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Review

DNA double-strand break formation and repair in *Tetrahymena* meiosis

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ABSTRACT

The molecular details of meiotic recombination have been determined for a small number of model organisms. From these studies, a general picture has emerged that shows that most, if not all, recombination is initiated by a DNA double-strand break (DSB) that is repaired in a recombinogenic process using a homologous DNA strand as a template. However, the details of recombination vary between organisms, and it is unknown which variant is representative of evolutionarily primordial meiosis or most prevalent among eukaryotes. To answer these questions and to obtain a better understanding of the range of recombination processes among eukaryotes, it is important to study a variety of different organisms. Here, the ciliate *Tetrahymena thermophila* is introduced as a versatile meiotic model system, which has the additional bonus of having the largest phylogenetic distance to all of the eukaryotes studied to date. Studying this organism can contribute to our understanding of the conservation and diversification of meiotic recombination processes.

Contents

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1. Introduction

1.1. Meiotic recombination

Meiosis is a specialized cell division in which germ progenitor cells reduce the somatic double chromosome set to a gametic single set during sexual reproduction in eukaryotes. The chromosomes of both original sets are randomly represented in the gametic set. In addition to this mixing of original sets, the chromosomes of the gametic set are mosaics that are assembled from portions of the homologous parental chromosomes. This "interhomolog recombination" provides the physical linkage of chromosome pairs and ensures their faithful segregation. Moreover, it contributes to the reshuffling of parental genes in the gametes and the genetic diversity of the offspring.

Each chromosome consists of two sister DNA molecules, the chromatids. At certain locations, one of the four chromatids of a chromosome pair experiences a programmed DNA double-strand break (DSB) that is caused by a dedicated transesterase, Spo11 (Fig. 1) (see Ref. [1]). This DSB is repaired using one of the sister- or homologous DNA strands as a template for repair synthesis. The repair process may lead to the exchange of strands in a subset of events. During meiosis, several regulatory mechanisms ensure that this exchange takes place more often with a homologous chromatid than a sister chromatid (see Ref. [2] for a detailed review on these steps of recombination).

DNA flanking the DSB is resected to expose single-strand (ss) 3′ overhangs. These ssDNA tracts are first bound by proteins that stabilize and reconfigure the DNA. Later, these proteins are replaced by the strand exchange proteins Rad51 and/or Dmc1. These DNA-protein complexes can invade homologous dsDNA tracts, search for complementary base sequences and initiate strand exchange (see Ref. [3]). A subset of DSB repair events via the resulting transient DNA joint molecules (JMs) is accompanied by reciprocal strand exchange, i.e., crossing over (Fig. 1).

The predominant crossover (CO) pathway in most organisms is described by the canonical DSB repair model. Its molecular details have been worked out in the budding yeast, *Saccharomyces cerevisiae* (for a review see Ref. [4]). In this so-called class I pathway, JMs are converted into interfering (mutually suppressive) COs in the context of the synaptonemal complex (SC) (see Ref. [5]). It requires the ZMM (for Zip1, 2, 3, Msh4, 5 and Mer3) group of proteins [6,7]. The resolution of JMs is probably exerted by the MutLγ (Mlh1-Mlh3) nuclease complex with the help of Exo1 [8–10]. An additional, minor (class II) pathway, employing the Mus81-Mms4(Eme1) endonuclease complex, leads to a small number of additional, non-interfering, COs without the help of ZMM proteins (see Refs. [11,12], and literature cited therein) in budding yeast, *Arabidopsis* and mouse [13–16]. The class II pathway is believed to be evolutionarily older because it does not depend on a SC and makes use of fewer meiosis-specific factors. In the fission yeast, which does not form a SC and produces only non-interfering COs [17], this pathway is predominant (see Ref. [18]). However, most eukaryotes also possess the more sophisticated class I pathway, which allows for a more efficient control of COs, and they use both pathways in parallel [12].

1.2. Studying meiosis *in* Tetrahymena

Tetrahymena thermophila is a free-living freshwater ciliated protist. It can propagate vegetatively and by sexual reproduction when cells of complementing sexes mate. Like other ciliates, it has two functionally distinct nuclei per cell (see Ref. [19]). One is the transcriptionally silent micronucleus with $2n = 10$ chromosomes, which functions as the germline. It undergoes meiosis, and the meiotic products of mating partners fuse into zygote nuclei, which are passed on to sexual progeny. The polyploid macronucleus represents the soma. Protein-encoding genes are only transcribed from the macronucleus. It disintegrates in sexual cells and is reconstituted in progeny cells from new micronuclei.

Tetrahymena has many benefits as an experimental model system for molecular and genetic studies [20]. It is easy to culture, and thanks to its inducible and highly synchronous meiosis, *Tetrahymena* is the only organism besides the yeasts, where bulk isolation of meiotic DNA for molecular assays of DSBs is possible. Its nuclei and chromosomes are amenable to cytological analysis. Additionally, due to its capability of performing both vegetative propagation and sexual reproduction, meiosis-defective mutants can be easily maintained [21].

