The role of DNA topoisomerase 1α (AtTOP1α) in regulating arabidopsis meiotic recombination and chromosome segregation (#100215)

First submission

Guidance from your Editor

Please submit by **28 May 2024** for the benefit of the authors (and your token reward) .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files
Download and review all files from the materials page.

12 Figure file(s)
3 Raw data file(s)
3 Other file(s)

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

The role of DNA topoisomerase 1α (AtTOP1α) in regulating arabidopsis meiotic recombination and chromosome segregation

Ibrahim Eid Elesawi [Corresp., 1, 2], **Ahmed M. Hashem** [1, 3], **Li Yao** [1], **Mohamed Maher** [2], **Abdallah A. Hassanin** [4], **Diaa Abd El-Moneim** [5], **Fatmah A. Safhi** [6], **Nora M. Al Aboud** [7], **Salha Mesfer ALshamrani** [8], **Wael F. Shehata** [9, 10], **Chen Chunli** [1, 11]

[1] College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

[2] Agricultural Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

[3] Biotechnology Department, Faculty of Agriculture, Al-Azhar University, Cairo 11651, Egypt

[4] Genetics Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

[5] Department of Plant Production, (Genetic Branch), Faculty of Environmental and Agricultural Sciences, Arish University, El-Arish, El-Arish, Egypt

[6] Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia, Riyadh, Riyadh, Saudi Arabia

[7] Department of Biology Faculty of Science, Umm Al-Qura University, Makkah, Makkah, Saudi Arabia

[8] Department of Biology, College of Science, University of Jeddah, Jeddah, Jeddah, Saudi Arabia

[9] College of Agriculture and Food Sciences, Department of Agricultural Biotechnology, King Faisal University, Al-Ahsa, Al-Ahsa, Saudi Arabia

[10] College of Environmental Agricultural Science, Plant Production Department, Arish University, Arish, North Sinai, Egypt

[11] National Key Laboratory for Germplasm Innovation and Utilization for Fruit and Vegetable Horticultural Crops, Huazhong Agricultural University, Wuhan, Hubei 430070, China

Corresponding Author: Ibrahim Eid Elesawi
Email address: ibrahimeid@zu.edu.eg

Meiosis is a critical process in sexual reproduction, and errors during this cell division can significantly impact fertility. Successful meiosis relies on the coordinated action of numerous genes involved in DNA replication, strand breaks, and subsequent rejoining. DNA topoisomerase enzymes play a vital role by regulating DNA topology, alleviating tension during replication and transcription. To elucidate the specific function of DNA topoisomerase 1α (*AtTOP1α*) in male reproductive development of Arabidopsis thaliana, we investigated meiotic cell division in Arabidopsis flower buds. Combining cytological and biochemical techniques, we aimed to reveal the novel contribution of *AtTOP1α* to meiosis. Our results demonstrate that the absence of *AtTOP1α* leads to aberrant chromatin behavior during meiotic division. Specifically, the top1α1 mutant displayed altered heterochromatin distribution and clustered centromere signals at early meiotic stages. Additionally, this mutant exhibited disruptions in the distribution of 45s rDNA signals and a reduced frequency of chiasma formation during metaphase I, a crucial stage for genetic exchange. Furthermore, the atm-2×top1a1 double mutant displayed even more severe meiotic defects, including incomplete synapsis, DNA fragmentation, and the presence of

polyads. These observations collectively suggest that *AtTOP1α* plays a critical role in ensuring accurate meiotic progression, promoting homologous chromosome crossover formation, and potentially functioning in a shared DNA repair pathway with ATAXIA TELANGIECTASIA MUTATED (ATM) in Arabidopsis microspore mother cells.

The Role of DNA Topoisomerase 1α (AtTOP1α) in Regulating Arabidopsis Meiotic Recombination and Chromosome Segregation

Ibrahim Eid Elesawi[1,2]*, Ahmed M. Hashem[1,3], Li Yao[1], Mohamed Maher[2], Abdallah A. Hassanin[4], Diaa Abd El-Moneim[5],Fatmah A. Safhi[6], Nora M. Al Aboud[7], Salha Mesfer ALshamrani[8], Wael F. Shehata[9, 10], and ChunliChen[1,11]

[1]College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
[2]Agricultural Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

[3]Biotechnology Department, Faculty of Agriculture, Al-Azhar University, Cairo 11651, Egypt

[4]Genetics Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt;

[5]Department of Plant Production, (Genetic Branch), Faculty of Environmental and Agricultural Sciences, Arish University, El-Arish 45511, Egypt

[6]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia

[7]Department of Biology Faculty of Science, Umm Al-Qura University, Makkah, Saudi Arabia

[8]Department of Biology, College of Science, University of Jeddah, Jeddah, Saudi Arabia 21959

[9]King Faisal University, College of Agriculture and Food Sciences, Department of Agricultural Biotechnology, Al-Ahsa 31982, Saudi Arabia; wshehata@kfu.edu.sa; [10]Arish University, College of Environmental Agricultural Science, Plant Production Department, P.O. Box: 45511 North Sinai, Egypt

[11]National Key Laboratory for Germplasm Innovation and Utilization for Fruit and Vegetable Horticultural Crops, Huazhong Agricultural University, Wuhan, Hubei 430070, China.

*Correspondence: ibrahimeid@zu.edu.eg (I.E.E.)

Abstract

Meiosis is a critical process in sexual reproduction, and errors during this cell division can significantly impact fertility. Successful meiosis relies on the coordinated action of numerous genes involved in DNA replication, strand breaks, and subsequent rejoining. DNA topoisomerase enzymes play a vital role by regulating DNA topology, alleviating tension during replication and transcription. To elucidate the specific function of DNA topoisomerase 1α (*AtTOP1α*) in male reproductive development of Arabidopsis thaliana, we investigated meiotic cell division in Arabidopsis flower buds. Combining cytological and biochemical techniques, we aimed to reveal the novel contribution of *AtTOP1α* to meiosis. Our results demonstrate that the absence of *AtTOP1α* leads to aberrant chromatin behavior during meiotic division. Specifically, the top1α1 mutant displayed altered heterochromatin distribution and clustered centromere signals at early meiotic stages. Additionally, this mutant exhibited disruptions in the distribution of 45s rDNA signals and a reduced frequency of chiasma formation during metaphase I, a crucial stage for genetic exchange. Furthermore, the atm-2×top1a1 double mutant displayed even more severe meiotic defects, including incomplete synapsis, DNA fragmentation, and the presence of polyads. These observations collectively suggest that *AtTOP1α* plays a critical role in ensuring accurate meiotic progression, promoting homologous chromosome crossover formation, and potentially functioning in a shared DNA repair pathway with ATAXIA TELANGIECTASIA MUTATED (ATM) in Arabidopsis microspore mother cells.

Keywords: ATM; centromere; DNA topoisomerase 1α; FISH; meiosis; 45s rDNA

1. Introduction

Sexual reproduction is the dominant mode for most eukaryotic organisms, although the reasons behind this prevalence remain a subject of investigation (Ma and Shi, 2014; Plötner, 2023). Plants produce several flowers with readily detectable meiotic chromosomes, making them an ideal model for investigating the cellular processes that underpin meiosis (Prusicki *et al.*, 2021). In flowering plants, meiosis takes place within the anthers and ovules, a critical process for sexual reproduction in eukaryotes (Cui *et al.*, 2024). Meiosis facilitates the formation of haploid gametes through two meiotic divisions (meiosis I and II) following a single round of DNA replication (Zickler and Kleckner, 2023). This reduction in chromosome number from diploid (2n) to haploid (1n) is essential for the fusion of gametes during fertilization, restoring the diploid state in the offspring (Wang *et al.*, 2021). The formation of a homologous chromosome connection, which ensures proper chromosome segregation during meiosis I, represents a critical step in prophase I. Recombination nodules (RNs) and synaptonemal complexes (SCs) are complexes associated with chromosome synapsis and recombination that can be seen in a variety of organisms (You *et al.*, 2024). During prophase I of meiosis, homologous chromosomes undergo recombination, enabling the reciprocal exchange of genetic material between non-sister chromatids, thus becoming an essential source of genetic diversity (Hofstatter *et al.*, 2021). During meiotic prophase I, programmed DSBs occur in homologous chromosomes, catalyzed by SPO11 protein complexes (Ito and Shinohara, 2023). During meiosis, DSBs are processed to generate 3'ssDNA with a free 3' hydroxyl end. These 3'ssDNA tails can initiate a DNA strand invasion step, where they anneal with homologous sequences on the partner chromosome. This homologous recombination process is essential for proper chromosome segregation during meiosis and enable crossover (Cos) or non-crossover repairs (Thangavel *et al.*, 2023; Yamaya *et al.*, 2023). Approximately 200 meiotic DSBs are produced in *Arabidopsis*, and only 10 are repaired as crossovers (Wang *et al.*, 2021).

Following the occurrence of DNA breakage, SPO11 forms covalent bonds with the 5′ end of the DSBs; it's important to note that SPO11 is subsequently removed before the repair of the DNA end. The RAD51 and DMC1 proteins are responsible for capturing and rebuilding nucleoprotein filaments, specifically targeting non-sister chromatids. Functional meiotic proteins for DNA repair and other factors mediate the second-end DSB capture, ligation, DNA synthesis, and CO-generating commitment (Ito and Shinohara, 2023).

Most organisms exhibit two distinct categories of crossover events (COs). Class I COs are characterized by their susceptibility to a phenomenon known as crossover interference. This phenomenon describes the reduced probability of additional COs occurring in close proximity to an existing CO. The ZMM proteins, specifically Zip1-3, Zip4/Spo22, and Msh4/Msh5, play a crucial role in mediating class I CO formation (Xin *et al.*, 2021). Class II COs are non-interfering and rely on the Mus81 endonuclease (Desjardins *et al.*, 2022). In addition to crossovers, meiotic DSB repair can utilize non-crossover processes like synthesis-dependent strand annealing (Lorenz and Mpaulo, 2022; Sen *et al.*, 2023). In a subset of organisms, at least one crossover (CO) event per homologous chromosome pair is essential for accurate chromosome segregation during meiosis. However, a significant portion of double-strand breaks (DSBs) are repaired through mechanisms that do not result in CO formation, or even by homologous recombination with sister chromatids (Wang *et al.*, 2015; Lloyd, 2023).

Crossover recombinations create genetic variation as well as chiasmata formation. The chiasmata connect homologous chromosomes, thereby ensuring proper chromosome alignment

and separation during meiosis (Kumar Koul and Nagpal, 2023). Therefore, it is necessary that crossover creation is closely controlled and that only a small number of DSBs are used to form mature crossovers (Alavattam *et al.*, 2021). Recombination events are not randomly distributed, as can be seen in human, mouse, insect, yeast, and plant meiosis; moreover, crossovers close to centromeric and pericentromeric regions, and some telomeric regions, are suppressed, most probably to decrease the aneuploid threat (Kim and Choi, 2022; Ito and Shinohara, 2023).

ATM acts as a critical regulator in the DNA damage response pathway, promoting repair through phosphorylation of key proteins involved in various cellular processes, including cell cycle arrest checkpoints (Groelly *et al.*, 2023; Herbst *et al.*, 2024). The protein kinase ATM acts as a critical regulator and sensor of DSBs during meiosis in various organisms. In Arabidopsis specifically, ATM plays a pivotal role, impacting fertility and influencing numerous processes essential for successful meiotic completion. These processes include DSB formation and processing, DNA repair, the establishment of non-interference crossovers, and the assembly of the synaptonemal complex (SC) (Kurzbauer *et al.*, 2021; Herbst *et al.*, 2024).

