Marine Life Science & Technology (2022) 4:595–608
https://doi.org/10.1007/s42995-022-00149-8

REVIEW

A practical reference for studying meiosis in the model ciliate *Tetrahymena thermophila*

Miao Tian[1,2,4] · Xia Cai[1,2] · Yujie Liu[1,2] · Mingmei Liucong[1,2] · Rachel Howard-Till[3]

Received: 5 March 2022 / Accepted: 28 September 2022 / Published online: 22 November 2022

Abstract

Meiosis is a critical cell division program that produces haploid gametes for sexual reproduction. Abnormalities in meiosis are often causes of infertility and birth defects (e.g., Down syndrome). Most organisms use a highly specialized zipper-like protein complex, the synaptonemal complex (SC), to guide and stabilize pairing of homologous chromosomes in meiosis. Although the SC is critical for meiosis in many eukaryotes, there are organisms that perform meiosis without a functional SC. However, such SC-less meiosis is poorly characterized. To understand the features of SC-less meiosis and its adaptive significance, the ciliated protozoan *Tetrahymena* was selected as a model. Meiosis research in *Tetrahymena* has revealed intriguing aspects of the regulatory programs utilized in its SC-less meiosis, yet additional efforts are needed for obtaining an in-depth comprehension of mechanisms that are associated with the absence of SC. Here, aiming at promoting a wider application of *Tetrahymena* for meiosis research, we introduce basic concepts and core techniques for studying meiosis in *Tetrahymena* and then suggest future directions for expanding the current *Tetrahymena* meiosis research toolbox. These methodologies could be adopted for dissecting meiosis in poorly characterized ciliates that might reveal novel features. Such data will hopefully provide insights into the function of the SC and the evolution of meiosis from a unique perspective.

Keywords Meiosis · Ciliate · *Tetrahymena* · Synaptonemal complex · Cytogenetics

Introduction

In most investigated organisms, meiosis is a specialized cell division that turns diploid germ cells into haploid gametes for sexual reproduction. Advances in meiosis research have been directly aiding the elucidation of causes of infertility and birth defects in humans (Ioannou et al. 2019; Veitia 2020), as well as promoting crop breeding (Lambing and Heckmann 2018; Taagen et al. 2020) and improving the understanding of speciation and evolution (Smagulova et al. 2016; Webster and Hurst 2012). Because of its importance, meiosis has been extensively studied since the early 1900s, although predominantly in a few model eukaryotes. These studies have revealed that the conserved meiosis program begins with a round of chromosome replication, followed by the formation of programmed DNA double-strand breaks (DSBs). Next, broken chromosomes are repaired preferentially using the counterpart of intact homologous chromosomes (homologs) as templates, such that crossovers (COs) can form between homologs. COs not only re-shuffle alleles, which is the major advantage of meiosis over mitosis in generating genetic diversity, but more importantly, they provide physical connections between homologs so that they can be faithfully segregated into gametes that each contain a haploid genome (see Ohkura 2015).

The synaptonemal complex (SC) is a zipper-like tripartite protein structure formed between aligned homologs during meiotic prophase I of most organisms (Fig. 1A). It is crucial for several meiotic events, including regulating

Edited by Jiamei Li.

Special topic: Ciliatology.

✉ Miao Tian
miao.tian@ouc.edu.cn

[1] Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao 266003, China

[2] Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China

[3] Department of Molecular and Cellular Biology, University of California Davis, Davis, CA, USA

[4] Institute of Human Genetics, CNRS, University of Montpellier, 34090 Montpellier, France

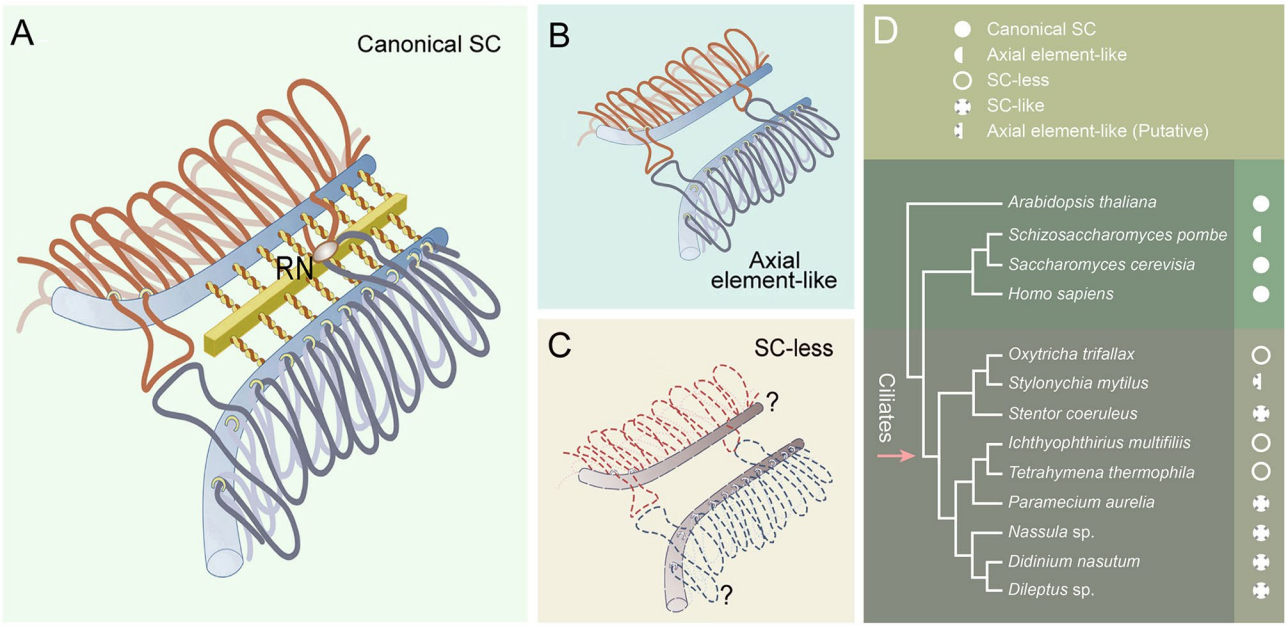

Fig. 1 Diagrams of the canonical synaptonemal complex (SC), other relevant structures, and their presence and absence in organisms of different taxa. **A** A diagram of the SC. Orange and grey (or blue) lines are homologous chromosomes, they are anchored at the axial/lateral elements (blue cylinder; yellow rings are cohesin complexes) and form loops. Within the region with full synapsis, lateral elements, transverse elements (yellow–red twists), and central elements (yellow bar) form the canonical tripartite structure. Homologous recombination takes place within the fully synapsed region and is often observed as an intensive node (aka recombination node, RN) under electron microscopy. **B** Fission yeast lacks a canonical SC; however, it has homologs of axial/lateral elements core components, and they are crucial for meiotic recombination. **C** In *Tetrahymena*, a SC or SC-like structure has not been detected. Moreover, it is unclear whether *Tetrahymena* meiotic chromosomes organize as highly structured loops. **D** The synaptonemal complex is absent in multiple organisms of different taxa (i.e., organisms labeled with half-circles or open circles). The solid white circle and half-circle with black bars indicate canonical SC-like tripartite structure or axial element-like liner structure was observed in corresponding organisms by electron microscopy, respectively. The phylogenetic tree (Neighbor-Joining method) was constructed using *18S* rRNA sequences

the number of DSBs (Lee et al. 2021), enforcing homologous pairing, promoting the interhomolog recombinational repair of DSBs, and maintaining obligate CO formation, thus ensuring faithful chromosome segregation (see Zickler and Kleckner 2015). Consequently, disruption of SC components or assembly leads to abnormal or abolished CO formation (Capilla-Perez et al. 2021; MacQueen et al. 2002; Wang et al. 2010) and causes infertility in mice and humans (de Vries et al. 2005; Fan et al. 2021).

Strikingly, there are a few organisms from different taxa lacking SCs that still perform meiosis successfully (Loidl 2016; Shah et al. 2020) (Fig. 1B–D). The discoveries of SC-less meiosis raise interesting evolutionary questions: Why is SC-dependent meiosis more prevalent than SC-less meiosis? What is the adaptive significance of SC-less meiosis? What selection forces shape SC-less meiosis and SC-dependent meiosis? Answers to these questions will advance understanding of the origin and evolution of the SC, an important goal of meiosis research.

Among the organisms with SC-less meiosis, the fission yeast *Schizosaccharomyces pombe* and the ciliated protozoan *Tetrahymena thermophila* (herein referred to as *Tetrahymena*) are mostly investigated (see Loidl 2006, 2021; Loidl and Lorenz 2016; Yamada et al. 2018). Despite lacking a canonical SC, *S. pombe* still possesses a linear proteinaceous structure that shares some morphological and functional features with the canonical SC components (see Kariyazono et al. 2019; Loidl 2016) (Fig. 1B). By contrast, *Tetrahymena* lacks most proteins with any homology to SC components, and a linear structure has not been detected in the meiotic nucleus by electron microscopy (Chi et al. 2014; Wolfe et al. 1976) (Fig. 1C). In this regard, *Tetrahymena* could be a better model than *S. pombe* for exploring SC-independent meiosis.

Tetrahymena is a convenient and genetically tractable single-celled model organism. Using *Tetrahymena* as a model, recent works in the field of chromatin biology have revealed functions of the epigenetic DNA modification N6-methyladenine (Cheng et al. 2019; Sheng et al. 2021; Wang et al. 2019; Zhao et al. 2021), small RNA-mediated transposable element repression (Xu et al. 2021; Zhao et al. 2019), and functions of a conserved methyltransferase in DNA replication and transcription (Zhao et al. 2020). Notably, it is also a favorable model for studying meiosis, in particular.