Mating pairs undergo simultaneous meioses [22], and the progression of meiosis can easily be followed and staged. Perhaps the most remarkable feature of *Tetrahymena* meiosis is the extreme elongation of nuclei during prophase to approximately twice the length of the cell (Fig. 2). Within an elongated nucleus, chromosomes are arranged in a polarized manner, resembling the widely-conserved bouquet where all telomeres gather in a limited area at the nuclear periphery [23]. However, in *Tetrahymena*, in addition to telomere clustering at one end of the nucleus, the centromeres are attached to the opposite end. This ultimate bouquet arrangement promotes the juxtaposition of homologous regions and, thereby, homologous pairing and crossing over [24,25]. Cytological studies failed to detect a SC [25–27]. Following this unusual pairing stage, nuclei shorten and five condensed bivalents become discernible at the diplotene/diakinesis stage (Fig. 2), which is followed by first and second meiotic divisions within the intact nuclear envelope.

BLAST searches revealed homologs of several conserved meiotic proteins, however, in accordance with cytological evidence, no homologs of SC proteins were detected [27,28]. The identification of meiotic genes is greatly facilitated by the published expression profiles of ~27,000 genes throughout the *Tetrahymena* life cycle ([29] and http://tfgd.ihb.ac.cn/). Knockouts of genes that are highly upregulated during the time of meiosis revealed that only a minority have functions in meiosis per se (unpublished data), but the majority are important for germline-soma nuclear differentiation in the meiotic progeny [30]. This suggests a repertoire of meiotic genes that is smaller than in other model organisms, and hence the presence of a simplified meiotic process in *Tetrahymena*.

The understanding of molecular recombination in *Tetrahymena* meiosis is still fragmentary, but the function of several recombination genes was established in mutant studies (Fig. 2). These studies showed that, at the molecular level, the early steps in the meiotic recombination pathway are similar to those in canonical model organisms, whereas the later stages seem to deviate. In the following, we give a synoptic view of the events from DSB formation to crossing over, as is understood to date (Fig. 3).

2. Stages of recombination

2.1. DSB initiation

A conserved role for *Tetrahymena SPO11* in inducing DSBs was revealed by the occurrence of transient Spo11-dependent chromosome breakage, as observed by pulsed-field gel electrophoresis [31], and by the Spo11-dependent nuclear localization of DSB markers, such as γ-H2A.X [28] and Dmc1 [32] (Fig. 2). Moreover, the fragmentation of meiotic chromosomes in DSB repair-deficient mutants is suppressed in double mutants with *spo11*. Finally, meiotic repair synthesis, as visualized by BrdU incorporation, occurs only in the presence of *SPO11* [24]. By counting nuclear Dmc1 foci, it was estimated that >170 DSBs occur in wild-type meiosis [32]. As in