DNA topoisomerases play a critical role in developing appropriate DNA structure and topology, fixing kinks and torsions, and dealing with tight-twisted intermediates produced during replication and transcription.DNA topoisomerases influence DNA replication processes by reducing stress (Durand-Dubief *et al.*, 2011; Gamarra and Narlikar, 2021; McKie *et al.*, 2021). In *Arabidopsis*, there are two topoisomerase type I genes, *TOP1α* and *TOP1β*, which are tandemly located on the 5 chromosome. The TOP1α and TOP1β proteins share 60% sequence similarity (Takahashi *et al.*, 2002). The downregulation of *TOP1β* has no detectable effects; however, *TOP1α* knockout disrupts primordium initiation in shoot and floral meristem tissues, affecting plant phyllosphere guidance and overall plant architecture (Dinh *et al.*, 2014; Zhang *et al.*, 2022b). Furthermore, TOP1α functions in several developmental processes. For instance, photosynthesis-derived sugars promote TOP1α expression at the root tip to regulate TARGET OF RAPAMYCIN (TOR) expression and directly or indirectly maintain the quiescent center (QC), columella stem cell (CSC) identity and columella (COL) development (Zhang *et al.*, 2022a); it is involved in determining seed size (Wang *et al.*, 2024); it regulates Polycomb-group (PcG) proteins by changing nucleosome density in *Arabidopsis*(Liu *et al.*, 2014; Yang *et al.*, 2022); it influences floral transition by regulating the expression of *FLC* gene and close homologs; it regulates a central flowering repressor by influencing transcription machinery and histone modification (Gong *et al.*, 2017; Zhong *et al.*, 2019); and the inhibition of *TOP1α* results in defects in fertilization and spore formation in *Physcomitrium patens* (*P. patens*) while inhibition of *TOP1β* has no effect(Gu *et al.*, 2022). TOP1α also emerges as a key regulator of RNA-DNA hybrids (R-loops) within the nucleus in response to stress signals. Deficiency in TOP1α function leads to accumulation of R-loops in plant nuclear chromatin (Shafiq *et al.*, 2017; Kyung *et al.*, 2022).

This study uncovers novel functions of TOP1α during meiosis in *Arabidopsis thaliana* and underscores its importance in meiotic recombination. Notably, the absence of TOP1α led to reduced fertility and a decline in genetic crossing over. Moreover, the top1α1 and atm double mutant exhibited exacerbated meiotic defects, including incomplete synapsis and DNA fragmentation, culminating in the formation of ployads. In conclusion, our findings suggest that *AtTOP1α* is essential for proper meiotic progression.

2. Materials and Methods

2.1. Plant materials, crosses, and growth conditions

A. thaliana (Col-0) plants were used in this study. The *top1α1* contained a T-DNA insertion in the eighth intron (Takahashi *et al.*, 2002), and was obtained from the Taku Takahashi lab. The atm-2 mutant (SALK_006953) was obtained from the Salk Institute for Biological Studies. The double mutant was generated in our laboratory by crossing*top1α1* and *atm-2* mutants. Plants were maintained at ~22 °C and 60% humidity with light cycles of 16 h light/8 h dark.

2.2. PCR genotyping and semi-quantitative PCR

Plant genotyping was performed using PCR with specific primers detailed in Table S1. These primers included top1α_LP, RP and LB; atm_LP, RP and SALK LBb1.3. All plant lines were in the Columbia (Col-0) genetic background. RNA extraction from seedlings was achieved using TRIzol reagent (Invitrogen). Subsequently, first-strand cDNA synthesis was carried out from 3 μg of total RNA using 200 units (U) of M-MLV reverse transcriptase (Invitrogen). *ACTIN8* served as the reference gene for normalization of gene expression.

2.3. Pollen grains, mature anther staining, and dissected tetrads

Pollen viability and mature anther phenotypes were assessed using Alexander stain according to the Gu method (Liu *et al.*, 2023). Briefly, Briefly, flower buds at stage 12 of anther development were fixed in 1 mL of Carnoy's solution (6:3:1 ethanol: chloroform: acetic acid, v/v) for a minimum of two hours. Following fixation, the Carnoy's solution was removed, and the flower buds were dissected under a dissecting microscope. To differentiate viable from non-viable pollen grains, individual anthers were incubated with Alexander stain for 30 minutes at room temperature (25°C). Subsequently, the anthers were mounted on a slide and visualized using an Olympus BX53F microscope (Olympus, Tokyo, Japan). This staining technique enabled the discrimination of viable pollen grains (magenta-red) from non-viable ones (blue or green) (Chang *et al.*, 2014; Mao-Sen *et al.*, 2021).

2.4. Iodine pollen starch test

Pollen viability was assessed using a starch staining technique following the methodology of (Chang *et al.*, 2021). Inflorescences were fixed and stored in 70% (*v*/*v*) ethanol. Flower buds were dissected under a dissecting microscope for anther isolation. Six anthers per flower were crushed on a microscope slide in a droplet of 1% (w/v) iodine-potassium iodide (I2/KI) solution. Excess solution was carefully withdrawn using forceps. The preparation was then observed under a light microscope at room temperature (25°C) after coverslip placement.

2.5. Separation and detection of tetrads

Inflorescences with unopened flowers were fixed with Carnoy’s fixative (3:1 100% ethanol, glacial acetic acid) overnight at 25°C. Older buds outside the inflorescence containing obvious yellow anthers were removed, while sepals were left intact. The prepared inflorescence was washed three times with water. Immature buds were dissected using a fine needle on a glass slide under a binocular microscope. Immature anthers were isolated from other floral organs in two drops of water. The anthers were then squeezed with sharp forceps to release the tetrads. The slide was air-dried, and tetrads were stained for 2–3 minutes with 0.01% basic fuchsine (1:3 3.0 g basic fuchsine in 100 mL 95% ethanol, 5% aqueous phenol). The slide was covered with a coverslip, and the red-stained tetrads were observed under a light microscope following previously recommended procedures (Prusicki *et al.*, 2021).

2.6. Chromosome distribution for meiotic chromosome detection with DAPI

The chromosome spread was determined by applying the protocol described previously (Wang *et al.*, 2014; Prusicki *et al.*, 2021). The inflorescences with unopened flowers were fixed with Carnoy's solution at room temperature overnight. We rinsed the buds three times every 10 min with 10 mM citrate solution. Then, we removed old buds containing yellow anthers that could be seen before adding the digestion-by-digestion cocktail (0.3% pectolyase, 0.3% cellulase, and 0.5% cytohelicase in 10 mM citrate buffer, pH 4.5) at 37 °C for 80 min. Then, we washed the set buds five times, every five minutes, with 10 mM citrate buffer, after stopping digestion on ice for five minutes. Following that, we placed a bud on the slide to dissect it, obtain anthers, and remove of the rest of the flower. Anthers were mashed with sharp forceps in a drop of 60% acetic acid. Next, we moved the slide to a heat block, incubated it at 45 °C for 30 s, and added another drop for another 30 s. Then, 20 µL of freezing Carnoy's fixative was added to a center sample to spread the meiotic chromosomes on the slide, which were left to air dry for approximately 5 min. We added 5 µL of DAPI to the slide, covered with a coverslip, and observed the chromosomes under a fluorescent microscope.

2.7. Fluorescence *In Situ* Hybridization (FISH)

Chromosomes were separated according to (Wang *et al.*, 2014). Next, 100 µL of 70% formamide was added to each dry slide dissolved in 2 × SSC buffer; then, the slide was covered with a parafilm piece and incubated in an oven at 80–90 °C for 5 min. We dehydrated the slides by treating them with cold alcoholic dilutions at −20 °C (70, 80, 90, and 100%ethanol) for 5 min for each dilution, and then left the slides to air dry. Following that, 10 µL of solution was mixed by combining 2µL of the labeled probe (centromere or/and telomere or/and 45s rDNA probe, designed as shown in Table S2) with 8 µL the hybridization cocktail for each slide. We poured 10 µL mixture for each slide and covered with a coverslip; slides were incubated at 85 °C for 5 min to denature the mixture and then cooled directly on ice for 5 min. The slices were incubated in a slide box with high humidity to prevent the slices from drying out at 37°C overnight while avoiding bright light. The covers were removed in a 2 × SSC buffer and then washed in 2 × SSC buffer three times for 15 min each time, and then one time with 1 × PBS buffer for 5 min. The slides were air-dried, 10 µL DAPI was applied on each slide, and we covered with a coverslip. The slides were observed under a fluorescent microscope.

3. Results

3.1. The absence of *TOP1α* resulted in reduced fertility and impaired the process of meiosis

To investigate how the absence of functional *AtTOP1α* affects pollen viability and anther starch accumulation, we used Alexander Red staining to assess pollen viability and PCR analysis to confirm the genotype of *top1α1* mutants. Our results revealed that the wild-type (WT) anthers were full of pollen compared to *top1α1* anthers, which had few pollen grains (Figure1A and 1B, respectively). We also examined the tetrad stages. While the WT tetrads had four microspores (Figure 1C), tetrads contained polyads with small microspores, suggesting the occurrence of a meiotic abnormality. A total of 50 observations were conducted, revealing that 6% of the observed tetrads had abnormalities (Figure 1D, E). The homozygous mutants developed normally but with early flowering and differences in leaf shape and internodal distance (Takahashi *et al.*, 2002). Since the T-DNA insertion disrupts *AtTOP1α* gene expression, a continuous mRNA transcript cannot be generated across the insertion site. Therefore, the

expression level of AtTOP1α cannot be determined using the presented semi-quantitative PCR (Figure 1F) and qPCR (Figure 1G) data.

We also investigated pollen fertility using I2/KI staining and observed many immature pollen grains in the mutant; some of the top1α1 pollen grains were larger than wild-type pollen (Figure S1).

3.2. The disruption of TOP1α influenced the behavior of chromosomes during prophase

To assess chromosomal behavior during male meiosis in WT and top1α1 mutants, we employed DAPI staining. This revealed that meiosis in the mutants appeared largely normal compared to WT, with only minor variations observed in some stages (Figure 2). Both WT and *top1α1* meiotic chromosomes condensed at leptotene and displayed generally identical morphologies (Figure 2A and 2A1, respectively). However, in *top1α1* at zygotene and pachytene, a chromosome displayed bright large blocks (large distances of heterochromatin) (Figure 2B1 and 2C1, respectively).WT meiocytes had compact homologs at diplotene (Figure 2D), but the *top1α1* chromosomes were crisp, with thinner regions than usual (Figure 2D1). In diakinesis, the WT showed five complete bivalents (Figure 2E), while some entanglements existed between the bivalents in *top1α1* (Figure 2E1). At metaphase I, the WT bivalents were regular and lined up (Figure 2F), while the *top1α1* bivalents were often thin with a few crossovers and two or more *top1α1* chromosomes frequently linked together (Figure 2F1). After meiotic II division, chromosome crowding was observed in *top1α1* at anaphase I and telophase I (Figure 2G1 and2H1, respectively). The presence of conglomerates during meiosis I was consistent with the lack of *AtTOP1α* function, which plays a critical role in developing appropriate DNA structure and topology, fixing kinks, torsions, and dealing with tight-twisted intermediates produced during replication.

3.3. TOP1α is required to untangle chromosomes at the centromere regions

The centromere is a region on each linear chromosome necessary for correct chromosome segregation. The centromere is the smallest chromatin element necessary for the kinetochore's normal formation, a protein-rich structure connecting microtubules to chromosomes (Lawrimore and Bloom, 2022).

To test whether the absence of the TOP1α function affects the disentanglement at the chromosome level during meiosis I, we performed FISH using a centromere probe on top1α1 meiotic cells to examine chromosome pairing and synapsis, to see whether *top1α1* bivalents occurred between homologs or non-homologs. FISH analyses with a centromere probe exhibited a similar number of signals in WT and *top1α1* at leptotene, with wild-type cells displayed 10 centromere foci, while top1α1 cells had 8-10 foci (Figure 3A and 3B, respectively).

At zygotene and pachytene, Col-0 WT cells had 10 (n = 85) and 5 (n = 120) centromeric foci, respectively (Figure 3C,E); in contrast, there were fewer and larger foci in *top1α1* at zygotene, ranging between 6 (~16%, 28/175), 5 (~26%, 45/175), 4 (~23%, 40/175), 3 (~23%, 40/175), 2 (~9%, 15/175), and 1 (~3%, 5/175) (Figure 3D). Several *top1α1* cells at the zygotene stage also displayed signal accumulation and conglomerates in unusual locations compared to WT (Figure S2).

The number of chromosome signals decreased at the pachytene stage. The number of foci ranged from 5 (~11.5%, 30/260), 4 (~19%, 50/260), 3 (~52%, 135/260), 2 (~13.5, 35/260), to 1

(~4%, 10/260), with a large signal (Figure 3F), which was confirmed by multiple cell analysis of *top1α1*at the pachytene stage (Figure S3).