Like budding yeast, billions of *Tetrahymena* cells can be obtained—axenically—within one liter of simple and inexpensive medium (Cassidy-Hanley 2012). Moreover, *Tetrahymena* meiosis can be easily triggered by mixing starved cells of different mating types. Hence, a large quantity of meiotic cells can be routinely generated with simple laboratory setups. Despite that *Tetrahymena* cells of different mating types can exchange genetic information in a sexual manner, they reproduce asexually. Therefore, *Tetrahymena* mutants with disrupted meiosis can be stably maintained by asexual reproduction. Of note, *Tetrahymena* has several distinct advantages over budding yeast for studying meiosis: first, the meiotic nucleus is about thirty times larger than that of budding yeast, which is easier for microscopic examination. Second, the meiotic nucleus undergoes notable morphological alterations in meiotic prophase I, hence, different sub-stages can be easily determined (Fig. 2). Finally, *Tetrahymena* diverged early in evolution from commonly used models for studying meiosis (e.g., yeasts and mice), hence, studying meiosis using *Tetrahymena* provides exclusive

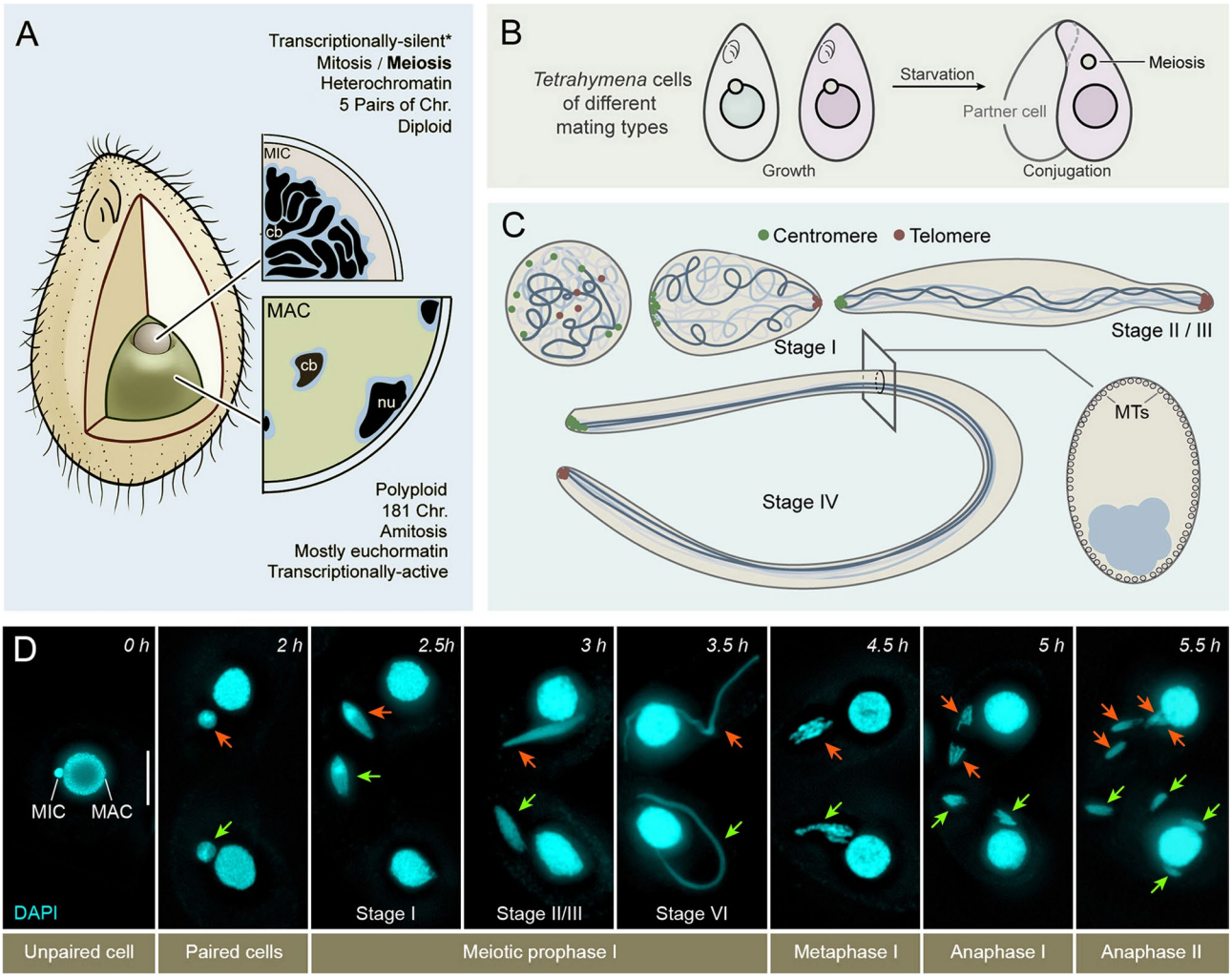

Fig. 2 The bi-nucleated *Tetrahymena* and the dynamic morphological alterations of its germline nucleus in the meiotic prophase I. **A** The somatic macronucleus (MAC) and the germline micronucleus (MIC) are different in transcription activity, nuclear division programs, chromatin status, chromosome numbers, and ploidy levels. The asterisk (*) indicates that the MIC is transcribed mostly, if not exclusively, in meiosis. 'cb' stands for chromatin body. 'nu' stands for nucleolus. MAC and MIC chromatin structures are adapted from published electron micrographs (Wolfe et al. 1976). **B** *Tetrahymena* meiosis can be easily induced by mixing starved cells of different mating types. **C** In meiotic prophase I, the MIC undergoes dynamic nuclear morphological alterations. The chromosomes are also rearranged, with centromeres and telomeres clustered at opposite poles of the elongated nucleus. MTs stands for microtubules. **D** Representative 4′,6-diamidino-2-phenylindole (DAPI)-stained single cell and paired cells at different stages of meiosis. The numbers indicate hours after mixing cells. It is worth noting that the progression of meiosis presented here was obtained with cells maintained at 30 ℃. The actual progression of meiosis could be affected by many factors, including mating efficiency, temperature, aeration. Arrows of different colors denote meiotic nuclei of different cells. Scale bar: 5 μm

information to advance the comprehensive understanding of the diversification and conservation of meiosis (see Loidl 2021; Loidl and Lorenz 2016).

Because many aspects of *Tetrahymena* are biologically unique from commonly used model organisms, specialized methods have been developed or adapted for studying its meiosis. However, these techniques are scattered in methods sections of different research articles. Given the above arguments for its study, we see a need for these methods to be combined into an instructional handbook for studying *Tetrahymena* meiosis. Hence, in this review we summarized concepts and techniques for studying *Tetrahymena* meiosis, from culturing cells, inducing meiosis, cytological staining, and finally to analyzing protein function. Moreover, typical meiotic failures and their causes are enumerated at the end of this article. We hope that this review will serve as a basic reference for studying meiosis in *Tetrahymena* and other ciliates whose meiotic chromatin seems to have intriguing features but were never investigated with modern techniques (Gong et al. 2020; Jiang et al. 2019; Raĭkov 1982).

Culturing strains and inducing meiosis

Tetrahymena is a typical ciliate housing two types of nuclei within a cell (Fig. 2A). They differ in many aspects, including morphology, transcriptional activity, cell cycle, and even histone composition (see Chalker et al. 2013; Ruehle et al. 2016; Wei et al. 2022). Like other ciliates (Li et al. 2021; Zheng et al. 2021), the large polyploid macronucleus (MAC) of *Tetrahymena* has highly fragmented chromosomes. The MAC contains the somatic genome, transcribes mRNAs, and thus determines phenotype. The small diploid micronucleus (MIC) has five pairs of chromosomes and contains the germline genome. However, it is transcriptionally silent, except during meiotic prophase I, when the transcription products are non-coding RNAs (Chalker and Yao 2001; Schoeberl et al. 2012; Sugai and Hiwatashi 1974; Zhao et al. 2019). The MAC divides amitotically and does not undergo meiosis. By contrast, the MIC divides mitotically and undergoes meiosis upon mating of *Tetrahymena* cells (i.e., conjugation). Notably, in meiotic prophase, the intranuclear microtubules (MTs) drive the stretching of the MIC over 20 times its length (> 2 × length of the cell). Meanwhile, centromeres and telomeres cluster at opposite poles of the elongated nucleus and form an 'ultimate bouquet'(Loidl et al. 2012; Luo et al. 2020). Such spatial constraints are crucial for pairing of homologous chromosomes in *Tetrahymena* (Fig. 2B–D)(Tian et al. 2020).

The highly in-bred *Tetrahymena thermophila* B strains are commonly used wild-type strains with well-defined genetic backgrounds (Byrne et al. 1978; Cassidy-Hanley 2012; Frankel et al. 1993; Mayo and Orias 1981). Because conjugation of WT B strains B2086 and CU428 yields a relatively high percentage of progeny cells (in our hands, up to 72% of paired conjugants were able to produce viable progeny), they are usually used (and are also recommended) for meiosis research. Both strains are available from the national *Tetrahymena* Stock Center, located at Cornell University (Chalker 2012). High-quality MIC and MAC genome sequences have been generated (Eisen et al. 2006; Hamilton et al. 2016; Sheng et al. 2020; Wang et al. 2021), gene expression and protein phosphorylation datasets are also available from publicly accessible databases (http://ciliate.org/; http://tfgd.ihb.ac.cn/) (Coyne et al. 2008; Miao et al. 2009; Stover et al. 2006; Tian et al. 2014; Xiong et al. 2011, 2012, 2013). It is also worth noting that macronuclear genomes of ten closely related *Tetrahymena* species are available in the *Tetrahymena* comparative genome database (Xiong et al. 2019; Yang et al. 2019) (http://ciliate.ihb.ac.cn/tcgd/), which serves as a resource to determine conserved, and therefore possibly functionally critical segments of *Tetrahymena* meiosis proteins.

A variety of simple, budget-friendly peptone-based media, as well as chemically defined media, are available for axenically culturing *Tetrahymena* (Cassidy-Hanley et al. 1997). For meiosis research, the peptone-based modified Neff medium is preferable over the commonly used nutrient-rich SPP medium. In Neff, cells propagate sufficiently rapidly (ca. 3.5 h per generation), accumulate at high density (up to 3×10^6 cells/ml), and can be maintained for at least a week at the stationary phase. In SPP, however, cells have a short stationary phase and then deteriorate rapidly.