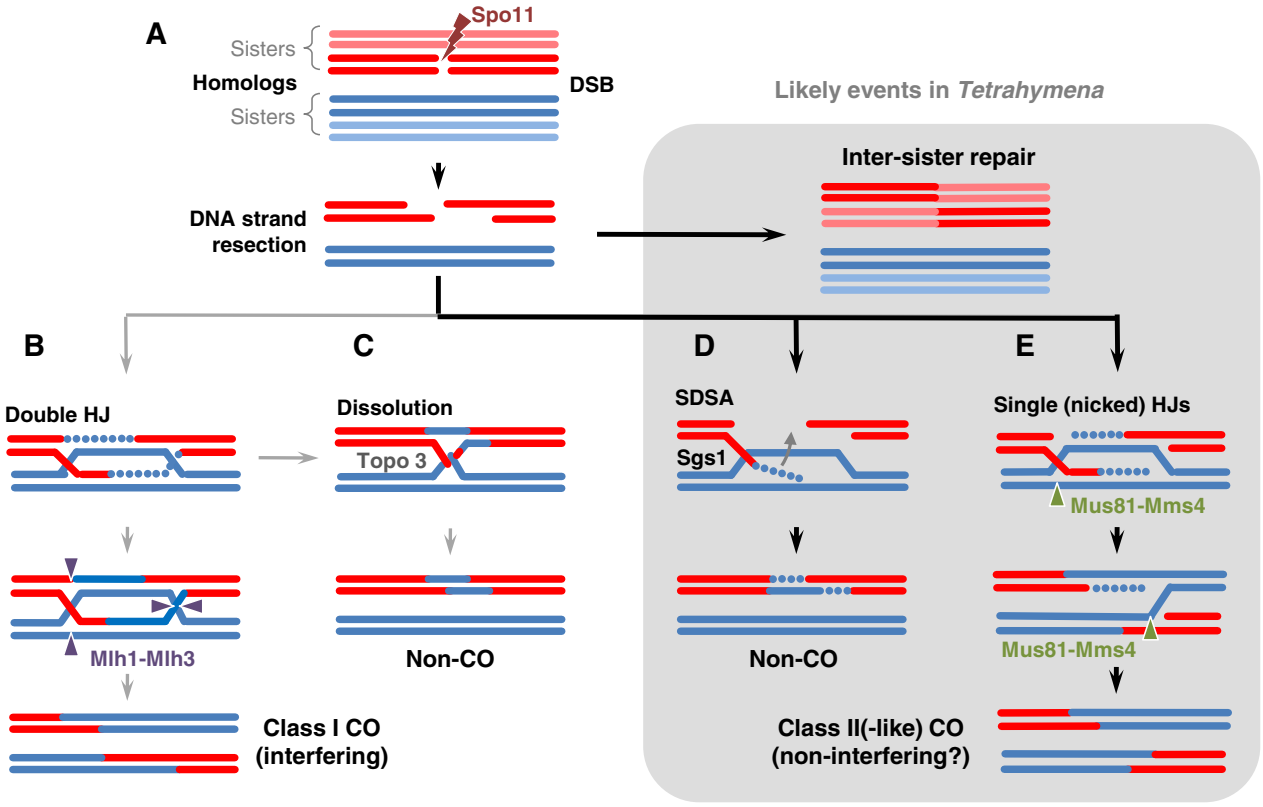

Fig. 1. Models of meiotic crossing over (CO). (A) All meiotic recombination is initiated by Spo11-generated DSBs, which are then extended to gaps with 3′-ssDNA overhangs. An unknown proportion of DSBs is repaired using the sister chromatid as the template for repair DNA synthesis. (B) A version of the canonical DSB interhomology repair model featuring double Holliday junctions (HJs). For simplicity, strand invasion and second end capture are shown as simultaneous occurrences. Only the CO-mode of resolution by the asymmetrical cleavage of the HJs (probably by Mlh1-Mlh3) is shown. COs formed along this class I pathway are interfering and SC-dependent. (C) The dissolution of a JM formed via a double HJ. (D) Synthesis-dependent strand annealing (SDSA) occurs when ssDNA with a newly synthesized DNA tract is displaced from the template strand and bridges and repairs the gap. This is a major pathway for non-CO recombinational DSB repair in most organisms and probably contributes to the repair of meiotic DSBs in *Tetrahymena* as well. (E) A simple version of the Mus81-Mms4-dependent CO model, featuring nicked single-HJ JMs. (See Ref. [92] for an alternative that invokes the activity of a putative D-loop nickase to account for the fact that mostly single HJs accumulate in a *mus81*Δ fission yeast mutant.) Also here, strand invasion by both ends is depicted as a single step. This class II pathway is generally assumed to generate non-interfering COs. A modified version of this pathway produces most, if not all, of the COs in *Tetrahymena*. It is not yet known if *Tetrahymena* COs are interfering.

most other model organisms, except budding yeast and *Caenorhabditis elegans* [33], Mre11 is not required for DSB formation [31]. While several poorly or non-conserved proteins have been found in the yeasts, animals and plants to serve as auxiliary factors that enable Spo11 to induce DSBs (see Refs. [33–36]), no DSB cofactors are yet known in *Tetrahymena*.

Notably, in *Tetrahymena*, DSBs trigger the elongation of meiotic nuclei via an ATR-dependent DNA-damage-response mechanism [28,37]. Within the elongated nuclei, homologous chromosomes become prealigned (see Section 1.2), and this increases the chance of interhomologous compared to intersister interactions. Thus, this unprecedented consequence of DSB induction ensures that an appropriate number of DSBs are repaired via the homolog [24].

2.2. DSB end processing

To make DSB ends capable of invading duplex DNA, Spo11 that is covalently bound to the 5′ end must be removed, and 3′ ss-overhangs must be generated. In budding yeast, it is believed that a nick in the 5′ strand proximal to the DSB is created by Mre11 in conjunction with Sae2(Com1). This is then extended toward the end by the 3′ → 5′ exonuclease activity of Mre11 until the remaining DNA oligo detaches with Spo11. The short 3′ overhang thus produced is further extended in the opposite direction by the 5′ → 3′ exonuclease Exo1 [38]. When *Tetrahymena*'s homologs of Sae2(Com1) and Mre11 were deleted, DSBs were not repaired, yet reduced loading of Dmc1 still occurred [31]. This suggests that a limited resection of the flanking DNA tracts occurs. Only when both Mre11 and Exo1 are missing is Dmc1 no longer loaded [39]. This is consistent with the yeast model of their combined activities for ssDNA extension in both directions. However, in yeast, Mre11 and Sae2 are essential for releasing Spo11 from DSBs and hence to start resection, whereas in *Tetrahymena* Exo1 is still able to resect if both nucleases are missing. Thus, Spo11 release seems to be exerted by another protein in *Tetrahymena* [39].