At diakinesis, the WT had five pairs of homologous chromosomes and five signals (Figure 3G). In contrast, the *top1α1* chromosomes were grouped and overlapping, with signals concentrated in two locations (Figure 3H). At metaphase I, in WT, five pairs of centromere foci on five bivalents were positioned on a single line (Figure 3I), while in *top1α1*, chromosomes were in abnormal alignment, and there was a tangle between non-homologous chromosomes but also 10 foci (Figure 3J), revealing a non-homologous association. Results for WT and *top1α1*cells from anaphase I to telophase II were similar (Figure 3). These observations indicate that *top1α1* had abnormalities in pairing and synapsis around the centromeres.

In many organisms, telomeres are arranged as "a bouquet" on the nuclear envelope to promote pairing and synapsis. While *Arabidopsis* does not have a traditional bouquet, it is believed that the same outcome is achieved by clumping telomeres around the nucleolus before leptotene (Wang *et al.*, 2012; Shakirov *et al.*, 2022; Silkova *et al.*, 2022).

We observed this "clumping" pattern in both WT and top1α1 telomere foci at leptotene (Figure S4A and S4B, respectively), at zygotene (Figure S4C and S4D, respectively), and at pachytene (Figure S4E and S4F, respectively), and we found WT and top1α1 both had similar numbers of signals (9–10, 10 and 10 telomere foci, respectively).

3.4. Disruption of TOP1α affected 45s DNA localization in A. thaliana

In *Arabidopsis* (Col-0), 45S rDNA is located insubtelomeric groups on the second and the fourth chromosomes (Delorme-Hinoux *et al.*, 2023).Copied 45S rDNA units create the nucleolus organizer regions (NORs) (Fultz *et al.*, 2023). We conducted FISH analysis targeting 45S rDNA to examine pairing in *top1α1* plants compared to WT. The 45s rDNA fociin WT and *top1α1* were identical at leptotene and zygotene (Figure 4A, 4C and 4B, 4D, respectively). At pachytene, the WT had a single intense signal (Figure 4E), while the majority of the *top1α1* samples had two separate signals (Figure 4F), which indicates partial separation of the 45S rDNA regions. This result shows that homolog chromosomes in *top1α1* were incorrectly paired and synapsed easily at the 45S rDNA region. We also observed the presence of signals on three bivalents in *top1α1* at diakinesis and metaphase I (Figure 4H and 4J, respectively) and more images at metaphase I (Figure S5), to clarify these defects in the *top1α1*.

3.5. TOP1α promotes crossover in *A. thaliana*

Chiasma visualization during diplotene and diakinesis in Arabidopsis thaliana is hindered by the difficulty in differentiating between true chiasmata and non-recombinant twists of homologous chromosomes. Additionally, the close association of nucleolus organizer regions (NORs) with the nucleolus in the short arms of chromosomes 2 and 4 impedes chiasma identification in these regions. Therefore, despite the condensed state of chromosomes at metaphase I, this stage offers a more suitable platform for chiasma scoring in Arabidopsis (López *et al.*, 2011; Sims *et al.*, 2021). We investigated and documented chiasma frequency in wild-type (WT) and top1α1 mutant pollen mother cells (PMCs) at metaphase I using fluorescence in situ hybridization (FISH) labeling with a centromeric probe, following established protocols (Wang *et al.*, 2023). Analysis revealed a consistent pattern of five bivalents formed by the five chromosome pairs in all examined cells. These bivalents could be categorized as rods or rings. Rods displayed chiasmata in only one chromosome arm, while rings exhibited

chiasmata in both arms. Figure 5 demonstrates a significant difference in chiasma number between WT and top1α1. The mutant exhibited an increased frequency of cells containing 5, 6, or 7 chiasmata compared to WT. Conversely, WT displayed a higher frequency of cells with more than 7 chiasmata. This suggests that functional TOP1α does not influence crossovers in Arabidopsis. Based on these findings, we conclude that TOP1α promotes crossover events in Arabidopsis.

The atm mutant is known to have defects in meiosis and produces fewer numbers of seeds (Zhao *et al.*, 2023). While the pachytene stage in atm PMCs appears normal(Kurzbauer *et al.*, 2018), diakinesis shows intertwining between the non-homologous chromosomes (Figure 6E).At metaphase I, multivalent links involving three or more chromosomes form alongside univalents (Figure 6F).Bridges between chromosome groups and chromosome fragmentation are observed throughout anaphase I (Figure 6G and H), metaphase II (Figure 6I), anaphase II (Figure 6J), and telophase II (Figure 6K). Telophase II cells also exhibit unequal chromosome numbers (Figure 6G, 6I, 6J, 6K).

We developed the *atm×top1α1* double mutant and examined its chromosomal behavior. Compared tothe single-atm mutant, we observed incomplete synapsis of homologous chromosomes at zygotene and pachytene (Figure 7A–C and 7D,E, respectively).Additionally, some DNA fragments fail to segregate to cell corners at anaphase I (Figure 7F).These defects lead to the production of multiple polyads (five unbalanced microspores) at telophase II in the double mutant (Figure 7G–I).

4. Discussion

The defects in meiotic processes can cause genome instability or even death in proliferating cells (Hou *et al.*, 2021). Here, we demonstrate that *Arabidopsis* TOP1α is required for chromosome relaxation during meiosis. Large distances of heterochromatin at early stages (zygotene and pachytene), also chromosome compacted (Figure 2B1 and 2C1, respectively), were observed in top1α1 mutant. This observation aligns with the known function of TOP1α in resolving torsional stress during DNA transactions (Tan and Tse-Dinh, 2024).

In mammalian cells, centromeric chromatin distribution is regulated epigenetically (Morrison and Thakur, 2021). When we performed FISH analysis with a centromere probe, we observed clusters of signals at the heterochromatin sites in zygotene and pachytene (Figure 3). This was evident in most cells in both zygotene (Figure S2) and pachytene (Figure S3). Therefore, we suggest that TOP1α has an essential role in regulating the centromeric region, which controls the regulation of multiple functions.

In addition, a defect in 45s was observed when using a 45s rDNA probe with FISH (Figures 4 and S5). From pachytene to late stages, the signal was divided into two halves at pachytene in the top1α1 mutant and observed on three bivalents in the top1α1 mutant, instead of two in the WT diakinesis and metaphase I. These findings also suggest that TOP1α plays a role in the proper organization of centromeric and pericentromeric regions. Meiotic centromeres tend to disassemble, but transient proximity to telomeres ensures they reassemble (Klutstein *et al.*, 2015). TOP1α affected the reassembly of the centromeric regions after prior disassembly (Hou *et al.*, 2021; Shao *et al.*, 2021).

Similar to yeast and humans, *Arabidopsis* exhibits at least two functionally distinct classes of crossover (CO) events (France *et al.*, 2021; Singh *et al.*, 2023). Class I CO is sensitive to

interference and requires the ZMM proteins Zip1, Zip2, Zip3, Zip 4/Spo22, and Msh4/Msh5 (Dluzewska *et al.*, 2023). In contrast, Class II COs depend on MUS81 and are interference-insensitive (Li *et al.*, 2021). We observed reductions in chiasma frequency in *top1α1* compared with the WT (Figure 5); this loss of COs is consistent with the region's instability between the centromere and telomere (de Massy, 2013; Kim and Choi, 2022; Ito and Shinohara, 2023). To examine CO formation in *top1a1*, we applied DAPI staining combined withFISH to metaphase I chromosome spreads to observe chiasmata in chromosome pairs (Armstrong, 2013).The chiasmata count indicated a decrease in the total COs in *top1α1* compared to the WT (Figure 5). We, therefore, conclude that TOP1α affects crossover occurrence through the regulation of DNA torsion. DNA topoisomerases are critical enzymes responsible for relaxing supercoiled DNA during DNA duplication, transcription, and other cellular transactions by decreasing the strain in DNA. These enzymes allow the passage from single-strand to double-strand DNA and vice versa (Soren *et al.*, 2020; Duprey and Groisman, 2021; Jian and Osheroff, 2023).

In various organisms, ATM functions as a negative regulator of meiotic double-strand break (DSB) initiation (Kurzbauer *et al.*, 2021; Láscarez-Lagunas *et al.*, 2022). We suggest that exacerbated defects observed in the double mutant which exhibited incomplete synapsis and chromosome fragmentation compared to the atm-2 single mutant, were due to the absence of the functional TOP1α affecting DNA repair (Figure 7). The double mutant also exhibited polyad at telophase II (Figure 7G–I), demonstrating that TOP1α and ATM play a critical role in DNA repair. Our study sheds light on the intricate roles of ATM and TOP1α in the context of meiotic DNA repair. These two genes, while sharing a common involvement in DNA repair processes, exhibit distinct mechanisms and operate within different cellular pathways. ATM is primarily recognized for its pivotal role in the DNA damage response, functioning as a sentinel for detecting and initiating signaling cascades in response to DSBs. In contrast, the TOP1α role centers on resolving topological challenges that emerge during DNA replication and transcription processes (Zhang *et al.*, 2022c).

A notable aspect of our findings is the potential for synergistic interactions between ATM and TOP1α within the context of the double mutant. Although these genes may not be explicitly aligned within the same canonical DNA repair pathway, the cumulative loss of ATM and TOP1α in the double mutant suggests the possibility of a more pronounced defect in meiotic DNA repair. This observation hints at the likelihood of a functional interplay or redundancy between these genes, whereby their combined absence yields a notable impact on the repair machinery.

5. Conclusions

This study provides valuable insights into the involvement of DNA topoisomerase 1α (AtTOP1 α) in male reproductive development in *A. thaliana*. The findings demonstrate that *AtTOP1α* plays a role in the meiotic division process, as its absence leads to aberrant chromatin behaviors and various defects during meiosis. Specifically, the top1α1 mutant displays notable variation in heterochromatin distances, clusters of centromere signals, defective distribution of 45s rDNA signals, and reduced frequency of chiasma formation. Furthermore, the *atm-2×top1a1* double mutant exhibits additional defects, including incomplete synapsis, DNA fragmentation, and the occurrence of polyads.

Our results highlight the critical role of DNA topoisomerase 1α in ensuring accurate meiotic completion and promoting crossover formation. Moreover, it suggests that *AtTOP1α* functions in conjunction with ATM in the same DNA repair pathway. This study contributes to

our understanding of the molecular mechanisms underlying male reproductive development and sheds light on potential targets for further research. It is important to acknowledge that the precise nature of this interaction and whether ATM and TOP1α converge on common downstream effectors necessitate further in-depth investigation. While our study provides a foundational exploration of the roles played by these genes in meiotic DNA repair, we recognize the need for more comprehensive mechanistic studies to dissect their individual contributions and uncover any potential crosstalk. These future investigations will be essential for elucidating the exact mechanisms underpinning the observed phenotypes and refining our understanding of the complex interplay between ATM and TOP1α in DNA repair during meiosis.

Future research should investigate the specific mechanisms by which *AtTOP1α* regulates chromatin behaviors during meiotic division. Additionally, investigating the interaction between *AtTOP1α* and ATM in DNA repair pathways could provide further insights into their cooperative functions. Furthermore, the investigation of the transcriptome and analysis of gene expression associated with meiosis in single-mutant *top1α1, atm*, and double-mutant *atm-2×top1a1* in anthers at various stages of meiosis can provide valuable insights into the role of TOP1 in crossover processes. Exploring the impact of *AtTOP1α* on other aspects of plant development and fertility would broaden our understanding of its overall significance.

Overall, this study highlights the importance of DNA topoisomerase 1α in male reproductive development and opens up avenues for future research to unravel its precise roles and mechanisms in plant reproduction.