Proper aeration is crucial for obtaining cell cultures with highly synchronized meiotic cells. This can be achieved by starving and mixing equal quantity of starved cells with different mating types in shallow, flat bottom vessels. For example, a 10 cm Petri-dish can be used for a 10 ml small scale culture, and plastic boxes (e.g., the IKEA SAMLA box, 39 cm [L] × 28 cm [W] × 14 cm [H]) can be used for larger scale cultures, up to 300 ml. Cell density should also be controlled to ensure efficient mattings. In our hands, over 90% conjugation efficiency could be achieved regularly by mixing cells starved in 10 mmol/L Tris–HCl (pH 7.5) at a density of 3.5×10^5 cells/ml (or $O.D_{540nm} \approx 0.5$, see Supplementary Table S1 for an O.D and cell density conversion chart). Additionally, the presence of $CdCl_2$ (a chemical for activating the most commonly used inducible promoter, *MTT1*) also reduces the efficiency of conjugation. Thus, it has to be removed (preferably) by pelleting cells and resuspending them in fresh 10 mmol/L Tris–HCl (pH 7.5). Alternatively, $CdCl_2$ needs to be drastically reduced (< 0.1 μg/ul) before the induction of conjugation.

Manipulating meiotic processes

Well-established genetic tools exist for *Tetrahymena* genetic manipulation (Akematsu et al. 2018; Chalker 2012; Hayashi and Mochizuki 2015; Howard-Till et al. 2013; Iwamoto et al. 2014; Qiao et al. 2022; Ruehle et al. 2016). These have been used for studying the importance of a number of genes for meiotic processes. In addition, several meiotic processes can be conveniently inhibited using commercially available chemicals. For example, meiotic nuclear elongation is effectively repressed by 10 μg/ml of nocodazole, an effective and reversible microtubule depolymerizing drug (Loidl et al. 2012). It has been successfully used for revealing the critical roles of intranuclear microtubules in promoting centromere clustering and faithful homolog pairing in meiosis. The *Tetrahymena* meiotic DNA damage response (DDR) is dependent on the conserved ataxia telangiectasia-mutated and Rad3-related (ATR) kinase, a member of the phosphatidylinositol 3-kinase (PI3K) family of proteins (Mochizuki et al. 2008; Tian and Loidl 2018). PI3K inhibitors, 10 mmol/L caffeine, or 2 μmol/L wortmannin have been successfully applied to block the meiotic DDR and the subsequent DNA damage repair in *Tetrahymena* meiotic cells (Loidl and Mochizuki 2009). In addition, multiple DNA damage-inducing agents (e.g., cisplatin and methyl methane sulfonate) have been used for the induction of ectopic DNA damage in *Tetrahymena* (Loidl and Mochizuki 2009), which lead to the conclusion that DNA damage triggers the nuclear stretching and clustering of centromeres and telomeres via the DDR pathway.

Cytological techniques

Conventional fixation for cytology

Fixing cells for 30 min in 3.7% formaldehyde and 0.5% Triton X-100 has an overall good performance in preserving both cortical membrane structure and nuclear morphology (Loidl and Scherthan 2004) (Fig. 2D). Using cells fixed by this method (hereinafter referred to as 'conventional fixation method'), proteins localized to the cortical membrane, cytoplasm (except for microtubules), or nucleus can usually be stained by immunostaining.

Protein localization

Protein localization data are crucial for inferring whether and how a protein is involved in regulating meiosis. In *Tetrahymena*, protein localization is investigated either in live cells or in chemically fixed cells (Fig. 3A). The outer layer of *Tetrahymena* consists of juxtaposed layers of soft membranes (Frankel 2000) which allows the usage of cell-permeable dyes (e.g., Hoechst 33342) for live-cell imaging. By immobilizing cells with either a custom microcompressor (Yan et al. 2014), 3% low-melting-temperature agarose (Kobayashi et al. 2016), nickel chloride (Jiang et al. 2015), or simply compressing cells with a coverslip, dynamic localization of fluorescent-fusion proteins can be investigated and documented (Iwamoto et al. 2015). Fluorescent-tagged proteins could also be directly visualized in cells fixed with 3.7% formaldehyde and 10% methanol (Kataoka and Mochizuki 2015). However, care should be taken as artificial green fluorescence signals from nuclei appear after exposing cells to a strong exciting light source.

In addition to the direct detection by fusing a protein of interest (hereinafter referred to as POI) to a fluorescent protein, indirect immunostaining is commonly used for protein localization (see Pina et al. 2022). Briefly, cells are first fixed and permeabilized with chemicals and then POI is probed with antibodies. However, it is worth noting that the fixation method may vary according to the properties of the POI (Dave et al. 2009; Howard-Till et al. 2011).

Many proteins exist as two major fractions, a freely diffusible fraction and an immobilized fraction that is tightly associated with specific subcellular domains or structures (e.g., chromatin, nuclear matrix, cytoskeleton). The two forms may actively exchange within cells. To specifically visualize the immobilized form, soluble cytoplasmic and nucleoplasmic proteins need to be removed. This can be done by first extracting cells with 1% Triton X-100 (supplemented with 0.37% formaldehyde) on ice, then completing fixation by adding extra formaldehyde to a final concentration of 3.7% (Fig. 3B). Using this in situ fractionation method (aka 'detergent spreading method', 'prefixation detergent treatment' in published works) (Ali et al. 2018; Howard-Till et al. 2011; Lukaszewicz et al. 2010; Tian et al. 2019), Howard-Till and colleagues revealed that the meiotic DSB repair recombinase, Dmc1, localizes in the nucleus in both diffused form and chromatin-associated form (Howard-Till et al. 2011). While Dmc1 localizes to the nucleus in a DSB-independent manner, the association of Dmc1 with chromatin is dependent on DSB formation (Howard-Till et al. 2011).

Histone variants and histone modifications play crucial roles in meiosis (Diagouraga et al. 2018; Yadav and Claeys Bouuaert 2021). In *Tetrahymena*, they are best visualized using cells fixed with mercuric chloride and organic solvents (e.g., methanol or ethanol). Two versions of mercuric chloride-based fixative are currently used for cell fixation. One of them is the standard Schaudinn's fixative (Mochizuki et al. 2008). It is a mixture of 2 parts of saturated $HgCl_2$, 1 part of ethanol, and 1% acetic acid. After fixing cells with this method at 25 ℃, cells are further washed with ice-cold 70% ethanol, then resuspended in an appropriate amount

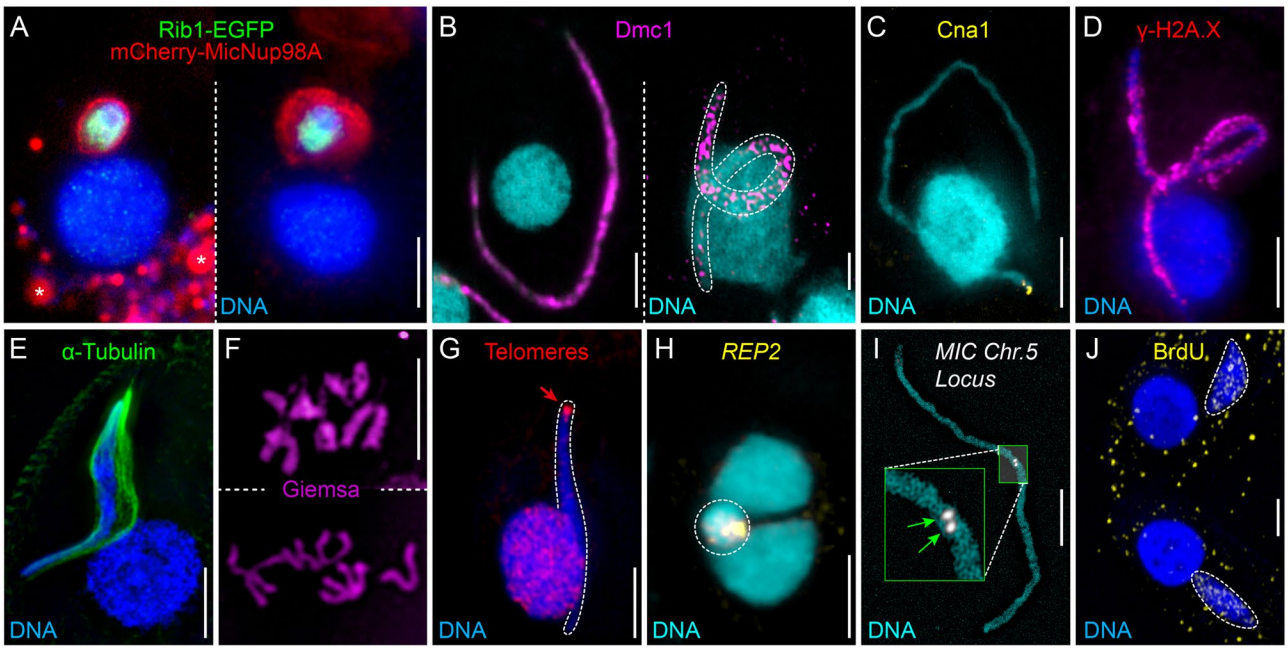

Fig. 3 Representative images of cytological stainings. **A** Fluoresence imaging of EGFP-/mCherry-fusion proteins in a live cell (left panel) and a methanol fixed cell (right panel). Rib1 is a MIC transcription regulator, MicNup98A is a MIC-specific nuclear pore complex subunit. The asterisk (*) indicates commonly seen autofluorescence in *Tetrahymena* cells, presumably from food vacuoles. **B** Detection of total Dmc1 protein in a cell fixed with the conventional fixation method (left panel) and chromatin-bound Dmc1 fraction in a cell fixed with an in situ fractionation method using detergent extraction of soluble proteins (right panel, see text for details). **C** Immunostaining of the centromeric histone H3 variant, Cna1, in a cell prepared with Schaudinn's fixative. **D** Immunostaining of the DNA double-strand break marker, γ-H2A.X, in a cell prepared with the modified Schaudinn's fixative. **E** Immunostaining of microtubules in a cell fixed in modified PEM buffer. **F** Staining of condensed chromosomes at meiotic metaphase I Upper panel: Bivalents; Lower panel: Univalents. **G–I** Labelling of telomeric repeats, MIC-specific repetitive sequences (*REP2*), and a MIC chromosome 5 locus (ca. 38 kb) using fluorescence in situ hybridization. **J** Labeling of newly synthesized DNA during the meiotic DSB repair using BrdU. Scale bars: 5 μm

of methanol/acetic acid mixture (in 2:1 ratio) and dropped onto a slide. After fixation, cells burst yet the nuclei remain intact (Fig. 3C). Such treatment makes chromatin more accessible for antibody binding, and it is excellent for staining the centromere-specific histone H3 variant (Cna1) using a custom antibody (Cervantes et al. 2006). Another method uses a modified and simplified Schaudinn's fixative. It has been successfully applied for staining γ-H2A.X (Tian and Loidl 2019) (Fig. 3D). Briefly, cells are fixed with a solution of 0.26% saturated $HgCl_2$ and 0.14% ethanol, then washed twice with ice cold methanol, and then dropped onto a slide.