2.3. DNA strand exchange

Most eukaryotes employ two RecA homologs during meiosis, where they form nucleoprotein filaments with ssDNA and promote its invasion into a homologous dsDNA and subsequent strand exchange (reviewed in [3]). *Tetrahymena* possesses a single paralogue each of the ubiquitous Rad51 and the meiosis-specific Dmc1. Immunostaining and protein tagging demonstrated that Dmc1 forms strong DSB-dependent foci on chromatin in elongating prophase nuclei (Fig. 2), whereas weak Rad51 foci appear only in shortening nuclei after maximal elongation [32]. In the absence of Dmc1, efficient Rad51-dependent repair takes place, but the chromosomes remain univalent, suggesting that repair occurs via the sister chromatid. The interhomolog repair deficit in *dmc1*Δ meiosis is consistent with a requirement of Dmc1 to promote the homolog as the preferred recombination partner. Indeed it has been

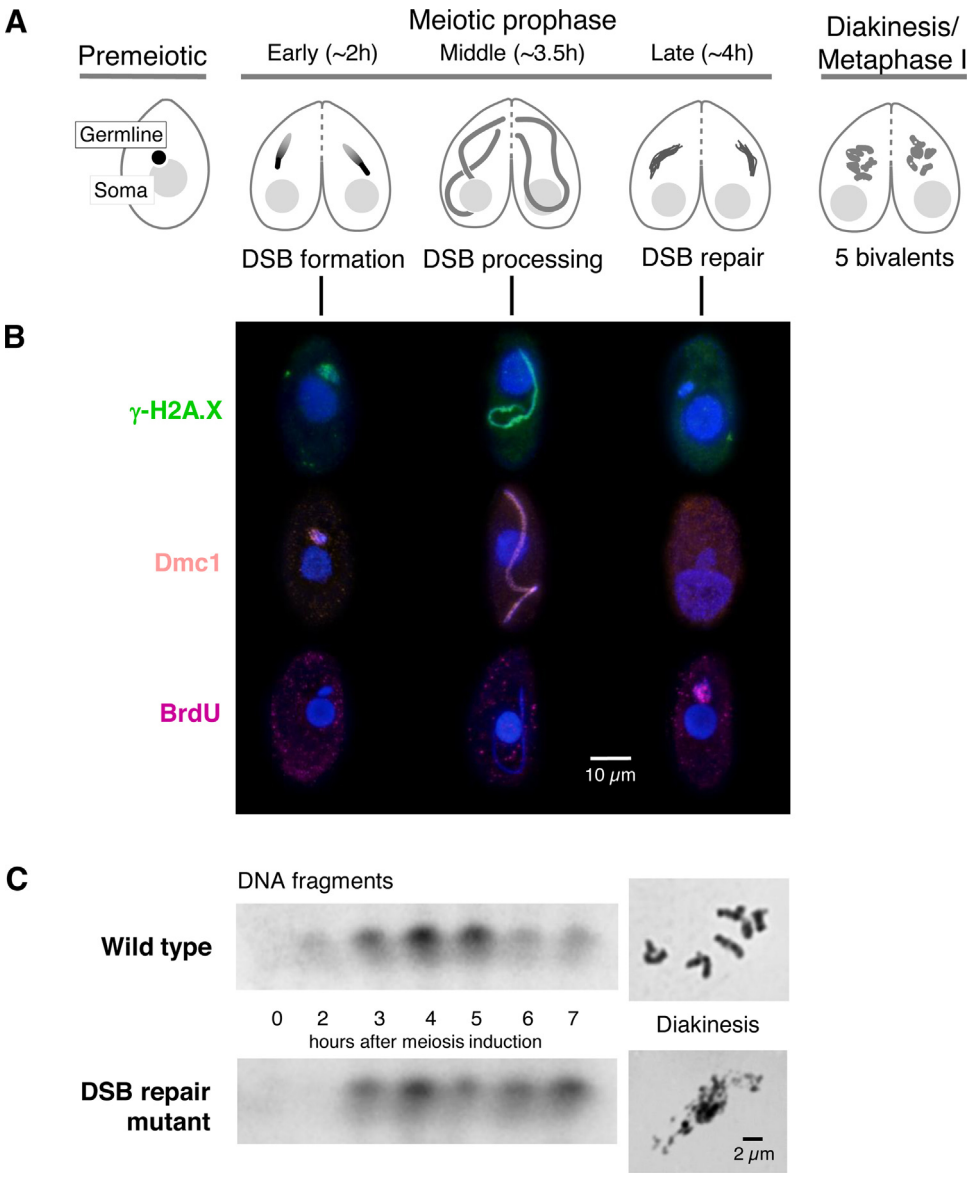

Fig. 2. Cytological and molecular processes in *Tetrahymena* meiosis. (A) Cells of different mating types mate upon starvation. The germline nuclei of mating partners undergo synchronous meioses. Meiotic nuclei elongate during meiotic prophase. This elongation is triggered by meiotic DSBs. Chromosome pairing takes place in elongated nuclei [25,28]. At the end of prophase, the chromosomes condense into bundles of strands and, finally, become visible as five bivalents. (B) From the beginning of the elongation (~2 h after meiosis induction) to maximal elongation (~3.5 h), γ-H2A.X and Dmc1 foci localize to nuclei. During nuclear shortening near the end of prophase (~4 h), DSBs are repaired. Repair synthesis is visualized by the incorporation of BrdU. (C) Meiotic DSBs produce fragmented chromosomes that migrate in a pulsed field gel. (Under the running conditions applied here, fragments of different sizes migrate as a distinct band.) In the wild type DSBs appear transiently, whereas in a DSB repair mutant (here: *sae2/com1*Δ—[31]) they persist. While in the wild type distinct bivalents are formed at diplotene/diakinesis, the failure to repair DSBs results in a mass of chromatin fragments at the corresponding stage.