Author Contributions

Conceptualization, I.E.E., L.Y., and C.C.; methodology, I.E.E., L.Y., and C.C.; software, I.E.E., L.Y., A.A.H., A.M.H., M.H., F.A.S., D.A-E., S.M.A., N.M.A., W.F.S., and C.C.; validation, I.E.E., L.Y., A.A.H., A.M.H., M.H., F.A.S., D.A-E., S.M.A., N.M.A., W.F.S., and C.C.; formal analysis, I.E.E., L.Y., A.A.H., A.M.H., M.H., F.A.S., D.A-E., S.M.A., N.M.A., W.F.S., and C.C.; investigation, I.E.E., L.Y., M.H., and C.C.; resources, I.E.E., L.Y., A.A.H., A.M.H., M.H., F.A.S., D.A-E., S.M.A., N.M.A., W.F.S., and C.C.; data curation, I.E.E., L.Y., A.A.H., A.M.H., M.H., F.A.S., D.A-E., S.M.A., N.M.A., W.F.S., and C.C.; writing—original draft preparation, I.E.E., L.Y., A.A.H., A.M.H., M.H., F.A.S., D.A-E., S.M.A., N.M.A., W.F.S., and C.C.; writing—review and editing, I.E.E., L.Y., A.A.H., A.M.H., M.H., F.A.S., D.A-E., S.M.A., N.M.A., W.F.S., and C.C.; visualization, I.E.E., L.Y., A.A.H., A.M.H., M.H., F.A.S., D.A-E., S.M.A., N.M.A., W.F.S., and C.C.; supervision, L.Y., and C.C.; project administration, L.Y., F.A.S., and C.C.; funding acquisition, L.Y., F.A.S., and C.C. All authors have read and agreed to the published version of the manuscript.

Funding

This work was funded by the National Natural Science Foundation of China (No.31970525), the Advanced Foreign Experts Project (G2023157014L), and the Cultivating Fund Project of Hubei Hongshan Laboratory (2022hspy002). This work was also supported by the Princess Nourah bint Abdulrahman University (PNURSP2024R318), and the Deanship of Scientific Research, King Faisal University, Saudi Arabia

Conflicts of Interest

The authors declare no conflicts of interest.

References

Alavattam, K.G., Maezawa, S., Andreassen, P.R., Namekawa, S.H. 2021. Meiotic sex chromosome inactivation and the XY body: a phase separation hypothesis. Cellular and Molecular Life Sciences 79, 18. 10.1007/s00018-021-04075-3

Armstrong, S., 2013. Spreading and fluorescence in situ hybridization of male and female meiocyte chromosomes from Arabidopsis thaliana for cytogenetical analysis, Plant Meiosis. Springer, pp. 3-11.

Chang, F., Zhang, Z., Jin, Y., Ma, H., 2014. Cell biological analyses of anther morphogenesis and pollen viability in arabidopsis and rice, Flower Development. Springer, pp. 203-216.

Chang, S., Li, C., Jiang, Y., Long, Y., Li, Y., Yin, J. 2021. Characteristics of the pollen morphology and viability of Bougainvillea (Nyctaginaceae). Scientia Horticulturae 277, 109732. 10.1016/j.scienta.2020.109732

Cui, D.-L., Xu, C.-X., Wang, P., Gao, T.-Y., Wang, B., Yu, T.-Y. 2024. Male gametogenesis in flowering plants. Frontiers in Sustainable Food Systems 7, 1333544.

de Massy, B. 2013. Initiation of meiotic recombination: how and where? Conservation and specificities among eukaryotes. Annu. Rev. Genet 47, 563-599. 10.1146/annurev-genet-110711-155423

Delorme-Hinoux, V., Mbodj, A., Brando, S., De Bures, A., Llauro, C., Covato, F., Garrigue, J., Guisset, C., Borrut, J., Mirouze, M., Reichheld, J.P., Sáez-Vásquez, J. 2023. 45S rDNA Diversity In Natura as One Step towards Ribosomal Heterogeneity in Arabidopsis thaliana. Plants (Basel) 12. 10.3390/plants12142722

Desjardins, S.D., Simmonds, J., Guterman, I., Kanyuka, K., Burridge, A.J., Tock, A.J., Sanchez-Moran, E., Franklin, F.C.H., Henderson, I.R., Edwards, K.J., Uauy, C., Higgins, J.D. 2022. FANCM promotes class I interfering crossovers and suppresses class II non-interfering crossovers in wheat meiosis. Nature Communications 13, 3644. 10.1038/s41467-022-31438-6

Dinh, T.T., Gao, L., Liu, X., Li, D., Li, S., Zhao, Y., O'Leary, M., Le, B., Schmitz, R.J., Manavella, P.A., Li, S., Weigel, D., Pontes, O., Ecker, J.R., Chen, X. 2014. DNA topoisomerase 1α promotes transcriptional silencing of transposable elements through DNA methylation and histone lysine 9 dimethylation in Arabidopsis. PLOS Genet 10, e1004446. 10.1371/journal.pgen.1004446

Dluzewska, J., Dziegielewski, W., Szymanska-Lejman, M., Gazecka, M., Henderson, I.R., Higgins, J.D., Ziolkowski, P.A. 2023. MSH2 stimulates interfering and inhibits non-interfering crossovers in response to genetic polymorphism. Nat Commun 14, 6716. 10.1038/s41467-023-42511-z

Duprey, A., Groisman, E.A. 2021. The regulation of DNA supercoiling across evolution. Protein Sci 30, 2042-2056. 10.1002/pro.4171

Durand-Dubief, M., Svensson, J.P., Persson, J., Ekwall, K. 2011. Topoisomerases, chromatin and transcription termination. Transcription 2, 66-70. 10.4161/trns.2.2.14411

France, M.G., Enderle, J., Röhrig, S., Puchta, H., Franklin, F.C.H., Higgins, J.D. 2021. ZYP1 is required for obligate cross-over formation and cross-over interference in Arabidopsis. Proceedings of the National Academy of Sciences 118, e2021671118. 10.1073/pnas.2021671118

Fultz, D., McKinlay, A., Enganti, R., Pikaard, C.S. 2023. Sequence and epigenetic landscapes of active and silent nucleolus organizer regions in Arabidopsis. Sci Adv 9, eadj4509. 10.1126/sciadv.adj4509

Gamarra, N., Narlikar, G.J. 2021. Collaboration through chromatin: motors of transcription and chromatin structure. J Mol Biol 433, 166876. 10.1016/j.jmb.2021.166876

Gong, X., Shen, L., Peng, Y.Z., Gan, Y., Yu, H. 2017. DNA Topoisomerase Iα Affects the Floral Transition. Plant Physiol 173, 642-654. 10.1104/pp.16.01603

Groelly, F.J., Fawkes, M., Dagg, R.A., Blackford, A.N., Tarsounas, M. 2023. Targeting DNA damage response pathways in cancer. Nature Reviews Cancer 23, 78-94. 10.1038/s41568-022-00535-5

Gu, N., Chen, C., Kabeya, Y., Hasebe, M., Tamada, Y. 2022. Topoisomerase 1α is required for synchronous spermatogenesis in Physcomitrium patens. New Phytol 234, 137-148. 10.1111/nph.17983

Herbst, J., Li, Q.-Q., De Veylder, L. 2024. Mechanistic insights into DNA damage recognition and checkpoint control in plants. Nature Plants 10, 539-550. 10.1038/s41477-024-01652-9

Hofstatter, P.G., Thangavel, G., Castellani, M., Marques, A.J. 2021. Meiosis progression and recombination in holocentric plants: what is known? Frontiers in Plant Science 12, 658296. 10.3389/fpls.2021.658296

Hou, H., Kyriacou, E., Thadani, R., Klutstein, M., Chapman, J.H., Cooper, J.P. 2021. Centromeres are dismantled by foundational meiotic proteins Spo11 and Rec8. Nature 591, 671-676. 10.1038/s41586-021-03279-8

Ito, M., Shinohara, A. 2023. Chromosome architecture and homologous recombination in meiosis. Frontiers in Cell and Developmental Biology 10, 1097446. 10.3389/fcell.2022.1097446

Jian, J.Y., Osheroff, N. 2023. Telling Your Right Hand from Your Left: The Effects of DNA Supercoil Handedness on the Actions of Type II Topoisomerases. Int J Mol Sci 24. 10.3390/ijms241311199

Kim, H., Choi, K. 2022. Fast and Precise: How to Measure Meiotic Crossovers in Arabidopsis. Mol Cells 45, 273-283. 10.14348/molcells.2022.2054

Klutstein, M., Fennell, A., Fernández-Álvarez, A., Cooper, J.P. 2015. The telomere bouquet regulates meiotic centromere assembly. Nat. Cell Biol 17, 458-469. 10.1038/ncb3132

Kumar Koul, K., Nagpal, R. 2023. Female meiosis in plants, and differential recombination in the two sexes: a perspective. The Nucleus 66, 195-203. 10.1007/s13237-023-00417-7

Kurzbauer, M.-T., Pradillo, M., Kerzendorfer, C., Sims, J., Ladurner, R., Oliver, C., Janisiw, M.P., Mosiolek, M., Schweizer, D., Copenhaver, G.P. 2018. Arabidopsis thaliana FANCD2 promotes meiotic crossover formation. Plant Cell 30, 415-428. 10.1105/tpc.17.00745

Kurzbauer, M.T., Janisiw, M.P., Paulin, L.F., Prusén Mota, I., Tomanov, K., Krsicka, O., Haeseler, A.V., Schubert, V., Schlögelhofer, P. 2021. ATM controls meiotic DNA double-strand break formation and recombination and affects synaptonemal complex organization in plants. Plant Cell 33, 1633-1656. 10.1093/plcell/koab045

Kyung, J., Jeon, M., Lee, I. 2022. Recent advances in the chromatin-based mechanism of FLOWERING LOCUS C repression through autonomous pathway genes. Front Plant Sci 13, 964931. 10.3389/fpls.2022.964931

Láscarez-Lagunas, L.I., Nadarajan, S., Martinez-Garcia, M., Quinn, J.N., Todisco, E., Thakkar, T., Berson, E., Eaford, D., Crawley, O., Montoya, A., Faull, P., Ferrandiz, N., Barroso, C., Labella, S., Koury, E., Smolikove, S., Zetka, M., Martinez-Perez, E., Colaiácovo, M.P. 2022. ATM/ATR kinases link the synaptonemal complex and DNA double-strand break repair pathway choice. Curr Biol 32, 4719-4726.e4714. 10.1016/j.cub.2022.08.081

Lawrimore, J., Bloom, K. 2022. Shaping centromeres to resist mitotic spindle forces. Journal of Cell Science 135, jcs259532. 10.1242/jcs.259532

Li, X., Zhang, J., Huang, J., Xu, J., Chen, Z., Copenhaver, G.P., Wang, Y. 2021. Regulation of interference-sensitive crossover distribution ensures crossover assurance in Arabidopsis. Proc Natl Acad Sci USA 118. 10.1073/pnas.2107543118

Liu, Q., Yang, J., Wang, X., Zhao, Y., 2023. Studies on Pollen Morphology, Pollen Vitality and Preservation Methods of Gleditsia sinensis Lam. (Fabaceae), Forests.

Liu, X., Gao, L., Dinh, T.T., Shi, T., Li, D., Wang, R., Guo, L., Xiao, L., Chen, X. 2014. DNA topoisomerase I affects polycomb group protein-mediated epigenetic regulation and plant development by altering nucleosome distribution in Arabidopsis. Plant cell 26, 2803-2817. 10.1105/tpc.114.124941

Lloyd, A. 2023. Crossover patterning in plants. Plant Reproduction 36, 55-72. 10.1007/s00497-022-00445-4

López, E., Pradillo, M., Oliver, C., Romero, C., Cuñado, N., Santos, J.L. 2011. Looking for natural variation in chiasma frequency in Arabidopsis thaliana. J. Exp. Bot 63, 887-894. 10.1093/jxb/err319

Lorenz, A., Mpaulo, S.J. 2022. Gene conversion: a non-Mendelian process integral to meiotic recombination. Heredity 129, 56-63. 10.1038/s41437-022-00523-3

Ma, H., Shi, Q. 2014. Meiosis. J Genet Genomics 41, 83-186.

Mao-Sen, L., Shih-Hsuan, T., Ting-Chu, C., Mei-Chu, C. 2021. Visualizing Meiotic Chromosome Pairing and Segregation in Interspecific Hybrids of Rice by Genomic in situ Hybridization. Rice Science 28, 69-80. 10.1016/j.rsci.2020.11.008

McKie, S.J., Neuman, K.C., Maxwell, A. 2021. DNA topoisomerases: Advances in understanding of cellular roles and multi-protein complexes via structure-function analysis. Bioessays 43, e2000286. 10.1002/bies.202000286

Morrison, O., Thakur, J., 2021. Molecular Complexes at Euchromatin, Heterochromatin and Centromeric Chromatin, International Journal of Molecular Sciences.