Unlike homologous pairing in humans and yeasts, stretching intranuclear microtubules drive *Tetrahymena* meiotic nuclear elongation and promote the alignment of homologs (Kushida et al. 2015; Wolfe et al. 1976). However, the gene(s) directly responsible for regulating the dynamic alteration of the meiotic intranuclear microtubules remains unknown, hence efforts are needed to solve this enigma. To visualize intranuclear microtubules, fixation methods must be modified to preserve microtubule integrity (Fujiu and Numata 2000; Kushida et al. 2015; Loidl et al. 2012). Briefly, cells are first treated with a modified PEM buffer (0.145 mol/L NaCl, 7.4 mmol/L Na_2HPO_4, 2.6 mmol/L NaH_2PO_4, 2 mmol/L EGTA, 5 mmol/L $MgSO_4$, pH 7.2) at 25 ℃, then fixed by adding formaldehyde to a final concentration of 1.8%. Subsequently, cells are collected, resuspended in the modified PEM buffer supplemented with 0.1 mol/L glycine, and spread onto poly-L lysine coated slides for immunostaining with an anti-tubulin antibody (Fig. 3E).

Analyzing chromosome morphology

Chiasmata, the physical linkages formed between homologs, are cytological markers of interhomolog COs. The number and distribution of chiasmata is a valuable cytological readout for evaluating meiotic recombination. However, as nuclear structures are well preserved under the conventional fixation condition, individual bivalents or chromosomes cannot be clearly distinguished as they are closely juxtaposed. Thus, the standard Schaudinn's fixation is needed to slightly disassociate chromosomes (Fig. 3F). Moreover, instead of DAPI (which bleaches during examination using fluorescence microscopy), Giemsa is commonly used for staining

acidic chromatin to investigate chromosome configurations (Lukaszewicz et al. 2015; Shodhan et al. 2014; Tian and Loidl 2019). Chromosomes fixed in this manner can be viewed and imaged using conventional bright-field light microscopy, which is also an advantage over using fluorescent DNA stains.

DNA analysis

Meiotic chromosomes undergo dynamic alterations in multiple ways. First, homologs become closely juxtaposed and alleles are faithfully aligned. Second, DNA double-strand breaks (DSBs) form and are repaired; during this process, new DNA is synthesized around DSB sites. All these events can be studied cytologically in *Tetrahymena* by the following methods.

Fluorescent in situ hybridization

Homologous pairing can be monitored by measuring the distance between alleles labeled using fluorescent in situ hybridization (FISH). FISH can be used to label long (ca. 100 kb) or repetitive DNA segments (e.g., telomere repeats, transposable elements; Fig. 3G-I) using cells fixed with the conventional fixation method (Howard-Till and Loidl 2018; Tian et al. 2020). However, unwanted background staining often impairs visualization of weak signals in conventionally fixed cells. Therefore, we recommend releasing nuclei from the cells by disrupting cells and fixing nuclei using a methanol-based Carnoy's fixative (methanol, chloroform, acetic acid: 6:3:1; Fig. 3I) (Mochizuki et al. 2008). In short, cells are first fixed with the Carnoy's fixative at 25 ℃ for an hour and then washed with 70% ethanol and dropped on a slide. According to our experience, optimal FISH is usually obtained using a slide that is air-dried in a fume hood at 25 ℃. The standard FISH is a rather laborious procedure, thus a more time-efficient and labor-saving FISH method (e.g., Tn5 FISH (Niu et al. 2020)) might be adapted for *Tetrahymena*.

DSB detection

Although the presence of DSBs can be assayed indirectly by immuno-staining against γ-H2A.X or the Dmc1 recombinase (discussed above, Fig. 3D), agarose gel electrophoresis provides a direct indication of chromosome fragmentation resulting from the formation of DSBs. However, it demands a large number of meiotic cells with good synchronicity and purity (Lukaszewicz et al. 2010). Because billions of synchronized *Tetrahymena* meiotic cells can be obtained by an entry-level laboratory (see section "Culturing strains and inducing meiosis"), it is one of the few organisms in which meiotic DSB formation and repair could be investigated with this method.

Sizes of the five *Tetrahymena* meiotic chromosomes range from 25.5 to 36.2 Mb (Hamilton et al. 2016). Hence, even using pulsed-field gel electrophoresis for resolving high molecular weight DNA, intact meiotic chromosomes are too large to migrate into the 1% agarose gel. However, smaller chromosome fragments resulting from DSB can migrate into the gel. Therefore, the appearance and disappearance of low molecular weight DNA bands serves as direct indications of the formation and repair of meiotic DSBs (Lukaszewicz et al. 2010).

To avoid potential artificial chromosome fragmentation during genomic DNA preparation, *Tetrahymena* cells are first embedded in an agarose gel plug, then chromosomes are crudely extracted in situ by digestion with protease K and RNase A (Lukaszewicz et al. 2010). It is worth noting that, owing to the co-existence of MAC and MIC chromosomes in the agarose gel plug, fragmented MIC chromosomes are masked by MAC chromosomes, whose sizes range from 38 Kb to 3.2 Mb (Sheng et al. 2020; Wang et al. 2021). Therefore, Southern hybridization is needed to label the MIC-derived chromosomes, using a MIC-specific DNA probe (e.g., Tlr elements (Wuitschick et al. 2002)). The sizes and intensities of the gel bands provide quantitative data to evaluate the frequency and the level of DSBs, respectively (Akematsu et al. 2017; Lukaszewicz et al. 2010; Tian and Loidl 2018).

Monitoring DNA repair synthesis

During homologous recombination, new DNA needs to be synthesized to fill gaps that are resected around DSB sites. In *Tetrahymena*, such DNA repair synthesis can be monitored by labeling newly synthesized DNA with a thymidine analog, bromodeoxyuridine (BrdU, Fig. 3J) and then probing the incorporated BrdU with an Anti-BrdU antibody (Loidl and Scherthan 2004). BrdU staining can be performed on cells fixed with the conventional fixation method. However, as BrdU is not accessible for antibodies without denaturing the incorporated DNA, cells for BrdU staining need to be pretreated (e.g., by heating, to denature DNA) before immunostaining (Loidl and Scherthan 2004).

Identification of protein–protein interactions

Emerging data suggest that some meiosis genes evolve rapidly (Dapper and Payseur 2019; Grishaeva and Bogdanov 2014). In different organisms, some proteins with similar functions in meiosis show little similarity at the sequence level (Tian and Loidl 2018). Therefore, only a few highly conserved meiosis genes were identified in *Tetrahymena* based on sequence homology (Chi et al. 2014; Mochizuki et al. 2008). The rest of *Tetrahymena* meiosis genes have mostly been identified by a systematic knockout screen of

genes expressed primarily in meiosis (see Loidl 2021). Most of these genes lack any known protein domain or signature motif. Therefore, in silico analysis provides little useful information to infer their molecular functions. For this reason, the functions of these novel proteins are often determined by investigating their protein partners, which may be characterized proteins or their homologs.

Immunoprecipitation coupled mass spectrometry (IP-MS) is a commonly used technique for identifying protein partners of POIs in vivo. Briefly, cells are harvested, lysed, and incubated with antibody-conjugated microbeads. Ideally, the POI and its protein partners bind to the beads, and unbound proteins are washed away. The bound proteins are then identified by mass spectrometry-based proteomic analysis.

Unlike some commonly used model organisms, currently there are only a few antibodies that can be used for IP of *Tetrahymena* meiosis proteins (e.g., Dmc1, Cna1, Mms4) (Cervantes et al. 2006; Howard-Till et al. 2011; Lukaszewicz et al. 2013). Therefore, IP is often performed using transgenic cells expressing epitope-tagged proteins. So far, many epitope tags have been successfully used for the IP of *Tetrahymena* proteins. For instance, HA-tag, EGFP-tag, mCherry-tag, and a Flag-ZZ fusion tag (Akematsu et al. 2020; Jiang et al. 2013; Kataoka et al. 2010; Tian et al. 2020). The HA tag is the smallest tag with nine amino acids; thus, theoretically, the fusion of an HA-tag with the POI would introduce minimal alteration. For this reason, cells expressing HA-fusion proteins are often used for IPs in *Tetrahymena*.

To purify HA-fusion proteins, cells are first lysed with mild detergent (e.g., 0.1–1% Triton X-100) and then incubated with microbeads conjugated with anti-HA antibodies. The beads are then washed to remove unbound proteins, and HA peptides are used to specifically elute the POI and its protein partners. According to our experience, lysate prepared from over 50 million cells (ca. 200 ml of conjugating cells) is sufficient for IPs using anti-HA beads (Tian and Loidl 2019). If there are limitations for obtaining an adequate number of cells, POI can be fused with an EGFP tag and then purified with the GFP-Trap® beads. Because of the superior affinity of GFP-Trap® breads to GFP (equilibrium dissociation constant: 10^{-12} mol/L), a sufficient amount of EGFP-tagged protein and its partners can be purified with the beads from as little as 2.5 million cells (unpublished data). However, in addition to the size of the EGFP-tag, a drawback of this method is that the captured POI cannot be specifically eluted from the beads, hence it may have higher background compared to the peptide elution strategy used with anti-HA beads. Nonetheless, high confidence partners of the POI have been identified by analyzing duplicated IP samples using dedicated proteomic analysis algorithms for identifying protein–protein interactions, for instance, SAINTexpress (Teo et al. 2014). Due to the page limitation, detailed protocols are not included in this review, but they will be uploaded onto the following webpage: https://www.protocols.io/workspaces/miao_tian.