shown that Dmc1 is better than Rad51 in searching similar but non-identical DNA sequences at DSBs [40]. In the absence of Rad51, metaphase I chromosomes were fragmented and pulsed-field gel electrophoresis revealed that DNA from meiotic nuclei remained permanently broken [32]. On the other hand, Dmc1 focus formation was found to be independent of the presence of Rad51. This suggests that in contrast to a recent model [41], Dmc1 nucleoprotein filaments can form without the participation of Rad51, but are incompetent for strand exchange.

2.4. The timing of DSB formation and repair

γ-H2A.X localization and nuclear elongation (marking incipient DSB formation) begin ~2 h after meiosis induction. Dmc1 foci appear at this time and then persist for ~2 h during the nuclear elongation stage. When nuclei shorten again, Dmc1 foci are quickly lost, whereas weak Rad51 foci transiently appear. BrdU incorporation experiments show that meiotic DSB-dependent DNA synthesis occurs in these shortening nuclei (Fig. 2). Dmc1 and BrdU foci do not appear together in the nucleus, indicating that once the repair of DSBs is initiated, it is fast and synchronous. It probably occurs within a narrow interval of 30 min after the completion of homologous pairing [24]. This means that COs and non-COs are formed simultaneously, which parallels the situation in *C. elegans* [42] and contrasts with the observation in budding yeast that non-COs are produced earlier than COs [5,43].

Early in *Tetrahymena* meiosis, i.e., in elongating nuclei, DSBs are required for the production of ssDNA ends that serve as probes for the precise sequence matching of homologs [37]. Later, a subset of DSBs will be converted to COs. To allow for homology