Plötner, M. 2023. New insights into the molecular basis of gametogenesis of the hybridogenetic water frog Pelophylax esculentus. 10.18452/25739

Prusicki, M.A., Balboni, M., Sofroni, K., Hamamura, Y., Schnittger, A. 2021. Caught in the act: Live-cell imaging of plant meiosis. Frontiers in Plant Science 12, 718346. 10.3389/fpls.2021.718346

Sen, S., Dodamani, A., Nambiar, M., 2023. Chapter Seven - Emerging mechanisms and roles of meiotic crossover repression at centromeres, in: Cole, F. (Ed.), Current Topics in Developmental Biology. Academic Press, pp. 155-190.

Shafiq, S., Chen, C., Yang, J., Cheng, L., Ma, F., Widemann, E., Sun, Q. 2017. DNA Topoisomerase 1 Prevents R-loop Accumulation to Modulate Auxin-Regulated Root Development in Rice. Mol Plant 10, 821-833. 10.1016/j.molp.2017.04.001

Shakirov, E.V., Chen, J.J.-L., Shippen, D.E. 2022. Plant telomere biology: The green solution to the end-replication problem. The Plant Cell 34, 2492-2504. 10.1093/plcell/koac122

Shao, F., Liu, X., Zhang, X., Wang, Q., Wang, W. 2021. Methylation of 45S Ribosomal DNA (rDNA) Is Associated with Cancer and Aging in Humans. Int J Genomics 2021. 10.1155/2021/8818007

Silkova, O.G., Loginova, D.B., Zhuravleva, A.A., Shumny, V.K. 2022. Bouquet Formation Failure in Meiosis of F(1) Wheat-Rye Hybrids with Mitotic-Like Division. Plants (Basel) 11. 10.3390/plants11121582

Sims, J., Schlögelhofer, P., Kurzbauer, M.T. 2021. From Microscopy to Nanoscopy: Defining an Arabidopsis thaliana Meiotic Atlas at the Nanometer Scale. Front Plant Sci 12, 672914. 10.3389/fpls.2021.672914

Singh, D.K., Lian, Q., Durand, S., Fernandes, J.B., Chambon, A., Hurel, A., Walkemeier, B., Solier, V., Kumar, R., Mercier, R. 2023. HEIP1 is required for efficient meiotic crossover implementation and is conserved from plants to humans. Proceedings of the National Academy of Sciences 120, e2221746120. 10.1073/pnas.2221746120

Soren, B.C., Dasari, J.B., Ottaviani, A., Iacovelli, F., Fiorani, P. 2020. Topoisomerase IB: a relaxing enzyme for stressed DNA. Cancer Drug Resist 3, 18-25. 10.20517/cdr.2019.106

Takahashi, T., Matsuhara, S., Abe, M., Komeda, Y. 2002. Disruption of a DNA topoisomerase I gene affects morphogenesis in Arabidopsis. Plant Cell 14, 2085-2093. 10.1105/tpc.001925

Tan, K., Tse-Dinh, Y.C. 2024. Variation of Structure and Cellular Functions of Type IA Topoisomerases across the Tree of Life. Cells 13. 10.3390/cells13060553

Thangavel, G., Hofstatter, P.G., Mercier, R., Marques, A. 2023. Tracing the evolution of the plant meiotic molecular machinery. Plant Reproduction 36, 73-95. 10.1007/s00497-022-00456-1

Wang, C., Chen, Z., Copenhaver, G.P., Wang, Y. 2024. Heterochromatin in plant meiosis. Nucleus 15, 2328719. 10.1080/19491034.2024.2328719

Wang, S., Zickler, D., Kleckner, N., Zhang, L. 2015. Meiotic crossover patterns: obligatory crossover, interference and homeostasis in a single process. Cell cycle 14, 305-314. 10.4161/15384101.2014.991185

Wang, T., Wang, H., Lian, Q., Jia, Q., You, C., Copenhaver, G.P., Wang, C., Wang, Y. 2023. HEI10 is subject to phase separation and mediates RPA1a degradation during meiotic interference-sensitive crossover formation. Proc Natl Acad Sci U S A 120, e2310542120. 10.1073/pnas.2310542120

Wang, Y., Cheng, Z., Huang, J., Shi, Q., Hong, Y., Copenhaver, G.P., Gong, Z., Ma, H. 2012. The DNA Replication Factor RFC1 Is Required for Interference-Sensitive Meiotic Crossovers in Arabidopsis thaliana. PLoS Genet 8, e1003039. 10.1371/journal.pgen.1003039

Wang, Y., Cheng, Z., Lu, P., Timofejeva, L., Ma, H., 2014. Molecular cell biology of male meiotic chromosomes and isolation of male meiocytes in Arabidopsis thaliana, Flower Development. Springer, pp. 217-230.

Wang, Y., van Rengs, W.M., Zaidan, M.W.A.M., Underwood, C.J. 2021. Meiosis in crops: from genes to genomes. Journal of experimental botany 72, 6091-6109. 10.1093/jxb/erab217

Xin, X., Li, X., Zhu, J., Liu, X., Chu, Z., Shen, J., Wu, C. 2021. OsMLH1 interacts with OsMLH3 to regulate synapsis and interference-sensitive crossover formation during meiosis in rice. J Genet Genomics 48, 485-496. 10.1016/j.jgg.2021.04.011

Yamaya, K., Wang, B., Memar, N., Odiba, A.S., Woglar, A., Gartner, A., Villeneuve, A.M. 2023. Disparate roles for C. elegans DNA translocase paralogs RAD-54. L and RAD-54. B in meiotic prophase germ cells. Nucleic Acids Research 51, 9183-9202. 10.1093/nar/gkad638

Yang, T., Wang, D., Tian, G., Sun, L., Yang, M., Yin, X., Xiao, J., Sheng, Y., Zhu, D., He, H., Zhou, Y. 2022. Chromatin remodeling complexes regulate genome architecture in Arabidopsis. The Plant Cell 34, 2638-2651. 10.1093/plcell/koac117

You, H., Tang, D., Liu, H., Zhou, Y., Li, Y., Shen, Y., Gong, Z., Yu, H., Gu, M., Jiang, J. 2024. Chromosome ends initiate homologous chromosome pairing during rice meiosis. Plant Physiology, kiae152. 10.1093/plphys/kiae152

Zhang, H., Guo, L., Li, Y., Zhao, D., Liu, L., Chang, W., Zhang, K., Zheng, Y., Hou, J., Fu, C., Zhang, Y., Zhang, B., Ma, Y., Niu, Y., Zhang, K., Xing, J., Cui, S., Wang, F., Tan, K., Zheng, S., Tang, W., Dong, J., Liu, X. 2022a. TOP1α fine-tunes TOR-PLT2 to maintain root tip homeostasis in response to sugars. Nat. Plants. 10.1038/s41477-022-01179-x

Zhang, H., Guo, L., Li, Y., Zhao, D., Liu, L., Chang, W., Zhang, K., Zheng, Y., Hou, J., Fu, C., Zhang, Y., Zhang, B., Ma, Y., Niu, Y., Zhang, K., Xing, J., Cui, S., Wang, F., Tan, K., Zheng, S., Tang, W., Dong, J., Liu, X. 2022b. TOP1α fine-tunes TOR-PLT2 to maintain root tip homeostasis in response to sugars. Nature Plants 8, 792-801. 10.1038/s41477-022-01179-x

Zhang, H., Tang, Z., Zhang, Y., Liu, L., Zhao, D., Liu, X., Guo, L., Dong, J. 2022c. TOP1α suppresses lateral root gravitropism in Arabidopsis. Plant Signal Behav 17, 2098646. 10.1080/15592324.2022.2098646

Zhao, J., Gui, X., Ren, Z., Fu, H., Yang, C., Wang, W., Liu, Q., Zhang, M., Wang, C., Schnittger, A., Liu, B. 2023. ATM-mediated double-strand break repair is required for meiotic genome stability at high temperature. Plant J 114, 403-423. 10.1111/tpj.16145

Zhong, P., Li, J., Luo, L., Zhao, Z., Tian, Z. 2019. TOP1α regulates FLOWERING LOCUS C expression by coupling histone modification and transcription machinery. Development 146. 10.1242/dev.167841

Zickler, D., Kleckner, N. 2023. Meiosis: dances between homologs. Annual Review of Genetics 57, 1-63. 10.1146/annurev-genet-061323-044915

Figure legends

Figure 1. *top1α1* mutation reduces pollen viability, disrupts meiosis. (**A**)The anther of the wild-type stained with Alexander Red to assess pollen viability exhibited a density of pollen grains. (**B**) The anthers of the top1α1 mutant displayed a significantly lower abundance of pollen grains. (**C**) Wild-type tetrads had four microspores. (**D**,**E**) *top1α1* contained polyads with small microspores and displayed meiotic defects with 6% tetrad abnormality. Gene expression of *AtTOP1α* gene in both wild type and *top1α1* line using semi-quantitative PCR (**F**) and qPCR (**G**). Scale bars= 50 μm (**A**,**B**) and 20 μm (**C**–**E**).

Figure 2. DAPI staining of meiotic chromosomes revealed abnormal chromosomal behavior in top1α1 mutants during prophase I.Wild-type chromosome behavior is shown in panels A-K, while panels A1-K1 correspond to top1α1. Unlike the wild-type, top1α1 displayed abnormal chromosome features from zygotene to metaphase I, including the formation of multivalent links between chromosomes instead of normal bivalents and interlocks between homologous chromosomes. Chromosomes were stained with DAPI. Scale bar = 10μm.

Figure 3. *top1α1* induces unbalanced chromosome segregation. FISH analysis with a centromere probe. (A-P) Wild-type (A, C, E, G, I, K, M, O) and top1α1 (B, D, F, H, J, L, N, P) meiotic cells at leptotene (A, B), zygotene (C, D), pachytene (E, F), diakinesis (G, H), metaphase I (I, J), anaphase I (K, L), metaphase II (M, N), and anaphase II (O, P) stages.FISH with a centromere probe revealed a similar number of signals in wild-type and top1α1 cells at leptotene (10 in wild-type and 8-10 in top1α1). In wild-type cells, the number of centromeric foci decreased from 10 at zygotene to 5 at pachytene. In contrast, top1α1 cells displayed fewer and larger foci at zygotene (4 foci) and pachytene (3 foci). While both wild-type and top1α1 diakinesis and metaphase I cells had the expected 5 pairs (10 signals) of centromeres, top1α1 cells frequently exhibited univalent and multivalent chromosomes. No significant differences were observed between WT and top1α1 cells from anaphase I to telophase II. Blue: DAPI staining of chromosomes; Green: centromere FISH signals. n = number of cells observed with the corresponding phenotype. Scale bar = 10 μm.

Figure 4. FISH with a 45S rDNA probe revealed the same number of signals in wild-type and *top1α1*plantsat leptotene and zygotene. At pachytene, the WT showed a single intense 45S rDNA signal, while top1α1 displayed two separate signals. At metaphase I, WT signals were located on two bivalent chromosomes (2-4), whereas top1α1 signals were on two bivalents and a univalent chromosome. Both WT and top1α1 cells displayed similar foci at diakinesis, anaphase I, and anaphase II. Blue indicates DAPI-stained chromosomes; red indicates 45S rDNA FISH signals. Scale bar = 10 μm.

Figure 5. Comparing the average number of chiasmata formations per one PMC in the wild-type and *top1α1* at metaphase I.

Figure 6. The meiotic chromosomal behavior of atm (by DAPI). (**A**) Leptotene, (**B**) zygotene, (**C**) pachytene. (**D**) At diplotene, the beginning of the emergence of interlocking and overlapping between non-homologous chromosomes. (**E**) At diakinesis, intertwined between the non-homologous chromosomes. (**F**) At metaphase I, atm formed a multivalent link between three or more chromosomes and univalent link. From anaphase I (G,H) through telophase II (K), bridges between chromosome groups and chromosome fragmentation are observed. Telophase II cells also exhibit unequal chromosome numbers. White indicates chromosomes stained with DAPI. Scale bar = 20μm. Red arrows indicate incomplete migration of chromosomes.