Typical signs of abnormal meiosis

Thanks to the large sizes of germline chromosomes and their characteristic changes in morphology, *Tetrahymena* cells with defective meiosis can be identified by checking nuclear or chromosome configurations by DNA staining, or immunostaining of robust meiotic proteins or modified histones (see Loidl and Lorenz 2016). Attention should also be given to the timing or duration of meiosis in mutant cells compared to wild-type. Therefore, cytological time courses of mating cells at 1–2 h intervals can be useful to determine whether meiosis is progressing normally, and at what stage cells are delayed or arrested. Here, we summarize typical phenotypes of aberrant meiotic cells and underlying genetic bases, such that one could compare a novel meiotic mutant to published ones. The mutants are classified into different groups according to their representative nuclear morphological features:

MIC stays round

More specifically, the MIC chromatin, as visualized with DAPI or other DNA stains, remains round. So far, this 'round MIC' phenotype has only been observed in cells with a defective centromeric Histone H3 variant, Cna1, which leads to the dissociation of chromatin from the intracellular microtubules (Loidl et al. 2012). Consequently, MIC chromatin remains round due to the lack of pulling force from the microtubules (Fig. 4A). Finding additional mutants with similar phenotypes would be highly desirable, as this could identify components that serve to link centromeres to one pole of the elongating nucleus.

MIC stays like a teardrop

Mutants with this phenotype mate and form stable pairs, like WT cells. Their MICs migrate from the MAC pocket towards the direction of the mating junction; however, they remain next to the mating junction and elongate only slightly, like teardrops (Fig. 4B). This is a typical phenotype of defective meiosis initiation. Deletion of a meiosis-specific cyclin (Cyc2 (Xu et al. 2016)), Cyclin-dependent kinase (Cdk3 (Xu et al. 2019; Yan et al. 2016)), or Zinc-finger domain-containing protein, Zfp1, leads to this phenotype (Zhang et al. 2022a). Nevertheless, the underlying molecular mechanism remains elusive.

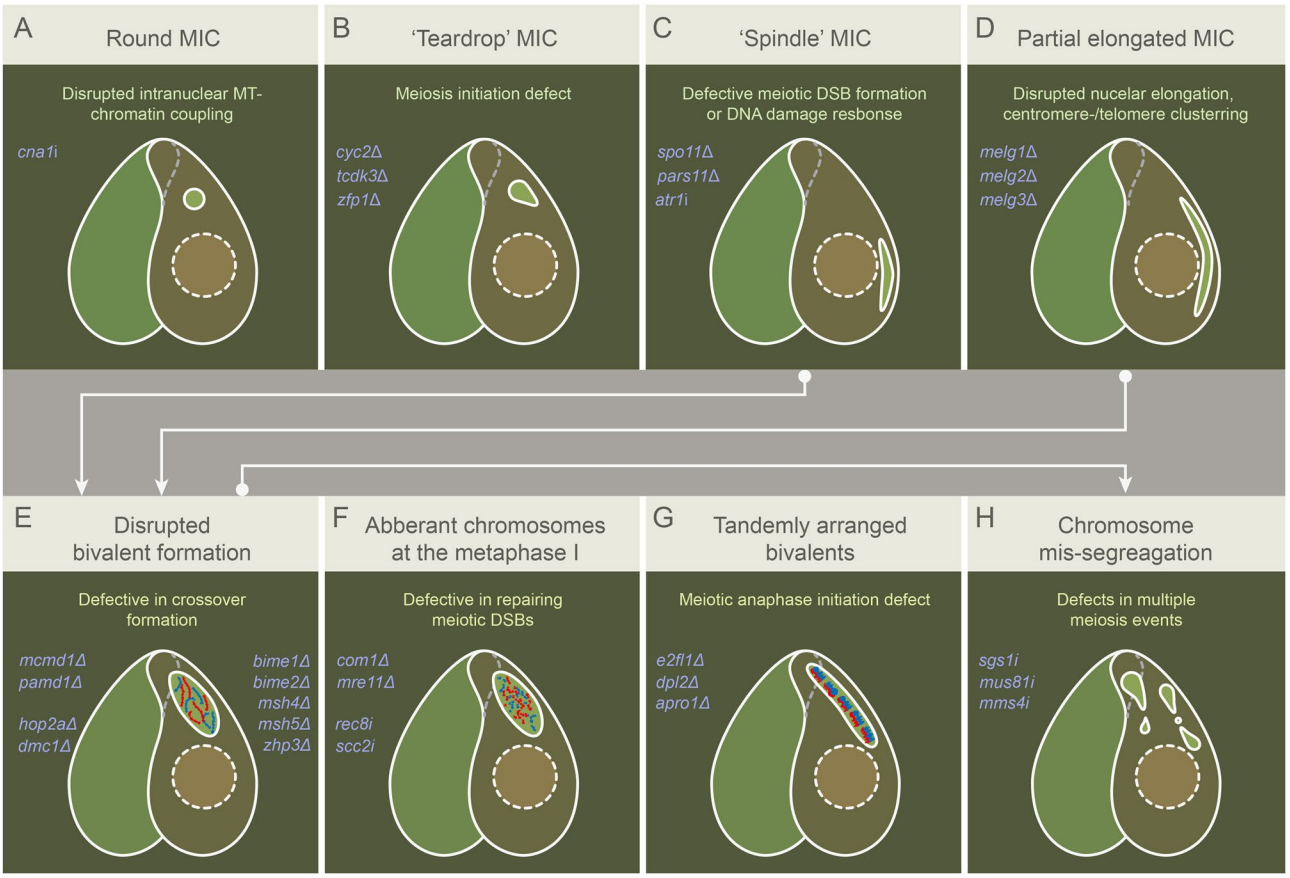

Fig. 4 Schematic summaries of representative nuclear and chromosome morphologies in cells with disrupted meiosis. **A–H** Aberrant phenotypes are arranged according to the progression of meiosis. MACs are depicted as pink circles with dashed outlines; MICs are depicted with light yellow circles with a solid outline. Blue and red lines are homologous chromosomes. Arrows indicate disruption of certain meiosis genes leads to aberrant phenotypes in the early and late stages of meiosis

MIC elongates slightly, like a spindle

This phenotype has been observed in cells that lack meiotic DSB formation (i.e., *spo11*Δ, *pars11*Δ) or DDR (i.e., *ATR1* knockdown cells) (Mochizuki et al. 2008; Tian and Loidl 2018). The major differences between this 'spindle' phenotype and the above described 'teardrop' phenotype are that the MICs are longer and, instead of remaining next to the conjugation junction, they tend to migrate towards the posterior of the MAC (Fig. 4C).

MIC elongates partially

In mutants with defective nuclear elongation *per se* and/or disrupted centromere or telomere clustering, meiotic MICs are shorter than the length of the cell (Fig. 4D). Nonetheless, their MICs are longer than that of cells without meiotic DSBs or DDR. It is worth noting that, the mutant with disrupted telomere clustering has these characteristic pointy MICs (Tian et al. 2020).

Bivalent formation defects

The association of condensed homologs as bivalents is a consequence of the formation of meiotic COs. Using Giemsa staining, the thick bivalent form can be easily differentiated from the thin univalent form (i.e., individual chromosomes lacking pairing partners; Fig. 4E). Mutants with defects either in DSB formation (Mochizuki et al. 2008; Tian and Loidl 2018), interhomolog recombination (Howard-Till et al. 2011; Mochizuki et al. 2008; Tian and Loidl 2019), or CO formation (Shodhan et al. 2014, 2017a, b) are characteristically incapable of forming five bivalents at metaphase I.

Fragmented or aberrant chromosomes at metaphase I

The appearance of fragmented chromosomes at meiotic metaphase I indicates that meiotic DSBs are not repaired properly (Lukaszewicz et al. 2010) (Fig. 4F). This phenotype commonly presents as grainy, punctate DAPI staining

in what should be the metaphase stage of meiosis. Fragmentation occurs in cells in which DSB ends are not properly processed for DNA repair (e.g., cells lacking *COM1* or *MRE11* (Lukaszewicz et al. 2010)), in cells with defective DNA damage response (e.g., *ATR1* knockdown cells (Tian and Loidl 2018)), or cells lacking or misregulating the cohesin complex (Ali et al. 2018; Howard-Till et al. 2013). It is worth noting that the lack of the meiosis-specific recombinase Dmc1 does not lead to chromosome fragmentation, as meiotic DSBs are effectively repaired by the Rad51-mediated DNA repair pathway in *Tetrahymena* (Howard-Till et al. 2011). In the latter case, DSBs are likely repaired using sister chromatids as templates, and crossovers are not formed. Less fragmented, but nevertheless aberrant chromosomes were observed in mutants with an abnormally elevated level of DSBs (Tian and Loidl 2018). The absence of condensin can also present aberrant chromosomal morphologies similar to fragmentation, due to lack of condensation and decatenation (Howard-Till and Loidl 2018).

Meiosis arrests at the onset of the first meiotic division, condensed bivalents are arranged tandemly

Such a phenotype has been identified in three mutants (Fig. 4G). They are either lacking a meiosis-specific E2F family transcription factor (E2fl1) or its protein partner (Dpl2 (Zhang et al. 2017, 2018)) or another protein without any detectable domain (named Apro1, for anaphase promoting 1 (Tian et al. 2022)). All three proteins localize exclusively in the MAC of conjugating cells. Hence, they possibly regulate yet unknown genes required for the initiation of subsequent chromosome segregation.

Chromosome mis-segregation in anaphase

Disruption of many genes will eventually cause chromosome segregation defects, which may present as unequal segregation, lagging chromosomes, or extensive bridging during anaphase (Fig. 4H). However, except for the characteristic collapsed anaphase I phenotype observed in CO resolution factor mutants (Lukaszewicz et al. 2013) and the potentially entangled chromosomes incapable of segregating in cells with a disrupted condensin complex (Howard-Till and Loidl 2018), it is difficult to determine which pathway is disrupted by solely considering the mis-segregation phenotype. Therefore, if defective divisions are observed, it is generally advised to examine earlier steps in meiosis. Many of the mutants described above will eventually attempt meiotic divisions, more or less successfully, since *Tetrahymena* seems to have no hard checkpoints. Even mutants that do not form crossovers are capable of occasionally producing a seemingly functional haploid gamete by random chance.

Conclusion and perspectives

Various cytogenetic and biochemistry techniques have been developed or adapted for studying *Tetrahymena* meiosis. By utilizing this initial version of the meiosis research toolbox, many details of *Tetrahymena's* SC-less meiosis have been decoded (see Loidl 2021). Collectively, mechanisms regulating *Tetrahymena's* 'simplified' meiosis are believed to mirror core features of a hypothetical proto-meiosis and thus, may facilitate the understanding of the origin and evolution of meiosis (see Loidl 2021).