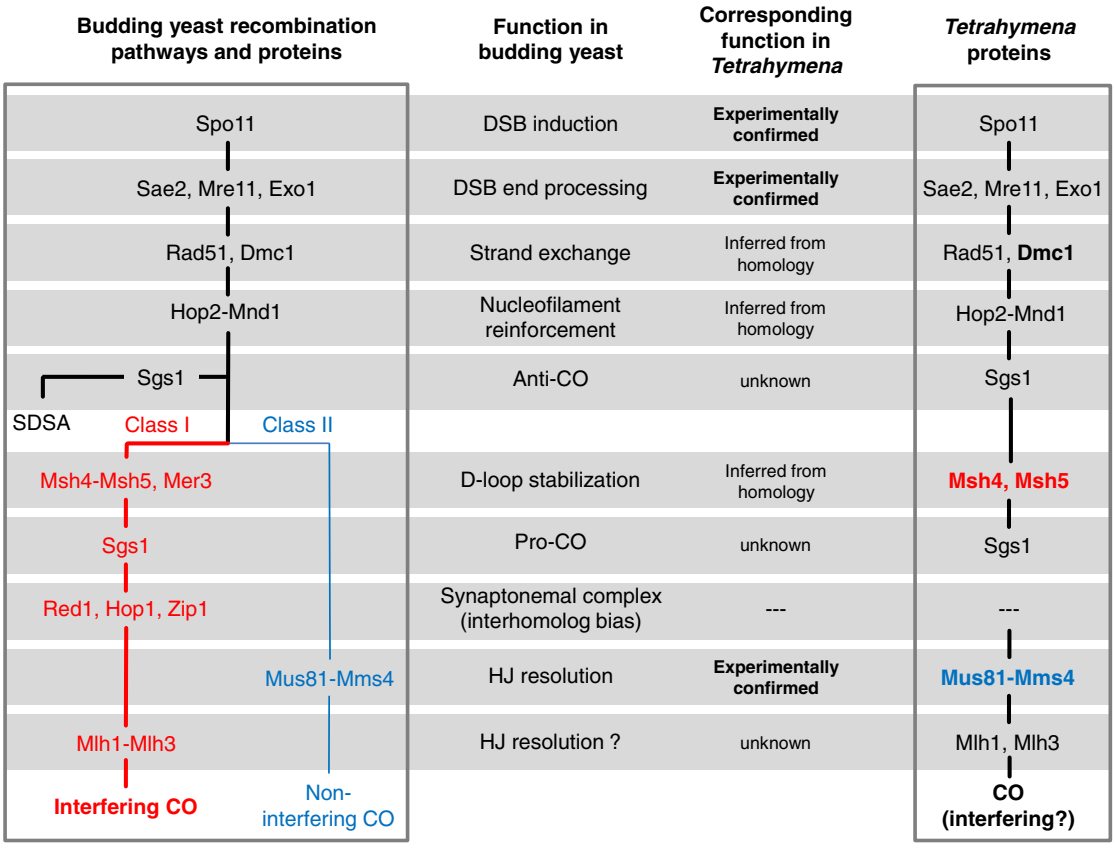

Fig. 3. The factors involved in early events in meiotic recombination, DSB formation and D-loop formation, are very similar between model organisms. The downstream events may be more variable. In the budding yeast, like in most other eukaryotes, the majority of COs are interfering and are produced in a pathway (class I) that is also associated with SC formation. A minor class II pathway also exists. In *Tetrahymena*, most, if not all, COs depend on the class II resolvase complex Mus81-Mms4. Wild-type CO levels also require Msh4 and Msh5, which previously have been considered to be part of the class I pathway. It is not clear if they operate in the same pathway as Mus81-Mms4 or whether they are part of another, SC-independent, pathway.

searching and CO, however, immediate DSB repair via the sister must be suppressed. It is possible that Rad51 is absent in elongating nuclei, which would explain the inability to repair DSBs precociously (see Section 2.3). Alternatively, repair may also be postponed by the destabilization of early intersister D-loops by Sgs1 (see Section 2.6.3).

2.5. Crossing over

Tetrahymena possesses *MUS81* and *MMS4* homologs, and when either is knocked down, bivalents are unable to separate [44]. This suggests that JMs are not resolved. This conclusion was confirmed by the behavior of meiotic DNA on a pulsed-field gel; DNA from *mus81*(RNAi) meiotic cells, even when cut into restriction fragments of ca. 1.5 Mb would not enter the gel, and it did so only upon treatment with RuvC [44]. Because RuvC is known to resolve Holliday junctions and related branched DNA structures, non-separable DNA was interpreted as persistent JMs. Thus, as in fission yeast (which also does not possess a canonical SC—see Ref. [45]), most if not all crossing over depends on Mus81-Mms4 (Fig. 1).

In vitro studies of yeasts revealed that Mus81-Mms4 acts as a single heterodimer [46] and has a preferential nuclease activity on displacement loops (D-loops) or nicked Holliday junctions [47,48]. It was inferred that, unlike class I COs, Mus81-dependent class II COs do not involve the coordinated cleavage of pairs of classic Holliday junctions containing four uninterrupted strands [18]. Indeed, in fission yeast, electron microscopy revealed single (nicked?) HJs as the main meiotic recombination intermediate [49], and it is not unlikely that this also holds true for *Tetrahymena* (Fig. 1).

Intriguingly, *Tetrahymena* possesses homologs of Mlh1 and Mlh3, which likely provide the endonuclease activity needed for resolving recombination intermediates in the class I pathway (see Section 1.1). The expression patterns of *TMLH1* and *MLH3* genes are consistent with their role in meiosis, however, deletion of either did not create a notable meiotic defect [44,50]. Thus, they may play a role in a process that coincides with meiosis, or they are required for only a small proportion of recombination events.

2.6. Pro-crossover factors

2.6.1. Hop2

Hop2 functions in a complex with Mnd1, and they have a dual role in stabilizing Rad51- and Dmc1-ssDNA nucleoprotein filaments and in enhancing their ability to capture duplex DNA (see Refs. [51,52,3]). *Tetrahymena* possesses meiosis-specific and ubiquitously expressed paralogues of both *HOP2* and *MND1* [28]. In the absence of meiotic Hop2, DSBs are repaired but chromosomes remain univalent at metaphase I. This behavior is different from *HOP2* deletions in budding yeast [53], *Arabidopsis* [54], or mouse [55], where DSBs accumulate. However, it parallels the situation in a non-null *Arabidopsis hop2* mutant that permits Rad51-dependent DSB repair via the sister, but not Dmc1-dependent repair via the homolog [54]. It is conceivable that in *Tetrahymena*, meiotic Hop2 is specific for Dmc1 nucleoprotein filaments and that in its absence, the ubiquitous version of Hop2 works together with Rad51 in intersister repair.

2.6.2. Msh4, Msh5

The ZMM proteins Msh4 and Msh5 form a heterodimer that binds and stabilizes DNA strand-exchange intermediates to promote class I meiotic crossing over [16,56]. *Tetrahymena* possesses homologs of both Msh4 and Msh5 (Fig. 3), and bivalent formation is reduced in *msh4*Δ and *msh5*Δ mutants. This reduction is likely due to a smaller proportion of DSBs being converted into COs [50]. In the absence of both Msh4 and Sgs1, bivalent formation was restored, suggesting that Msh4 (and Msh5) may protect recombination intermediates against the anti-CO activity of Sgs1 (see Section 2.6.3), similar to budding yeast [57]. This activity of Msh4 and Msh5 in *Tetrahymena* is unexpected because it is presumably missing the other ZMM proteins, and so it was proposed that Msh4 and Msh5 work outside of the conventional class I pathway in this organism (see Section 3.2) [50].