Figure 7. The meiotic chromosomal behavior *atm×top1α1*(by DAPI). (**A**–**C**) Zygotene, (**D**,**E**) pachytene, (**D**) anaphase I, (**G**–**I**) telophase II. White indicates chromosomes stained with DAPI. Scale bar = 10 μm. (**A**–**E**) Red arrows indicate incomplete chromosomal duplication. (**G**–**I**) The red arrows indicate the presence of chromosome pieces that did not migrate to one of the daughter cells.

Figure S1. Decreased pollen viability in *top1α1*. Pollen viability was determined using the I_2KI staining process. Microscopic analysis revealed large number of pollen grains that did not reach

full maturity. Red arrows indicate abnormal pollens and blue arrows indicate normal pollens in top1α1. Scale bar = 50 μm.

Figure S2. TOP1α is essential for chromosome segregation at centromeres during prophase I. This figure demonstrates the variation in centromere signal numbers in the *top1α1* at the zygotene stage. *top1α1*exhibits fewer and larger foci at zygotene, ranging from 6 to 1 focus per cell. (A) 6 foci (~16%, 28/175), (B) 5 foci (~26%, 45/175), (C) 4 foci (~23%, 40/175), (D) 3 foci (~23%, 40/175), (E) 2 foci (~9%, 15/175), (F) 1 focus (~3%, 5/175). White and blue indicate chromosomes stained with DAPI; green indicates the centromere signals of FISH. Scale bar = 20μm.

Figure S3. FISH analysis using a centromere probe reveals variation in centromere signal numbers in the *top1α1*mutant at the pachytene stage. Compared to zygotene, the number of centromere signals in *top1α1* cells decreased at pachytene. The foci range from 5 to 1 focus per cell. (A) 5 foci (~11.5%, 30/260), (B) 4foci(~19%, 50/260), (C) 3 foci (~52%, 135/260), (D) 2 foci (~13.5, 35/260), (E) 1 focus (~4%, 10/260). White and blue indicate chromosomes stained with DAPI; green indicates the centromere signals of FISH. Scale bar = 20 μm.

Figure S4.FISH analysis with a telomere prober eveals similar telomere signals in wild-type and *top1α1* cells at leptotene, zygotene, and pachytene stages. No significant difference in the number of telomere foci (9-10, 10, and 10, respectively) was observed between wild-type and top1α1 cells (n=45). (A, C, and E) show wild-type, (B, D, and F) show *top1α1*.Blue indicates chromosomes stained with DAPI; red: indicates the telomere signals of FISH. Scale bar=10 μm.

Figure S5.FISH analysis with a 45s rDNA reveals localization defects in top1α1 cells at metaphase I. In *top1α1* cells, signals were found on only three bivalents (~90%, 45/50), suggesting a defect in 45s rDNA localization. White and blue indicate chromosomes stained with DAPI; red: indicates the 45s signals of FISH. Scale bar = 10μm.

Figure 1

top1α1 plants exhibited reduced fertility and induced abnormal meiotic products.

The anther of the wild-type exhibited a density of pollen grains (**A**), while the anthers of the top1α1 mutant displayed a significantly lower abundance of pollen grains(**B**). Unlike the wild-type, *top1α1,* tetrads with four microspores contained polyads with small microspores, suggesting the occurrence of a meiotic abnormality. A total of 50 observations were conducted, revealing that 6% of the observed tetrads had abnormalities. A wild-type of tetrad with four microspores (**C**). *top1α1* polyads with additional small microspores (**D**,**E**). *AtTOP1α* mRNA is disrupted across the T-DNA insertion sites in the *top1α1* line (**F**,**G**). Scale bars= 50 μm (**A**,**B**) and 20 μm (**C**–**E**).

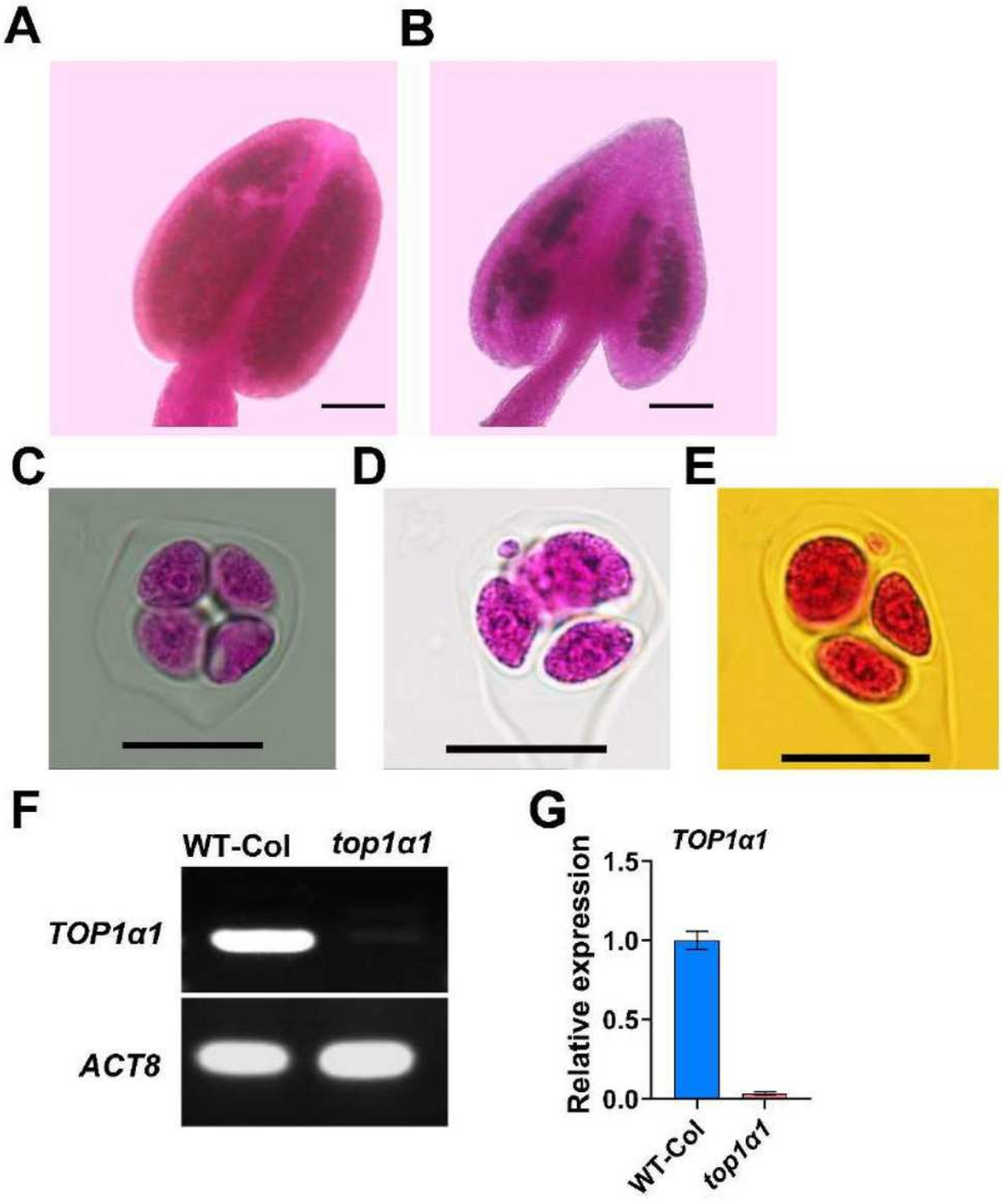

A
B
C
D
E
F
WT-Col
top1α1
TOP1α1
ACT8
G
TOP1α1
Relative expression
1.5
1.0
0.5
0.0
WT-Col
top1α1

Figure 2

DAPI staining of meiotic chromosomes revealed abnormal chromosomal behavior in top1α1 mutants during prophase I.

Wild-type chromosome behavior is shown in panels A-K, while panels A1-K1 correspond to top1α1. Unlike the wild-type, top1α1 displayed abnormal chromosome features from zygotene to metaphase I, including the formation of multivalent links between chromosomes instead of normal bivalents and interlocks between homologous chromosomes. Chromosomes were stained with DAPI. Scale bar = 10μm.

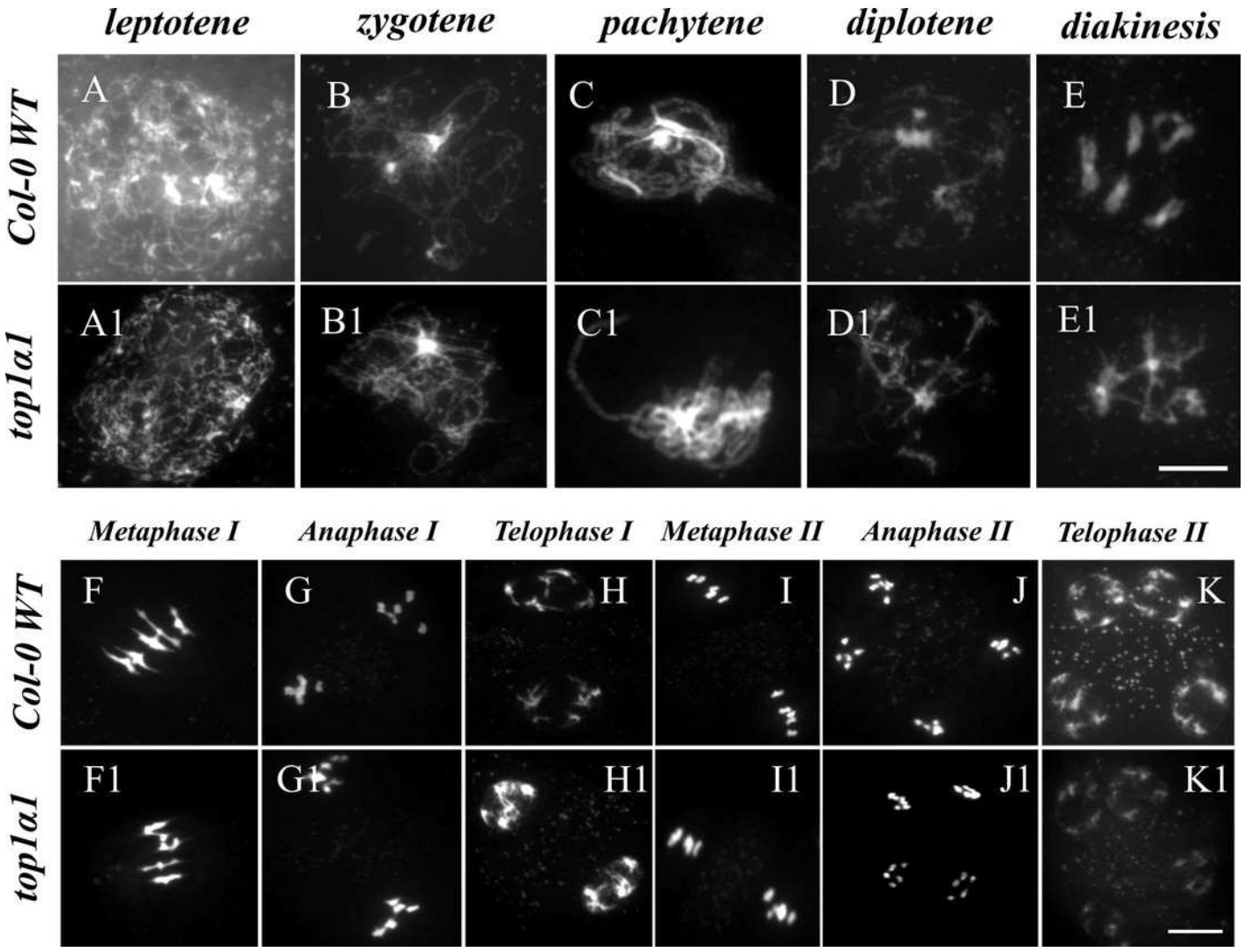


Figure 3

top1α1 induces unbalanced chromosome segregation. FISH analysis with a centromere probe.