The rapid development of next-generation sequencing (NGS) technologies has led to the generation of numerous tools for tackling fundamental questions concerning meiosis that could not be solved easily with classical, low-resolution techniques (Brick et al. 2018; Gittens et al. 2019; Lam et al. 2017; Mimitou and Keeney 2018; Paiano et al. 2020). Although novel techniques for identification of DSB sites are still emerging (e.g., Protec-seq (Prieler et al. 2021)), none of them can be directly used for studying *Tetrahymena* meiosis. Consequently, the proof for the existence of meiotic DSB hotspots (i.e., a locus with a high frequency of DSB formation events) and CO hotspots in *Tetrahymena* remains elusive. This has greatly hindered the characterization of chromatin features of the DSB site, the understanding of DSB end processing mechanisms, and the correlation between DSB hotspots and CO hotspots in SC-independent meiosis. Hence, adding NGS-based techniques into the current *Tetrahymena* meiosis toolbox is needed.

The utilization of high-throughput, genome-wide techniques is largely limited by the lack of an effective method for purifying the meiotic MICs from the cell lysate, which contains MACs, non-meiotic MICs, and meiotic MICs. We believe that this could be solved by combining utilization of a recently optimized MIC preparation method (Duan et al. 2021) with the widely-applied fluorescent-activated cell sorting technique (i.e., for sorting labelled meiotic MICs from non-meiotic MICs and MACs).

Pioneer works revealed structural features in the meiotic nucleus of various ciliates (Raĭkov 1982; Zhang et al. 2022b). Notably, these features are highly diverse in different ciliates. For instance, an SC seems to be present in some ciliates but absent in others (Fig. 2D). However, these features have not been investigated with molecular biology techniques (e.g., immunostaining, FISH). Because the cellular structures of ciliates are largely conserved, the *Tetrahymena* meiosis research toolbox might be used for studying regulatory mechanisms in other ciliates.

In conclusion, we believe that investigating the enigmatic features of the SC-less meiosis using novel techniques in *Tetrahymena* would help to understand the

biological significance of the SC from an opposite perspective. Meanwhile, a comprehensive characterization of SC-dependent and SC-independent meiosis of different ciliates will undoubtedly shed light on the evolutionary significance of different types of meiosis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42995-022-00149-8.

Acknowledgements We thank Dr. Josef Loidl (University of Vienna) for his critical comments on the manuscript. We also thank Drs Weibo Song and Long Zhao (Ocean University of China) for their great help in figure illustration and fluorescence microscopy, respectively. We appreciate Dr. Ying Yan (Ocean University of China) for sharing a valuable out-of-print monograph. Moreover, we also appreciate the computing resources provided by IEMB-1, a high-performance computing cluster operated by the Institute of Evolution & Marine Biodiversity. This work is supported by the Fundamental Research Funds for the Central Universities (No. 202241003, to Dr. Miao Tian), by the Natural Science Foundation of Shandong Province (ZR2022JQ13, to Dr. Miao Tian), and by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement (No. 101024333). Dr. Rachel Howard-Till is supported by NIH RO1GM127571.

Author contributions MT conceived and wrote the original manuscript, and prepared most figures; RHT contributed to writing the manuscript; MT collected and analyzed most data; XC, and YL provided the necessary assistance in data collection and interpretation; CL contributed to the illustration of some figures. All authors provided critical feedback and helped shape the manuscript.

Data availability All data generated or analysed during this study are included in this published article (and its supplementary information file).

Declarations

Conflict of interest The authors declare no conflicts of interest.

Animal and human rights statements We declare that all applicable international, national, and or institutional guidelines for sampling, care, and experimental use of organisms for the study have been followed and all necessary approvals have been obtained.

References

Akematsu T, Fukuda Y, Garg J, Fillingham JS, Pearlman RE, Loidl J (2017) Post-meiotic DNA double-strand breaks occur in *Tetrahymena*, and require Topoisomerase II and Spo11. Elife 6:e26176

Akematsu T, Findlay A, Fukuda Y, Pearlman RE, Loidl J, Orias E, Hamilton EP (2018) Resistance to 6-methylpurine is conferred by defective adenine phosphoribosyltransferase in *Tetrahymena*. Genes 9:179

Akematsu T, Sanchez-Fernandez R, Kosta F, Holzer E, Loidl J (2020) The transmembrane protein Semi1 positions gamete nuclei for reciprocal fertilization in *Tetrahymena*. iScience 23:100749

Ali EI, Loidl J, Howard-Till RA (2018) A streamlined cohesin apparatus is sufficient for mitosis and meiosis in the protist *Tetrahymena*. Chromosoma 127:421–435

Brick K, Pratto F, Sun CY, Camerini-Otero RD, Petukhova G (2018) Analysis of meiotic double-strand break initiation in mammals. Meth Enzymol 601:391–418

Byrne BC, Brussard TB, Bruns PJ (1978) Induced resistance to 6-methylpurine and cycloheximide in *Tetrahymena*. I. Germ line mutants of *T. thermophila*. Genetics 89:695–702

Capilla-Perez L, Durand S, Hurel A, Lian Q, Chambon A, Taochy C, Solier V, Grelon M, Mercier R (2021) The synaptonemal complex imposes crossover interference and heterochiasmy in *Arabidopsis*. Proc Natl Acad Sci USA 118:e2023613118

Cassidy-Hanley DM (2012) *Tetrahymena* in the laboratory: strain resources, methods for culture, maintenance, and storage. Methods Cell Biol 109:237–276

Cassidy-Hanley D, Bowen J, Lee JH, Cole E, VerPlank LA, Gaertig J, Gorovsky MA, Bruns PJ (1997) Germline and somatic transformation of mating *Tetrahymena thermophila* by particle bombardment. Genetics 146:135–147

Cervantes MD, Xi X, Vermaak D, Yao MC, Malik HS (2006) The CNA1 histone of the ciliate *Tetrahymena thermophila* is essential for chromosome segregation in the germline micronucleus. Mol Biol Cell 17:485–497

Chalker DL (2012) Transformation and strain engineering of *Tetrahymena*. Methods Cell Biol 109:327–345

Chalker DL, Yao MC (2001) Nongenic, bidirectional transcription precedes and may promote developmental DNA deletion in *Tetrahymena thermophila*. Genes Dev 15:1287–1298

Chalker DL, Meyer E, Mochizuki K (2013) Epigenetics of ciliates. Cold Spring Harb Perspect Biol 5:a017764

Cheng T, Wang YY, Huang J, Chen X, Zhao XL, Gao S, Song WB (2019) Our recent progress in epigenetic research using the model ciliate, *Tetrahymena thermophila*. Mar Life Sci Technol 1:4–14

Chi J, Mahe F, Loidl J, Logsdon J, Dunthorn M (2014) Meiosis gene inventory of four ciliates reveals the prevalence of a synaptonemal complex-independent crossover pathway. Mol Biol Evol 31:660–672

Coyne RS, Thiagarajan M, Jones KM, Wortman JR, Tallon LJ, Haas BJ, Cassidy-Hanley DM, Wiley EA, Smith JJ, Collins K, Lee SR, Couvillion MT, Liu Y, Garg J, Pearlman RE, Hamilton EP, Orias E, Eisen JA, Methe BA (2008) Refined annotation and assembly of the *Tetrahymena thermophila* genome sequence through EST analysis, comparative genomic hybridization, and targeted gap closure. BMC Genom 9:562

Dapper AL, Payseur BA (2019) Molecular evolution of the meiotic recombination pathway in mammals. Evolution 73:2368–2389

Dave D, Wloga D, Sharma N, Gaertig J (2009) DYF-1 is required for assembly of the axoneme in *Tetrahymena thermophila*. Eukaryot Cell 8:1397–1406

de Vries FA, de Boer E, van den Bosch M, Baarends WM, Ooms M, Yuan L, Liu JG, van Zeeland AA, Heyting C, Pastink A (2005) Mouse Sycp1 functions in synaptonemal complex assembly, meiotic recombination, and XY body formation. Genes Dev 19:1376–1389

Diagouraga B, Clement JAJ, Duret L, Kadlec J, de Massy B, Baudat F (2018) *PRDM9* methyltransferase activity is essential for meiotic DNA double-strand break formation at its binding sites. Mol Cell 69:853-865.e856

Duan L, Cheng T, Wei F, Qiao Y, Wang C, Warren A, Niu J, Wang Y (2021) New contribution to epigenetic studies: isolation of micronuclei with high purity and DNA integrity in the model ciliated protist *Tetrahymena Thermophila*. Eur J Protistol 80:125804

Eisen JA, Coyne RS, Wu M, Wu D, Thiagarajan M, Wortman JR, Badger JH, Ren Q, Amedeo P, Jones KM, Tallon LJ, Delcher AL, Salzberg SL, Silva JC, Haas BJ, Majoros WH, Farzad M, Carlton JM, Smith RK Jr, Garg J et al (2006) Macronuclear genome

sequence of the ciliate *Tetrahymena thermophila*, a model eukaryote. PLoS Biol 4:e286

Fan S, Jiao Y, Khan R, Jiang X, Javed AR, Ali A, Zhang H, Zhou J, Naeem M, Murtaza G, Li Y, Yang G, Zaman Q, Zubair M, Guan H, Zhang X, Ma H, Jiang H, Ali H, Dil S et al (2021) Homozygous mutations in C14orf39/SIX6OS1 cause non-obstructive azoospermia and premature ovarian insufficiency in humans. Am J Hum Genet 108:324–336

Frankel J (2000) Cell biology of *Tetrahymena thermophila*. Methods Cell Biol 62:27–125

Frankel J, Jenkins LM, Nelsen EM, Stoltzman CA (1993) Hypoangular: a gene potentially involved in specifying positional information in a ciliate, *Tetrahymena thermophila*. Dev Biol 160:333–354

Fujiu K, Numata O (2000) Reorganization of microtubules in the amitotically dividing macronucleus of *Tetrahymena*. Cell Motil Cytoskel 46:17–27

Gittens WH, Johnson DJ, Allison RM, Cooper TJ, Thomas H, Neale MJ (2019) A nucleotide resolution map of Top2-linked DNA breaks in the yeast and human genome. Nat Commun 10:4846

Gong RT, Jiang YH, Vallesi A, Gao YY, Gao F (2020) Conjugation in *Euplotes raikovi* (Protista, Ciliophora): new insights into nuclear events and macronuclear development from micronucleate and amicronucleate cells. Microorganisms 8:162

Grishaeva TM, Bogdanov YF (2014) Conservation and variability of synaptonemal complex proteins in phylogenesis of eukaryotes. Int J Evol Biol 2014:856230