2.6.3. Sgs1

The RecQ family helicase Sgs1 is assumed to act at two steps in the recombination process [58]. First, it channels a substantial proportion of meiotic DSBs into a non-CO pathway, and second, it may contribute directly to CO formation by being part of the class I CO pathway [8] (Fig. 3). In the absence of *Tetrahymena*'s Sgs1, homologous chromosomes failed to separate and JMs accumulated in a pulsed-field gel in exactly the same way as in the absence of Mus81 [44]. This could be explained by the anti-CO activity of Sgs1 (see Section 2.7). However, a direct function in helping JM resolution by Mus81-Mms4 is also conceivable. In *Tetrahymena*, Sgs1 appears in nuclei prior to DSB repair. Therefore, it was speculated that it may have yet another role, namely, in destabilizing early intersister D-loops (at a time in prophase when homologs are not yet paired) and thereby recycling DSBs for homology testing and, eventually, interhomolog recombination [44]. This is consistent with the observation that early "scout" DSBs in budding yeast are repaired mostly via the sister and later DSBs have an interhomolog bias [59].

2.7. Alternative DSB repair pathways

In *Tetrahymena*, there are >170 DSBs, but the estimated number of chiasmata is much lower [50]. Therefore, the majority of DSB repair events must lead to non-CO outcomes. Nonhomologous end-joining (NHEJ) likely does not contribute to a large extent because over 100 BrdU foci are formed per meiotic nucleus [50], which indicates the recombinational repair of the majority of, if not all, DSBs. While Tetrahymena's homologs of NHEJ factors Lig4, Ku70 and Ku80 are expressed roughly at the time of meiosis, they may not function in meiotic DNA repair but in DNA rearrangements during sexual progeny development [60]. Moreover, NHEJ is suppressed in *C. elegans* [61,62] and *Drosophila* [63] meiosis and is generally unfavored whenever homologous repair is possible [64,65].

Most likely, a large proportion of DSBs are repaired by intersister recombination (Fig. 1) because *Tetrahymena* lacks a dedicated meiosis-specific chromosome axis, which is one of the barriers to intersister recombination, as is known from other organisms [2]. Moreover, in the absence of Dmc1, DSBs are efficiently repaired, but mostly univalents are found at metaphase I [32]. BrdU incorporation demonstrated that this repair involves DNA synthesis (unpublished result).

Another possible non-CO exit is through synthesis-dependent strand annealing (SDSA) [66] (Fig. 1). In addition to Sgs1 (which also has pro-CO activity in budding yeast—see Section 2.6.3), *Tetrahymena* possesses a homolog of another protein that is known to operate in the SDSA pathway, FANCM [67,68]. Like *SGS1, FANCM* gene expression is upregulated in meiotic cells and in replication-stressed cells of *Tetrahymena*, which indicates that it has a role in DNA repair [69]. The anaphase arrest observed in *sgs1*Δ mutant meiosis [44] could be explained by the anti-CO activity of Sgs1. In the absence of Sgs1, the CO machinery could be overtaxed and unable to resolve excess or aberrant JMs. Indeed, *S. cerevisiae* Sgs1 has been demonstrated to remove aberrant recombination intermediates during meiosis, which requires the action of Mus81-Mms4 in the absence of Sgs1 [70]. On the other hand, when the FANCM protein was depleted by RNAi, no obvious meiotic defect was observed in *Tetrahymena* (A. Shodhan and J. Loidl, unpublished). Therefore, also considering the unresolved question of *SGS1*'s role in DSB repair (see Section 2.6.3), the existence or extent of meiotic SDSA in *Tetrahymena* is uncertain.

Finally, double HJ dissolution also contributes to non-COs in model organisms [71]. This process decatenates hemicatenated DNA strands that are flanked by intact double HJs (Fig. 1). In *S. cerevisiae,* it requires type IA topoisomerase Top3 together with its accessory factor Rmi1, as well as the Sgs1 helicase. It is unknown whether double HJ intermediates exist in *Tetrahymena* meiosis, and if so, they may not be processed by Mus81-Mms4 and subject to dissolution. A topoisomerase that could exert this function at the appropriate time has not yet been identified in *Tetrahymena*, but it possesses a *RMI1* homologthat is upregulated in meiosis and in replication-stressed cells [69]. Recently, it was shown in budding yeast that the Top3-Rmi1 complex acts in all of the meiotic pro-CO and non-CO recombination processes except the class II pathway, mostly in collaboration with Sgs1 [43,72]. Therefore, it will be interesting to study the possible involvement of *Tetrahymena* Rmi1 in the processing of meiotic recombination intermediates.

3. Crossover control

3.1. The question of CO interference

CO (or chiasma) interference is the suppression of neighboring crossovers with the result that double events are rarely found close to each other. The consequence is a more even distribution of COs and chiasmata along (and also between) chromosomes than expected if they were localizing at random (for review see Ref. [73]). Wherever tested, interfering COs and SCs occurred together, and the functional link may be that the SC provides a platform for the conversion of interfering CO precursors into chiasmata. A more direct role of the SC or its lateral elements as structures along which an interference signal is transmitted was proposed for mammals and *C. elegans* [74,75], whereas in budding yeast, interference may be the result of multiple layers of control [76].