(A-P) Wild-type (A, C, E, G, I, K, M, O) and top1α1 (B, D, F, H, J, L, N, P) meiotic cells at leptotene (A, B), zygotene (C, D), pachytene (E, F), diakinesis (G, H), metaphase I (I, J), anaphase I (K, L), metaphase II (M, N), and anaphase II (O, P) stages.FISH with a centromere probe revealed a similar number of signals in wild-type and top1α1 cells at leptotene (10 in wild-type and 8-10 in top1α1). In wild-type cells, the number of centromeric foci decreased from 10 at zygotene to 5 at pachytene. In contrast, top1α1 cells displayed fewer and larger foci at zygotene (4 foci) and pachytene (3 foci). While both wild-type and top1α1 diakinesis and metaphase I cells had the expected 5 pairs (10 signals) of centromeres, top1α1 cells frequently exhibited univalent and multivalent chromosomes. No significant differences were observed between WT and top1α1 cells from anaphase I to telophase II. Blue: DAPI staining of chromosomes; Green: centromere FISH signals. n = number of cells observed with the corresponding phenotype. Scale bar = 10 μm.

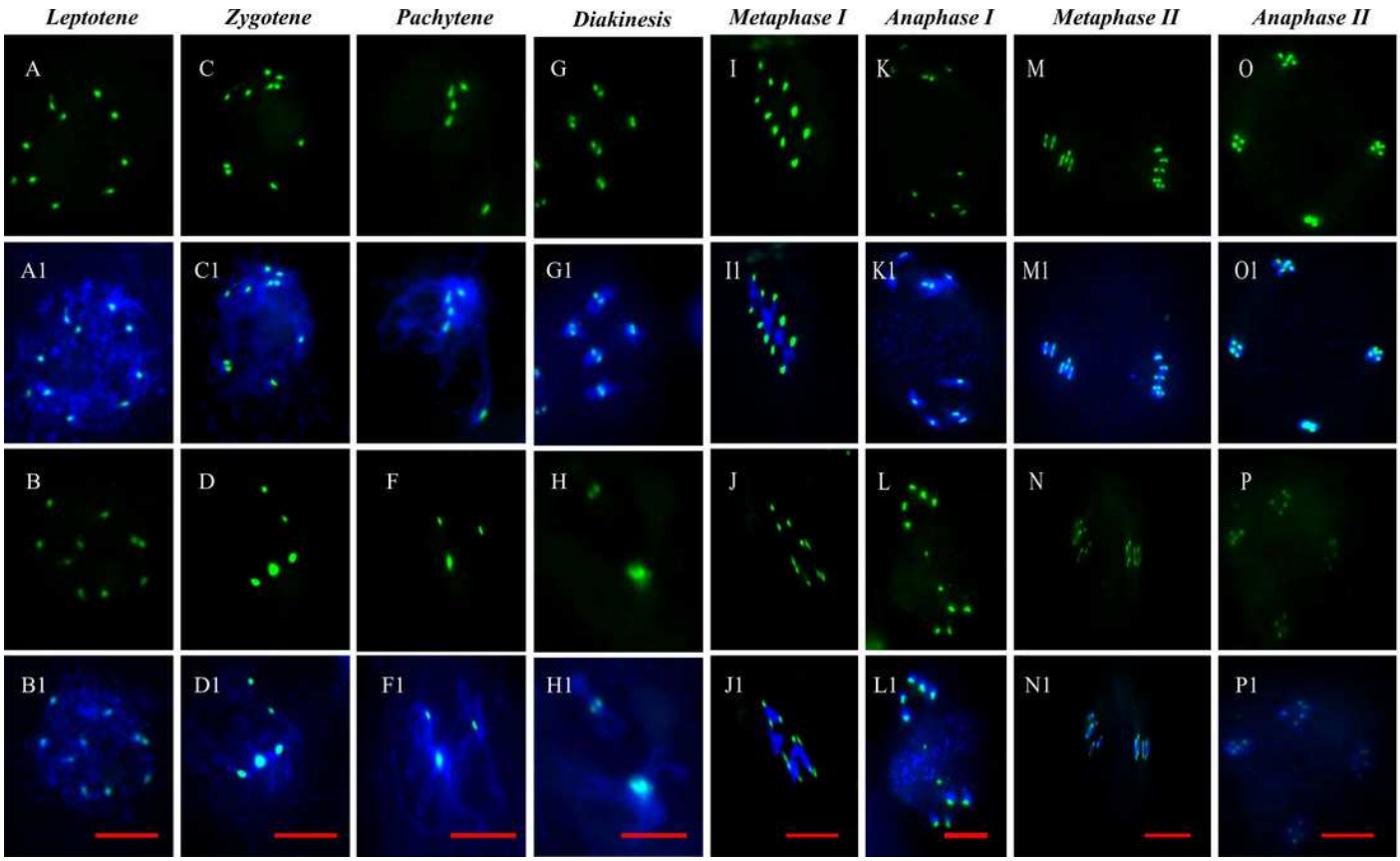

Leptotene
Zygotene
Pachytene
Diakinesis
Metaphase I
Anaphase I
Metaphase II
Anaphase II
A
C
G
I
K
M
O
A1
C1
G1
I1
K1
M1
O1
B
D
F
H
J
L
N
P
B1
D1
F1
H1
J1
L1
N1
P1

Figure 4

FISH with a 45S rDNA probe revealed the same number of signals in wild-type and *top1α1*plantsat leptotene and zygotene.

At pachytene, the WT showed a single intense 45S rDNA signal, while top1α1 displayed two separate signals. At metaphase I, WT signals were located on two bivalent chromosomes (2-4), whereas top1α1 signals were on two bivalents and a univalent chromosome. Both WT and top1α1 cells displayed similar foci at diakinesis, anaphase I, and anaphase II. Blue indicates DAPI-stained chromosomes; red indicates 45S rDNA FISH signals. Scale bar = 10 μm.

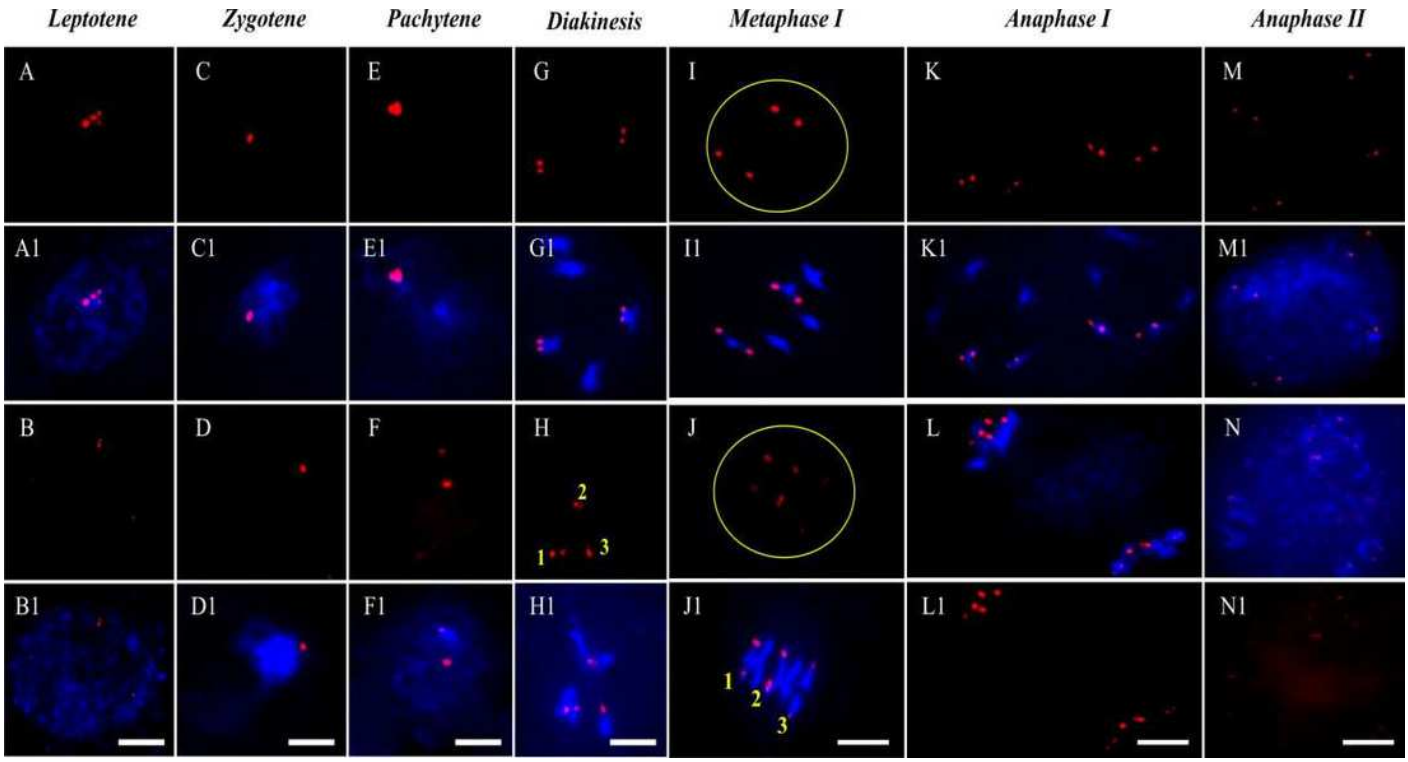
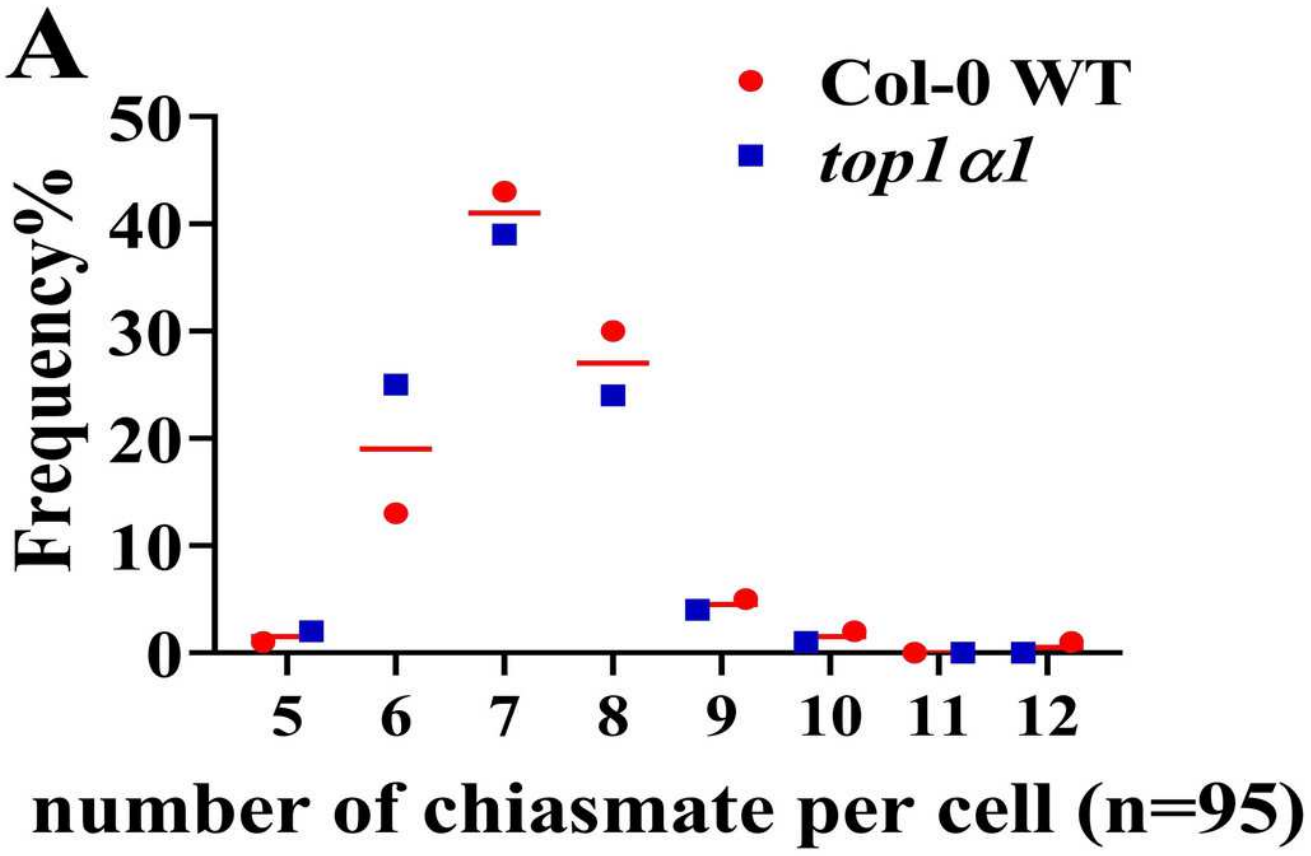


Figure 5

Comparing the average number of chiasmata formations per one PMC in the wild-type and *top1α1* at metaphase I.