Hamilton EP, Kapusta A, Huvos PE, Bidwell SL, Zafar N, Tang H, Hadjithomas M, Krishnakumar V, Badger JH, Caler EV, Russ C, Zeng Q, Fan L, Levin JZ, Shea T, Young SK, Hegarty R, Daza R, Gujja S, Wortman JR et al (2016) Structure of the germline genome of *Tetrahymena thermophila* and relationship to the massively rearranged somatic genome. Elife 5:19090

Hayashi A, Mochizuki K (2015) Targeted gene disruption by ectopic induction of DNA elimination in *Tetrahymena*. Genetics 201:55–64

Howard-Till R, Loidl J (2018) Condensins promote chromosome individualization and segregation during mitosis, meiosis, and amitosis in *Tetrahymena thermophila*. Mol Biol Cell 29:466–478

Howard-Till RA, Lukaszewicz A, Loidl J (2011) The recombinases Rad51 and Dmc1 play distinct roles in DNA break repair and recombination partner choice in the meiosis of *Tetrahymena*. PLoS Genet 7:e1001359

Howard-Till RA, Lukaszewicz A, Novatchkova M, Loidl J (2013) A single cohesin complex performs mitotic and meiotic functions in the protist *Tetrahymena*. PLoS Genet 9:e1003418

Ioannou D, Fortun J, Tempest HG (2019) Meiotic nondisjunction and sperm aneuploidy in humans. Reproduction 157:R15–R31

Iwamoto M, Mori C, Hiraoka Y, Haraguchi T (2014) Puromycin resistance gene as an effective selection marker for ciliate *Tetrahymena*. Gene 534:249–255

Iwamoto M, Koujin T, Osakada H, Mori C, Kojidani T, Matsuda A, Asakawa H, Hiraoka Y, Haraguchi T (2015) Biased assembly of the nuclear pore complex is required for somatic and germline nuclear differentiation in *Tetrahymena*. J Cell Sci 128:1812–1823

Jiang J, Miracco EJ, Hong K, Eckert B, Chan H, Cash DD, Min B, Zhou ZH, Collins K, Feigon J (2013) The architecture of *Tetrahymena* telomerase holoenzyme. Nature 496:187–192

Jiang YY, Lechtreck K, Gaertig J (2015) Total internal reflection fluorescence microscopy of intraflagellar transport in *Tetrahymena thermophila*. Methods Cell Biol 127:445–456

Jiang Y, Zhang T, Vallesi A, Yang X, Gao F (2019) Time-course analysis of nuclear events during conjugation in the marine ciliate *Euplotes vannus* and comparison with other ciliates (Protozoa, Ciliophora). Cell Cycle 18:288–298

Kariyazono R, Oda A, Yamada T, Ohta K (2019) Conserved HORMA domain-containing protein Hop1 stabilizes interaction between proteins of meiotic DNA break hotspots and chromosome axis. Nucleic Acids Res 47:10166–10180

Kataoka K, Mochizuki K (2015) Phosphorylation of an HP1-like protein regulates heterochromatin body assembly for DNA elimination. Dev Cell 35:775–788

Kataoka K, Schoeberl UE, Mochizuki K (2010) Modules for C-terminal epitope tagging of *Tetrahymena* genes. J Microbiol Meth 82:342–346

Kobayashi S, Iwamoto M, Haraguchi T (2016) Live correlative light-electron microscopy to observe molecular dynamics in high resolution. Microscopy 65:296–308

Kushida Y, Takaine M, Nakano K, Sugai T, Numata O (2015) Dynamic change of cellular localization of microtubule-organizing center during conjugation of ciliate *Tetrahymena thermophila*. Zool Sci 32:25–32

Lam I, Mohibullah N, Keeney S (2017) Sequencing Spo11 oligonucleotides for mapping meiotic DNA double-strand breaks in yeast. Methods Mol Biol 1471:51–98

Lambing C, Heckmann S (2018) Tackling plant meiosis: from model research to crop improvement. Front Plant Sci 9:829

Lee MS, Higashide MT, Choi H, Li K, Hong S, Lee K, Shinohara A, Shinohara M, Kim KP (2021) The synaptonemal complex central region modulates crossover pathways and feedback control of meiotic double-strand break formation. Nucleic Acids Res 49:7537–7553

Li C, Chen X, Zheng W, Doak TG, Fan G, Song W, Yan Y (2021) Chromosome organization and gene expansion in the highly fragmented genome of the ciliate *Strombidium stylifer*. J Genet Genomics 48:908–916

Loidl J (2006) *S. pombe* linear elements: the modest cousins of synaptonemal complexes. Chromosoma 115:260–271

Loidl J (2016) Conservation and variability of meiosis across the eukaryotes. Annu Rev Genet 50:293–316

Loidl J (2021) *Tetrahymena* meiosis: Simple yet ingenious. PLoS Genet 17:e1009627

Loidl J, Lorenz A (2016) DNA double-strand break formation and repair in *Tetrahymena* meiosis. Semin Cell Dev Biol 54:126–134

Loidl J, Mochizuki K (2009) *Tetrahymena* meiotic nuclear reorganization is induced by a checkpoint kinase-dependent response to DNA damage. Mol Biol Cell 20:2428–2437

Loidl J, Scherthan H (2004) Organization and pairing of meiotic chromosomes in the ciliate *Tetrahymena thermophila*. J Cell Sci 117:5791–5801

Loidl J, Lukaszewicz A, Howard-Till RA, Koestler T (2012) The *Tetrahymena* meiotic chromosome bouquet is organized by centromeres and promotes interhomolog recombination. J Cell Sci 125:5873–5880

Lukaszewicz A, Howard-Till RA, Novatchkova M, Mochizuki K, Loidl J (2010) *MRE11* and *COM1/SAE2* are required for double-strand break repair and efficient chromosome pairing during meiosis of the protist *Tetrahymena*. Chromosoma 119:505–518

Lukaszewicz A, Howard-Till RA, Loidl J (2013) Mus81 nuclease and Sgs1 helicase are essential for meiotic recombination in a protist lacking a synaptonemal complex. Nucleic Acids Res 41:9296–9309

Lukaszewicz A, Shodhan A, Loidl J (2015) Exo1 and Mre11 execute meiotic DSB end resection in the protist *Tetrahymena*. DNA Repair 35:137–143

Luo Z, Hu T, Jiang H, Wang R, Xu Q, Zhang S, Cao J, Song X (2020) Rearrangement of macronucleus chromosomes correspond to TAD-like structures of micronucleus chromosomes in *Tetrahymena thermophila*. Genome Res 30:406–414

MacQueen AJ, Colaiacovo MP, McDonald K, Villeneuve AM (2002) Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in *C. elegans*. Genes Dev 16:2428–2442

