cell (Supplementary Fig. 1). This