A
Col-0 WT
top1α1
Frequency%
50
40
30
20
10
0
5
6
7
8
9
10
11
12
number of chiasmate per cell (n=95)

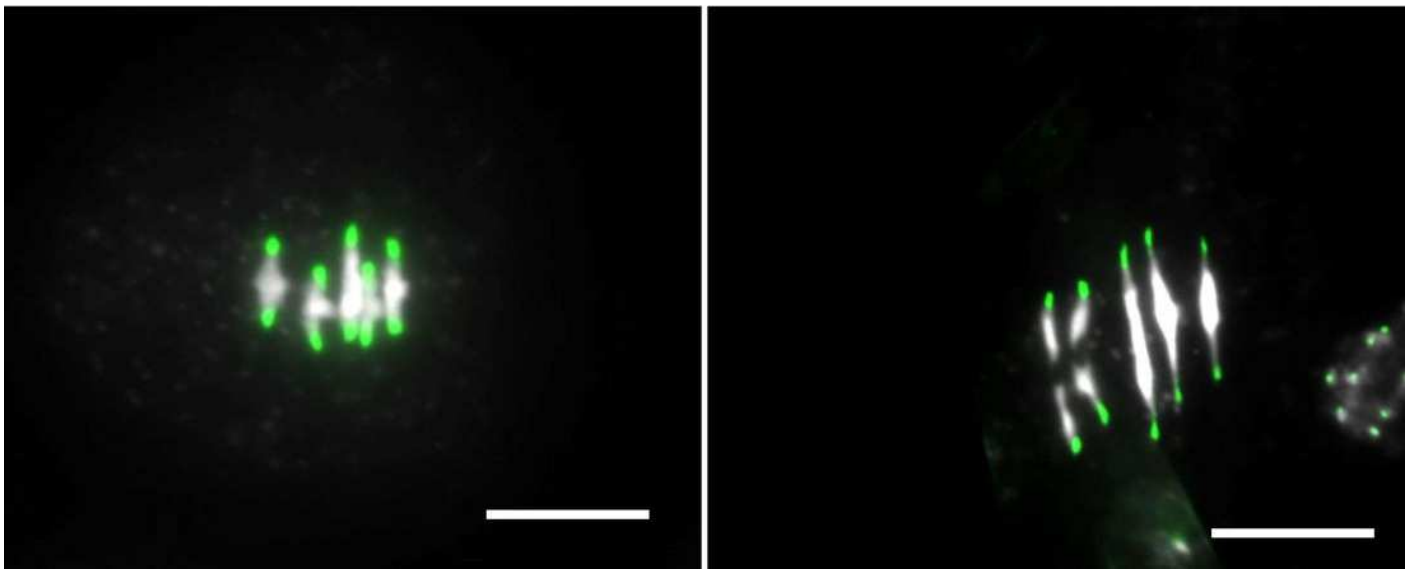


Figure 6

The meiotic chromosomal behavior of atm(by DAPI).

(**A**) Leptotene, (**B**) zygotene, (**C**) pachytene. (**D**) At diplotene, the beginning of the emergence of interlocking and overlapping between non-homologous chromosomes. (**E**) At diakinesis, intertwined between the non-homologous chromosomes. (**F**) At metaphase I, atm formed a multivalent link between three or more chromosomes and univalent link. From anaphase I (G,H) through telophase II (K), bridges between chromosome groups and chromosome fragmentation are observed. Telophase II cells also exhibit unequal chromosome numbers. White indicates chromosomes stained with DAPI. Scale bar = 20μm. Red arrows indicate incomplete migration of chromosomes.

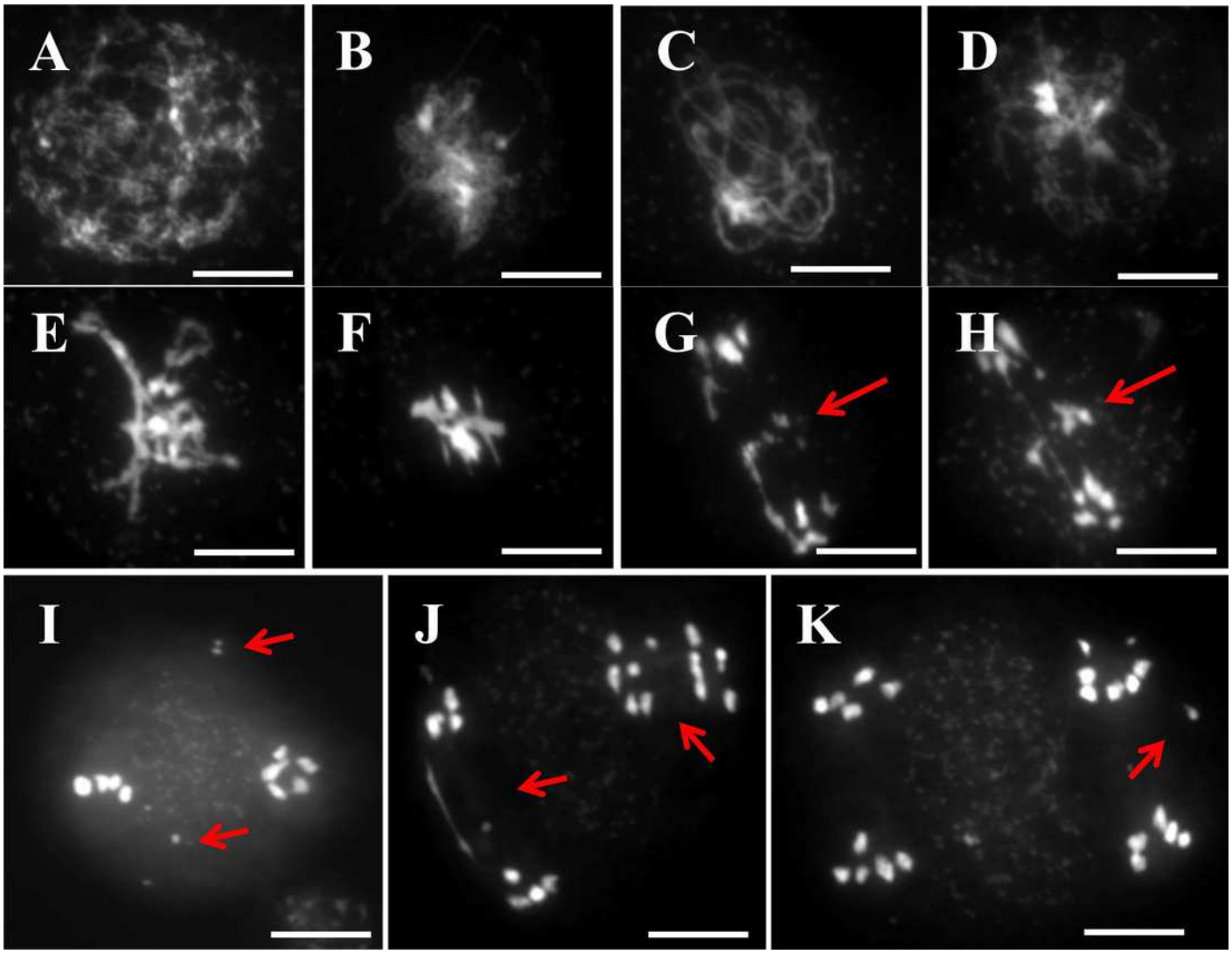
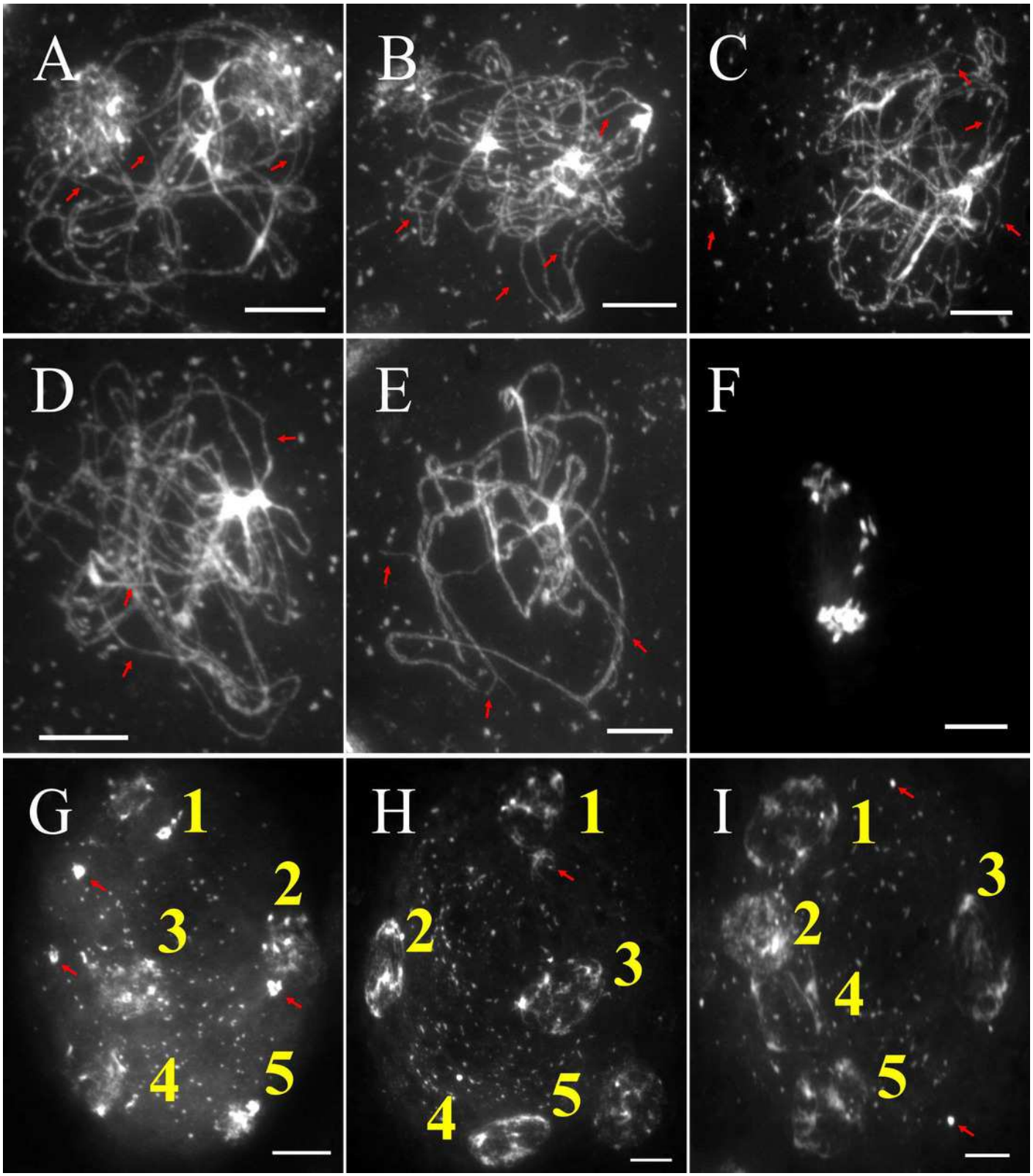

A
B
C
D
E
F
G
H
I
J
K

Figure 7

The meiotic chromosomal behavior *atm*×*top1α1*(by DAPI).

(**A**–**C**) Zygotene, (**D**,**E**) pachytene, (**D**) anaphase I, (**G**–**I**) telophase II. White indicates chromosomes stained with DAPI. Scale bar = 10 μm. (**A**–**E**) Red arrows indicate incomplete chromosomal duplication. (**G**–**I**) The red arrows indicate the presence of chromosome pieces that did not migrate to one of the daughter cells.


A
B
C
D
E
F
G
1
2
3
4
5
H
1
2
3
4
5
I
1
2
3
4
5