Because *Tetrahymena* does not form a SC and uses a Mus81-dependent CO pathway, the question arises as to whether its COs are subject to interference. Currently, the state of genetic mapping does not allow for the detection of interference, and the number and distribution of chiasmata are difficult to determine due to the small size of the chromosomes. However, the search for a cytological CO marker in *Tetrahymena* is ongoing, and the analysis of the CO distribution may help to validate the generality of the co-occurrence of SC and CO interference.

3.2. Tetrahymena *crossing over does not conform to the two-pathway paradigm*

Because a SC has not been detected, and meiotic recombination depends largely on Mus81/Mms4, it was posited that the class II pathway is the predominant, if not the only, CO pathway in *Tetrahymena* [44]. However, homologs of the ZMM proteins Msh4 and Msh5 contribute to full CO formation, and Mlh1 and Mlh3 may also have functions in meiosis. It is not yet clear, however, if they do so in the context of a hybrid pathway that also uses the class II components Mus81 and Mms4 or whether they participate in a yet unidentified parallel pathway that has some class I char-

acteristics, but does not involve SC formation. The presence of a mixed pathway in *Tetrahymena* may not be unique, because in *C. elegans* a large proportion of SC-associated interfering COs relies on the activity of Mus81 [77–79], and Msh4 and Msh5 may promote Mus81-dependent COs in mice [80].

3.3. *Why is* Tetrahymena *meiosis "simple"?*

Tetrahymena shares the predominance of the Mus81-dependent COs with fission yeast. It appears that in these two organisms, which independently lost the SC during evolution, a small set of mitotic repair proteins and slightly modified paralogs thereof is sufficient for executing meiotic recombination. The special circumstances that caused or allowed them to abandon the sophisticated class I pathway remain speculative. The answer may lie in the still obscure function of the SC. Considering a role in CO interference and/or as a barrier to intersister recombination (see Section 3.1), and considering that full synapsis could be a signal to cease DSB formation [81–83], it seems that the SC allows for a more efficient control of COs. A low chromosome number (fission yeast: $2n = 6$ and *Tetrahymena*: $2n = 10$) together with a particularly efficient device for homology searching and alignment (the "horsetail" in fission yeast [84] and the "ultimate bouquet" in *Tetrahymena* [24]) may contribute to the reliable allocation of chiasmata among chromosomes without an elaborate control mechanism.

4. Conclusions and outlook

The elucidation of CO- and non-CO-factors and pathways in *Tetrahymena* is far from complete; certainly there are many players still remaining to be identified. But beyond getting a better view of the meiotic process in this organism itself, its study may contribute to our general understanding of meiotic recombination. Future work may, among others, address the following questions:

What are the requirements for DSB formation? Is a loop-axis organization of chromatin necessary to initiate DSBs (see Ref. [85])? What if, as is the case in *Tetrahymena*, such an axis is not provided by the lateral elements of a SC?

How is the genome-wide distribution of DSBs or recombination events regulated, and what defines DSB or CO hotspots? In *Tetrahymena*, the germline-limited fraction (encompassing ca. 30%) of the genome is believed to be refractory to meiotic DSBs [86]. Apart from an interest in possible causes, studies in this evolutionarily distant system may help to further explore the range of strategies that have evolved to control the localization of DSBs [33].

Are the two ends of a DSB differentiated? The question of the asymmetric loading of Rad51 and Dmc1 to the two DSB ends is still unsolved (see Ref. [41]), but it has been postulated that the two ends have functionally and temporally distinct activities during recombination [87]. A possible preliminary to such different behavior may be the release of Spo11-oligos of different sizes as was found in budding yeast and the mouse [88], but not in fission yeast [89,90]. In addition, how common is asymmetric trimming? *Tetrahymena* should have its say in this question.

How is the labor divided between Rad51 and Dmc1 in meiotic DNA strand exchange? In some organisms, Rad51 may have a primarily regulatory function, whereas in others where Dmc1 is missing, it must be capable of forming extensive nucleofilaments. On the other hand, in *Tetrahymena*, Rad51 nucleofilaments are very short or unstable. How does Rad51-dependent meiotic recombination work in this organism?

How is CO formation related to the SC? Several studies have suggested that by its role in interference and/or other constraints on crossing over [91], the SC is a safeguard against too many or potentially harmful defective COs. Paradoxically, SC-less *Tetrahymena* may contribute to the answer by revealing if and to what extent its CO formation is less rigidly controlled.

With the development of additional tools for studying meiosis in this fascinating organism, *Tetrahymena* will broaden the range of model systems among which the conserved and derived aspects of meiosis can be compared and the evolutionary flexibility of this process can be studied. Although *Tetrahymena*'s rather simple meiotic process is not primordial, it may give an impression of how ancient meiosis at the root of eukaryotic radiation may have worked.

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