Mayo KA, Orias E (1981) Further evidence for lack of gene expression in the *Tetrahymena* micronucleus. Genetics 98:747–762
Miao W, Xiong J, Bowen J, Wang W, Liu YF, Braguinets O, Grigull J, Pearlman RE, Orias E, Gorovsky MA (2009) Microarray analyses of gene expression during the *Tetrahymena thermophila* life cycle. PLoS ONE 4:e4429
Mimitou EP, Keeney S (2018) S1-seq assay for mapping processed DNA ends. Meth Enzymol 601:309–330
Mochizuki K, Novatchkova M, Loidl J (2008) DNA double-strand breaks, but not crossovers, are required for the reorganization of meiotic nuclei in *Tetrahymena*. J Cell Sci 121:2148–2158
Niu J, Zhang X, Li G, Yan P, Yan Q, Dai Q, Jin D, Shen X, Wang J, Zhang MQ, Gao J (2020) A novel cytogenetic method to image chromatin interactions at subkilobase resolution: Tn5 transposase-based fluorescence in situ hybridization. J Genet Genomics 47:727–735
Ohkura H (2015) Meiosis: an overview of key differences from mitosis. Cold Spring Harb Perspect Biol 7:a015859
Paiano J, Wu W, Yamada S, Sciascia N, Callen E, Cotrim AP, Deshpande RA, Maman Y, Day A, Paull TT, Nussenzweig A (2020) *ATM* and *PRDM9* regulate *SPO11*-bound recombination intermediates during meiosis. Nat Commun 11:857
Pina R, Santos-Diaz AI, Orta-Salazar E, Aguilar-Vazquez AR, Mantellero CA, Acosta-Galeana I, Estrada-Mondragon A, Prior-Gonzalez M, Martinez-Cruz JI, Rosas-Arellano A (2022) Ten approaches that improve immunostaining: a review of the latest advances for the optimization of immunofluorescence. Int J Mol Sci 23:1426
Prieler S, Chen D, Huang LZ, Mayrhofer E, Zsoter S, Vesely M, Mbogning J, Klein F (2021) Spo11 generates gaps through concerted cuts at sites of topological stress. Nature 594:577–582
Qiao Y, Cheng T, Zhang J, Alfarraj SA, Tian M, Liu Y, Gao S (2022) Identification and utilization of a mutated 60S ribosomal subunit coding gene as an effective and cost-efficient selection marker for *Tetrahymena* genetic manipulation. Int J Biol Macromol 204:1–8
Raĭkov IB (1982) The protozoan nucleus, morphology and evolution. Springer-Verlag, Wien
Ruehle MD, Orias E, Pearson CG (2016) *Tetrahymena* as a unicellular model eukaryote: genetic and genomic tools. Genetics 203:649–665
Schoeberl UE, Kurth HM, Noto T, Mochizuki K (2012) Biased transcription and selective degradation of small RNAs shape the pattern of DNA elimination in *Tetrahymena*. Genes Dev 26:1729–1742
Shah S, Chen Y, Bhattacharya D, Chan CX (2020) Sex in *Symbiodiniaceae dinoflagellates*: genomic evidence for independent loss of the canonical synaptonemal complex. Sci Rep 10:9792
Sheng Y, Duan L, Cheng T, Qiao Y, Stover NA, Gao S (2020) The completed macronuclear genome of a model ciliate *Tetrahymena thermophila* and its application in genome scrambling and copy number analyses. Sci China Life Sci 63:1534–1542
Sheng Y, Pan B, Wei F, Wang Y, Gao S (2021) Case study of the response of N^6-methyladenine DNA modification to environmental stressors in the unicellular eukaryote *Tetrahymena thermophila*. eSphere 6:e0120820
Shodhan A, Lukaszewicz A, Novatchkova M, Loidl J (2014) Msh4 and Msh5 function in SC-independent chiasma formation during the streamlined meiosis of *Tetrahymena*. Genetics 198:983–993
Shodhan A, Kataoka K, Mochizuki K, Novatchkova M, Loidl J (2017a) A Zip3-like protein plays a role in crossover formation in the SC-less meiosis of the protist *Tetrahymena*. Mol Biol Cell 28:825–833
Shodhan A, Novatchkova M, Loidl J (2017b) *BIME2*, a novel gene required for interhomolog meiotic recombination in the protist model organism *Tetrahymena*. Chromosome Res 25:291–298
Smagulova F, Brick K, Pu Y, Camerini-Otero RD, Petukhova GV (2016) The evolutionary turnover of recombination hot spots contributes to speciation in mice. Genes Dev 30:266–280
Stover NA, Krieger CJ, Binkley G, Dong Q, Fisk DG, Nash R, Sethuraman A, Weng S, Cherry JM (2006) *Tetrahymena* Genome Database (TGD): a new genomic resource for *Tetrahymena thermophila* research. Nucleic Acids Res 34:D500–D503
Sugai T, Hiwatashi K (1974) Cytologic and autoradiographic studies of the micronucleus at meiotic prophase in *Tetrahymena pyriformis*. J Protozool 21:542–548
Taagen E, Bogdanove AJ, Sorrells ME (2020) Counting on crossovers: controlled recombination for plant breeding. Trends Plant Sci 25:455–465
Teo G, Liu G, Zhang J, Nesvizhskii AI, Gingras AC, Choi H (2014) SAINTexpress: improvements and additional features in Significance Analysis of INTeractome software. J Proteomics 100:37–43
Tian M, Loidl J (2018) A chromatin-associated protein required for inducing and limiting meiotic DNA double-strand break formation. Nucleic Acids Res 46:11822–11834
Tian M, Loidl J (2019) An MCM family protein promotes interhomolog recombination by preventing precocious intersister repair of meiotic DSBs. PLoS Genet 15:e1008514
Tian M, Chen X, Xiong Q, Xiong J, Xiao C, Ge F, Yang F, Miao W (2014) Phosphoproteomic analysis of protein phosphorylation networks in *Tetrahymena thermophila*, a model single-celled organism. Mol Cell Proteomics 13:503–519
Tian M, Mochizuki K, Loidl J (2019) Non-coding RNA transcription in *Tetrahymena* meiotic nuclei requires dedicated mediator complex-associated proteins. Curr Biol 29:2359–2370
Tian M, Agreiter C, Loidl J (2020) Spatial constraints on chromosomes are instrumental to meiotic pairing. J Cell Sci 133:jcs253724
Tian M, Mochizuki K, Loidl J (2022) Arrested crossover precursor structures form stable homologous bonds in a *Tetrahymena* meiotic mutant. PLoS ONE 17:e0263691
Veitia RA (2020) Primary ovarian insufficiency, meiosis and DNA repair. Biomed J 43:115–123
Wang M, Wang K, Tang D, Wei C, Li M, Shen Y, Chi Z, Gu M, Cheng Z (2010) The central element protein ZEP1 of the synaptonemal complex regulates the number of crossovers during meiosis in rice. Plant Cell 22:417–430
Wang Y, Sheng Y, Liu Y, Zhang W, Cheng T, Duan L, Pan B, Qiao Y, Liu Y, Gao S (2019) A distinct class of eukaryotic MT-A70 methyltransferases maintain symmetric DNA N^6-adenine methylation at the ApT dinucleotides as an epigenetic mark associated with transcription. Nucleic Acids Res 47:11771–11789
Wang G, Wang S, Chai X, Zhang J, Yang W, Jiang C, Chen K, Miao W, Xiong J (2021) A strategy for complete telomere-to-telomere assembly of ciliate macronuclear genome using ultra-high coverage nanopore data. Comput Struct Biotechnol J 19:1928–1932
Webster MT, Hurst LD (2012) Direct and indirect consequences of meiotic recombination: implications for genome evolution. Trends Genet 28:101–109
Wei F, Pan B, Diao J.H, Wang Y.Y, Sheng Y.L, Gao S (2022) The micronuclear histone H3 clipping in the unicellular eukaryote *Tetrahymena thermophila*. Mar Life Sci Technol. https://doi.org/10.1007/s42995-022-00151-0
Wolfe J, Hunter B, Adair WS (1976) A cytological study of micronuclear elongation during conjugation in *Tetrahymena*. Chromosoma 55:289–308
Wuitschick JD, Gershan JA, Lochowicz AJ, Li S, Karrer KM (2002) A novel family of mobile genetic elements is limited to the germline genome in *Tetrahymena thermophila*. Nucleic Acids Res 30:2524–2537

Xiong J, Yuan D, Fillingham JS, Garg J, Lu X, Chang Y, Liu Y, Fu C, Pearlman RE, Miao W (2011) Gene network landscape of the ciliate *Tetrahymena thermophila*. PLoS ONE 6:e20124

Xiong J, Lu X, Zhou Z, Chang Y, Yuan D, Tian M, Wang L, Fu C, Orias E, Miao W (2012) Transcriptome analysis of the model protozoan, *Tetrahymena thermophila*, using deep RNA sequencing. PLoS ONE 7:e30630

Xiong J, Lu Y, Feng J, Yuan D, Tian M, Chang Y, Fu C, Wang G, Zeng H, Miao W (2013) *Tetrahymena* functional genomics database (TetraFGD): an integrated resource for *Tetrahymena* functional genomics. Database 2013:bat008

Xiong J, Yang W, Chen K, Jiang C, Ma Y, Chai X, Yan G, Wang G, Yuan D, Liu Y, Bidwell SL, Zafar N, Hadjithomas M, Krishnakumar V, Coyne RS, Orias E, Miao W (2019) Hidden genomic evolution in a morphospecies-the landscape of rapidly evolving genes in *Tetrahymena*. PLoS Biol 17:e3000294

Xu Q, Wang R, Ghanam AR, Yan G, Miao W, Song X (2016) The key role of *CYC2* during meiosis in *Tetrahymena thermophila*. Protein Cell 7:236–249

Xu J, Li X, Song W, Wang W, Gao S (2019) Cyclin Cyc2p is required for micronuclear bouquet formation in *Tetrahymena thermophila*. Sci China Life Sci 62:668–680

Xu J, Zhao XL, Mao FBA, Basrur V, Ueberheide B, Chait BT, Allis CD, Taverna SD, Gao S, Wang W, Liu YF (2021) A polycomb repressive complex is required for RNAi-mediated heterochromatin formation and dynamic distribution of nuclear bodies. Nucleic Acids Res 49:5407–5425

Yadav VK, Claeys Bouuaert C (2021) Mechanism and control of meiotic DNA double-strand break formation in *S. cerevisiae*. Front Cell Dev Biol. 9:642737

Yamada S, Kugou K, Ding DQ, Fujita Y, Hiraoka Y, Murakami H, Ohta K, Yamada T (2018) The conserved histone variant H2A.Z illuminates meiotic recombination initiation. Curr Genet 64:1015–1019

Yan Y, Jiang L, Aufderheide KJ, Wright GA, Terekhov A, Costa L, Qin K, McCleery WT, Fellenstein JJ, Ustione A, Robertson JB, Johnson CH, Piston DW, Hutson MS, Wikswo JP, Hofmeister W, Janetopoulos C (2014) A microfluidic-enabled mechanical microcompressor for the immobilization of live single- and multi-cellular specimens. Microsc Microanal 20:141–151

Yan GX, Zhang J, Shodhan A, Tian M, Miao W (2016) Cdk3, a conjugation-specific cyclin-dependent kinase, is essential for the initiation of meiosis in *Tetrahymena thermophila*. Cell Cycle 15:2506–2514

Yang W, Jiang C, Zhu Y, Chen K, Wang G, Yuan D, Miao W, Xiong J (2019) *Tetrahymena* comparative genomics database (TCGD): a community resource for *Tetrahymena*. Database 2019:baz029

Zhang J, Tian M, Yan GX, Shodhan A, Miao W (2017) E2fl1 is a meiosis-specific transcription factor in the protist *Tetrahymena thermophila*. Cell Cycle 16:123–135

Zhang J, Yan G, Tian M, Ma Y, Xiong J, Miao W (2018) A DP-like transcription factor protein interacts with E2fl1 to regulate meiosis in *Tetrahymena thermophila*. Cell Cycle 17:634–642

Zhang J, Tian M, Chen K, Yan G, Xiong J, Miao W (2022a) Zfp1, a Cys2His2 zinc finger protein is required for meiosis initiation in *Tetrahymena thermophila*. Cell Cycle 21:1422–1433

Zhang X, Lu XT, Chi Y, Jiang YH, Wang CD, Al-Farraj SA, Vallesi A, Gao F (2022b) Timing and characteristics of nuclear events during conjugation and genomic exclusion in *Paramecium multimicronucleatum*. Mar Life Sci Technol 4:317–328

Zhao X, Xiong J, Mao F, Sheng Y, Chen X, Feng L, Dui W, Yang W, Kapusta A, Feschotte C, Coyne RS, Miao W, Gao S, Liu Y (2019) RNAi-dependent polycomb repression controls transposable elements in *Tetrahymena*. Genes Dev 33:348–364

Zhao XL, Li Y, Duan LL, Chen X, Mao FBA, Juma M, Liu YF, Song WB, Gao S (2020) Functional analysis of the methyltransferase SMYD in the single-cell model organism *Tetrahymena thermophila*. Mar Life Sci Technol 2:109–122

Zhao L, Gao F, Gao S, Liang Y, Long H, Lv Z, Su Y, Ye N, Zhang L, Zhao C, Wang X, Song W, Zhang S, Dong B (2021) Biodiversity-based development and evolution: the emerging research systems in model and non-model organisms. Sci China Life Sci 64:1236–1280

Zheng WB, Wang CD, Lynch M, Gao S (2021) The compact macronuclear genome of the ciliate *Halteria grandinella*: a transcriptome-like genome with 23,000 nanochromosomes. mBio 12:e01964-20

Zickler D, Kleckner N (2015) Recombination, pairing, and synapsis of homologs during meiosis. Cold Spring Harb Perspect Biol 7:a016626